UNIVERSITAT DE BARCELONA

Variabilidad Farmacodinámica en la Respuesta a Clopidogrel: Mecanismos Implicados y Uso de Inhibidores Más Potentes del Receptor Plaquetario $P2Y_{12}$ en Pacientes con Enfermedad Coronaria

Jorge Carlos Espinós Pérez

Universidad de Barcelona

Facultad de Medicina – Campus de Ciencias de la Salud de Bellvitge

Departamento de Ciencias Clínicas

Variabilidad Farmacodinámica en la Respuesta a Clopidogrel: Mecanismos Implicados y Uso de Inhibidores Más Potentes del Receptor Plaquetario $P2Y_{12}$ en Pacientes con Enfermedad Coronaria

Tesis doctoral presentada por

JOSÉ LUIS FERREIRO GUTIÉRREZ

para optar al grado de Doctor en Medicina

Directores: **Dr. DOMINICK J. ANGIOLILLO** y **Dr. ÁNGEL CEQUIER FILLAT**

Barcelona, 1 de diciembre de 2015

El Dr. Ángel Cequier Fillat, Director del Área de Enfermedades del Corazón del Hospital Universitario de Bellvitge y Profesor de la Facultad de Medicina de la Universidad de Barcelona, y el Dr. Dominick J. Angiolillo, Director of Cardiovascular Research y Assistant Professor of Medicine en la University of Florida College of Medicine-Jacksonville (Jacksonville, Florida, USA)

HACEN CONSTAR

Que José Luis Ferreiro Gutiérrez, licenciado en Medicina, ha realizado bajo su dirección el trabajo de investigación para elaborar su Tesis Doctoral titulada **"Variabilidad Farmacodinámica en la Respuesta a Clopidogrel: Mecanismos Implicados y Uso de Inhibidores Más Potentes del Receptor Plaquetario $P2Y_{12}$ en Pacientes con Enfermedad Coronaria"**, la consideran finalizada y autorizan su presentación para ser defendida ante el tribunal que corresponda para optar al Grado de Doctor en Medicina

En Barcelona, 1 de diciembre de 2015

Dr. Ángel Cequier Fillat

Dr. Dominick J. Angiolillo

A Mariana, mi esposa, y a José Luis, mi hijo, las personas más importantes de mi vida... mi inspiración y motivación

A mis padres y mi hermano, por su amor y apoyo incondicional... por estar siempre ahí

AGRADECIMIENTOS

¿Qué es la vida? Una ilusión,
una sombra, una ficción,
y el mayor bien es pequeño:
que toda la vida es sueño,
y los sueños, sueños son.

(Segismundo en "La vida es sueño")
Pedro Calderón de la Barca

Es difícil no cometer un descuido al intentar recordar a todas las personas que han contribuido a mi desarrollo personal o profesional y que han podido aportar de algún modo a la realización de esta tesis, pero voy a intentarlo con la mejor intención. Por todo ello: "Si no están todos los que son, mis disculpas; pero sin duda todos los que están, son."

Quiero expresar mi agradecimiento sincero:

A mis directores de tesis, quienes además de dirigirla con dedicación han contribuido de manera capital a mi formación clínica, en investigación y en otros muchos aspectos importantes para mi carrera profesional; he aprendido, y sigo aprendiendo, mucho de vosotros. Al Dr. Angiolillo por ser mi mentor en el "mundo de las plaquetas", por todo el tiempo y esfuerzo que me ha dedicado con extrema generosidad, por el impulso que le ha dado a mi carrera y, lo más importante, por ser un tipo excepcional y convertirse en mi amigo; Dominick, siempre podrás contar conmigo. Al Dr. Cequier por animarme a emprender un camino distinto, por haber creído en mí y por apoyarme de manera personal e institucional para crear el Laboratorio de Investigación Cardiovascular y confiarme su dirección.

A todo el personal del Cardiovascular Research Center y de la Division of Cardiology de la University of Florida College of Medicine – Jacksonville por su profesionalidad, por el magnífico trato personal y por su colaboración en todos los proyectos en los que participé. Un agradecimiento especial a Bhaloo Desai y Jennifer Zhai, por portarse maravillosamente bien conmigo, y a los otros fellows de investigación con los que compartí penas y alegrías. A mi gran amigo el Dr. Masafumi Ueno, por ser mi inseparable compañero de fatigas durante mi estancia en Jacksonville y "my Japanese brother" para siempre; Masa, siempre podrás contar conmigo.

A todo el personal (médicos, enfermeras, auxiliares…) del Área de Enfermedades del Corazón del Hospital Universitario de Bellvitge por la formación, el trato humano y la ayuda recibida todos estos años.

A todo el personal de la Unidad de Hemodinámica del Hospital Universitari de Bellvitge, por convertir el trabajo diario en un auténtico placer. Al Dr. Gómez-Hospital, por sus enseñanzas y su confianza, y a todos mis compañeros sin excepción (los actuales y

los pasados, como el Dr. Jara), por sus enseñanzas y su compañerismo, con un recuerdo particular al Dr. Gómez-Lara y al Dr. Roura, ya son muchos años juntos. A la enfermería y auxiliares de la unidad por ser como sois y por colaborar en mis proyectos de investigación de manera desinteresada. A todos ellos, por aguantar mis "escasos" momentos de "ligero" mal humor.

A todos los que hacen posible el funcionamiento del Laboratorio de Investigación Cardiovascular; a Gaby, por su dedicación, a la Dra. Marcano, la primera fellow en esta aventura, y a todos los residentes que colaboran.

A mis compañeros de residencia, principalmente a "Los Animales de Bellota", a mis compañeros de pool y a mis compañeros (R grandes y pequeños) en Cardiología, por vuestra ayuda, enseñanzas y cariño.

A mis compañeros de facultad, destacando a mis amigos de "Las 5 Jotas", el Dr. Sánchez, el Dr. Sabadell, el Dr. Cortés y el Dr. Vilaseca; siempre podréis contar conmigo.

A mis compañeros en los equipos de balonmano, por aportarme valores que sólo el deporte puede ofrecer.

A mis amigos de siempre, al "BEJA" (Paco, Rober, Diego), y a otros como Josep, por los momentos vividos y los que vendrán, además de por haberse adaptado a mi perenne falta de disponibilidad. Un agradecimiento especial a Paco y Mari Carmen, por estar siempre ahí.; siempre podréis contar conmigo.

A mi familia, gallega y castellana, porque nunca hay que olvidar los orígenes. A mi familia política peruana, por su magnífica acogida y hacerme sentir parte de sus vidas.

A mis padres, por todo el cariño que me han dado, por los valores que me han transmitido y por el esfuerzo y los sacrificios que han realizado para que yo tuviera siempre lo que necesitaba. A mi hermano, por su apoyo y por estar siempre ahí cuando era necesario. A ellos, por ser capaces de aguantar mi temperamento y porque soy absolutamente consciente de lo afortunado que soy por tenerlos.

A mi mujer, Mariana, y a mi hijo, José Luis, porque vosotros le dais sentido a todo.

ÍNDICE

LISTADO DE ABREVIATURAS

AAS	Ácido acetilsalicílico
ADP	Adenosín difosfato
ARI	Arteria responsable del infarto
ATP	Adenosín trifosfato
CYP	Citocromo P450
DAP	Doble antiagregación plaquetaria
DM	Diabetes mellitus
GP	Glucoproteína
HTPR	Respuesta subóptima al tratamiento
IAMCEST	Infarto agudo de miocardio con elevación del segmento ST
IBP	Inhibidor de la bomba de protones
ICP	Intervencionismo coronario percutáneo
IPA	Inhibición de la agregación plaquetar
IRC	Insuficiencia renal crónica
LPA	Agregación plaquetar tardía
LTA	Agregometría óptica
MEA	Agregometría de electrodos múltiples
MDR1	Transportador de resistencia a múltiples fármacos
MPA	Agregación plaquetar máxima
PRI	Índice de reactividad $P2Y_{12}$
PRU	Unidades de reacción $P2Y_{12}$
SCA	Síndrome coronario agudo

SCASEST Síndrome coronario agudo sin elevación del segmento ST

TRAP Péptido agonista del receptor de trombina

TxA_2 Tromboxano A_2

VASP Fosfoproteína estimulada por vasodilatador

LIST OF ABBREVIATIONS

ACS	Acute coronary syndrome
ADP	Adenosine diphosphate
ASA	Aspirin (Acetylsalicylic acid)
ATP	Adenosine triphosphate
CKD	Chronic kidney disease
CYP	Cytochrome P450
DAPT	Dual antiplatelet therapy
DM	Diabetes mellitus
GP	Glycoprotein
HTPR	High on-treatment platelet reactivity
IPA	Inhibition of platelet aggregation
IRA	Infarct-related artery
LPA	Late platelet aggregation
LTA	Light transmittance aggregometry
MDR1	Multidrug resistance transporter
MEA	Multiple electrode aggregometry
MPA	Maximal platelet aggregation
PCI	Percutaneous coronary intervention
PPI	Proton-pump inhibitor
PRI	$P2Y_{12}$ reactivity index
PRU	$P2Y_{12}$ reaction units
NSTE-ACS	Non-ST-segment elevation acute coronary syndrome

STEMI ST-segment elevation myocardial infarction

TRAP Thrombin-receptor agonist peptide

TxA_2 Thromboxane A_2

VASP Vasodilator-stimulated phosphoprotein

1. INTRODUCCIÓN

Tan largo me lo fiáis.

(DON JUAN TENORIO EN "EL BURLADOR DE SEVILLA")

TIRSO DE MOLINA

La aterosclerosis es la principal causa subyacente de enfermedad arterial coronaria o cardiopatía isquémica, siendo un proceso inflamatorio crónico que produce un estrechamiento progresivo de las arterias coronarias [1,2]. En caso de producirse la rotura o erosión de una placa aterosclerótica se inician una serie de mecanismos que dan lugar a dicho nivel a la formación de trombo, fenómeno en el que las plaquetas juegan un papel esencial [3,4]. Esta rotura puede ocurrir de manera espontánea, como en un síndrome coronario agudo (SCA), o iatrogénica, como tras la realización de un intervencionismo coronario percutáneo (ICP).

Las plaquetas, además de ser el primer paso en la hemostasia primaria, son un elemento clave en el desarrollo de las complicaciones aterotrombóticas derivadas de la aterosclerosis, interviniendo a través de un proceso que consta clásicamente de tres fases: adhesión, activación y agregación [3,4]. En breve, tras la rotura o erosión de una placa aterosclerótica, quedan expuestas o son liberadas determinadas substancias (son particularmente relevantes en todo el proceso de trombosis el colágeno, el factor tisular y el factor de von Willebrand) que promoverán el reclutamiento y adhesión de las plaquetas circulantes a la zona de rotura de placa o daño endotelial, lo que es seguido de la activación y agregación de las mismas [5], destacando que el componente plaquetar es el más numeroso y relevante en la fase inicial de formación del trombo [4,5]. Así pues, dado que la función plaquetar juega un papel preponderante y absolutamente esencial en la producción de eventos aterotrombóticos, esto subraya la importancia del uso de los fármacos antiagregantes plaquetarios en

los pacientes con un SCA o en los que se realiza un ICP, representando la piedra angular del tratamiento en estos escenarios.

Cada una de las fases implicadas en el funcionalismo plaquetar (adhesión, activación y agregación) constituye una posible diana para el desarrollo de fármacos antitrombóticos. Los inhibidores de la adhesión plaquetaria se encuentran todavía en fase de investigación y actualmente no hay ninguno autorizado para uso clínico [6]. Los inhibidores de la glucoproteína (GP) IIb/IIIa son fármacos endovenosos que bloquean el paso final común de la agregación plaquetaria (la unión del receptor GP IIb/IIIa a fibrinógeno, factor de von Willebrand, fibronectina y protrombina) y cuyo uso clínico está limitado a la fase aguda del tratamiento de pacientes con un SCA de alto riesgo en los que se realiza un ICP, especialmente si existe una gran carga trombótica o utilizados en situaciones “de rescate” [7]. Como veremos en detalle a continuación, son los inhibidores de los procesos de activación plaquetaria los que constituyen la piedra angular del tratamiento y la prevención a corto y largo plazo de la recurrencia de episodios isquémicos en los pacientes con SCA, incluidos la angina inestable, el síndrome coronario agudo sin elevación del segmento ST (SCASEST) y el infarto de miocardio con elevación del segmento ST (IAMCEST), o en los que se realiza un ICP [7-9].

Existen en la actualidad dos grupos de inhibidores de la activación plaquetaria autorizados en la práctica clínica para el tratamiento y la prevención de la recurrencia de episodios isquémicos en el contexto de SCA o ICP: a) antagonistas de la vía del tromboxano A_2 (TxA_2): el ácido acetilsalicílico (AAS), un inhibidor irreversible de la ciclooxigenasa-1 mediante la acetilación selectiva

de un residuo de serina en la posición 529 (Ser529) que impide la formación de TxA_2 [10], es el único fármaco disponible de este grupo, cuya eficacia en el seno de la enfermedad coronaria ha sido ampliamente demostrada [11,12]; y b) antagonistas del receptor plaquetario de adenosín difosfato (ADP) $P2Y_{12}$: ticlopidina, clopidogrel, prasugrel y ticagrelor. La doble antiagregación plaquetaria (DAP) con AAS y un inhibidor del receptor $P2Y_{12}$ es actualmente el tratamiento antiplaquetario de elección en todo el espectro de pacientes con un SCA o en los que se practica ICP [7-9].

1.1. Receptor purinérgico plaquetario $P2Y_{12}$: Generalidades

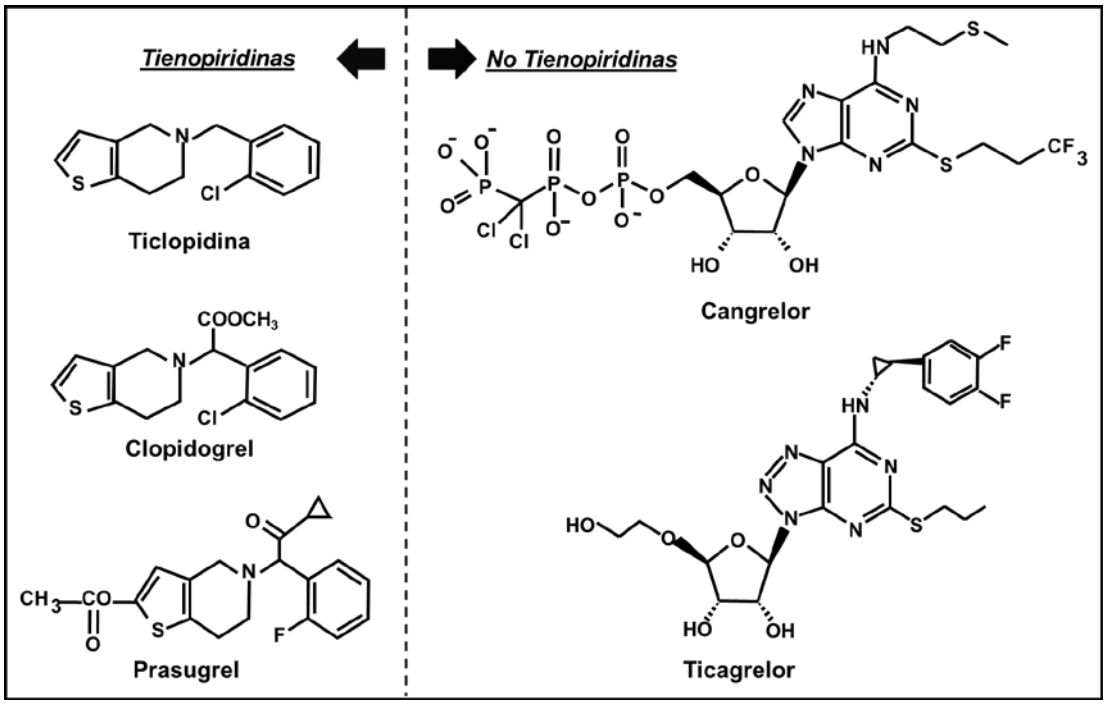
Los receptores purinérgicos con expresión plaquetar conocidos en la actualidad son los siguientes: $P2X_1$, $P2Y_1$ y $P2Y_{12}$. El $P2X_1$, cuyo agonista fisiológico es el adenosín trifosfato (ATP), es un canal catiónico regulado por ligando que interviene en el cambio de la forma de las plaquetas a través de un flujo de entrada de calcio extracelular, además de facilitar una amplificación de las respuestas mediadas por otros agonistas [13]. El ADP ejerce su acción sobre las plaquetas a través de los receptores purinérgicos de siete dominios transmembrana acoplados a proteína G $P2Y_1$ y $P2Y_{12}$, de los que es el agonista fisiológico [14]. La activación del receptor $P2Y_1$ produce un cambio transitorio de forma de las plaquetas, movilización del calcio intracelular y liberación de gránulos de otros mediadores para finalmente, iniciar una fase transitoria y débil de agregación plaquetaria [14]. Aunque son necesarios ambos receptores P2Y para producir una agregación completa [15], los efectos del ADP en la función plaquetar se producen predominantemente a través de la

vía de señalización del receptor $P2Y_{12}$. En resumen, la activación de esta vía causa una serie de procesos intracelulares que conducen a movilización del calcio, liberación del contenido granular, generación de TxA_2 y finalmente activación del receptor de la GP IIb/IIIa, lo que resulta en una amplificación de la agregación plaquetaria y en la estabilización del agregado plaquetario [12,15,16]. En consecuencia, el bloqueo del receptor $P2Y_{12}$ es crucial para inhibir la activación y agregación plaquetarias y tratar de impedir, por ende, la formación de trombo plaquetario.

Los fármacos antagonistas del receptor $P2Y_{12}$ disponibles actualmente son de administración oral y se pueden agrupar en: a) ticlopidina, clopidogrel y prasugrel, tres generaciones del grupo farmacológico de las tienopiridinas, antagonistas indirectos (profármacos que precisan de biotransformación hepática para convertirse en su metabolito activo) que inhiben de manera irreversible el receptor $P2Y_{12}$; y b) ticagrelor, una ciclopentiltriazolopirimidina, que inhibe el receptor de manera directa (sin necesidad de conversión de un profármaco en un metabolito activo) y reversible (Figura 1). La inhibición de la vía del receptor $P2Y_{12}$ es una diana terapéutica establecida en pacientes con enfermedad coronaria, cuya importancia queda confirmada por el beneficio clínico demostrado, en asociación con AAS, desde los primeros estudios realizados con ticlopidina (el primer antagonista del receptor $P2Y_{12}$ comercializado) a mediados de los años 90 [17]. La ticlopidina, una tienopiridina de primera generación, en combinación con AAS demostró ser superior a la monoterapia con AAS y a la anticoagulación añadida a AAS en el contexto de ICP en cuanto a reducción de eventos isquémicos [18-21]. Debido

a ciertos problemas de seguridad, principalmente tasas elevadas de neutropenia, la ticlopidina fue pronto reemplazada ampliamente por clopidogrel, una tienopiridina con una eficacia similar y un mejor perfil de seguridad [22].

Figura 1. Estructura química de los inhibidores del receptor plaquetario $P2Y_{12}$



1.2. Clopidogrel: Variabilidad de respuesta

El clopidogrel, una tienopiridina de segunda generación, es un profármaco que, como todos los miembros de su grupo, precisa de una biotransformación hepática para ser convertido en su metabolito activo, que es el que finalmente se une de manera irreversible al receptor $P2Y_{12}$ y lo bloquea. Aproximadamente el 15% del clopidogrel absorbido en el torrente sanguíneo (el

85% restante es hidrolizado por esterasas plasmáticas e inactivado) se metaboliza en el hígado por un doble proceso de oxidación en el que intervienen varias isoformas del citocromo P450 (CYP), para convertirse en su metabolito activo [23]. Dado que la inhibición del receptor $P2Y_{12}$ es irreversible, los efectos del clopidogrel persisten durante toda la vida de la plaqueta (7-10 días). El clopidogrel presenta un inicio de acción lento, requiriendo el empleo de una dosis de carga (generalmente 300 ó 600 mg) para acortarlo cuando se precisa una inhibición plaquetar rápida, como ocurre en el contexto de un SCA o un ICP, continuándose el tratamiento con una dosis de mantenimiento de 75 mg al día. En la práctica clínica se ha generalizado el uso de una dosis de carga de 600 mg, al presentar un efecto más rápido y potente que la de 300 mg [24-26], estando también avalada esta estrategia en las guías de práctica clínica [7-9].

Desde su aprobación para uso clínico en 1997, el clopidogrel sustituyó rápidamente a la ticlopidina debido fundamentalmente a su mejor perfil de seguridad, fundamentalmente en lo referente a toxicidad hematológica [22], contando además con la ventaja de poder conseguir un inicio de acción más rápido gracias a la administración de una dosis de carga [27]. Durante más de una década, hasta la aparición de los nuevos y más potentes antagonistas del receptor $P2Y_{12}$ que se comentarán posteriormente, el protagonismo del clopidogrel en asociación con AAS en los contextos clínicos del ICP y los SCA fue indiscutible. De hecho, la DAP con AAS y clopidogrel fue considerada en ese periodo el *"standard of care"* del tratamiento antiagregante oral en dichos escenarios debido al claro beneficio observado en numerosos ensayos clínicos

a gran escala en cuanto a reducción de eventos adversos isquémicos, incluyendo la trombosis del stent [28-32]. A pesar del beneficio observado con este régimen terapéutico, un número importante de pacientes continúa presentando eventos isquémicos en el seguimiento, lo que se ha atribuido en parte al fenómeno conocido como variabilidad de respuesta al tratamiento con clopidogrel.

El principal problema de clopidogrel es, por tanto, su gran variabilidad interindividual de respuesta en los sujetos tratados, lo que conlleva que exista un porcentaje de pacientes relativamente elevado (entre el 5 y el 40%, dependiendo de las características de la población, del test de función plaquetar utilizado y de los valores de corte empleados) que presentan una respuesta disminuida o subóptima al fármaco, también llamada en ocasiones “resistencia” [33]. La trascendencia de la variabilidad de respuesta al clopidogrel se pone de manifiesto en el hecho de que multitud de estudios han evidenciado una asociación entre respuesta pobre o subóptima al fármaco y eventos cardiovasculares isquémicos en el seguimiento [33].

1.2.1. Mecanismos de variabilidad de respuesta

Se han identificado múltiples mecanismos que contribuyen a la variabilidad de respuesta del clopidogrel, pudiendo clasificarse dentro de tres categorías: factores genéticos, celulares y clínicos (Figura 2).

Numerosos estudios farmacogenéticos han evaluado las variantes alélicas o polimorfismos de diferentes genes involucrados en la farmacocinética

y la farmacodinámica del clopidogrel, entre los que se encuentran genes que codifican proteínas participantes en la absorción, el metabolismo hepático y la actividad biológica (receptores de membrana) del clopidogrel. El gen ABCB1 codifica la glucoproteína P intestinal denominada MDR1 (transportador de resistencia a múltiples fármacos o *multidrug resistance transporter*), que interviene en la absorción de clopidogrel. Se ha observado en algún estudio a gran escala que los pacientes homozigotos (portadores de dos variantes alélicas) para un polimorfismo del ABCB1 podrían tener un riesgo superior de eventos cardiovasculares al año de seguimiento tras un infarto de miocardio por el que habían recibido tratamiento con clopidogrel [34]. Esto se ha atribuido a que la presencia de dos variantes alélicas del ABCB1 puede reducir la generación del metabolito activo tras la administración de una dosis de carga del fármaco [35], aunque no está comprobado que ese mismo polimorfismo de ABCB1 se asocie claramente con la respuesta farmacodinámica a clopidogrel [36]. Varias isoformas del sistema CYP participan en el doble proceso de oxidación hepática por el que clopidogrel se transforma en su metabolito activo. En concreto, las isoenzimas CYP3A4, CYP3A5, CYP2C9 y CYP1A2 intervienen sólo en uno de los pasos, mientras que las isoenzimas CYP2B6 y CYP2C19 participan en ambos [23]. Se han descrito en estudios mecanísticos polimorfismos de CYP3A4, CYP3A5, CYP2C9 y CYP2C19 [37-41] que podrían tener un papel en la variabilidad de respuesta a clopidogrel, aunque los estudios farmacogenéticos a gran escala únicamente han podido observar una asociación consistente con eventos clínicos de ciertos polimorfismos de CYP2C19. De hecho, numerosos estudios han mostrado una intensa relación

entre las variantes alélicas de pérdida de función de CYP2C19 (principalmente la CYP2C19*2) y la menor formación del metabolito activo, lo que conlleva una menor inhibición plaquetaria y, finalmente, una peor evolución clínica en cuanto a un aumento del riesgo de eventos isquémicos [36,42-44]. En el otro extremo, la presencia de la variante alélica de ganancia de función CYP2C19*17 se ha asociado (aunque con menor consistencia) con mayor producción de metabolito activo, mayor inhibición de la agregación plaquetaria inducida por clopidogrel y a un aumento del riesgo de sangrados [45]. Polimorfismos de genes que codifican receptores de la membrana plaquetaria, como P2YR12 (receptor $P2Y_{12}$ de ADP), ITGB3 (receptor GPIIb/IIIa de fibrinógeno), ITGA2 (receptor GPIa de colágeno) o PAR-1 (receptor activado por proteasa 1 de trombina), se han señalado también en algunos estudios a pequeña escala como posibles determinantes de la respuesta a clopidogrel, aunque la evidencia al respecto es poco consistente [46].

En lo que respecta a los factores celulares, se han postulado varios que pueden afectar al efecto antiagregante inducido por clopidogrel. Un recambio (*"turnover"*) plaquetar acelerado, típico de los pacientes con diabetes mellitus (DM), se representa por la presencia de plaquetas reticuladas (inmaduras), que poseen una mayor reactividad. Algunos estudios han asociado un mayor porcentaje de plaquetas reticuladas circulantes con una menor respuesta al clopidogrel en pacientes con enfermedad coronaria [47,48]. Se ha propuesto también que la regulación al alza de las vías de señalización plaquetarias, fundamentalmente de la iniciada en los receptores $P2Y_{12}$, podría estar involucrada en un empeoramiento de la respuesta al clopidogrel, especialmente

en pacientes con DM [49]. Finalmente, el diferente grado de actividad metabólica basal del sistema CYP es un factor celular que puede condicionar la transformación de clopidogrel en su metabolito activo y, consecuentemente, su actividad [50].

Múltiples factores clínicos han sido asociados con una mayor agregabilidad plaquetar y una respuesta insuficiente al clopidogrel. Profundizar en el conocimiento de los mismos es de notable importancia, ya que es posible actuar sobre algunos de estos mecanismos (no así en los factores genéticos y difícilmente en los celulares), con los que disminuiría su impacto. Entre los factores clínicos asociados con la respuesta a clopidogrel se encuentran evidentemente el cumplimiento terapéutico, uno de los más relevantes [51], y una posología correcta del fármaco [33]. Existen también características clínicas que afectan a la reactividad plaquetaria y a la respuesta al clopidogrel, como la obesidad [52,53], la DM [54-56] o la presencia de un SCA [57,58]. Cabe destacar las dos últimas por su gran importancia pronóstica, siendo factores clara y fuertemente asociados con una mayor agregabilidad plaquetar y una peor respuesta a los fármacos antiplaquetarios [54-58]. La presencia de un SCA condiciona *per se* un empeoramiento de la respuesta a clopidogrel, siendo relevante además que en el IAMCEST se observa una mayor prevalencia de respuesta subóptima al fármaco que en las otras formas de SCA [57]. Este aspecto resulta de interés dado que con los programas de angioplastia primaria el tiempo entre la administración de los fármacos antiagregantes orales en pacientes con IAMCEST y la realización del ICP se acorta notablemente, lo que puede empeorar todavía más la inhibición

plaquetar conseguida con clopidogrel en el momento periintervencionismo debido a que es un fármaco con un inicio de acción lento [33], con el impacto pronóstico que ello conlleva. En lo que respecta a los pacientes con DM, numerosos mecanismos debidos a anomalías metabólicas y celulares típicas de esta patología acaban conduciendo a una hiperreactividad plaquetar que es, a su vez, uno de los determinantes del estado protrombótico característico de estos pacientes y que juega un papel esencial en la aterosclerosis acelerada y el alto riesgo de complicaciones aterotrombóticas que presentan [59]. Los mecanismos que intervienen en la disfunción plaquetar de los pacientes diabéticos (llegándose a denominar “la plaqueta diabética”) y que acaba produciendo una adhesión, activación y agregación intensificadas, se pueden agrupar en cuatro categorías etiopatogénicas, según se deban a: a) hiperglicemia, b) déficit de acción de la insulina, c) condiciones metabólicas asociadas, o d) otras anormalidades celulares [60-63]. En resumen ese fenotipo plaquetar hiperreactivo provoca una respuesta inadecuada a los fármacos antiagregantes, fundamentalmente a clopidogrel [64,65], lo que contribuye al riesgo aumentado de eventos isquémicos que presentan los pacientes con DM y al menor beneficio relativo que obtienen de las terapias antiagregantes en comparación con los sujetos no diabéticos [65].

La transformación hepática por el sistema del CYP de clopidogrel es un paso crítico para conseguir su efecto antiplaquetario. Por tanto, los fármacos que son activados o metabolizados por las isoformas del CYP involucradas en dicha biotransformación podrían potencialmente interferir en la generación de metabolito activo y, por tanto, en la acción antiplaquetaria de clopidogrel. Varios

estudios, principalmente farmacodinámicos, han señalado una potencial interacción farmacológica de clopidogrel con algunos fármacos usados habitualmente en el tratamiento de pacientes con enfermedad coronaria y que podrían, por tanto, disminuir la potencia antiagregante del fármaco: a) estatinas lipófilas (aunque con resultados discordantes en estudios farmacodinámicos y sin evidencia de asociarse con una evolución clínica adversa en análisis post-hoc de ensayos clínicos o registros de gran tamaño) [66-70]; b) antagonistas del calcio (tipo dihidropiridinas, metabolizados por el CYP3A4) [71,72] y c) inhibidores de la bomba de protones (IBPs).

La posible interacción farmacológica entre los IBPs y el clopidogrel es de particular relevancia por la frecuencia con que se combinan ambos tipos de fármacos, ya que los IBPs se prescriben de manera rutinaria en pacientes con DAP con la intención de prevenir hemorragias gastrointestinales. De hecho, los primeros estudios que reportaron en pacientes con SCA que el tratamiento simultáneo con IBPs y clopidogrel se asociaba de manera significativa con un incremento de eventos cardiovasculares comparado con los pacientes que no tomaban ningún IBP [73,74] provocaron una importante preocupación en la comunidad científica precisamente por la gran frecuencia con que están prescritos ambos fármacos en pacientes con un SCA o sometidos a ICP. El mecanismo sugerido para explicar la interacción entre clopidogrel y los IBPs es una inhibición competitiva a nivel de la isoenzima CYP2C19. De hecho, los resultados más consistentes respecto a esta interacción se han obtenido con omeprazol, el más utilizado de los IBPs, que es metabolizado principalmente por la isoenzima CYP2C19. En concreto, se observó en estudios funcionales

que la administración de omeprazol disminuye el efecto antiagregante de clopidogrel [75] y los primeros análisis *post hoc* de ensayos clínicos y registros a gran escala que evaluaron esta interacción mostraron inicialmente que el uso de omeprazol podía asociarse con una peor evolución clínica en pacientes bajo tratamiento con clopidogrel tras un SCA [73,74]. Sin embargo, los resultados de otros estudios mecanísticos que han evaluado otros IBPs como pantoprazol (metabolizado principalmente por la isoenzima CYP2C9) no permiten establecer conclusiones definitivas sobre si esta interacción farmacodinámica es un efecto de clase (se produce con todos los IBPs) o se produce únicamente con algún fármaco de este grupo (p.ej. omeprazol). Adicionalmente, se planteó que separar el momento de la administración del IBP y clopidogrel podría evitar la interacción farmacológica dado que las concentraciones plasmáticas de clopidogrel y, por ejemplo, omeprazol son casi indetectables a las 6-8 horas tras su toma.

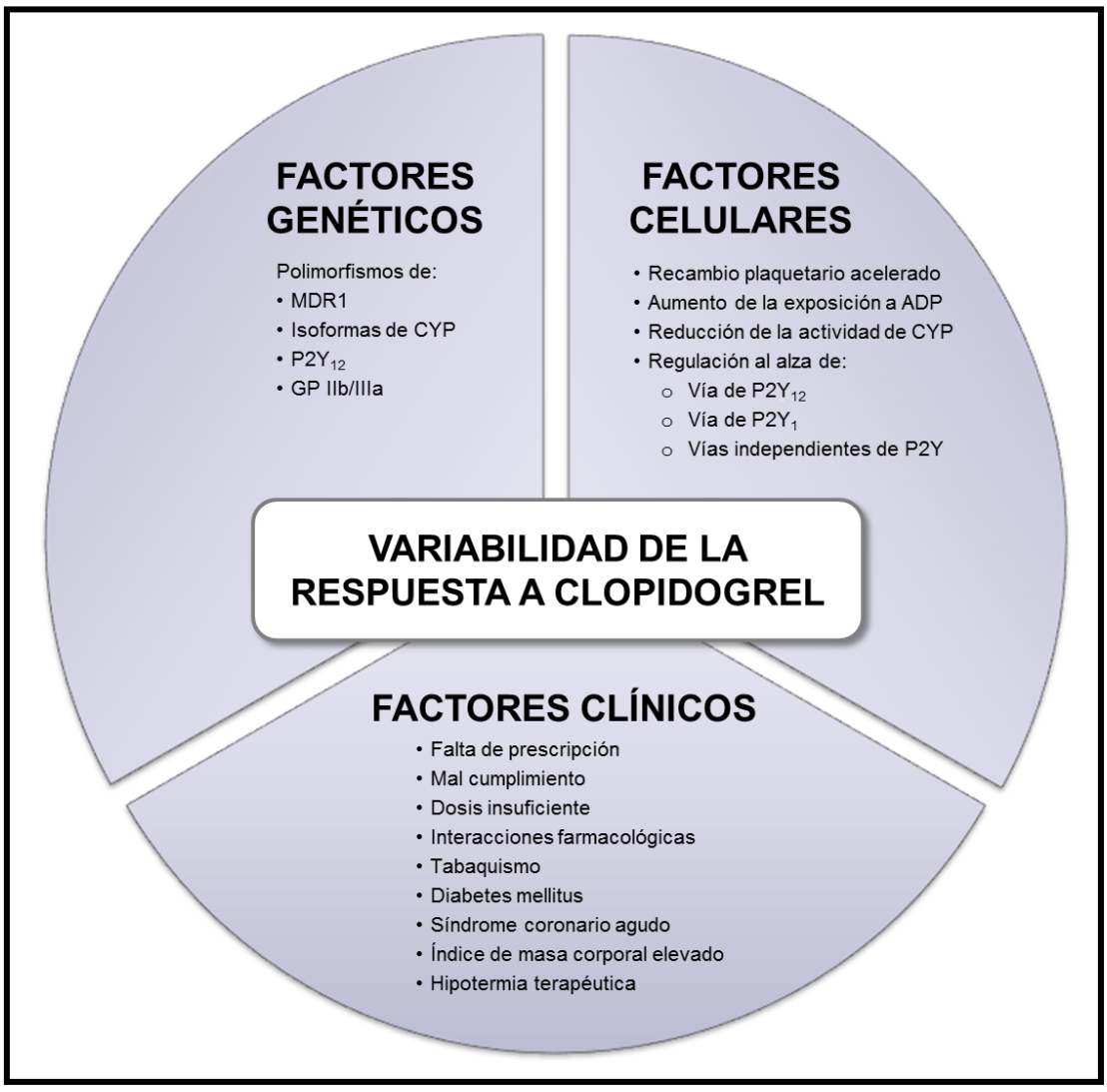
El tabaquismo también podría estar asociado con la variabilidad de respuesta a clopidogrel. Sin tratarse de una interacción medicamentosa como tal, el mecanismo causante también estaría relacionado con la generación del metabolito activo de clopidogrel por el sistema CYP. El consumo de cigarrillos es un potente inductor de la isoforma CYP1A2, por lo que podría aumentar la biotransformación del clopidogrel [76]. Algunos estudios han observado que un hábito tabáquico intenso potencia los efectos antiagregantes de clopidogrel [77], lo que podría conllevar un aumento del beneficio clínico de los pacientes tratados con clopidogrel, como se ha observado en ciertos análisis *post hoc* de ensayos clínicos [78,79]. Sin embargo, cabe recordar que el tabaquismo es un

notable factor de riesgo de procesos cardiovasculares aterotrombóticos y dejar de fumar es una recomendación de clase I para la prevención secundaria de episodios isquémicos en pacientes con enfermedad coronaria. Sin embargo, si existe un impacto del consumo de tabaco sobre la eficacia antiagregante de clopidogrel no está completamente confirmado, ya que en los estudios funcionales que lo han sugerido no se evaluó de manera objetiva y cuantitativa el consumo de tabaco (determinando algún metabolito estable de la nicotina como la cotinina).

Finalmente, otro factor clínico a destacar que podría jugar un papel en la variabilidad de respuesta a clopidogrel y condicionar resultados clínicos es la utilización de la hipotermia terapéutica. La hipotermia leve (32-34ºC) se emplea en supervivientes a una parada cardiaca (siendo la causa más frecuente un SCA) que persisten en situación de coma con la intención fundamental de mejorar el pronóstico vital y neurológico de estos pacientes [80]. Investigaciones recientes sugieren que podría inducir un aumento en la reactividad plaquetar y una reducción de la respuesta a los antiagregantes orales, fundamentalmente a clopidogrel [81,82]. Esto último podría tener repercusiones clínicas ya que se ha reportado en algunas series de casos un aumento del riesgo de trombosis del stent en pacientes sometidos a hipotermia tras revascularización coronaria con angioplastia primaria, a pesar del tratamiento con DAP [83]. Sin embargo, si existe realmente un efecto de la hipotermia en rango terapéutico sobre la reactividad plaquetar y, si éste puede tener repercusiones clínicas, es decir, si afecta el riesgo de eventos

aterotrombóticos de estos pacientes es actualmente objeto de controversia y debate.

Figura 2. Mecanismos implicados en la variabilidad de respuesta a clopidogrel



ADP: adenosín difosfato; CYP: citocromo P450; GP: glucoproteína; MDR1: transportador de resistencia a múltiples fármacos

1.3. Antagonistas potentes del receptor $P2Y_{12}$

El impacto pronóstico de una respuesta subóptima al clopidogrel enfatiza la necesidad de buscar y utilizar nuevas estrategias antiagregantes que consigan un bloqueo más potente del receptor $P2Y_{12}$ con una menor variabilidad de respuesta (un efecto más consistente), especialmente en pacientes de alto riesgo, como aquéllos con un SCA sometidos a ICP. En general, se han propuesto tres estrategias para superar el problema de la variabilidad de respuesta al clopidogrel: a) aumentar la dosis de clopidogrel; b) añadir un tercer fármaco antiagregante a la combinación de AAS y clopidogrel; y c) usar nuevos antagonistas del receptor $P2Y_{12}$ más potentes.

Pese a una discreta mejoría farmacodinámica [84-86], ni aumentar la dosis de clopidogrel ni añadir un tercer agente antiplaquetario oral (p.ej. cilostazol) han conseguido demostrar de manera fehaciente mejorías netas relevantes a nivel clínico y no se han implantado estas estrategias de forma habitual en la práctica clínica [87,88]. En cambio, sí ha funcionado el uso de nuevos fármacos de administración oral bloqueadores del receptor $P2Y_{12}$ como prasugrel y ticagrelor, que tienen en común poseer un efecto fundamentalmente más potente, pero también más rápido y con menor variabilidad que el clopidogrel [33]. La eficacia superior de estos fármacos en el SCA, fundamentalmente en el contexto de ICP, ha sido demostrada en ensayos clínicos a gran escala [89,90], por lo que han sido autorizados para uso clínico y se recomiendan por encima de clopidogrel en las guías actuales de práctica clínica [7-9].

El prasugrel, como todas las tienopiridinas, es un profármaco de administración oral que requiere biotransformación hepática para producir un metabolito activo, que es el que ejerce un bloqueo irreversible del receptor $P2Y_{12}$ [33]. La conversión en metabolito activo de prasugrel es más eficiente que la de clopidogrel, por lo que, dada la equipotencia de ambos metabolitos activos, la inhibición plaquetaria conseguida con prasugrel es superior, además de ser más rápida y con menor variabilidad [91]. El beneficio de prasugrel comparado con clopidogrel se demostró en el ensayo TRITON-TIMI 38 (Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel-Thrombolysis in Myocardial Infarction), realizado en pacientes con un SCA de riesgo moderado a alto en los que se indicaba ICP [89]. En dicho ensayo, el uso de prasugrel se asoció de forma significativa con una reducción relativa del 19% de eventos adversos isquémicos (variable combinada de muerte cardiovascular, infarto de miocardio no fatal e ictus no fatal), a costa de un ligero aumento del riesgo de hemorragias mayores no relacionadas con bypass coronario según criterio TIMI (Thrombolysis in Myocardial Infarction). El beneficio de prasugrel fue particularmente importante, además sin evidenciarse un aumento del riesgo de sangrado, en los pacientes con un IAMCEST [92] y en los diabéticos [93].Por otro lado, la eficacia de ambos fármacos fue similar en los pacientes de bajo peso (<60 kg) o de edad avanzada (≥75 años), y prasugrel presentó un efecto clínico neto negativo en los sujetos con antecedente de ictus. El uso de prasugrel está aprobado en pacientes con SCA en los que se realiza ICP, debiendo administrarse tras

conocer la anatomía coronaria en los pacientes con SCASEST, mientras que el pretratamiento está permitido en el IAMCEST [7-9].

El ticagrelor es el primer fármaco desarrollado de un nuevo grupo, las ciclopentiltriazolopirimidinas, que inhibe de manera directa (sin necesidad de un metabolito activo) y reversible el receptor $P2Y_{12}$. En comparación con clopidogrel, presenta un efecto antiagregante más potente y con menor variabilidad, un inicio de acción más rápido y una desaparición más temprana de su efecto (3-5 días), debido a su reversibilidad y a una semivida corta (requiere administración dos veces al día), aunque aproximadamente el 30-40% de su efecto es atribuible a metabolitos activos generados a nivel hepático [94,95]. La eficacia y seguridad de ticagrelor comparado con clopidogrel se evaluó en el ensayo PLATO (Platelet Inhibition and Patient Outcomes), realizado en pacientes con un SCA de riesgo moderado a alto, con o sin elevación del segmento ST [90]. En este estudio, el tratamiento con ticagrelor se asoció con una mejoría significativa en las tasas de eventos isquémicos (reducción relativa del 16%), sin aumentar el riesgo de hemorragia mayor según la definición del estudio, pero sí incrementando discretamente las hemorragias mayores no relacionadas con bypass coronario (aumento similar en cifras absolutas, un 0,6%, al usar la misma definición que en el ensayo pivotal con prasugrel). El beneficio observado con ticagrelor fue consistente en los pacientes en los que se planeó una estrategia invasiva inicial [96] y en aquéllos en los que se optó inicialmente por una estrategia no invasiva [97], mostrando un beneficio particular en los pacientes con insuficiencia renal

crónica (IRC) [98]. El ticagrelor está aprobado para uso clínico en pacientes con SCA, tanto los manejados médicamente como en los que se realiza ICP.

La eficacia superior de prasugrel o ticagrelor sobre clopidogrel mostrada en los ensayos de fase III descritos anteriormente debe interpretarse *sensu stricto* como aplicable a la población que presente las mismas características que las de las incluidas en cada estudio. Sin embargo, se ha mostrado un beneficio particular de estos fármacos en ciertos subgrupos de pacientes que clásicamente se asocian con una mayor reactividad plaquetar y una mayor prevalencia de respuesta subóptima a clopidogrel, además de con un peor pronóstico, como son los pacientes con IAMCEST, DM o incluso IRC. Este hecho estaría sugiriendo que un antagonismo más potente de la vía del receptor $P2Y_{12}$ podría ayudar a superar la hiperreactividad plaquetar característica de estos subgrupos de riesgo, alcanzar el nivel de inhibición plaquetar deseado y contribuir así a mejorar su evolución clínica.

El agente que consigue la inhibición más potente (ampliamente superior al 90%) de la vía iniciada en el receptor $P2Y_{12}$ es el cangrelor, un análogo de ATP, que es un fármaco de administración intravenosa que inhibe de manera reversible y directa (sin necesidad de metabolito activo) dicho receptor (Figura 1) [99]. Otras propiedades farmacológicas de interés de cangrelor son: a) inicio de acción rápido, alcanzando las concentraciones estables en unos minutos; b) efectos dosis-dependientes y, por tanto, predecibles; y c) desaparición rápida de la acción, puesto que tiene una semivida extremadamente breve (3-6 min) a causa de una rápida inactivación por ectonucleotidasas plasmáticas, con lo que la función plaquetar vuelve a su nivel basal en unos 30-60 minutos tras parar la

infusión [99]. El programa CHAMPION (Cangrelor versus standard tHerapy to Achieve optimal Management of Platelet InhibitiON) de ensayos de fase III tuvo como objetivo evaluar la eficacia y seguridad de cangrelor en pacientes en los que se realizaba ICP, la mayoría de ellos tras un SCA. Los primeros ensayos que compararon cangrelor (siempre administrado antes de iniciar el ICP) con clopidogrel, este último administrado antes del procedimiento en el CHAMPION-PCI e inmediatamente después en el CHAMPION-PLATFORM, fueron suspendidos prematuramente por futilidad, no mostrando diferencias significativas entre los dos fármacos a la hora de reducir las tasas de la variable de valoración principal (combinación de muerte por cualquier causa, infarto de miocardio o revascularización guiada por la presencia de isquemia a las 48 horas) [100,101]. Sin embargo, un análisis conjunto de los resultados de ambos estudios usando la definición universal de infarto de miocardio en lugar de la originalmente empleada en estos ensayos sí mostró que el uso de cangrelor se asociaba con una reducción significativa en las tasas de la variable de valoración principal descrita anteriormente [102]. Adicionalmente, los resultados del ensayo CHAMPION-PHOENIX mostraron un beneficio de cangrelor comparado con clopidogrel en cuanto a reducción de eventos isquémicos (variable combinada de muerte por cualquier causa, infarto de miocardio, revascularización guiada por la presencia de isquemia o trombosis de stent a las 48 horas) en pacientes sometidos a ICP (con angina estable o SCA) [103]. Estas nuevas evidencias reactivaron el interés por cangrelor, que ha sido recientemente aprobado para uso clínico tanto en Europa como en USA en el contexto de ICP, con la particularidad en Europa de que debe ser administrado

en pacientes que no han recibido pretratamiento antes del procedimiento con un antagonista del receptor $P2Y_{12}$ (clopidogrel, prasugrel o ticagrelor) o en aquéllos en los que el tratamiento con uno de estos agentes no es posible o deseable [104]. Dado que cangrelor es el antagonista del receptor $P2Y_{12}$ más potente desarrollado, se trata de una opción sumamente atractiva para intentar superar el fenotipo plaquetar hiperreactivo que caracteriza a ciertos subgrupos de riesgo, como serían los pacientes con DM.

1.4. Justificación

Es importante señalar que, pese al desarrollo de nuevos fármacos (prasugrel y ticagrelor) más potentes y que han demostrado una mayor eficacia clínica en los pacientes con SCA, el clopidogrel es todavía el antagonista del receptor $P2Y_{12}$ más usado en nuestro medio [105]. Además, debe considerarse que prasugrel y ticagrelor están aprobados para uso clínico en SCA, pero no así en pacientes con cardiopatía isquémica estable en los que se realiza ICP, donde clopidogrel sigue siendo la primera opción de tratamiento, siempre asociado a AAS [7-9]. Por tanto, la existencia en nuestro medio de una proporción importante de pacientes con SCA o en los que se realiza ICP que reciben tratamiento con clopidogrel, pudiendo estar un porcentaje de ellos en mayor riesgo de padecer eventos isquémicos por presentar una respuesta subóptima al fármaco, evidencia la vigencia del problema y el interés por profundizar en el conocimiento de los mecanismos potencialmente asociados a una respuesta subóptima a clopidogrel y si el uso de fármacos que consiguen un bloqueo más potente de la vía iniciada en el receptor $P2Y_{12}$ puede superar

el efecto de dichos mecanismos y conseguir un nivel de inhibición plaquetar adecuado.

Como se ha comentado anteriormente, un conocimiento exhaustivo de los factores clínicos asociados con una mayor agregabilidad plaquetar y una respuesta insuficiente al clopidogrel es de una especial relevancia porque es a este nivel donde es más factible poder realizar acciones terapéuticas que minimicen su impacto deletéreo. Es por este motivo que el trabajo de esta tesis ha sido encaminado en su mayor parte a profundizar en el conocimiento de diferentes factores clínicos que pueden contribuir de manera importante a una reducción de la eficacia antiagregante de clopidogrel y, por tanto, empeorar la evolución de los pacientes con SCA o en los que se realiza ICP que estén bajo tratamiento con dicho fármaco como parte de la DAP. En concreto, los mecanismos estudiados son: a) interacción farmacológica con omeprazol, un IBP metabolizado principalmente por CYP2C19, evaluando su administración separada o concomitante con clopidogrel (artículo I); b) interacción farmacológica con pantoprazol, un IBP que no se metaboliza principalmente por CYP2C19, evaluando su administración separada o concomitante con clopidogrel (artículo II); c) efecto del consumo de tabaco, evaluado de manera objetiva y cuantitativa según los niveles de cotinina (un producto de degradación estable de la nicotina), en la eficacia de clopidogrel en una cohorte de pacientes con DM (artículo III); d) impacto de la presencia de un IAMCEST sobre la eficacia inicial de clopidogrel, en concreto en el momento de iniciar un procedimiento de angioplastia primaria (estudio IV); y e) efecto de la hipotermia leve en rango terapéutico sobre la eficacia de clopidogrel (artículo V).

Adicionalmente, se ha evaluado también si el uso *in vitro* de cangrelor, el antagonista más potente del receptor $P2Y_{12}$, puede conseguir un nivel de inhibición plaquetar similar en pacientes con y sin DM, es decir, si un bloqueo muy potente de la vía iniciada en el receptor $P2Y_{12}$ puede superar la disfunción plaquetar característica de los pacientes con DM, una patología que aglutina varios de los mecanismos que contribuyen a tener una hiperreactividad plaquetar y una peor respuesta a los fármacos antiagregantes (artículo VI).

Por último, fruto también del trabajo relacionado con esta tesis doctoral se han publicado varios artículos de revisión, de los que se han adjuntado los más relevantes por encuadrarse perfectamente en el tema general desarrollado en la tesis, por su impacto bibliométrico y fundamentalmente por la capital importancia clínica de los aspectos desarrollados de manera exhaustiva en dichas revisiones, que son: a) antagonistas del receptor $P2Y_{12}$, con especial atención a los mecanismos de variabilidad de respuesta a clopidogrel (artículo VII); b) la disfunción plaquetar y terapia antiagregante en los pacientes con DM y SCA (artículo VIII); y c) perspectivas futuras de la terapia antiagregante, con especial atención a los fármacos comercializados recientemente o todavía en desarrollo (artículo IX).

1.5. Escenario del proyecto

La realización de esta tesis doctoral se enmarca dentro de un plan estratégico de colaboración entre el Área de Enfermedades del Corazón del Hospital Universitario de Bellvitge y el Cardiovascular Research Center de la

University of Florida College of Medicine - Jacksonville. Este proyecto dio inicio con la estancia del doctorando durante dos años en calidad de *"Research Fellow"* en la mencionada University of Florida bajo la tutela del Dr. Dominick Angiolillo (codirector de esta tesis) con el objeto de recibir en este centro de reconocido prestigio una formación altamente cualificada en investigación traslacional relacionada con fármacos antitrombóticos. Fruto de esta estancia y de la colaboración entre ambas instituciones se han realizado los artículos incluidos en esta tesis.

Posteriormente a la reincorporación del doctorando al Hospital Universitario de Bellvitge, esta estrategia continuó con la creación en el Área de Enfermedades del Corazón, dirigida por el Dr. Cequier (director de esta tesis) del Laboratorio de Investigación Cardiovascular, dirigido por el Dr. Ferreiro (doctorando de esta tesis), que está especializado en investigación traslacional en el campo del funcionalismo plaquetar y la respuesta a fármacos antitrombóticos, con un especial interés en estrategias de tratamiento individualizado. La creación de este Laboratorio ha permitido continuar la colaboración entre las dos instituciones y generar unas importantes sinergias que han contribuido al diseño y desarrollo con éxito de proyectos de investigación traslacional y clínica.

2. HIPÓTESIS

El que quiere en esta vida todas las cosas a su gusto, tendrá muchos disgustos en su vida.

FRANCISCO DE QUEVEDO

La hipótesis principal de esta tesis doctoral es que el efecto antiagregante de clopidogrel se ve modificado por los siguientes mecanismos: a) empeorado por la administración de omeprazol, fundamentalmente cuando se administran ambos fármacos de forma concomitante, mientras que no hay interacción farmacológica con pantoprazol; b) aumentado con el hábito tabáquico, con una relación dosis-respuesta; c) disminuido por la presencia de un IAMCEST; y d) reducido por la hipotermia leve en rango terapéutico generada *in vitro*.

Una segunda hipótesis de esta tesis es que la administración *in vitro* de cangrelor puede conseguir un nivel de inhibición plaquetar similar en pacientes con y sin DM, es decir, que un bloqueo muy potente del receptor $P2Y_{12}$ es capaz de superar el efecto de los diversos mecanismos que contribuyen a la hiperreactividad plaquetar característica de la población diabética.

3. OBJETIVOS

Cuando quiero que un asunto no se resuelva lo encomiendo a un comité.

NAPOLEÓN BONAPARTE

El objetivo general de esta tesis doctoral es profundizar en el conocimiento de varios mecanismos clínicos potencialmente asociados con una hiperreactividad plaquetar y una respuesta subóptima a clopidogrel, además de evaluar si el uso de un fármaco que consigue un bloqueo más potente de la vía iniciada en el receptor $P2Y_{12}$, puede superar el efecto de dichos mecanismos y conseguir un nivel de inhibición plaquetar adecuado en pacientes con un elevado riesgo de presentar respuesta subóptima a clopidogrel.

Para la consecución de este objetivo general se han realizado los estudios incluidos en esta tesis, con los siguientes objetivos específicos:

1. Analizar el impacto de la administración de omeprazol, un IBP metabolizado principalmente por CYP2C19, en la inhibición plaquetar inducida por clopidogrel, evaluando si existen diferencias cuando se administran ambos fármacos al mismo tiempo o separados entre 8 y 12 horas.
2. Examinar si la administración de pantoprazol, un IBP que no se metaboliza principalmente por CYP2C19, puede empeorar la inhibición plaquetar inducida por clopidogrel, evaluando si existen diferencias cuando se administran ambos fármacos al mismo tiempo o separados entre 8 y 12 horas.
3. Evaluar si el efecto del consumo de tabaco, medido según los niveles de cotinina sérica, tiene una relación dosis-respuesta sobre la inhibición plaquetar mediada por clopidogrel en una cohorte de pacientes con DM.

4. Determinar el porcentaje de pacientes con IAMCEST que presentan respuesta subóptima a clopidogrel (administrado en el momento del diagnóstico) cuando se inicia el procedimiento de angioplastia primaria y si dicha pobre respuesta se asocia con la permeabilidad inicial de la arteria responsable del infarto.
5. Analizar el efecto *in vitro* de la hipotermia leve en rango terapéutico sobre la respuesta farmacodinámica a clopidogrel y AAS en muestras de pacientes con IAMCEST en los que se realiza angioplastia primaria.
6. Evaluar la eficacia farmacodinámica *in vitro* de cangrelor en muestras de pacientes con y sin DM, comparando la inhibición plaquetar conseguida en ambos grupos, además de investigar si un bloqueo potente del receptor $P2Y_{12}$ con cangrelor puede conseguir una modulación de otras vías de señalización plaquetar o de procesos de generación de trombina dependientes de plaquetas.

4. PUBLICACIONES

Tu crítica majadera
de los dramas que escribí,
Pedancio, poco me altera;
más pesadumbre tuviera
si te gustaran a ti.

LEANDRO FERNÁNDEZ DE MORATÍN

4.1. Mecanismos implicados en la variabilidad de respuesta a clopidogrel

I. Pharmacodynamic effects of concomitant versus staggered clopidogrel and omeprazole intake: results of a prospective randomized crossover study.

Ferreiro JL, Ueno M, Capodanno D, Desai B, Dharmashankar K, Darlington A, Charlton RK, Bass TA, Angiolillo DJ.

Circ Cardiovasc Interv. 2010;3:436-41.

Pharmacodynamic Effects of Concomitant Versus Staggered Clopidogrel and Omeprazole Intake

Results of a Prospective Randomized Crossover Study

José L. Ferreiro, MD; Masafumi Ueno, MD; Davide Capodanno, MD; Bhaloo Desai, PhD; Kodlipet Dharmashankar, MD; Andrew Darlington, DO; Ronald K. Charlton, PhD; Theodore A. Bass, MD; Dominick J. Angiolillo, MD, PhD

Background—A drug interaction between clopidogrel and omeprazole resulting in impaired platelet inhibition has been reported. It has been suggested that staggering administration of clopidogrel and omeprazole may overcome this pharmacodynamic (PD) interaction.

Methods and Results—This prospective, open-label, 3-period, randomized crossover study was performed in 20 healthy volunteers. Subjects were randomly selected to receive omeprazole (40 mg daily) concomitantly (CONC) or staggered by 8 to 12 hours (STAG) for 1 week on a background of clopidogrel therapy in a crossover fashion, with a 2- to 4-week washout period between treatments. After another 2- to 4-week washout period, all subjects were treated for 1 week with clopidogrel alone. Clopidogrel was administered as a 600-mg loading dose followed by a 75-mg maintenance dose during all phases. PD effects were assessed by vasodilator-stimulated phosphoprotein phosphorylation assay, VerifyNow $P2Y_{12}$ system, and light transmittance aggregometry at baseline, 24 hours, and 1 week. The primary end point was the comparison of $P2Y_{12}$ reactivity index assessed by vasodilator-stimulated phosphoprotein phosphorylation assay at 1 week between CONC and STAG regimens. No significant difference in the primary end point was observed (least squares mean±SEM, 56.1±3.5% for CONC versus 61.6±3.4% for STAG; $P=0.08$). $P2Y_{12}$ reactivity index values were significantly lower in the clopidogrel regimen (48.8±3.4%) than in the CONC ($P=0.02$) and STAG ($P=0.001$) regimens. No PD differences were observed between regimens at baseline and 24 hours. Concordant results were obtained by $P2Y_{12}$-specific assessments using VerifyNow but not with light transmittance aggregometry.

Conclusions—Omeprazole impairs clopidogrel-induced antiplatelet effects in the maintenance phase of treatment irrespective of timing of their administration. **(*Circ Cardiovasc Interv*. 2010;3:436-441.)**

Key Words: clopidogrel ■ omeprazole ■ drug interactions

Numerous studies have shown a broad range in antiplatelet response profiles following treatment with clopidogrel, and patients with poor platelet inhibitory effects have an increased risk of recurrent atherothrombotic events.[1–4] Several mechanisms have been identified to explain the interindividual variability in clopidogrel-induced antiplatelet effects.[5,6] Among these, that secondary to a drug interaction between clopidogrel and proton pump inhibitors (PPIs) has been recently implicated.[7] In particular, pharmacodynamic (PD) studies have shown that omeprazole, which is the most broadly used PPI and primarily metabolized by the cytochrome P450 (CYP) 2C19 isoenzyme,[8] is associated with reduced platelet inhibitory effects induced by clopidogrel.[9,10] Because the CYP2C19 isoenzyme is involved in both oxidation steps required for clopidogrel prodrug to generate its active metabolite, which is responsible for irreversible blockade of the $P2Y_{12}$ receptor on the platelet surface,[11] any interference at this level may compromise the efficacy of the drug.

Clinical Perspective on p 441

Outcome studies have yielded conflicting results on the prognostic implications of concomitant clopidogrel and PPI use.[12–18] However, given the high frequency with which both drugs are prescribed, even effects of limited magnitude can affect a large number of patients. For these reasons, the Food and Drug Administration and the European Medicines Agency mandated that clopidogrel product information be updated to recommend avoidance of omeprazole.[19,20] This warning is in conflict with a recent expert consensus document on gastrointestinal risks for patients on antiplatelet therapy, which supports the coadministration of these 2 drugs.[21] Because both clopidogrel and omeprazole are rapidly

Received March 1, 2010; accepted August 6, 2010.

From the University of Florida College of Medicine-Jacksonville (J.L.F., M.U., D.C., B.D., K.D., A.D., T.A.B., D.J.A.) and Jacksonville Transplant Center-Shands Jacksonville (R.K.C.), Jacksonville, Fla.

Correspondence to Dominick J. Angiolillo, MD, PhD, University of Florida College of Medicine-Jacksonville, 655 West 8th St, Jacksonville, FL 32209. E-mail dominick.angiolillo@jax.ufl.edu

 DOI: 10.1161/CIRCINTERVENTIONS.110.957829

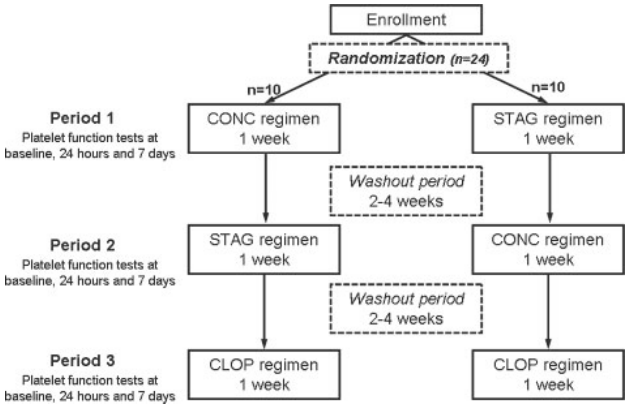


Figure 1. Flow diagram of the study design. CONC indicates clopidogrel 600-mg LD followed by 75-mg daily MD for 1 week in addition to omeprazole 40 mg daily, taking both drugs at the same time; STAG, clopidogrel 600-mg LD followed by 75-mg daily MD for 1 week in addition to omeprazole 40 mg daily, staggering 8 to 12 hours the administration of the drugs; and CLOP, clopidogrel 600-mg LD followed by 75-mg MD for 1 week without taking omeprazole.

metabolized, it has been hypothesized and recommended that staggering administration of these drugs may overcome their interaction.[22,23] However, despite this recommendation, to date no studies have validated this hypothesis. Therefore, the aim of this study was to evaluate whether the PD interaction between clopidogrel and omeprazole can be overcome by separating the intake of both drugs.

Methods

Subject Population and Study Design

This prospective, open-label, 2-sequence, 3-period, randomized crossover study was conducted in nonmedicated healthy male subjects aged 18 to 65 years. The study design is illustrated in Figure 1. Subjects were randomly selected in a 1:1 fashion to take omeprazole (40 mg daily) concomitantly (CONC regimen) or staggered by 8 to 12 hours (STAG regimen) for 1-week on a background of clopidogrel therapy. In particular, in the CONC regimen, both drugs were taken in the morning, whereas in the STAG regimen, clopidogrel was taken in the morning and omeprazole in the evening. After a 2- to 4-week washout period, subjects crossed over treatment regimen. After completing these 2 treatment phases, subjects underwent another washout period of 2 to 4 weeks and were treated for 1 week with clopidogrel alone, without receiving omeprazole therapy (CLOP regimen). The clopidogrel dosing regimen for all 3 phases was a 600-mg loading dose (LD) and a 75-mg maintenance dose (MD). Blood sampling for platelet function assessments were performed at all 3 phases of the study at the following time points: (1) baseline, (2) 24 hours after LD (before intake of study medication), and (3) 7 days (24 hours after the last MD). Clopidogrel was administered as 75-mg tablets of Plavix (Bristol-Myers Squibb/Sanofi Aventis, Bridgewater, NJ) and omeprazole as 20-mg tablets of Prilosec OTC (Proctor & Gamble, Cleveland, Ohio). In particular, 8 75-mg clopidogrel tablets were given for the LD and 1 tablet daily during the maintenance phase; 2 omeprazole tablets were given daily. The washout periods were included to minimize carryover effects between treatment regimens. Patient compliance was assessed by interview and pill counting.

The study complied with the Declaration of Helsinki and was approved by the Institutional Review Board of the University of Florida College of Medicine-Jacksonville. All subjects provided written informed consent. An independent data safety monitoring committee was instituted for adjudication of adverse clinical events.

Sample Collection and Platelet Function Assays

Blood samples for platelet function analyses were collected at scheduled time points before intake of study medication from an antecubital vein. The first 2 to 4 mL of blood were discarded to avoid spontaneous platelet activation. Samples were processed by laboratory personnel blinded to treatment. Platelet function assays included flow cytometric analysis of the status of phosphorylation of the vasodilator-stimulated phosphoprotein (VASP), VerifyNow $P2Y_{12}$ ($VN\text{-}P2Y_{12}$) system, and light transmission aggregometry (LTA).

VASP Assay

The VASP assay was used to determine the $P2Y_{12}$ reactivity index (PRI) according to standard protocols.[24,25] In brief, VASP phosphorylation (VASP-P) was measured by quantitative flow cytometry using commercially available labeled monoclonal antibodies (Biocytex Inc, Marseille, France). The PRI was calculated after measuring the mean fluorescence intensity (MFI) of VASP-P levels following challenge with prostaglandin E_1 (PGE_1) and PGE_1+ADP. PGE_1 increases VASP-P levels through stimulation of adenylate cyclase; ADP binding to purinergic receptors leads to inhibition of adenylate cyclase; thus, the addition of ADP to PGE_1-stimulated platelets reduces levels of PGE_1-induced VASP-P. The PRI was calculated as follows: [(MFI PGE1)−(MFI PGE1+ADP)/(MFI PGE1)]×100%. A reduced PRI is indicative of greater inhibition of the $P2Y_{12}$ signaling pathway.[24,25]

$VN\text{-}P2Y_{12}$ Assay

The $VN\text{-}P2Y_{12}$ assay is a rapid whole-blood point-of-care device and was used according to the instructions of the manufacturer (Accumetrics, Inc; San Diego, Calif) as previously described.[26] In brief, $VN\text{-}P2Y_{12}$ assay mimics turbidometric aggregation and uses disposable cartridges containing 20 μmol/L ADP and 22 nmol/L PGE_1. Aggregation testing using ADP as a sole agonist activates $P2Y_1$ and $P2Y_{12}$ purinergic signaling, whereas adding PGE_1 increases the specificity of the test for $P2Y_{12}$ signaling.[27] In a separate channel of the cartridge in which iso-TRAP (thrombin receptor activating peptide) is used as an agonist, a baseline value for platelet function is obtained, enabling assessment of platelet inhibition without having to wean the patient off antiplatelet treatment. The $VN\text{-}P2Y_{12}$ assay reports the results as $P2Y_{12}$ reaction units (PRU) and percent inhibition of platelet aggregation (%IPA), which is calculated as [(baseline PRU)/baseline]×100. In contrast to IPA values, which increase with decreasing platelet function, PRU values decrease with decreasing platelet function.

LTA

LTA was performed according to standard protocols as previously described.[25] In brief, platelet aggregation was assessed using platelet-rich plasma and platelet-poor plasma by the turbidometric method in a 2-channel aggregometer (Chrono-Log 490 Model; Chrono-Log Corp; Havertown, Penn). Light transmission was adjusted to 0% for platelet-rich plasma and 100% for platelet-poor plasma for each measurement. Maximal platelet aggregation (MPA) was induced by 5 μmol/L and 20 μmol/L ADP as agonist.

Study End Points and Sample Size Calculation

The primary end point of this study was the comparison of the PRI achieved at 1 week between the CONC and STAG treatment regimens. A sample size of 18 patients was required to be able to detect a 10% absolute difference in PRI between both regimens with 80% power and a 2-sided significance level of 0.05, assuming a 15% SD for the difference between regimens. Considering an approximate 25% dropout rate, randomization of up to 24 patients was allowed to ensure that PD data from 18 patients completing both treatment regimens were available. Other end points were (1) comparison of PRU and MPA (assessed by $VN\text{-}P2Y_{12}$ and LTA, respectively) between CONC and STAG at 1 week and (2) comparison of PRI, PRU, and MPA among the 3 regimens (CONC, STAG, and CLOP) at 24 hours and 1 week.

Table. Pharmacodynamic Measures 24 Hours After Clopidogrel LD

Assay	CONC	STAG	CLOP
LTA			
MPA (ADP 20 μmol/L)	36.1±4.9	35.8±4.9	36.8±4.9
MPA (ADP 5 μmol/L)	21.0±3.5	19.3±3.6	19.2±3.6
VN-P2Y$_{12}$			
PRU	128.7±18.2	129.6±18.2	129.8±18.3
%IPA	57.6±5.7	56.9±5.7	58.2±5.7
VASP			
PRI	57.9±4.7	61.7±4.7	58.7±4.7

Data are expressed as LSM±SEM. CONC indicates clopidogrel 600-mg LD followed by 75-mg MD for 1 week in addition to omeprazole 40 mg daily, taking both drugs at the same time; STAG, clopidogrel 600-mg LD followed by 75-mg MD for 1 week in addition to omeprazole 40 mg daily, staggering 8 to 12 hours the administration of the drugs; and CLOP, clopidogrel 600-mg LD followed by 75-mg MD for 1 week without taking omeprazole.

Statistical Analysis

Continuous variables are expressed as mean±SD. Normal distribution was evaluated for continuous variables with the Kolmogorov-Smirnov test. Categorical variables are expressed as frequencies and percentages. Only subjects who successfully completed the first 2 treatment periods of the study were considered for analysis. For the baseline characteristics, paired Student *t* test or Wilcoxon *t* test were used to compare continuous variables, and comparisons between categorical variables were performed using McNemar test or binomial exact test. All statistical comparisons of platelet function for the primary and secondary end points were conducted using linear mixed-effect models, with treatment, sequence, period, and treatment-by-period interaction (in order to test for carryover effects) as fixed effects; subject as a random effect; and baseline value of the corresponding platelet function test (PRI, PRU, or MPA) as a covariate. A 2-tailed $P<0.05$ was considered to indicate a statistically significant difference for all the analyses performed. Results are reported as least squares mean (LSM)±SEM. Statistical analysis was performed using SPSS version 16.0 software (SPSS Inc; Chicago, Ill).

Results

Twenty-four healthy male subjects aged 34.0±6.3 years with a body mass index of 24.9±2.9 kg/m^2 were randomly assigned as follows: 12 starting with the CONC regimen and 12 with the STAG regimen. Two subjects in each group withdrew consent after randomization; therefore, 20 subjects were available for analysis, all of whom completed the 3 periods of the study.

At baseline, there were no differences in any of the PD measures among the 3 regimens studied (data not shown). At 24 hours (after clopidogrel LD administration), there were also no differences in PD measures as summarized in Table. At 1 week, PRI values were numerically lower in the CONC than in the STAG regimen but without reaching statistical significance (LSM±SEM, 56.1±3.5% versus 61.6±3.4%; $P=0.08$ [primary end point]). The PRI was significantly lower following the CLOP regimen (48.8±3.4%) than both regimens in which omeprazole was administered irrespective of timing of administration (CONC, $P=0.02$; STAG, $P=0.001$). The least significant difference in PRI between the CLOP and CONC regimens and between the CLOP and STAG regimens was 7.3% (95%CI, 1.2% to 13.5%) and 12.8% (95% CI, 6.9% to 18.7%), respectively. Distribution of PRI values over the treatment periods is represented in Figure 2. PRI values separated after 24 hours, and PRI was decreased at 1-week in the CLOP regimen compared with the CONC and STAG regimens. No statistically significant differences were observed by sequence, period, or treatment-by-period interaction, thus suggesting no carryover effect.

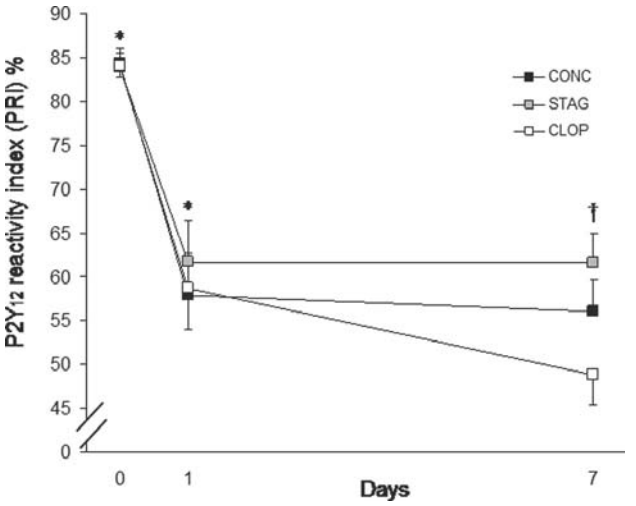


Figure 2. PRI across study time points. Values are expressed as LSM. Error bars indicate SEMs. CONC indicates clopidogrel 600-mg LD followed by 75-mg daily MD for 1 week in addition to omeprazole 40 mg daily, taking both drugs at the same time; STAG, clopidogrel 600-mg LD followed by 75-mg daily MD for 1 week in addition to omeprazole 40 mg daily, staggering 8 to 12 hours the administration of the drugs; and CLOP, clopidogrel 600-mg LD followed by 75-mg MD for 1 week without taking omeprazole. *Nonsignificant *P* for all comparisons at this time point. †CONC versus STAG, $P=0.08$; CONC versus CLOP, $P=0.02$; STAG versus CLOP, $P=0.001$.

Parallel findings were observed with the VN-P2Y$_{12}$ assay, either expressed as PRU or %IPA as shown in Figure 3A and 3B, respectively. In particular, PRU was significantly lower and %IPA significantly higher following the CLOP regimen than both regimens in which omeprazole was administered irrespective of timing of intake (CONC or STAG). Of note, compared with concomitant administration, staggering the intake of the drugs impaired clopidogrel-induced platelet inhibition measured as %IPA (least significant difference, 6.2%; 95% CI, 0.4% to 12.0%). However, this difference was not significant when VN-P2Y$_{12}$ assay values were expressed as PRU.

MPA values using 20 μmol/L ADP were similar in CONC and STAG regimens (43.0±4.6 versus 45.4±4.7; $P=0.56$). Although MPA values were lower in the CLOP regimen (38.1±4.6), this did not reach statistical significance compared with the CONC ($P=0.23$) and STAG ($P=0.09$) regimens. Similar findings were shown for MPA values following 5 μmol/L ADP (data not shown).

Discussion

Recent investigations have shown a PD interaction between clopidogrel and omeprazole, which translates into reduced platelet inhibition.[9,10] Although the clinical consequences of this interaction remain controversial,[7,12–18] this has led the Food and Drug Administration and European Medicines Agency to update the clopidogrel product information with a warning to avoid omeprazole therapy.[19,20] It has been hypothesized and recently recommended that staggering the admin-

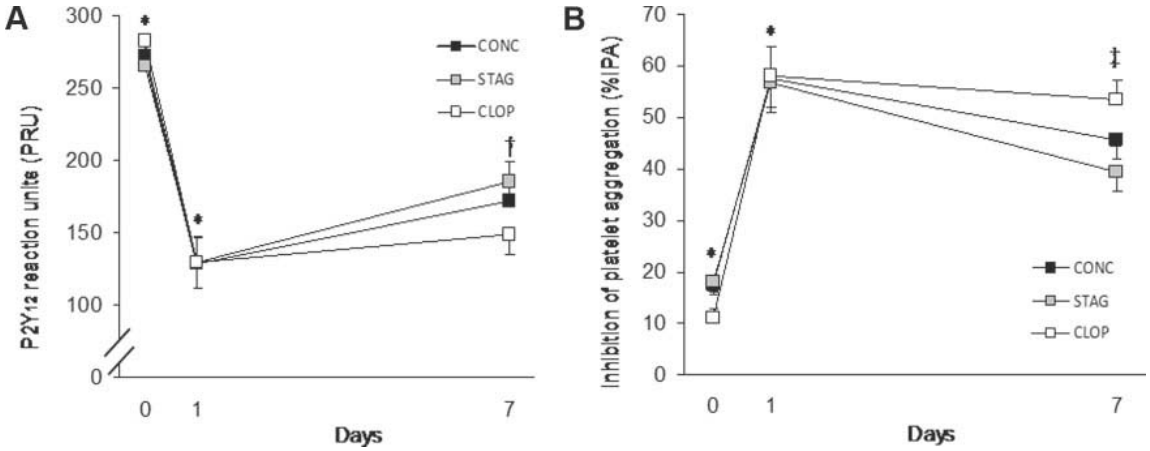


Figure 3. VerifyNow $P2Y_{12}$ testing across study time points. PRU (A) and %IPA (B) determined by the VerifyNow $P2Y_{12}$ assay. Values are expressed as LSM. Error bars indicate SEMs. CONC indicates clopidogrel 600-mg LD followed by 75-mg daily MD for 1 week in addition to omeprazole 40 mg daily, taking both drugs at the same time; STAG, clopidogrel 600-mg LD followed by 75-mg daily MD for 1 week in addition to omeprazole 40 mg daily, staggering 8 to 12 hours the administration of the drugs; and CLOP, clopidogrel 600-mg LD followed by 75-mg MD for 1 week without taking omeprazole. *Nonsignificant *P* for all comparisons at this time point. †CONC versus STAG, $P=0.23$; CONC versus CLOP, $P=0.05$; STAG versus CLOP, $P<0.01$. ‡CONC versus STAG, $P<0.05$; CONC versus CLOP, $P=0.01$; STAG versus CLOP, $P<0.001$.

istration of clopidogrel and omeprazole can prevent the interaction between these 2 drugs.[22,23] However, to date no reported studies have explored the PD effects of this regimen. The present PD study was specifically designed to test for this hypothesis. In our study, we confirmed that omeprazole is associated with reduced antiplatelet effects induced by clopidogrel in the maintenance phase of treatment. However, no differences were observed when comparing platelet reactivity after 1 week of taking both drugs simultaneously or staggered by 8 to 12 hours. These findings were supported by multiple platelet function assessments specific for $P2Y_{12}$ receptor signaling. Platelet reactivity assessed by non-$P2Y_{12}$-specific testing (ie, MPA defined by LTA) showed that although on-treatment platelet reactivity was higher with omeprazole, this did not reach statistical significance. This finding is in line with previous experiences[28] and is explained by the fact that LTA using ADP stimuli is reflective of both $P2Y_1$ and $P2Y_{12}$ signaling and, thus, not fully specific for clopidogrel effects.[24–27] Further, our data showed a PD interaction only in the maintenance phase of treatment, whereas no differences in platelet reactivity were observed in the acute phase following LD administration. These findings may be due to a high LD of clopidogrel, which may overcome this interaction. This is in line with previous observations showing that lipophylic statins may impair clopidogrel response when standard dosing regimens are used,[29] although this is not observed with high LDs.[30]

The concerns surrounding the drug interaction between clopidogrel and omeprazole have led to developing a hypothesis of strategies to overcome this phenomenon. Among these, separating the timing of administration of the 2 drugs has been suggested.[22,23] However, our observation showing that clopidogrel effects are reduced by omeprazole irrespective of timing of administration strongly argues against these recent recommendations. These findings may suggest that factors other than competitive inhibition at the level of CYP2C19 are involved in this interaction.[31] In fact, it cannot be excluded that an increase in gastric pH may alter clopidogrel absorption and decrease its bioavailability. Therefore, clopidogrel absorption could potentially be higher when both drugs are taken concomitantly instead of staggered because changes in gastric pH caused by PPIs might have not been fully achieved by the time clopidogrel is absorbed into the bloodstream. Although our study showed trends toward greater impairment in clopidogrel-induced antiplatelet effects with staggered versus concomitant treatment, this did not reach statistical significance for the primary end point and, therefore, cannot fully support this theory. However, our study findings clearly demonstrate the presence of a PD interaction between clopidogrel and omeprazole irrespective of timing of drug administration, which fall in favor of the precautions warranted by drug regulatory authorities on the use of these agents.[19,20] Whether the results obtained in our study would have been different using an omeprazole daily dose of 20 mg, which is commonly used in clinical practice, instead of 40 mg cannot be ascertained. However, the degree of impairment of clopidogrel-induced platelet inhibition associated with omeprazole at a dose of 40 mg, as used in our study, is similar to that obtained in other studies using a 20-mg daily dose of omeprazole.[9,10]

Although gastric pH is important in the drug absorption processes, if this was particularly relevant in modulating clopidogrel effects, it would be expected that other gastric-protecting agents could impair clopidogrel response as well. However, this is not fully supported by PD studies using PPIs other than omeprazole or with the histamine H_2-receptor antagonists.[32–34] These findings suggest that the PD interaction between clopidogrel and PPIs may be drug specific rather than a class effect and may imply several underlying contributing mechanisms. Indeed, interference at the level of the 2C19 isoenzyme represents one of the most accountable of these mechanisms. In fact, hepatic conversion of clopidogrel into its active metabolite, which occurs through a double oxidation process, is a critical step to achieving its

antiplatelet effects. Several CYP isoforms are involved in clopidogrel metabolism. In particular, CYP3A4, CYP3A5, CYP2C9, and CYP1A2 are involved in 1 oxidation step, whereas CYP2B6 and CYP2C19 are involved in both.[11] Thus, the pivotal role of CYP2C19 in both oxidation steps explains why substances, such as omeprazole, that interfere with its activity can modulate clopidogrel-induced antiplatelet effects.[11,31] This is also supported by the fact that genetic variants of the CYP2C19 enzyme associated with reduced functional activity have been associated with impaired platelet inhibition and clinical events in clopidogrel-treated patients.[35–38]

The major concern of the PD interaction described with omeprazole and clopidogrel is its potential to translate into an increased risk of atherothrombotic events. Although the specific thresholds of platelet reactivity associated with an increased risk of adverse events are not fully determined, absolute changes in platelet reactivity similar to that observed in our study have been shown to be associated in other PD studies with incremental cardiovascular risk.[5,6] However, studies evaluating the prognostic implications of clopidogrel and PPI use have shown conflicting findings.[12–18] This may be largely explained by the fact that most of these studies were retrospective in nature or based on post hoc assessments of clinical trials and, thus, are inadequate to draw definitive conclusions on the clinical implications of this interaction. Nevertheless, it is well established that patients who present with heightened platelet reactivity have an increased risk of ischemic events.[5,6] Indeed, further outcome studies, ideally integrated with PD assessments, are warranted to further elucidate the safety concerns surrounding the clopidogrel-omeprazole drug interaction.

Study Limitations

This study had an open-label design and was performed at a single center, which has its intrinsic limitations. It may be argued that the study was performed in healthy volunteers and that the data may not necessarily be extrapolated to patients with coronary artery disease. However, the objective of this study was to elucidate the PD interaction between clopidogrel and omeprazole in nonmedicated subjects because of the fact that many medications commonly prescribed in patients with coronary artery disease may interfere with the CYP system, thus leading to a potential bias in the PD findings. In addition, the lack of a pharmacokinetic evaluation limits the mechanistic interpretation of the study. Therefore, a study evaluating both pharmacokinetics and PD is needed to confirm the findings of our study.

Sources of Funding

This investigator-initiated study was funded by a grant from Sanofi Aventis/Bristol-Myers Squibb to the University of Florida College of Medicine-Jacksonville (Principal Investigator: Dominick J. Angiolillo, MD, PhD).

Disclosures

Dr. Ferreiro reports honoraria for lectures from Eli Lilly Co and Daiichi Sankyo, Inc. Drs Capodanno, Charlton, Darlington, Desai, Dharmashankar, Ferreiro, and Ueno have no conflicts of interest to report.

References

1. Matetzky S, Shenkman B, Guetta V, Shechter M, Bienart R, Goldenberg I, Novikov I, Pres H, Savion N, Varon D, Hod H. Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. *Circulation*. 2004;109: 3171–3175.
2. Hochholzer W, Trenk D, Bestehorn HP, Fischer B, Valina CM, Ferenc M, Gick M, Caputo A, Büttner HJ, Neumann FJ. Impact of the degree of peri-interventional platelet inhibition after loading with clopidogrel on early clinical outcome of elective coronary stent placement. *J Am Coll Cardiol*. 2006;48:1742–1750.
3. Marcucci R, Gori AM, Paniccia R, Giusti B, Valente S, Giglioli C, Buonamici P, Antoniucci D, Abbate R, Gensini GF. Cardiovascular death and nonfatal myocardial infarction in acute coronary syndrome patients receiving coronary stenting are predicted by residual platelet reactivity to ADP detected by a point-of-care assay: a 12-month follow-up. *Circulation*. 2009;119:237–242.
4. Sibbing D, Braun S, Morath T, Mehilli J, Vogt W, Schömig A, Kastrati A, von Beckerath N. Platelet reactivity after clopidogrel treatment assessed with point-of-care analysis and early drug-eluting stent thrombosis. *J Am Coll Cardiol*. 2009;53:849–856.
5. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Alfonso F, Macaya C, Bass TA, Costa MA. Variability in individual responsiveness to clopidogrel: clinical implications, management, and future perspectives. *J Am Coll Cardiol*. 2007;49:1505–1516.
6. Ferreiro JL, Angiolillo DJ. Clopidogrel response variability: current status and future directions. *Thromb Haemost*. 2009;102:7–14.
7. Ferreiro JL, Angiolillo DJ. Antiplatelet therapy: clopidogrel plus PPIs—a dangerous combination? *Nat Rev Cardiol*. 2009;6:392–394.
8. Li XQ, Andersson TB, Ahlström M, Weidolf L. Comparison of inhibitory effects of the proton pump-inhibiting drugs omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole on human cytochrome P450 activities. *Drug Metab Dispos*. 2004;32:821–827.
9. Gilard M, Arnaud B, Le Gal G, Abgrall JF, Boschat J. Influence of omeprazol on the antiplatelet action of clopidogrel associated to aspirin. *J Thromb Haemost*. 2006;4:2508–2509.
10. Gilard M, Arnaud B, Cornily JC, Le Gal G, Lacut K, Le Calvez G, Mansourati J, Mottier D, Abgrall JF, Boschat J. Influence of omeprazole on the antiplatelet action of clopidogrel associated with aspirin: the randomized, double-blind OCLA (Omeprazole CLopidogrel Aspirin) study. *J Am Coll Cardiol*. 2008;51:256–260.
11. Kazui M, Nishiya Y, Ishizuka T, Hagihara K, Farid NA, Okazaki O, Ikeda T, Kurihara A. Identification of the human cytochrome P450 enzymes involved in the two oxidative steps in the bioactivation of clopidogrel to its pharmacologically active metabolite. *Drug Metab Dispos*. 2010;38:92–99.
12. Aubert R, Epstein R, Teagarden J, Xia F, Yao J, Desta Z, Skaar T, Flockhart DA. Abstract 3998: proton pump inhibitors effect on clopidogrel effectiveness: the Clopidogrel Medco Outcomes study (abstract 3998). *Circulation.* 2008;118:S_815.
13. Dunn SP, Macaulay TE, Brennan DM. Abstract 3999: baseline proton pump inhibitor use is associated with increased cardiovascular events with and without the use of clopidogrel in the CREDO trial. *Circulation.* 2008;118:S_815.
14. Ho PM, Maddox TM, Wang L, Fihn SD, Jesse RL, Peterson ED, Rumsfeld JS. Risk of adverse outcomes associated with concomitant use of clopidogrel and proton pump inhibitors following acute coronary syndrome. *JAMA*. 2009;301:937–944.
15. Juurlink DN, Gomes T, Ko DT, Szmitko PE, Austin PC, Tu JV, Henry DA, Kopp A, Mamdani MM. A population-based study of the drug interaction between proton pump inhibitors and clopidogrel. *CMAJ*. 2009; 180:713–718.
16. O'Donoghue ML, Braunwald E, Antman EM, Murphy SA, Bates ER, Rozenman Y, Michelson AD, Hautvast RW, Ver Lee PN, Close SL, Shen L, Mega JL, Sabatine MS, Wiviott SD. Pharmacodynamic effect and clinical efficacy of clopidogrel and prasugrel with or without a proton-pump inhibitor: an analysis of two randomised trials. *Lancet.* 2009;374:989–997.
17. Rassen JA, Choudhry NK, Avorn J, Schneeweiss S. Cardiovascular outcomes and mortality in patients using clopidogrel with proton pump inhibitors after percutaneous coronary intervention or acute coronary syndrome. *Circulation*. 2009;120:2322–2329.
18. Bhatt DL. COGENT: a prospective, randomized, placebo-controlled trial of omeprazole in patients receiving aspirin and clopidogrel. Presented at:

Transcatheter Cardiovascular Therapeutics; September 24, 2009; San Francisco, CA.
19. Bhatt DL, Scheiman J, Abraham NS, Antman EM, Chan FK, Furberg CD, Johnson DA, Mahaffey KW, Quigley EM, Harrington RA, Bates ER, Bridges CR, Eisenberg MJ, Ferrari VA, Hlatky MA, Kaul S, Lindner JR, Moliterno DJ, Mukherjee D, Schofield RS, Rosenson RS, Stein JH, Weitz HH, Wesley DJ; American College of Cardiology Foundation; American College of Gastroenterology; American Heart Association. ACCF/ACG/AHA 2008 expert consensus document on reducing the gastrointestinal risks of antiplatelet therapy and NSAID use. *Am J Gastroenterol.* 2008;103:2890–2907.
20. Food and Drug Administration. Public-health advisory: updated safety information about a drug interaction between clopidogrel bisulfate (marketed as Plavix) and omeprazole (marketed as Prilosec and Prilosec OTC). http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2009/ucm191169.htm <https://swa.shandsjacksonville.com/exchweb/bin/redir.asp?URL=http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2009/ucm191169.htm> Published November 17, 2009. Accessed November 19, 2009.
21. European Medicines Agency. Public statement on possible interaction between clopidogrel and proton-pump inhibitors. http://www.ema.europa.eu/humandocs/PDFs/EPAR/Plavix/32895609en.pdf. Published May 29, 2009. Accessed November 30, 2009.
22. Juurlink DN. Proton pump inhibitors and clopidogrel: putting the interaction in perspective. *Circulation*. 2009;120:2310–2312.
23. Laine L, Hennekens C. Proton pump inhibitor and clopidogrel interaction: fact or fiction? *Am J Gastroenterol*. 2010;105:34–41.
24. Aleil B, Ravanat C, Cazenave JP, Rochoux G, Heitz A, Gachet C. Flow cytometric analysis of intraplatelet VASP phosphorylation for the detection of clopidogrel resistance in patients with ischemic cardiovascular diseases. *J Thromb Haemost*. 2005;3:85–92.
25. Angiolillo DJ, Shoemaker SB, Desai B, Yuan H, Charlton RK, Bernardo E, Zenni MM, Guzman LA, Bass TA, Costa MA. Randomized comparison of a high clopidogrel maintenance dose in patients with diabetes mellitus and coronary artery disease: results of the Optimizing Antiplatelet Therapy in Diabetes Mellitus (OPTIMUS) study. *Circulation*. 2007;115:708–716.
26. Angiolillo DJ, Costa MA, Shoemaker SB, Desai B, Bernardo E, Suzuki Y, Charlton RK, Zenni MM, Guzman LA, Bass TA. Functional effects of high clopidogrel maintenance dosing in patients with inadequate platelet inhibition on standard dose treatment. *Am J Cardiol*. 2008;101:440–445.
27. Fox SC, Behan MW, Heptinstall S. Inhibition of ADP-induced intracellular Ca2+ responses and platelet aggregation by the $P2Y_{12}$ receptor antagonists AR-C69931MX and clopidogrel is enhanced by prostaglandin E1. *Cell Calcium*. 2004;35:39–46.
28. Cuisset T, Frere C, Quilici J, Poyet R, Gaborit B, Bali L, Brissy O, Morange PE, Alessi MC, Bonnet JL. Comparison of omeprazole and pantoprazole influence on a high 150-mg clopidogrel maintenance dose the PACA (Proton Pump Inhibitors And Clopidogrel Association) prospective randomized study. *J Am Coll Cardiol*. 2009;54:1149–1153.
29. Lau WC, Waskell LA, Watkins PB, Neer CJ, Horowitz K, Hopp AS, Tait AR, Carville DG, Guyer KE, Bates ER. Atorvastatin reduces the ability of clopidogrel to inhibit platelet aggregation: a new drug-drug interaction. *Circulation*. 2003;107:32–37.
30. Müller I, Besta F, Schulz C, Li Z, Massberg S, Gawaz M. Effects of statins on platelet inhibition by a high loading dose of clopidogrel. *Circulation*. 2003;108:2195–2197.
31. Steinhubl SR. Genotyping, clopidogrel metabolism, and the search for the therapeutic window of thienopyridines. *Circulation*. 2010;121:481–483.
32. Small DS, Farid NA, Payne CD, Weerakkody GJ, Li YG, Brandt JT, Salazar DE, Winters KJ. Effects of the proton pump inhibitor lansoprazole on the pharmacokinetics and pharmacodynamics of prasugrel and clopidogrel. *J Clin Pharmacol*. 2008;48:475–484.
33. Small DS, Farid NA, Li YG, Ernest CS II, Payne CD, Salazar DE, Winters KJ. Effect of ranitidine on the pharmacokinetics and pharmacodynamics of prasugrel and clopidogrel. *Curr Med Res Opin*. 2008;24:2251–2257.
34. Siller-Matula JM, Spiel AO, Lang IM, Kreiner G, Christ G, Jilma B. Effects of pantoprazole and esomeprazole on platelet inhibition by clopidogrel. *Am Heart J*. 2009;157:148.e1–e5.
35. Collet JP, Hulot JS, Pena A, Villard E, Esteve JB, Silvain J, Payot L, Brugier D, Cayla G, Beygui F, Bensimon G, Funck-Brentano C, Montalescot G. Cytochrome P450 2C19 polymorphism in young patients treated with clopidogrel after myocardial infarction: a cohort study. *Lancet*. 2009;373:309–317.
36. Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT, Walker JR, Antman EM, Macias W, Braunwald E, Sabatine MS. Cytochrome p-450 polymorphisms and response to clopidogrel. *N Engl J Med*. 2009;360:354–362.
37. Simon T, Verstuyft C, Mary-Krause M, Quteineh L, Drouet E, Méneveau N, Steg PG, Ferrières J, Danchin N, Becquemont L; French Registry of Acute ST-Elevation and Non-ST-Elevation Myocardial Infarction (FAST-MI) Investigators. Genetic determinants of response to clopidogrel and cardiovascular events. *N Engl J Med.* 2009;360:363–375.
38. Marín F, González-Conejero R, Capranzano P, Bass TA, Roldán V, Angiolillo DJ. Pharmacogenetics in cardiovascular antithrombotic therapy. *J Am Coll Cardiol*. 2009;54:1041–1057.

CLINICAL PERSPECTIVE

A growing body of evidence has shown a broad variability in interindividual pharmacodynamic response profiles to the platelet inhibitor clopidogrel, and patients with reduced platelet inhibition have an increased risk of recurrent atherothrombotic events. Numerous factors may contribute to poor clopidogrel response. Among these, that secondary to a drug interaction with the proton pump inhibitor omeprazole has emerged. The prognostic implications associated with clopidogrel and omeprazole use are not fully elucidated. However, given the high frequency with which both these drugs are prescribed, even a small and limited impairment in clinical outcomes can potentially affect a large number of patients. The Food and Drug Administration and the European Medicines Agency have recently recommended avoidance of this drug combination. Nevertheless, because both clopidogrel and omeprazole are rapidly metabolized, many experts have hypothesized and proposed to stagger clopidogrel and omeprazole intake to minimize or even overcome their interaction. However, this strategy has not been validated yet and represents the rationale for the present study design. The findings of the present investigation demonstrate the presence of a pharmacodynamic interaction between clopidogrel and omeprazole when administered concomitantly as well as staggered. Given the presence of a pharmacodynamic interaction between omeprazole and clopidogrel irrespective of the timing of their administration, use of omeprazole should be avoided in clopidogrel-treated patients.

II. Pharmacodynamic evaluation of pantoprazole therapy on clopidogrel-effects: results of a prospective randomized crossover study.

Ferreiro JL, Ueno M, Tomasello SD, Capodanno D, Desai B, Dharmashankar K, Seecheran N, Kodali MK, Darlington A, Pham, JP, Tello-Montoliu A, Charlton RK, Bass TA, Angiolillo DJ.

Circ Cardiovasc Interv. 2011;4:273-9.

Pharmacodynamic Evaluation of Pantoprazole Therapy on Clopidogrel Effects

Results of a Prospective, Randomized, Crossover Study

José Luis Ferreiro, MD; Masafumi Ueno, MD; Salvatore D. Tomasello, MD; Davide Capodanno, MD; Bhaloo Desai, PhD; Kodlipet Dharmashankar, MD; Naveen Seecheran, MD; Murali K. Kodali, MD; Andrew Darlington, DO; John-Paul Pham, MD; Antonio Tello-Montoliu, MD, PhD; Ronald K. Charlton, PhD; Theodore A. Bass, MD; Dominick J. Angiolillo, MD, PhD

Background—Safety concerns have recently emerged based on a drug interaction between clopidogrel and proton pump inhibitors leading to reduced pharmacodynamic effects. However, whether such drug interaction is a class effect or a drug effect and if this can be modulated by timing of drug administration remains a matter of debate. The aim of this study was to assess the impact of high-dose pantoprazole therapy, a proton pump inhibitor with low potential to interfere with clopidogrel metabolism, administered concomitantly or staggered, on clopidogrel-mediated pharmacodynamic effects.

Methods and Results—This was a prospective, randomized, crossover study conducted in 20 healthy volunteers. Subjects were randomly assigned to receive pantoprazole (80 mg daily) administered concomitantly (CONC) or staggered by 8 to 12 hours (STAG) for 1 week on a background of clopidogrel therapy (600-mg loading dose followed by a 75-mg maintenance dose during all phases) in a crossover fashion with a 2- to 4-week washout period between treatments. All subjects had a 1-week treatment phase with a clopidogrel-only regimen with a 2- to 4-week washout period from randomization sequence. Platelet function was assessed by flow cytometric analysis of the status of phosphorylation of the vasodilator-stimulated phosphoprotein, light transmittance aggregometry after adenosine diphosphate stimuli, and VerifyNow $P2Y_{12}$ system at 3 time points: baseline, 24 hours after loading dose, and 1 week after maintenance dose. The primary end point was the comparison of $P2Y_{12}$ reactivity index assessed by vasodilator-stimulated phosphoprotein at 1 week. After 1 week, there were no significant difference in $P2Y_{12}$ reactivity index between the CONC and STAG regimens (least-squares mean±SEM, 56.0±3.9% versus 56.1±3.9%; $P=0.974$), as well as when compared with the clopidogrel-only regimen (61.0±3.9%; $P=0.100$ versus CONC and $P=0.107$ versus STAG). Further, no differences were observed at baseline and 24 hours between regimens. Concordant results were obtained by light transmittance aggregometry and VerifyNow $P2Y_{12}$ assays.

Conclusions—Pantoprazole therapy used at high doses is not associated with modulation of the pharmacodynamic effects of clopidogrel, irrespective of timing of drug administration.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT01170533.

(*Circ Cardiovasc Interv*. 2011;4:273-279.)

Key Words: clopidogrel ■ pantoprazole ■ proton pump inhibitors ■ drug interaction

Clopidogrel therapy in addition to aspirin is associated with a significant reduction in recurrent atherothrombotic events in high-risk settings, such as acute coronary syndromes or percutaneous coronary interventions.[1–3] However, numerous studies have shown that patients with high on-treatment platelet reactivity remain at increased risk of recurrent ischemic events.[4] Several factors have been associated with reduced pharmacokinetic and pharmacodynamic response profiles to clopidogrel.[5,6] Among these, a drug interaction between proton pump inhibitors (PPIs), in particular omeprazole, and clopidogrel has recently emerged.[7–9] This drug interaction probably is due to the common metabolic pathway of these agents, which involves the cytochrome P450 (CYP) 2C19 enzyme.[10,11] The CYP2C19 isoenzyme is

Received December 23, 2010; accepted March 17, 2011.

From the University of Florida College of Medicine–Jacksonville (J.L.F., M.U., S.D.T., D.C., B.D., K.D., N.S., M.K.K., A.D., J.-P.P., A.T.-M., R.K.C., T.A.B., D.J.A.); Jacksonville Transplant Center-Shands Jacksonville (R.K.C.), Jacksonville, FL; and the Cardiovascular Research Lab, Heart Diseases Institute, Hospital Universitari de Bellvitge, L'Hospitalet de Llobregat, Barcelona, Spain (J.L.F.).

Correspondence to Dominick J. Angiolillo, MD, PhD, University of Florida College of Medicine–Jacksonville, 655 W 8th St, Jacksonville, FL 32209. E-mail dominick.angiolillo@jax.ufl.edu

 DOI: 10.1161/CIRCINTERVENTIONS.110.960997

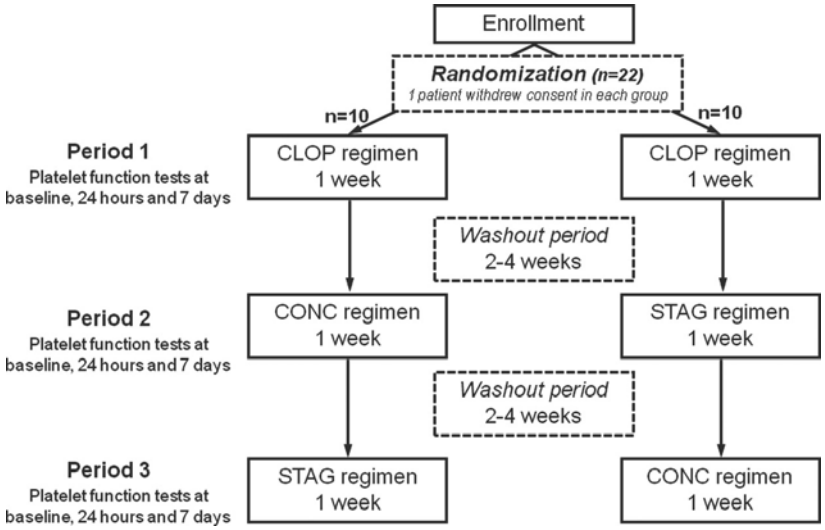


Figure 1. Flow diagram of the study design. CONC indicates clopidogrel 600-mg loading dose followed by 75-mg maintenance dose for 1 week, in addition to pantoprazole 80 mg daily, taking both drugs concomitantly; STAG, clopidogrel 600-mg loading dose followed by 75-mg maintenance dose for 1 week in addition to pantoprazole 80 mg daily, staggering 8 to 12 hours the administration of the drugs; and CLOP, clopidogrel 600-mg loading dose followed by 75-mg maintenance dose for 1 week in the absence of pantoprazole.

of particular importance because it is involved in both oxidation steps required for clopidogrel prodrug to generate its active metabolite.[11] Therefore, intrinsic (eg, genetic polymorphisms) or extrinsic (eg, drugs) factors modulating the activity of this enzyme may affect active metabolite levels and thus the platelet-inhibitory effects of clopidogrel.[5,6]

Clinical Perspective on p 279

Although the clinical implications of the clopidogrel-PPI interaction remains highly controversial,[12] pharmacokinetic and pharmacodynamic studies have consistently shown clopidogrel effects to be significantly altered by omeprazole, a PPI that is primarily metabolized by CYP2C19.[7–9] The concerns surrounding this interaction have prompted a box warning for the concomitant use of these drugs.[13,14] However, whether the clopidogrel-PPI interaction is a class effect or a drug-specific effect is still a matter of debate. In fact, the effects of other PPIs that are less influential on CYP2C19 activity have not been well explored and often controversial.[9,15,16] Further, the impact of timing of administration of these agents on pharmacodynamic effects has also been a topic of debate. Therefore, the aim of this pharmacodynamic study was to evaluate the impact of pantoprazole, a PPI with low potential to inhibit CYP2C19, on clopidogrel-induced antiplatelet effects and whether these may be affected by timing of administration of these agents.

Methods

Subject Population and Study Design

This was a prospective, open-label, 2-sequence, 3-period, randomized, crossover study conducted in nonmedicated healthy male subjects between the ages of 18 and 65 years. This investigation expands on a recently reported study by our group evaluating the pharmacodynamic effects of the clopidogrel-omeprazole drug interaction and how this may be affected by timing of drug intake and presents the same study entry criteria.[8] The study design of the present investigation is illustrated in Figure 1. Subjects were randomly assigned in a 1:1 fashion to take pantoprazole concomitantly (CONC regimen) or staggered by 8 to 12 hours (STAG regimen) for 1 week on a background of clopidogrel therapy. After a 2- to 4-week washout period, subjects crossed over treatment regimen. All subjects also had a 1-week treatment phase with clopidogrel alone, without receiving pantoprazole therapy (CLOP regimen), with a 2- to 4-week washout period from randomization sequence. The clopidogrel dosing regimen for all 3 phases was a 600-mg loading dose (LD) and a 75-mg maintenance dose (MD). Clopidogrel doses were chosen to reflect regimens most commonly used in clinical practice. Pantoprazole was used at a dose of 80 mg/daily. Pantoprazole dosing was higher than that conventionally recommended (40 mg/daily) to maximize any of its effects on CYP2C19. Blood sampling for platelet function assessments were performed at all 3 phases of the study at the following time points: (1) baseline, (2) 24 hours after LD (before intake of study medication), and (3) 7 days (24 hours after the last MD). Clopidogrel was administered as 75-mg tablets of Plavix (Bristol-Myers Squibb/Sanofi Aventis, Bridgewater, NJ) and pantoprazole as 40 mg-tablets of Protonix (Wyeth Pharmaceuticals, Philadelphia, PA). In particular, 8 75-mg Plavix tablets were given for the LD and 1 tablet daily during the maintenance phase, and 2 Protonix tablets were given daily. The washout periods were included to minimize carryover effects between treatment regimens. Patient compliance was assessed by interview and pill counting.

The study complied with the Declaration of Helsinki and was approved by the Institutional Review Board of the University of Florida College of Medicine–Jacksonville. All subjects provided written informed consent. An independent data safety monitoring committee was instituted for adjudication of adverse clinical events.

Sample Collection and Platelet Function Assays

Blood samples for platelet function analyses were collected at scheduled time points before intake of study medication from an antecubital vein. The first 2 to 4 mL of blood was discarded to avoid spontaneous platelet activation. Samples were processed by laboratory personnel blinded to treatment. Platelet function assays included flow cytometric analysis of the status of phosphorylation of the vasodilator-stimulated phosphoprotein (VASP), VerifyNow $P2Y_{12}$ ($VN\text{-}P2Y_{12}$) system, and light transmission aggregometry (LTA).

VASP Assay

The VASP assay was used to determine the $P2Y_{12}$ reactivity index (PRI) according to standard protocols.[17,18] In brief, VASP phosphorylation (VASP-P) was measured by quantitative flow cytometry using commercially available labeled monoclonal antibodies (Biocytex Inc, Marseille, France). The PRI was calculated after measuring the mean fluorescence intensity (MFI) of VASP-P levels after challenge with prostaglandin E_1 (PGE_1) and PGE_1+adenosine diphosphate (ADP). PGE_1 increases VASP-P levels through stimulation of adenylate cyclase; ADP binding to purinergic receptors leads to inhibition of adenylate cyclase; thus, the addition of ADP to PGE_1-stimulated platelets reduces levels of PGE_1-induced VASP-P. The PRI was calculated as follows: ([MFI PGE1]−[MFI

PGE1+ADP]/[MFI PGE1])×100%. A reduced PRI is indicative of greater inhibition of the $P2Y_{12}$ signaling pathway.[17,18]

VN-$P2Y_{12}$ Assay

The VN-$P2Y_{12}$ assay is a rapid whole-blood point-of-care device and was used according to the instructions of the manufacturer (Accumetrics, Inc, San Diego, CA) as previously described.[19] In brief, VN-$P2Y_{12}$ assay mimics turbidometric aggregation and uses disposable cartridges containing 20 μmol/L ADP and 22 nmol/L PGE_1. Aggregation testing using ADP as a sole agonist activates $P2Y_1$ and $P2Y_{12}$ purinergic signaling, whereas adding PGE_1 increases the specificity of the test for $P2Y_{12}$ signaling.[20] In a separate channel of the cartridge in which iso-TRAP is used as an agonist, a baseline value for platelet function is obtained, enabling assessment of platelet inhibition without having to wean the patient off antiplatelet treatment. The VN-$P2Y_{12}$ assay reports the results as $P2Y_{12}$ reaction units (PRU) and percent inhibition of platelet aggregation (%IPA), which is calculated as [(baseline−PRU)/baseline]×100. In contrast to IPA values, which increase with decreasing platelet function, PRU values decrease with decreasing platelet function.

Light Transmission Aggregometry

LTA was performed according to standard protocols as previously described.[18] In brief, platelet aggregation was assessed using platelet-rich plasma and platelet-poor plasma by the turbidometric method in a 2-channel aggregometer (Chrono-Log 490 Model, Chrono-Log Corp, Havertown, PA). Light transmission was adjusted to 0% for platelet-rich plasma and to 100% for platelet-poor plasma for each measurement. Maximal platelet aggregation (MPA) was induced by 5 μmol/L and 20 μmol/L ADP as agonist.

Study End Points and Sample Size Calculation

The primary end point of this study was the comparison of the PRI achieved at 1 week between the CONC and STAG treatment regimens. A sample size of 18 patients was required to be able to detect a 10% absolute difference in PRI between both regimens with 80% power and 2-sided significance level of 0.05, assuming a 15% standard deviation for the difference between regimens. Considering an approximate 15% dropout rate, random assignment of up to 22 patients was allowed to ensure that pharmacodynamic data from 18 patients completing both treatment regimens were available. Other end points included (1) comparison of PRU and MPA (assessed by VN-$P2Y_{12}$ and LTA, respectively) between CONC and STAG at 1 week; (2) comparison of PRI, PRU, and MPA between the 3 regimens (CONC, STAG, and CLOP) at 24 hours and 1 week.

Statistical Analysis

Continuous variables are expressed as mean±SD. Normal distribution was evaluated for continuous variables with the Kolmogorov-Smirnov test. Categorical variables are expressed as frequencies and percentages. Only subjects who successfully completed the first 2 treatment periods of the study were considered for analysis. All statistical comparisons of platelet function for the primary and secondary end points were conducted using linear mixed-effects models with treatment, sequence, period, and treatment×period (treatment by period interaction to test for carryover effects) as fixed effects, subject as a random effect, and baseline value of the corresponding platelet function test (PRI, PRU, or MPA) as a covariate. A 2-tailed probability value of <0.05 was considered to indicate a statistically significant difference for all the analyses performed. Results are reported as least-squares mean±SEM. Statistical analysis was performed using SPSS version 16.0 software (SPSS Inc, Chicago, IL).

Results

Twenty-two healthy male subjects ages 33.6±5.4 years with body mass index of 25.6±2.9 kg/m^2 were randomly assigned: 11 starting with the CONC regimen and 11 with the STAG regimen. One patient from each group withdrew consent after random assignment. Therefore, a total of 20 subjects were available for analysis, all of whom completed the 3 periods of the study.

Table. Pharmacodynamic Measures 24 Hours After Clopidogrel Loading Dose

Assay	CONC	STAG	CLOP
LTA			
MPA (ADP 20 μmol/L)	39.5±4.8	43.1±4.8	39.8±4.8
MPA (ADP 5 μmol/L)	27.9±3.9	31.1±3.9	27.9±3.9
VN-$P2Y_{12}$			
PRU	136.2±20.6	142.8±20.6	132.3±20.6
%IPA	55.5±6.5	51.0±6.5	53.2±6.5
VASP			
PRI	62.3±5.0	64.6±5.0	56.5±5.0

Values are expressed as least-squares mean±SEM.

CONC indicates clopidogrel 600-mg loading dose followed by 75-mg maintenance dose for 1 week, in addition to pantoprazole 80 mg daily, taking both drugs at the same time; STAG, clopidogrel 600-mg loading dose followed by 75-mg maintenance dose for 1 week, in addition to pantoprazole 80 mg daily, staggering 8 to 12 hours the administration of the drugs; LTA, light transmission aggregometry; MPA, maximal platelet aggregation; VN-$P2Y_{12}$, VerifyNow $P2Y_{12}$, IPA, percent inhibition of platelet aggregation; VASP, vasodilator-stimulated phosphoprotein; PRI, $P2Y_{12}$ reactivity index; and CLOP, clopidogrel 600-mg loading dose followed by 75-mg maintenance dose for 1 week, without taking pantoprazole.

There were no differences in any of the pharmacodynamic measures between the 3 regimens studied at baseline (data not shown) or at 24 hours after clopidogrel LD administration, as summarized in the Table. At 1 week, there were no significant difference in the primary end point, which showed similar PRI values with both CONC and STAG regimens (least-squares mean±SEM, 56.0±3.9% versus 56.1±3.9%; $P=0.974$;

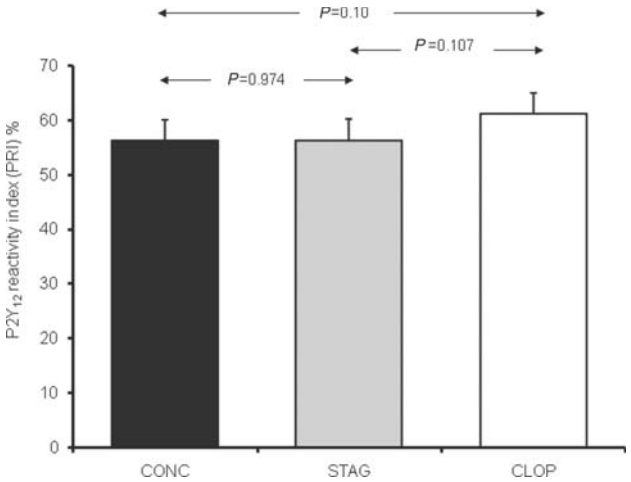


Figure 2. PRI after 1 week of therapy with the 3 regimens evaluated. PRI values are expressed as least-squares means. Error bars indicate standard errors of the mean. CONC indicates clopidogrel 600-mg loading dose followed by 75-mg maintenance dose for 1 week, in addition to pantoprazole 80 mg daily, taking both drugs concomitantly; STAG, clopidogrel 600-mg loading dose followed by 75-mg maintenance dose for 1 week, in addition to pantoprazole 80 mg daily, staggering 8 to 12 hours the administration of the drugs; CLOP, clopidogrel 600-mg loading dose followed by 75-mg maintenance dose for 1 week, in the absence of pantoprazole.

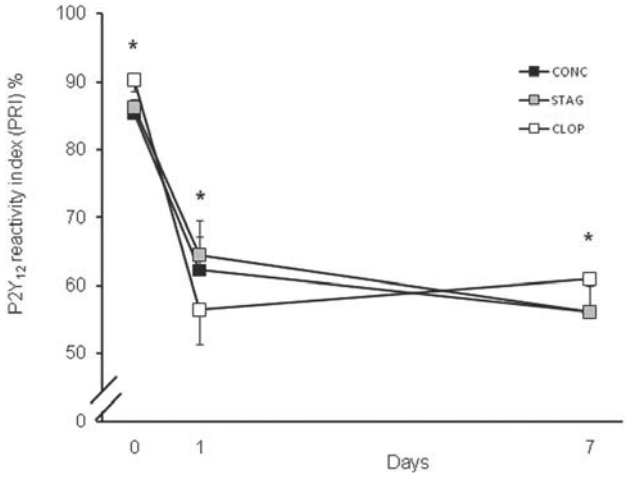


Figure 3. PRI determined by VASP assay across study time points. Values are expressed as least-squares means. Error bars indicate standard errors of the mean. CONC indicates clopidogrel 600-mg loading dose followed by 75-mg maintenance dose for 1 week, in addition to pantoprazole 80 mg daily, taking both drugs concomitantly; VASP, vasodilator-stimulated phosphoprotein; STAG, clopidogrel 600-mg loading dose followed by 75-mg maintenance dose for 1 week, in addition to pantoprazole 80 mg daily, staggering 8 to 12 hours the administration of the drugs; and CLOP, clopidogrel 600-mg loading dose followed by 75-mg maintenance dose for 1 week, in the absence of pantoprazole. *Nonsignificant probability value for all comparisons at this time point.

primary end point). A numerically higher PRI value was obtained with the CLOP regimen but without reaching statistical significance when compared with both regimens in which pantoprazole was administered irrespective of timing of administration (61.0±3.9%; $P=0.100$ versus CONC and $P=0.107$ versus STAG) (Figure 2). The lack of significance is also observed because the confidence intervals (CI) of the least-significant differences between the CLOP and CONC regimens and between the CLOP and STAG regimens include the 0 value: 4.9% (95% CI, −1.0% to 10.8%) and 4.8% (95% CI, −1.1% to 10.7%), respectively. Distribution of PRI values over the treatment periods are represented in Figure 3, showing that PRI values did not significantly separate at any time point between regimens. No statistically significant differences were observed by sequence, period, or the treatment-by-period interaction, which suggests no carryover effect.

Parallel findings were observed with the other platelet function tests performed. No significant difference for any comparison with any assay used was found. Results of the VN-$P2Y_{12}$ assay, either expressed as PRU or %IPA, are shown in Figure 4A and 4B, respectively. MPA values using 20 μmol/L ADP (CONC, 45.9±4.4; STAG, 44.2±4.4; CLOP, 43.5±4.4; no significant probability values for all comparisons) and 5 μmol/L ADP (CONC, 27.5±3.3; STAG, 31.4±3.3; CLOP, 39.6±3.3; no significant probability values for all comparisons) were also consistent.

Discussion

Recent studies have demonstrated a drug interaction between PPIs and clopidogrel.[7–9] Although the clinical implications associated with the reduced pharmacodynamic effects in clopidogrel-treated patients as a cause of this drug interaction remain controversial, this has prompted drug-regulating authorities in the United States and in Europe to provide a box warning for the use of these drugs, administered either concomitantly or staggered.[13,14] In fact, the impact of any negative interaction between PPIs and clopidogrel is of particular concern because of the high frequency with which these two drugs are coprescribed. Therefore, even a small increase in ischemic risk caused by this drug interaction may have significant consequences.[12] The mechanism underlying this drug interaction is a competitive inhibition at the level of the CYP2C19 isoenzyme, a critical step in the hepatic biotransformation of clopidogrel.[5,6,10,11] However, PPIs are recommended in patients at high risk for gastrointestinal bleed, such as those taking dual antiplatelet therapy with aspirin and clopidogrel.[21] This has prompted expert consensus to consider gastric protection strategies with lower potential to inhibit CYP2C19.[21,22] Most of the available pharmacodynamic data on the PPI-clopidogrel interaction is with omeprazole, a moderate CYP2C19 inhibitor.[7–9] Limited information is available on the pharmacodynamic effects of

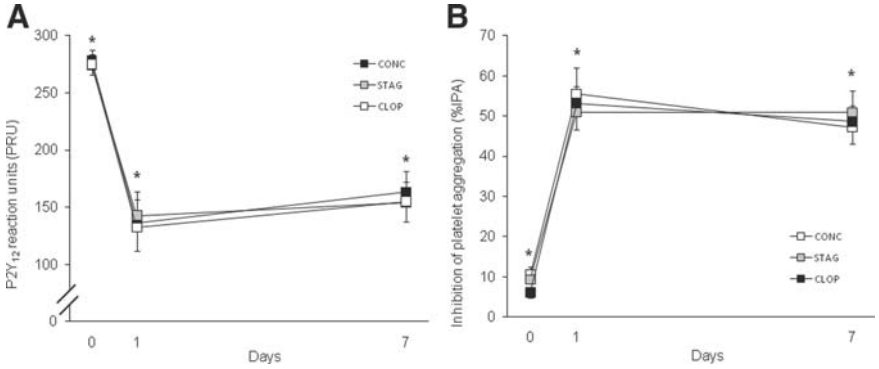


Figure 4. VerifyNow $P2Y_{12}$ measures across study time points. **A**, $P2Y_{12}$ reactivity units (PRU), and **B**, percentage of inhibition of platelet aggregation (%IPA) determined by the VerifyNow $P2Y_{12}$ assay after 1 week of therapy with the 3 regimens evaluated. Values are expressed as least-squares means. Error bars indicate standard errors of the mean. CONC indicates clopidogrel 600-mg loading dose followed by 75-mg maintenance dose for 1 week, in addition to pantoprazole 80 mg daily, taking both drugs concomitantly; STAG, clopidogrel 600-mg loading dose followed by 75-mg maintenance dose for 1 week, in addition to pantoprazole 80 mg daily, staggering 8 to 12 hours the administration of the drugs; and CLOP, clopidogrel 600-mg loading dose followed by 75-mg maintenance dose for 1 week, in the absence of pantoprazole. *Nonsignificant probability value for all comparisons at this time point.

other PPIs, such as pantoprazole, which has lower potential to inhibit CYP2C19. The results of this prospective, randomized, crossover study demonstrate the lack of any significant impairment in clopidogrel-induced pharmacodynamic effects as assessed by a multitude of assays with the use of pantoprazole administered either concomitantly or staggered. Of note, the dose of pantoprazole used in this study was higher than that conventionally used in practice, which provides further support to the conclusions of our investigation.

The results of our study are in line with prior pharmacodynamic investigations assessing the impact of pantoprazole on clopidogrel effects.[15,16,23,24] However, at difference with prior investigations, our study also investigated whether concomitant versus staggered treatment could have an impact on the pharmacodynamic findings. This is noteworthy because it has been suggested that staggering treatment may be a modality to overcome the PPI-clopidogrel drug interaction.[25,26] However, recent pharmacodynamic studies using omeprazole showed trends toward an increase in platelet reactivity with staggered PPI treatment.[8,9] These findings support the recommendation of drug-regulating authorities to avoid concomitant as well as staggered use of omeprazole in clopidogrel-treated patients.[13,14,21] Overall, these considerations underscore the importance of also comprehensively investigating the impact of timing of pantoprazole administration, as performed in the current investigation.

Understanding the clinical implications of the clopidogrel-PPI interaction remains a critical unmet need. Several observational studies have shown significant associations between PPI use and cardiovascular events.[27–31] However, other retrospective analyses (including observational and post hoc analyses of randomized trials)[32–34] and the only randomized, clinical trial evaluating the potential interaction between clopidogrel and a PPI (omeprazole) failed to show an increased risk of adverse cardiovascular events among PPI users, irrespective of the type of PPI.[35] With regard to pantoprazole, a population-based, nested, case-control study of patients receiving clopidogrel therapy after acute myocardial infarction showed that pantoprazole was not associated with an increase in cardiac events, whereas other PPIs were.[28] On the contrary, recently published retrospective cohort studies have shown that pantoprazole also adversely affects cardiovascular outcomes in clopidogrel users.[30,31] These conflicting findings suggest that PPI use might be a marker of unmeasured and uncontrolled confounding in observational studies because PPIs might be selectively prescribed to higher-risk patients, thus, potentially biasing the risk of ischemic outcomes. This is in line with a post hoc analysis of the Clopidogrel for the Reduction of Events During Observation (CREDO) trial, in which PPI use was associated with impaired clinical outcomes regardless whether or not the patients were receiving clopidogrel treatment.[36] Similar conclusions also derive from a post hoc analysis of the PLAtelet inhibition and patient Outcomes (PLATO) trial, in which the use of a PPI was independently associated with an increased risk of the composite of cardiovascular death, myocardial infarction, and stroke in patients receiving clopidogrel or ticagrelor, suggesting that PPI use is more likely a marker for, rather than a cause of, a higher risk of cardiovascular events.[37]

In conclusion, the present study demonstrates the lack of a pharmacodynamic interaction between clopidogrel and pantoprazole, a PPI with low potential to inhibit CYP2C19, supporting that the pharmacodynamic interaction between clopidogrel and PPIs is a drug-specific (eg, PPIs with moderate-high potential to inhibit CYP2C19) rather than a class effect. The lack of a pharmacodynamic interaction was observed irrespective of timing of administration of pantoprazole, which was given at a higher than standard dosing regimen. These results support recent recommendations suggesting that if a PPI is warranted in a patient at increased risk of a gastrointestinal bleed while receiving dual antiplatelet therapy, pantoprazole may be considered as a safer treatment option.

Study Limitations

This study had an open-label design and was performed at a single center and has intrinsic limitations. In addition, the study was performed in healthy volunteers, and it may be argued that the data may not necessarily be extrapolated to patients with coronary artery disease. However, the objective of this study was to elucidate the pharmacodynamic interaction between clopidogrel and pantoprazole, and being performed in nonmedicated subjects precludes any impact of medications commonly prescribed in patients with coronary artery disease that may interfere with the CYP system, which could potentially bias the pharmacodynamic findings. Although this study is supportive of the concept that the clopidogrel-PPI drug interaction is not a class effect and results of prior studies suggest that this is a drug effect,[8,9] head-to-head investigations comparing the effects of PPIs with different effects on CYP2C19 activity (eg, omeprazole versus pantoprazole) would provide more insights to this topic. In addition, it may be argued that the presence of CYP2C19 polymorphisms could have modified the pharmacodynamic response to clopidogrel. However, the influence of CYP2C19 loss-of-function allelic variations on clopidogrel-mediated antiplatelet effects is known to be relatively small (5% to 12%).[38,39] In addition, the small sample size of this pilot investigation and the fact that pantoprazole has limited interference, CYP2C19 activity makes it unlikely that CYP2C19 polymorphisms would have emerged as a variable modifying our pharmacodynamic findings. Of note, prior clinical investigations have failed to identify any impact of CYP2C19 polymorphisms on adverse outcomes of PPI-treated patients.[32,40] Also, a pharmacokinetic evaluation would have provided more insights on the lack of a metabolic interaction between pantoprazole and clopidogrel. Ultimately, whether the results obtained in our study would have been different using a pantoprazole daily dose of 40 mg, which is commonly used in clinical practice, instead of 80 mg cannot be ascertained. However, a pharmacodynamic interaction with a lower dose would be unlikely.

Sources of Funding

This investigator-initiated study was funded by a grant from Sanofi Aventis/Bristol-Myers Squibb to the University of Florida College of

Medicine–Jacksonville (Principal Investigator: Dominick J. Angiolillo, MD, PhD).

Disclosures

Dr Angiolillo (corresponding author) reports receiving honoraria for lectures from Bristol Myers Squibb, Sanofi-Aventis, Eli Lilly Co, and Daiichi Sankyo Inc; consulting fees from Bristol Myers Squibb, Sanofi-Aventis, Eli Lilly Co, Daiichi Sankyo Inc, The Medicines Company, Portola, Novartis, Medicure, Accumetrics, Arena Pharmaceuticals, Astra Zeneca, Merck, and Evolva; and research grants from Bristol Myers Squibb, Sanofi-Aventis, GlaxoSmithKline, Otsuka, Eli Lilly Co, Daiichi Sankyo Inc, The Medicines Company, Portola, Accumetrics, Schering-Plough, Astra-Zeneca, and Eisai. Dr Ferreiro reports honoraria for lectures from Eli Lilly Co and Daiichi Sankyo, Inc. Dr Bass reports honoraria for lectures from Eli Lilly Co and Daiichi Sankyo, Inc.

References

1. Yusuf S, Zhao F, Mehta SR, Chrolavicius S, Tognoni G, Fox KK; Clopidogrel in Unstable Angina to Prevent Recurrent Events Trial Investigators. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. *N Engl J Med*. 2001; 345:494–502.
2. Steinhubl SR, Berger PB, Mann JT III, Fry ET, DeLago A, Wilmer C, Topol EJ; CREDO Investigators. Clopidogrel for the Reduction of Events During Observation: Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: a randomized controlled trial. *JAMA*. 2002;288:2411–2420.
3. Sabatine MS, Cannon CP, Gibson CM, López-Sendón JL, Montalescot G, Theroux P, Claeys MJ, Cools F Hill KA, Skene AM, McCabe CH, Braunwald E; CLARITY-TIMI 28 Investigators. Addition of clopidogrel to aspirin and fibrinolytic therapy for myocardial infarction with ST-segment elevation. *N Engl J Med*. 2005;352:1179–1189.
4. Bonello L, Harhouri K, Sabatier F, Camoin-Jau L, Arnaud L, Baumstarck-Barrau K, Ait-Mokhtar O, Roubille F, Piot C, Lesavre N, Paganelli F, Dignat-George F. Level of adenosine diphosphate receptor P2Y12 blockade during percutaneous coronary intervention predicts the extent of endothelial injury, assessed by circulating endothelial cell measurement. *J Am Coll Cardiol*. 2010;56:1024–1031.
5. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Alfonso F, Macaya C, Bass TA, Costa MA. Variability in individual responsiveness to clopidogrel: clinical implications, management, and future perspectives. *J Am Coll Cardiol*. 2007;49:1505–1516.
6. Ferreiro JL, Angiolillo DJ. Clopidogrel response variability: current status and future directions. *Thromb Haemost*. 2009;102:7–14.
7. Gilard M, Arnaud B, Cornily JC, Le Gal G, Lacut K, Le Calvez G, Mansourati J, Mottier D, Abgrall JF, Boschat J. Influence of omeprazole on the antiplatelet action of clopidogrel associated with aspirin: the randomized, double-blind OCLA (Omeprazole CLopidogrel Aspirin) study. *J Am Coll Cardiol*. 2008;51:256–260.
8. Ferreiro JL, Ueno M, Capodanno D, Desai B, Dharmashankar K, Darlington A, Charlton RK, Bass TA, Angiolillo DJ. Pharmacodynamic effects of concomitant versus staggered clopidogrel and omeprazole intake: results of a prospective randomized crossover study. *Circ Cardiovasc Interv*. 2010;3:436–441.
9. Angiolillo DJ, Gibson CM, Cheng S, Ollier C, Nicolas O, Bergougnan L, Perrin L, Lacreta FP, Hurbin F, Dubar M. Differential effects of omeprazole and pantoprazole on the pharmacodynamics and pharmacokinetics of clopidogrel in healthy subjects: randomized, placebo-controlled, crossover comparison studies. *Clin Pharmacol Ther*. 2011;89: 65–74.
10. Li XQ, Andersson TB, Ahlström M, Weidolf L. Comparison of inhibitory effects of the proton pump-inhibiting drugs omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole on human cytochrome P450 activities. *Drug Metab Dispos*. 2004;32:821–827.
11. Kazui M, Nishiya Y, Ishizuka T, Hagihara K, Farid NA, Okazaki O, Ikeda T, Kurihara A. Identification of the human cytochrome P450 enzymes involved in the two oxidative steps in the bioactivation of clopidogrel to its pharmacologically active metabolite. *Drug Metab Dispos*. 2010;38:92–99.
12. Ferreiro JL, Angiolillo DJ. Antiplatelet therapy: Clopidogrel plus PPIs: a dangerous combination? *Nat Rev Cardiol*. 2009;6:392–394.
13. Food and Drug Administration. Information for Healthcare Professionals: Update to the labeling of clopidogrel bisulfate (marketed as Plavix) to alert healthcare professionals about a drug interaction with omeprazole (marketed as Prilosec and Prilosec OTC). 2009 Nov [cited 2009 Nov 17]. http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/DrugSafetyInformationforHeathcareProfessionals/ucm190787.htm.
14. European Medicines Agency. Public statement on possible interaction between clopidogrel and proton-pump inhibitors. 2009 May [cited 2009 May 29]. URL: http://www.ema.europa.eu/humandocs/PDFs/EPAR/Plavix/32895609en.pdf.
15. Siller-Matula JM, Spiel AO, Lang IM, Kreiner G, Christ G, Jilma B. Effects of pantoprazole and esomeprazole on platelet inhibition by clopidogrel. *Am Heart J*. 2009;157:148.e1–148.e5.
16. Cuisset T, Frere C, Quilici J, Poyet R, Gaborit B, Bali L, Brissy O, Morange PE, Alessi MC, Bonnet JL. Comparison of omeprazole and pantoprazole influence on a high 150-mg clopidogrel maintenance dose the PACA (Proton Pump Inhibitors And Clopidogrel Association) prospective randomized study. *J Am Coll Cardiol*. 2009;54:1149–1153.
17. Aleil B, Ravanat C, Cazenave JP, Rochoux G, Heitz A, Gachet C. Flow cytometric analysis of intraplatelet VASP phosphorylation for the detection of clopidogrel resistance in patients with ischemic cardiovascular diseases. *J Thromb Haemost*. 2005;3:85–92.
18. Angiolillo DJ, Shoemaker SB, Desai B, Yuan H, Charlton RK, Bernardo E, Zenni MM, Guzman LA, Bass TA, Costa MA. Randomized comparison of a high clopidogrel maintenance dose in patients with diabetes mellitus and coronary artery disease: results of the Optimizing Antiplatelet Therapy in Diabetes Mellitus (OPTIMUS) study. *Circulation*. 2007; 115:708–716.
19. Malinin A, Pokov A, Spergling M, Defranco A, Schwartz K, Schwartz D, Mahmud E, Atar D, Serebruany V. Monitoring platelet inhibition after clopidogrel with the VerifyNow-P2Y12(R) rapid analyzer: the VERIfy Thrombosis risk ASsessment (VERITAS) study. *Thromb Res*. 2007;119: 277–284.
20. Fox SC, Behan MW, Heptinstall S. Inhibition of ADP-induced intracellular Ca2+ responses and platelet aggregation by the P2Y12 receptor antagonists AR-C69931MX and clopidogrel is enhanced by prostaglandin E1. *Cell Calcium*. 2004;35:39–46.
21. Abraham NS, Hlatky MA, Antman EM, Bhatt DL, Bjorkman DJ, Clark CB, Furberg CD, Johnson DA, Kahi CJ, Laine L, Mahaffey KW, Quigley EM, Scheiman J, Sperling LS, Tomaselli GF. ACCF/ACG/AHA 2010 Expert Consensus Document on the Concomitant Use of Proton Pump Inhibitors and Thienopyridines: a focused update of the ACCF/ACG/AHA 2008 Expert Consensus Document on Reducing the Gastrointestinal Risks of Antiplatelet Therapy and NSAID Use. *Circulation*. 2010;122:2619–2633.
22. Bates ER, Wei L, Angiolillo DJ. Clopidogrel-drug interactions. *J Am Coll Cardiol*. 2011;57:1251–1263.
23. Sibbing D, Morath T, Stegherr J, Braun S, Vogt W, Hadamitzky M, Schömig A, Kastrati A, von Beckerath N. Impact of proton pump inhibitors on the antiplatelet effects of clopidogrel. *Thromb Haemost*. 2009; 101:714–719.
24. Neubauer H, Engelhardt A, Krüger JC, Lask S, Börgel J, Mügge A, Endres HG. Pantoprazole does not influence the antiplatelet effect of clopidogrel: a whole blood aggregometry study after coronary stenting. *J Cardiovasc Pharmacol*. 2010;56:91–97.
25. Laine L, Hennekens C. Proton pump inhibitor and clopidogrel interaction: fact or fiction? *Am J Gastroenterol*. 2010;105:34–41.
26. Juurlink DN. Proton pump inhibitors and clopidogrel: putting the interaction in perspective. *Circulation*. 2009;120:2310–2312.
27. Ho PM, Maddox TM, Wang L, Fihn SD, Jesse RL, Peterson ED, Rumsfeld JS. Risk of adverse outcomes associated with concomitant use of clopidogrel and proton pump inhibitors following acute coronary syndrome. *JAMA*. 2009;301:937–944.
28. Juurlink DN, Gomes T, Ko DT, Szmitko PE, Austin PC, Tu JV, Henry DA, Kopp A, Mamdani MM. A population-based study of the drug interaction between proton pump inhibitors and clopidogrel. *CMAJ*. 2009; 180:713–718.
29. Kreutz RP, Stanek EJ, Aubert R, Yao J, Breall JA, Desta Z, Skaar TC, Teagarden JR, Frueh FW, Epstein RS, Flockhart DA. Impact of proton pump inhibitors on the effectiveness of clopidogrel after coronary stent placement: the clopidogrel Medco outcomes study. *Pharmacotherapy*. 2010;30:787–796.

30. Stockl KM, Le L, Zakharyan A, Harada AS, Solow BK, Addiego JE, Ramsey S. Risk of rehospitalization for patients using clopidogrel with a proton pump inhibitor. *Arch Intern Med*. 2010;170:704–710.
31. Gaglia MA Jr, Torguson R, Hanna N, Gonzalez MA, Collins SD, Syed AI, Ben-Dor I, Maluenda G, Delhaye C, Wakabayashi K, Xue Z, Suddath WO, Kent KM, Satler LF, Pichard AD, Waksman R. Relation of proton pump inhibitor use after percutaneous coronary intervention with drug-eluting stents to outcomes. *Am J Cardiol*. 2010;105:833–838.
32. O'Donoghue ML, Braunwald E, Antman EM, Murphy SA, Bates ER, Rozenman Y, Michelson AD, Hautvast RW, Ver Lee PN, Close SL, Shen L, Mega JL, Sabatine MS, Wiviott SD. Pharmacodynamic effect and clinical efficacy of clopidogrel and prasugrel with or without a proton-pump inhibitor: an analysis of two randomised trials. *Lancet*. 2009;374:989–997.
33. Rassen JA, Choudhry NK, Avorn J, Schneeweiss S. Cardiovascular outcomes and mortality in patients using clopidogrel with proton pump inhibitors after percutaneous coronary intervention or acute coronary syndrome. *Circulation*. 2009;120:2322–2329.
34. Ray WA, Murray KT, Griffin MR, Chung CP, Smalley WE, Hall K, Daugherty JR, Kaltenbach LA, Stein CM. Outcomes with concurrent use of clopidogrel and proton-pump inhibitors: a cohort study. *Ann Intern Med*. 2010;152:337–345.
35. Bhatt DL, Cryer BL, Contant CF, Cohen M, Lanas A, Schnitzer TJ, Shook TL, Lapuerta P, Goldsmith MA, Laine L, Scirica BM, Murphy SA, Cannon CP; the COGENT Investigators. Clopidogrel with or without omeprazole in coronary artery disease. *N Engl J Med*. 2010;363:1909–1917.
36. Dunn SP, Macaulay TE, Brennan DM. Baseline proton pump inhibitor use is associated with increased cardiovascular events with and without the use of clopidogrel in the CREDO trial (abstract). *Circulation*. 2008;118:S815.
37. Goodman SG, Clare R, Pieper KS, James SK, Nicolau JC, Storey RF, Cantor WJ, Angiolillo DJ, Husted S, Cannon CP, Steg PG, Mahaffey KW, Kilhamn J, Harrington RA, Wallentin L. Proton pump inhibitor use is likely a marker for, rather than a cause of, a higher risk of cardiovascular events: insights from PLATO (abstract). *Circulation*. 2010;122:A12092.
38. Hochholzer W, Trenk D, Fromm MF, Valina CM, Stratz C, Bestehorn HP, Büttner HJ, Neumann FJ. Impact of cytochrome P450 2C19 loss-of-function polymorphism and of major demographic characteristics on residual platelet function after loading and maintenance treatment with clopidogrel in patients undergoing elective coronary stent placement. *J Am Coll Cardiol*. 2010;55:2427–2434.
39. Shuldiner AR, O'Connell JR, Bliden KP, Gandhi A, Ryan K, Horenstein RB, Damcott CM, Pakyz R, Tantry US, Gibson Q, Pollin TI, Post W, Parsa A, Mitchell BD, Faraday N, Herzog W, Gurbel PA. Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. *JAMA*. 2009;302:849–857.
40. Simon T, Steg PG, Gilard M, Blanchard D, Bonello L, Hanssen M, Lardoux H, Coste P, Lefèvre T, Drouet E, Mulak G, Bataille V, Ferrières J, Verstuyft C, Danchin N. Clinical events as a function of proton pump inhibitor use, clopidogrel use, and cytochrome P450 2C19 genotype in a large nationwide cohort of acute myocardial infarction: results from the French Registry of Acute ST-Elevation and Non-ST-Elevation Myocardial Infarction (FAST-MI) Registry. *Circulation*. 2011;123:474–482.

CLINICAL PERSPECTIVE

The prognostic implication of reduced pharmacodynamic efficacy of clopidogrel therapy as a result of a drug-drug interaction with proton-pump inhibitors (PPI) has not been elucidated fully. The regulatory authorities, in particular the Food and Drug Administration and the European Medicines Agency, have recommended avoidance of the combination of clopidogrel and omeprazole, the most commonly prescribed PPI. However, limited information is available on the effects of other PPIs, such as pantoprazole, which has lower potential to inhibit the CYP2C19 enzyme, on the pharmacodynamics of clopidogrel. The results of this prospective, randomized, crossover study demonstrate the absence of any significant impairment in clopidogrel-induced pharmacodynamic efficacy as assessed by several assays when pantoprazole is administered either concomitantly or staggered. Notably, this investigation used a dose of pantoprazole (80 mg) higher than that used in clinical practice to maximize any of its adverse effects on CYP2C19. Therefore, it is unlikely that a pharmacodynamic interaction would be observed with the lower dose used more commonly in clinical practice (eg, 40 mg). These observations are in line with the concept that a PPI-clopidogrel interaction is not a class-specific effect but rather a drug-specific effect affecting PPIs metabolized primarily by CYP2C19 (eg, omeprazole) and support recommendations suggesting that if a PPI is warranted in a patient at increased risk of a gastrointestinal bleed while receiving dual antiplatelet therapy, pantoprazole may be considered as a safe treatment option.

III. Cigarette smoking is associated with a dose-response effect in clopidogrel-treated patients with diabetes mellitus and coronary artery disease: results of a pharmacodynamic study.

Ueno M, Ferreiro JL, Desai B, Tomasello SD, Tello-Montoliu A, Capodanno D, Capranzano P, Kodali M, Dharmashankar K, Charlton RK, Bass TA, Angiolillo DJ.

JACC Cardiovasc Interv. 2012;5:293-300.

JACC: CARDIOVASCULAR INTERVENTIONS VOL. 5, NO. 3, 2012

ISSN 1936-8798/$36.00
PUBLISHED BY ELSEVIER INC. DOI: 10.1016/j.jcin.2011.09.027

Cigarette Smoking Is Associated With a Dose-Response Effect in Clopidogrel-Treated Patients With Diabetes Mellitus and Coronary Artery Disease

Results of a Pharmacodynamic Study

Masafumi Ueno, MD,*† José Luis Ferreiro, MD,* Bhaloo Desai, PHD,*
Salvatore D. Tomasello, MD,* Antonio Tello-Montoliu, MD, PHD,*
Davide Capodanno, MD,* Piera Capranzano, MD,* Murali Kodali, MD,*
Kodlipet Dharmashankar, MD,* Ronald K. Charlton, PHD,‡ Theodore A. Bass, MD,*
Dominick J. Angiolillo, MD, PHD*

Jacksonville, Florida; and Osakasayama, Japan

Objectives This study sought to assess the presence of a dose-response effect of cigarette smoking and its impact on high on-treatment platelet reactivity (HPR) in patients with diabetes mellitus treated with clopidogrel.

Background Cigarette smoking is an inducer of cytochrome P450 1A2, a hepatic enzyme involved in clopidogrel metabolism. If cigarette smoking is associated with a dose-response effect on pharmacodynamic measures in clopidogrel-treated patients is unknown.

Methods A total of 134 type 2 diabetes mellitus patients on maintenance aspirin and clopidogrel therapy were studied. Patients were divided into 3 groups according to cotinine levels: <3 ng/ml (nonsmokers), 3 to 199 ng/ml (light smokers), and ≥200 ng/ml (heavy smokers). Platelet function was assessed by light transmittance aggregometry, VerifyNow P2Y12 assay (Accumetrics, San Diego, California), and vasodilator-stimulated phosphoprotein. Rates of HPR were defined using established cutoff values.

Results A dose-response effect was observed for all pharmacodynamic parameters tested. Serum cotinine levels were inversely associated with platelet reactivity as assessed by light transmittance aggregometry using 5 and 20 μmol/l adenosine diphosphate ($p < 0.0001$ for all). Accordingly, platelet disaggregation increased with levels of serum cotinine ($p < 0.0001$). Similar results were found with $P2Y_{12}$ reaction units ($p < 0.0001$) and inhibition of platelet aggregation ($p = 0.005$) as defined by VerifyNow P2Y12 testing, and platelet reactivity index ($p = 0.002$) as assessed by vasodilator-stimulated phosphoprotein. Higher serum cotinine levels were significantly associated with lower rates of HPR, as defined according to various pharmacodynamic cutoff measures.

Conclusions Cigarette smoking is associated with a dose-response effect on clopidogrel-induced antiplatelet effects and lower rates of HPR in diabetes mellitus patients. (J Am Coll Cardiol Intv 2012;5:293–300) © 2012 by the American College of Cardiology Foundation

From the *Division of Cardiology, University of Florida College of Medicine–Jacksonville, Jacksonville, Florida; †Division of Cardiology, Faculty of Medicine, Kinki University, Osakasayama, Japan; and the ‡Jacksonville Transplant Center–Shands Jacksonville, Jacksonville, Florida. Dr. Ferreiro has received honoraria for lectures from Daiichi Sankyo, Inc. and Eli Lilly Co. Dr. Bass has reported receiving honoraria for lectures from Eli Lilly Co. and Daiichi Sankyo, Inc., consulting fees from Eli Lilly Co. and Daiichi Sankyo, Inc., and research grants from Baxter. Dr. Angiolillo has received honoraria for lectures from Bristol-Myers Squibb, Sanofi-Aventis, Eli Lilly Co., and Daiichi Sankyo, Inc., consulting fees from AstraZeneca, Bristol-Myers Squibb, Sanofi-Aventis, Eli Lilly Co., Daiichi Sankyo, Inc., The Medicines Company, Portola Pharmaceuticals, Novartis, Medicure, Accumetrics, Arena Pharmaceuticals, AstraZeneca, and Abbott Vascular; and research grants and funding from Bristol-Myers Squibb, Sanofi-Aventis, GlaxoSmithKline, Otsuka, Eli Lilly Co., Daiichi Sankyo, The Medicines Company, Portola Pharmaceuticals, Accumetrics, Schering-Plough, AstraZeneca, Eisai, the James and Esther King Biomedical Research Program, and the University of Florida College of Medicine–Jacksonville. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose. Drs. Ueno and Ferreiro contributed equally to this work.

Manuscript received July 27, 2011; revised manuscript received September 19, 2011, accepted September 28, 2011.

JACC: CARDIOVASCULAR INTERVENTIONS, VOL. 5, NO. 3, 2012
MARCH 2012:293–300

Numerous investigations have shown a broad variability in clopidogrel-induced antiplatelet effects, and patients with high on-treatment platelet reactivity (HPR) have an increased risk of recurrent atherothrombotic events (1,2). Multiple factors have been associated with the degree of platelet inhibition induced by clopidogrel. Among these, genetic and environmental factors modulating hepatic metabolism of clopidogrel appear to have a pivotal role (1,2). Clopidogrel is a prodrug that requires a 2-step oxidation by cytochrome P450 (CYP) isoenzymes to generate an active metabolite that in turn irreversibly inhibits the platelet $P2Y_{12}$ receptor (3). Cigarette smoking is a known inducer of CYP1A2, which is the predominant isoenzyme responsible for the first oxidative step in the conversion of clopidogrel into its active metabolite (4,5). Pharmacodynamic (PD) and clinical studies have shown that smokers treated with clopidogrel have enhanced platelet inhibition and derive higher relative benefit, as assessed by angiographic and clinical outcomes, than nonsmokers do (6–9). However, these studies identified the aforementioned effects in smokers consuming above a certain threshold of number of cigarettes and were not able to determine a dose-response effect in a continuous way. This may be attributed to the fact that these investigations were based on self-reported smoking, which is not an objective measure of the amount of nicotine exposure, as it depends for instance on the type and brand of cigarettes and smokers' habit (e.g., deep inhalation). In addition, because baseline characteristics are associated with variations in clopidogrel metabolism, it cannot be excluded that patient selection may have had an impact on these findings.

Abbreviations and Acronyms

ADP = adenosine diphosphate
CI = confidence interval
CYP = cytochrome P450
DM = diabetes mellitus
HPR = high on-treatment platelet reactivity
IPA = inhibition of platelet aggregation
LPA = late values of on-treatment platelet aggregation
LTA = light transmittance aggregometry
MFI = mean fluorescence intensity
MPA = maximal values of on-treatment platelet aggregation
OR = odds ratio
PD = pharmacodynamic
PGE_1 = prostaglandin E_1
PRI = platelet reactivity index
PRP = platelet-rich plasma
PRU = $P2Y_{12}$ reaction units
VASP-P = phosphorylation of vasodilator-stimulated phosphoprotein

In the present investigation, the impact of cigarette smoking on clopidogrel-induced antiplatelet effects was assessed by means of a more objective assessment based on levels of serum cotinine, the major stable degradation product of nicotine metabolism (10). Because clopidogrel metabolism is reduced among patients with diabetes mellitus (DM), which may contribute to their high prevalence of HPR while on clopidogrel therapy (11), this population was identified to test our study hypothesis. The aim of the present investigation was to assess if there is a dose-response effect of cigarette smoking, as assessed by serum cotinine levels, and how this affects rates of HPR in patients with DM on maintenance clopidogrel therapy.

Methods

Patient population. The present investigation is a cross-sectional observational study that evaluated the association between cigarette smoking and PD effects of clopidogrel. A database of patients who had undergone platelet function assessments at our Thrombosis Research Laboratory (University of Florida College of Medicine–Jacksonville) between 2006 and 2010 was used to identify eligible subjects for this investigation. Patients meeting study inclusion criteria, who also had a serum sample collected at the time of platelet function assessment to enable cotinine measurement, were identified. All patients had undergone percutaneous coronary intervention with stent implantation and were treated with dual antiplatelet therapy per standard of care. In particular, patients were eligible for the study if they had type 2 DM and were clinically stable while on maintenance dual antiplatelet therapy with aspirin (81 mg daily) and clopidogrel (75 mg daily) for at least 1 month. Patients needed to be on maintenance dual antiplatelet therapy for at least 1 month as prior investigations have shown that platelet reactivity is subject to variability in the earlier phases of treatment and reaches a steady-state phase following 1 month of therapy (12–14). Type 2 DM patients also needed to have been medically managed (oral or insulin therapy) for at least 2 months without changes in hypoglycemic treatment regimen. General major exclusion criteria included: known allergies to aspirin or clopidogrel; left ventricular ejection fraction <30%; blood dyscrasia; active bleeding or bleeding diathesis; gastrointestinal bleed within last 6 months; hemodynamic instability; cerebrovascular accident within 3 months; any malignancy; concomitant use of other antithrombotic drugs (oral anticoagulants, dipyridamole, ticlopidine, or cilostazol); recent treatment (<30 days) with a glycoprotein IIb/IIIa antagonist; platelet count $<100 \times 10^3/\mu l$; liver disease (baseline alanine transaminase >2.5× the upper limit of normal).

Patients were recruited at the Division of Cardiology of the University of Florida College of Medicine–Jacksonville. The study complied with the Declaration of Helsinki and was approved by the Institutional Review Board of the University of Florida College of Medicine–Jacksonville. All subjects provided written informed consent for platelet function assessments and for storage of serum samples. The authors had full access to the data and take full responsibility for its integrity. All authors have read and agreed to the manuscript as written.

Blood sampling and functional assessments. Peripheral venous blood samples were drawn with a loose tourniquet to

avoid artifacts through a short venous catheter inserted into a forearm vein. Samples were collected before administration of the morning dose of clopidogrel (trough levels). The first 2 to 4 ml of blood was discarded to avoid spontaneous platelet activation. Samples were processed within 1 h after blood drawing.

Light transmittance aggregometry. Platelet aggregation was performed using light transmittance aggregometry (LTA) according to standard protocols (15–17). In brief, platelet aggregation was assessed using platelet-rich plasma (PRP) by the turbidimetric method in a 2-channel aggregometer (Chrono-Log 490 Model, Chrono-Log Corp., Havertown, Pennsylvania). PRP was obtained as a supernatant after centrifugation of citrated blood at 800 revolutions/min for 10 min. The isolated PRP was kept at 37°C before use. Platelet-poor plasma was obtained by a second centrifugation of the blood fraction at 2,500 revolutions/min for 10 min. Light transmission was adjusted to 0% with PRP and to 100% for platelet-poor plasma for each measurement and assessed following challenge with adenosine diphosphate (ADP) (5 and 20 μmol/l) (15–17). Maximal (MPA) and late (LPA) values of on-treatment platelet aggregation were measured. Percentage of platelet disaggregation was derived from MPA and LPA values [disaggregation (%) = 100 × (1 − LPA/MPA)], as previously defined (15,16).

VerifyNow P2Y12 assay. The VerifyNow P2Y12 assay (Accumetrics, San Diego, California) is a rapid whole-blood point-of-care assay and was used according to the instructions of the manufacturer (16,17). The VerifyNow P2Y12 assay reports the results as $P2Y_{12}$ reaction units (PRU). This assay mimics turbidimetric aggregation and uses disposable cartridges containing 20 mmol/l ADP and 22 nmol/l prostaglandin E_1 (PGE_1). Aggregation testing using ADP as a sole agonist activates $P2Y_1$ and $P2Y_{12}$ purinergic signaling, whereas adding PGE_1 increases the specificity of the test for $P2Y_{12}$ signaling. In a separate channel of the cartridge in which iso-TRAP is used as an agonist, a baseline value for platelet function is obtained, enabling assessment of inhibition of platelet aggregation (IPA) without having to wean the patient off antiplatelet treatment.

$P2Y_{12}$ reactivity index. The platelet reactivity index (PRI) was calculated as a measure of the functional status of the $P2Y_{12}$ signaling pathway. PRI was determined through assessment of phosphorylation status of vasodilator-stimulated phosphoprotein (VASP-P), a key, specific intraplatelet mediator of $P2Y_{12}$ signaling, according to standard protocols (15–17). In brief, VASP-P was measured by quantitative flow cytometry (Beckman Coulter FC500, Miami, Florida) using commercially available labeled monoclonal antibodies (Biocytex Inc., Marseille, France). The PRI was calculated after measuring the mean fluorescence intensity (MFI) of VASP-P levels following challenge with PGE_1 and PGE_1 plus ADP. PGE_1 increases VASP-P levels through stimulation of adenylate cyclase, whereas ADP binding to purinergic receptors leads to inhibition of adenylate cyclase. Therefore, the addition of ADP to PGE_1-stimulated platelets reduces levels of PGE_1-induced VASP-P. The PRI was calculated as follows: ([MFI PGE_1] − [MFI PGE_1 + ADP]/[MFI PGE_1]) × 100. Elevated PRI values indicate up-regulation of the $P2Y_{12}$ signaling pathway (15–17).

Cotinine measurement. Cotinine levels were measured as a final batch assessment using stored serum samples collected at the time of platelet function assessment using the Cotinine Blood Test kit (Calbiotech, Spring Valley, California), a solid phase competitive enzyme-linked immunoadsorbent assay, as previously described (18). The samples and cotinine enzyme conjugate are added to the wells coated with anticotinine antibody. Cotinine in the samples competes with a cotinine enzyme conjugate for binding sites. Unbound cotinine and cotinine enzyme conjugate is washed off by a washing step. With the addition of the substrate, the intensity of color is inversely proportional to the concentration of cotinine in the samples obtained with the cotinine blood test. A standard curve is prepared relating color intensity to the concentration of the cotinine (18).

Definitions. Patients were divided into 3 groups according to serum cotinine levels measured by the cotinine enzyme-linked immunoadsorbent assay test. Serum cotinine levels <3, 3 to 199, and ≥200 ng/ml indicated nonsmoker, light smoker, and heavy smoker status, respectively (19–21).

HPR was defined using various previously defined cutoff levels that have been associated with an increased risk of recurrent ischemic events (1,15,22,23). These included the following cutoff values using LTA: MPA-ADP (20 μmol/l) >50% and MPA-ADP (5 μmol/l) >46%; VerifyNow P2Y12 assay: PRU >230 and IPA <40%; and VASP: PRI >50%.

Statistical analysis. Continuous variables were analyzed for a normal distribution with the Kolmogorov-Smirnov test and presented as mean ± SD or as median and interquartile range if a normal distribution was present or not, respectively. Student t test or Mann-Whitney U test were used for comparisons of continuous variables where appropriate. Categorical variables are expressed as frequencies and percentages. Categorical variables were tested using the chi-square test or Fisher exact test when at least 25% of values showed an expected cell frequency below 5. Analysis of variance with post hoc Bonferroni correction was used to compare continuous variables among more than 2 groups and correct for multiple comparisons. In addition, p values for trend when assessing platelet reactivity according to the smoking degree, which was considered as a categorical variable with an ordinal scale, were performed with a polynomial contrast with analysis of variance method, using median values of each category as coefficients. Comparisons between categorical variables were performed using McNemar test or binomial exact test. Control for potential confounders and analysis of independent correlates of HPR were performed with a logistic regression model, including

Table 1. Baseline Demographics and Clinical Characteristics

	Nonsmoker (n = 85)	Light Smoker (n = 27)	Heavy Smoker (n = 22)	p Value
Age, yrs	62.3 ± 9.0	64.0 ± 9.1	57.6 ± 9.1	0.04
Male	41 (48)	16 (59)	13 (59)	0.47
Race				0.67
Caucasian	52 (61)	20 (74)	16 (73)	
African American	29 (34)	6 (22)	5 (23)	
Hispanic	2 (2)	0	1 (5)	
Asian	2 (2)	1 (4)	0 (0)	
Risk factors/medical history				
Hyperlipidemia	79 (95)	25 (93)	20 (91)	0.72
Hypertension	82 (97)	27 (100)	22 (100)	0.41
Creatinine >1.5 mg/dl	11 (13)	3 (11)	0 (0)	0.35
Body mass index, kg/m^2	34.7 ± 7.7	31.7 ± 6.7	32.1 ± 7.7	0.13
Hemoglobin A_{1C}	7.7 ± 2.3	7.4 ± 1.5	7.6 ± 1.7	0.89
Prior myocardial infarction	50 (59)	16 (59)	18 (82)	0.13
Prior CABG	26 (31)	10 (37)	3 (14)	0.18
Prior stroke	5 (6)	2 (7)	2 (9)	0.87
Treatment				
Beta-blockers	71 (84)	21 (78)	15 (68)	0.27
Nitrates	27 (32)	10 (37)	7 (32)	0.87
ACE inhibitors/ARB	75 (88)	20 (74)	17 (77)	0.15
PPI	21 (25)	7 (26)	5 (23)	0.97
Statin	79 (93)	23 (85)	17 (77)	0.09
Insulin therapy	35 (41)	7 (26)	8 (36)	0.36

Values are mean ± SD or n (%).
ACE = angiotensin-converting enzyme; ARB = angiotensin II receptor blockers; CABG = coronary artery bypass graft; PPI = proton pump inhibitors.

age, insulin treatment, body mass index, creatinine >1.5 mg/dl, hemoglobin A_{1C}, use of statin, and proton pump inhibitors as covariates, and the degree of smoker (non-, light, or heavy smoker) as the independent categorical variable of interest, using nonsmoker as the reference category. Odds ratio (OR) and 95% confidence interval (CI) were calculated. All univariate variables $p < 0.1$ and those deemed of clinical interest were included in the statistical model. All probability values reported are 2-sided, and a value of $p < 0.05$ was considered significant. Statistical analysis was performed with SPSS software (version 15.0, SPSS Inc., Chicago, Illinois).

Results

A total of 134 type 2 DM patients with stable coronary artery disease on aspirin and clopidogrel therapy meeting study inclusion criteria were identified for this investigation. A total of 49 patients (37%) were active smokers. Patients were divided into 3 groups according to serum cotinine levels: <3 ng/ml (nonsmoker; n = 85), 3 to 199 ng/ml (light smoker; n = 27), and ≥200 ng/ml (heavy smoker; n = 22). Baseline demographics and clinical characteristics of the study population are provided in Table 1. Patients were similar for all baseline characteristics, except for a lower age in the heavy smoker group ($p = 0.04$).

A dose-response effect was observed for all pharmacodynamic parameters tested. Serum cotinine levels were inversely associated with levels of on-treatment platelet reactivity as assessed by LTA for both MPA and LPA values using 5 and 20 μmol/l ADP (p for trend <0.0001) (Fig. 1). Accordingly, platelet disaggregation increased with levels of serum cotinine (p for trend <0.0001; both 5 and 20 μmol/l ADP; data not shown). Similarly to the LTA findings, results obtained with the VerifyNow P2Y12 assay also showed a dose-response effect as measured by PRU (p for trend <0.0001) and IPA (p for trend = 0.002) values (Fig. 2). Ultimately, enhanced clopidogrel-induced antiplatelet effects with increased cotinine levels were observed using flow cytometric assessment of VASP to define PRI values (p for trend = 0.001) (Fig. 3).

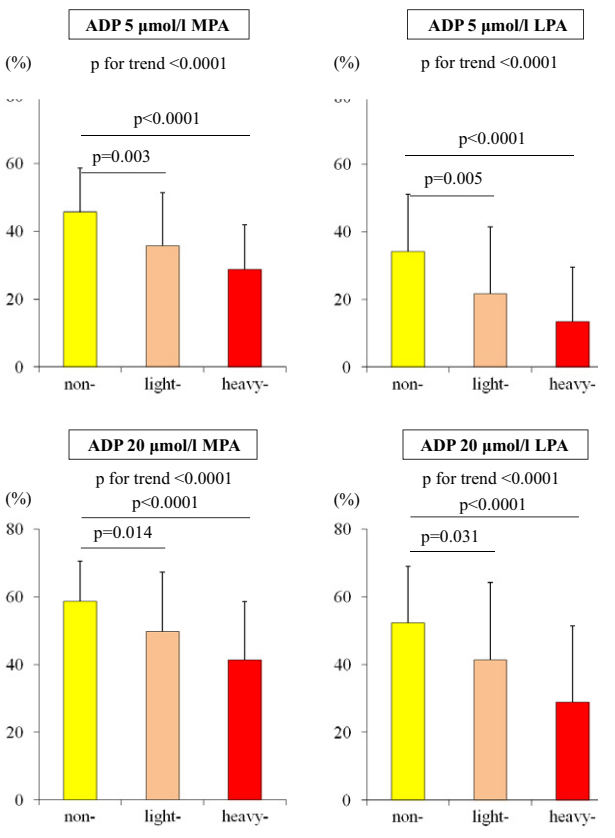


Figure 1. Platelet Reactivity Assessed by LTA According to the Degree of Smoking Defined by Cotinine Levels

Impact of the degree of smoking status to clopidogrel-induced antiplatelet effects on maximum and late 5 μmol/l and 20 μmol/l adenosine diphosphate (ADP)-induced platelet aggregation. Error bars indicate standard deviations of the mean. LPA = late value of on-treatment platelet aggregation; LTA = light transmittance aggregometry; MPA = maximal value of on-treatment platelet aggregation.

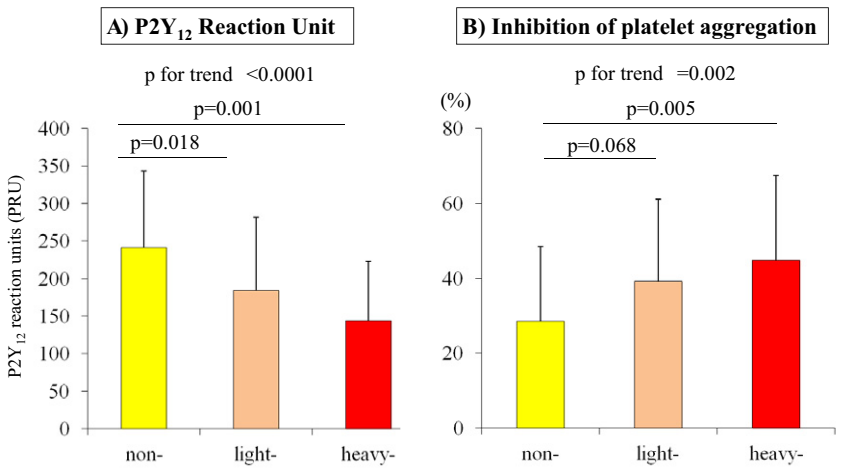


Figure 2. Platelet Reactivity Assessed by the VerifyNow P2Y$_{12}$ Assays According to the Degree of Smoking Defined by Cotinine Levels

Impact of the degree of smoking status to clopidogrel-induced antiplatelet effects on P2Y$_{12}$ reaction units (PRU) **(A)** and inhibition of platelet aggregation (IPA) **(B)**. Error bars indicate SD of the mean. VerifyNow P2Y$_{12}$ assays are a product of Accumetrics (San Diego, California).

The prevalence of HPR in the overall study population varied according to the definition used: MPA-ADP (20 μmol/l): 69%; MPA-ADP (5 μmol/l): 39%; PRU: 48%; IPA: 67%; PRI: 73%. Higher serum cotinine levels were significantly associated with lower rates of HPR as defined according to all pharmacodynamic cutoff measures (Fig. 4).

A multivariable logistic regression analysis showed that, compared with nonsmokers, light (adjusted OR: 0.24, 95% CI: 0.074 to 0.76, p = 0.015) and heavy smokers (adjusted OR: 0.10, 95% CI: 0.027 to 0.37, p = 0.001) were less likely to have HPR as assessed by LTA following 20 μmol/l ADP stimuli. Similar results were found with 5 μmol/l ADP stimuli (light smokers: adjusted OR: 0.47, 95% CI: 0.16 to 1.37, p = 0.17; heavy smokers: adjusted OR: 0.051, 95% CI: 0.006 to 0.43, p = 0.006), PRU values (light smokers: adjusted OR: 0.23, 95% CI: 0.063 to 0.85, p = 0.027; heavy smokers: adjusted OR: 0.24, 95% CI: 0.052 to 1.08, p = 0.063), IPA (light smokers: adjusted OR: 0.21, 95% CI: 0.062 to 0.73, p = 0.014; heavy smokers; adjusted OR: 0.14, 95% CI: 0.034 to 0.58, p = 0.006), and PRI values (light smokers: adjusted OR: 0.25, 95% CI: 0.067 to 0.94, p = 0.039; heavy smokers; adjusted OR: 0.24, 95% CI: 0.055 to 1.03, p = 0.054).

Discussion

Cigarette smoking has emerged as a factor associated with improved clopidogrel effects. This is supported by PD investigations as well as clinical outcome studies demonstrating better clopidogrel effects among smokers versus nonsmokers (6–9). However, to date, investigations have been based on self-reported smoking, which is a nonobjective way to quantify nicotine exposure. In turn, even though these seminal investigations were able to consistently define a threshold of smoking at least one-half pack/day to significantly affect the efficacy of clopidogrel, they were not able to ascertain the presence of a dose-response effect among smokers (6–9). Cotinine is the major degradation product of nicotine metabolism and has a serum half-life of about 17 h (being detectable up to 3 days after withdrawal), and its levels correlate with the amount of nicotine exposure (i.e., severity of smoking habit) (10). To the best of our knowledge, the present investigation is the first PD study to examine and demonstrate the presence of a dose-response effect of smoking on clopidogrel effects by using a more objective measure to quantify cigarette smoking as determined by assessing serum cotinine levels. In addition to demonstrating the impact of cotinine levels on the degree of platelet reactivity, our study showed a dose-response profile on the prevalence of rates of HPR. Importantly, our findings were consistent using multiple PD parameters and confirmed in multivariate analysis, which provided support to our study hypothesis.

Multiple factors have been associated with interindividual response profiles to clopidogrel therapy (1,2). Cigarette smoking has been recently added to the factors associated with improved clopidogrel effects (6–9). The enhanced PD effects observed among smokers and the lower prevalence of HPR, defined according to cutoff values associated with recurrent atherothrombotic events, can explain why these subjects derive more benefit from clopidogrel in preventing ischemic events than nonsmokers do (7–9). The enhanced platelet inhibitory effects induced by clopidogrel among smokers can also contribute to their increased potential for bleeding complications (9,24). Reduced ischemic event rates

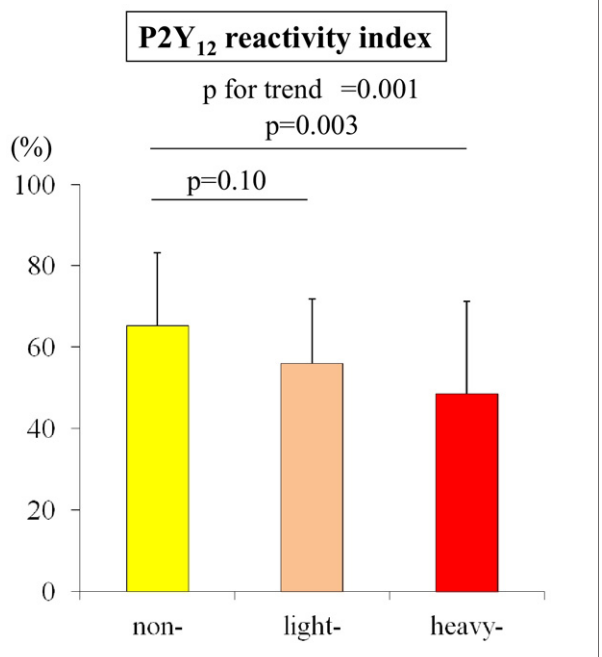


Figure 3. Platelet Reactivity Assessed by Phosphorylation Status of VASP According to the Degree of Smoking Defined by Cotinine Levels

Impact of the degree of smoking status to clopidogrel-induced antiplatelet effects on platelet reactivity index (PRI). Error bars indicate standard deviations of the mean. VASP = vasodilator-stimulated phosphoprotein.

and increased spontaneous bleeding have also been demonstrated with novel $P2Y_{12}$ inhibitors characterized by more potent PD effects (25,26). Several factors can explain the "smoker's paradox" observed among clopidogrel-treated patients. Cigarette smoking is a known inducer of CYP1A2, which is the predominant isoenzyme responsible for the first oxidative step in the conversion of clopidogrel into its active metabolite (3). Therefore, accelerating the first step of clopidogrel biotransformation would help prevent it from being shunted toward esterases mediating transformation into inactive metabolites (8). Importantly, CYP1A2 activity increases relative to the number of cigarettes smoked per day (27), which may explain the dose-response effect observed in our study.

Investigations have shown that smokers have higher $P2Y_{12}$ expression in platelet lysates than nonsmokers do (28). Therefore, it may be hypothesized that a high platelet surface $P2Y_{12}$ density may contribute to an increased risk of recurrent ischemic events among smokers, which can potentially be suppressed to a relatively greater extent by clopidogrel. Indeed, it may be argued that although several clinical studies assessing adjunctive treatment with clopidogrel in addition to aspirin in high-risk patients showed a greater relative clinical benefit in smokers than in nonsmokers (7–9), others have not (29). Differences in patient characteristics may contribute to these discrepancies as numerous clinical characteristics have shown to affect clopidogrel metabolism and ultimately its PD effects (1,2). The present investigation was selectively conducted in patients with DM, known to have high rates of HPR (15–17,30–33). Studies have shown that this may be attributed to reduced metabolic activity of the CYP system in DM patients, which in turn generates lower levels of active metabolites than are found in non-DM patients (11). Therefore, including a population, such as patients with DM, with reduced CYP metabolic activity can increase the likelihood of identifying a dose-response effect when analyzing the impact of a CYP inducer, such as cigarette smoking. In line with this observation, recent findings have shown that the smokers' paradox is limited only to patients with a specific *CYP1A2* genotype (34). However, the latter investigation did not discriminate the intensity of smoking in their patient population.

Despite the fact that clopidogrel effects are enhanced in smokers versus nonsmokers, cardiovascular event rates, including mortality, still remain markedly higher among smokers irrespective of type of antiplatelet treatment regimen used (35). Smoking is a major risk factor for atherothrombotic cardiovascular processes and smoking cessation is a class I recommendation for secondary prevention of ischemic events in patients with vascular disease (36). Whereas the optimal healthcare saving goal to reduce atherothrombotic risk is smoking cessation, this objective is not always achieved and many patients with established atherosclerotic disease continue smoking. Therefore, defining the optimal antiplatelet treatment strategy in these patients becomes of key importance. This is particularly relevant to those patients who do not have a clinical indication to be on dual antiplatelet therapy with aspirin and clopidogrel therapy according practice guidelines and who rely on a single antiplatelet agent, mostly aspirin, for their antithrombotic protection. Head-to-head comparisons between aspirin and clopidogrel for secondary prevention of recurrent ischemic events showed clopidogrel to be only marginally better than aspirin in the CAPRIE (Clopidogrel Versus Aspirin in Patients at Risk of Ischemic Events) trial (37). The benefit of clopidogrel was increased in higher risk subgroups, including patients with DM (38,39). Although, dedicated comparative assessments in smokers versus nonsmokers are lacking in this study, it may be hypothesized that aspirin may offer less antithrombotic protection than clopidogrel does, particularly in smokers. In fact, given the increased density of $P2Y_{12}$ receptors among smokers, clopidogrel may be a more effective platelet inhibitor (28). Therefore, understanding the differences in antithrombotic effects of aspirin compared with clopidogrel among smokers may help define the antiplatelet agent of choice when single therapy is indicated.

Study limitations. The present investigation is a cross-sectional observational study that evaluated the association

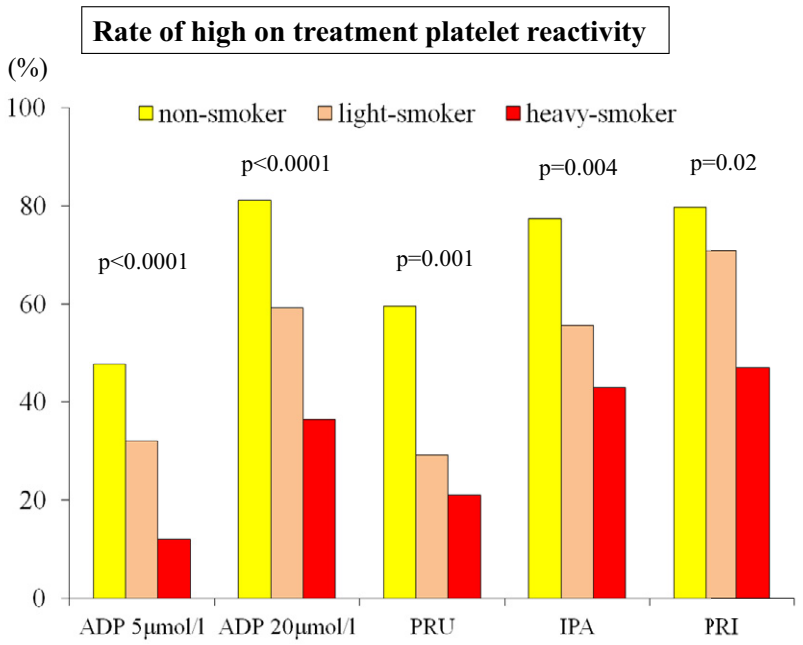


Figure 4. Rate of HPR According to the Degree of Smoking Defined by Cotinine Levels

Rates of high on-treatment platelet reactivity (HPR) assessed by different platelet function tests according to the degree of smoking defined by cotinine levels. Abbreviations as in Figures 1 to 3.

between cigarette smoking and PD effects of clopidogrel. A longitudinal study in which PD effects are measured in the same patient in the presence and absence of active cigarette smoking is needed to confirm a causative relationship between cigarette smoking and enhanced clopidogrel antiplatelet effects. The present investigation did not include pharmacokinetic assessments to determine clopidogrel active metabolite levels. In addition, the effects of smoking were not stratified according to individuals' genotype. The impact of cigarette smoking on pharmacokinetic and PD assessments, as well as if these may be affected by genotypes, is currently being investigated in a dedicated prospective trial (The Influence of Smoking Status on Prasugrel and Clopidogrel Treated Subjects Taking Aspirin and Having Stable Coronary Artery Disease; NCT01260584) that will provide further insights into this topic. A possible limitation of the present investigation is an overfitted covariate-adjusted model. However, in order to avoid spurious associations, we included in the analysis those variables that could represent potential confounders for the present analysis, as specified in the statistical analysis section.

Reprint requests and correspondence: Dr. Dominick J. Angiolillo, Division of Cardiology, University of Florida College of Medicine–Jacksonville, 655 West 8th Street, Jacksonville, Florida, 32209. E-mail: dominick.angiolillo@jax.ufl.edu.

REFERENCES

1. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, et al. Variability in individual responsiveness to clopidogrel: clinical implications, management, and future perspectives. J Am Coll Cardiol 2007;49:1505–16.
2. Ferreiro JL, Angiolillo DJ. Clopidogrel response variability: current status and future directions. Thromb Haemost 2009;102:7–14.
3. Farid NA, Kurihara A, Wrighton SA. Metabolism and disposition of the thienopyridine antiplatelet drugs ticlopidine, clopidogrel, and prasugrel in humans. J Clin Pharmacol 2010;50:126–42.
4. Zevin S, Benowitz NL. Drug interactions with tobacco smoking: an update. Clin Pharmacokinet 1999;36:425–38.
5. Bates ER, Lau WC, Angiolillo DJ. Clopidogrel-drug interactions. J Am Coll Cardiol 2011;57:1251–63.
6. Bliden KP, Dichiara J, Lawal L, et al. The association of cigarette smoking with enhanced platelet inhibition by clopidogrel. J Am Coll Cardiol 2008;52:531–3.
7. Saraff KY, Steinhubl SR, Hsu AP, Topol EJ. Smoking influences the effectiveness of dual antiplatelet therapy on long-term outcomes following percutaneous coronary intervention (abstr). J Am Coll Cardiol 2006;47:36B.
8. Desai NR, Mega JL, Jiang S, Cannon CP, Sabatine MS. Interaction between cigarette smoking and clinical benefit of clopidogrel. J Am Coll Cardiol 2009;53:1273–8.
9. Berger JS, Bhatt DL, Steinhubl SR, et al., for the CHARISMA Investigators. Smoking, clopidogrel, and mortality in patients with established cardiovascular disease. Circulation 2009;120:2337–44.
10. Wall MA, Johnson J, Jacob P, Benowitz NL. Cotinine in the serum, saliva, and urine of nonsmokers, passive smokers, and active smokers. Am J Public Health 1988;78:699–701.
11. Erlinge D, Varenhorst C, Braun OO, et al. Patients with poor responsiveness to thienopyridine treatment or with diabetes have lower levels of circulating active metabolite, but their platelets respond normally to active metabolite added ex vivo. J Am Coll Cardiol 2008;52:1968–77.

12. Gurbel PA, Bliden KP, Hiatt BL, O'Connor CM. Clopidogrel for coronary stenting: response variability, drug resistance, and the effect of pretreatment platelet reactivity. Circulation 2003;107:2908–13.
13. Price MJ, Berger PB, Teirstein PS, et al., for the GRAVITAS Investigators. Standard- vs high-dose clopidogrel based on platelet function testing after percutaneous coronary intervention: the GRAVITAS randomized trial. JAMA 2011;305:1097–105.
14. Campo G, Parrinello G, Ferraresi P, et al. Prospective evaluation of on-clopidogrel platelet reactivity over time in patients treated with percutaneous coronary intervention: relationship with gene polymorphisms and clinical outcomes. J Am Coll Cardiol 2011;57:2474–83.
15. Angiolillo DJ, Shoemaker SB, Desai B, et al. Randomized comparison of a high clopidogrel maintenance dose in patients with diabetes mellitus and coronary artery disease: results of the Optimizing Anti-Platelet Therapy in Diabetes Mellitus (OPTIMUS) study. Circulation 2007;115:708–16.
16. Angiolillo DJ, Capranzano P, Goto S, et al. A randomized study assessing the impact of cilostazol on platelet function profiles in patients with diabetes mellitus and coronary artery disease on dual antiplatelet therapy: results of the OPTIMUS-2 study. Eur Heart J 2008;29:2202–11.
17. Angiolillo DJ, Badimon JJ, Saucedo JF, et al. A pharmacodynamic comparison of prasugrel vs. high-dose clopidogrel in patients with type 2 diabetes mellitus and coronary artery disease: results of the Optimizing anti-Platelet Therapy In diabetes MellitUS (OPTIMUS)-3 trial. Eur Heart J 2011;32:838–46.
18. Ziegler UE, Kauczok J, Dietz UA, Reith HB, Schmidt K. Clinical correlation between the consumption of nicotine and cotinine concentrations in urine and serum by competitive enzyme-linked immunosorbent assay. Pharmacology 2004;72:254–9.
19. Benowitz NL, Bernert JT, Caraballo RS, Holiday DB, Wang J. Optimal serum cotinine levels for distinguishing cigarette smokers and nonsmokers within different racial/ethnic groups in the United States between 1999 and 2004. Am J Epidemiol 2009;169:236–48.
20. Sudbø J, Samuelsson R, Risberg B, et al. Risk markers of oral cancer in clinically normal mucosa as an aid in smoking cessation counseling. J Clin Oncol 2005;23:1927–33.
21. Hellerstein MK, Benowitz NL, Neese RA, et al. Effects of cigarette smoking and its cessation on lipid metabolism and energy expenditure in heavy smokers. J Clin Invest 1994;93:265–72.
22. Bonello L, Tantry US, Marcucci R, et al., for the Working Group on High On-Treatment Platelet Reactivity. Consensus and future directions on the definition of high on-treatment platelet reactivity to adenosine diphosphate. J Am Coll Cardiol 2010;56:919–33.
23. Valgimigli M, Campo G, de Cesare N, et al., for the 3T/2R Investigators. Intensifying platelet inhibition with tirofiban in poor responders to aspirin, clopidogrel, or both agents undergoing elective coronary intervention: results from the double-blind, prospective, randomized Tailoring Treatment with Tirofiban in Patients Showing Resistance to Aspirin and/or Resistance to Clopidogrel study. Circulation 2009;119:3215–22.
24. Ferreiro JL, Sibbing D, Angiolillo DJ. Platelet function testing and risk of bleeding complications. Thromb Haemost 2010;103:1128–35.
25. Wiviott SD, Braunwald E, McCabe CH, et al. Prasugrel versus clopidogrel in patients with acute coronary syndromes. N Engl J Med 2007;357:2001–15.
26. Wallentin L, Becker RC, Budaj A, et al., for the PLATO Investigators. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. N Engl J Med 2009;361:1045–57.
27. Tantcheva-Poór I, Zaigler M, Rietbrock S, Fuhr U. Estimation of cytochrome P-450 CYP1A2 activity in 863 healthy Caucasians using a saliva-based caffeine test. Pharmacogenetics 1999;9:131–44.
28. Shanker G, Kontos JL, Eckman DM, Wesley-Farrington D, Sane DC. Nicotine upregulates the expression of P2Y12 on vascular cells and megakaryoblasts. J Thromb Thrombolysis 2006;22:213–20.
29. Sibbald M, Yan AT, Huang W, et al. Association between smoking, outcomes, and early clopidogrel use in patients with acute coronary syndrome: insights from the Global Registry of Acute Coronary Events. Am Heart J 2010;160:855–61.
30. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, et al. Platelet function profiles in patients with type 2 diabetes and coronary artery disease on combined aspirin and clopidogrel treatment. Diabetes 2005;54:2430–5.
31. Angiolillo DJ, Bernardo E, Sabaté M, et al. Impact of platelet reactivity on cardiovascular outcomes in patients with type 2 diabetes mellitus and coronary artery disease. J Am Coll Cardiol 2007;50:1541–7.
32. Ferreiro JL, Angiolillo DJ. Diabetes and antiplatelet therapy in acute coronary syndrome. Circulation 2011;123:798–813.
33. Ueno M, Ferreiro JL, Tomasello SD, et al. Functional profile of the platelet $P2Y_{12}$ receptor signaling pathway in patients with type 2 diabetes mellitus and coronary artery disease. Thromb Haemost 2011;105:730–2.
34. Park KW, Park JJ, Jeon KH, et al. Enhanced clopidogrel responsiveness in smokers: smokers' paradox is dependent on cytochrome P450 CYP1A2 status. Arterioscler Thromb Vasc Biol 2011;31:665–71.
35. Ockene IS, Miller NH, for the American Heart Association Task Force on Risk Reduction. Cigarette smoking, cardiovascular disease, and stroke: a statement for healthcare professionals from the American Heart Association. Circulation 1997;96:3243–7.
36. Smith SC Jr., Allen J, Blair SN, et al. AHA/ACC guidelines for secondary prevention for patients with coronary and other atherosclerotic vascular disease: 2006 update. J Am Coll Cardiol 2006;47:2130–9.
37. CAPRIE Steering Committee. A randomised, blinded, trial of Clopidogrel versus Aspirin in Patients at Risk of Ischemic Events (CAPRIE). Lancet 1996;348:1329–39.
38. Bhatt DL, Marso SP, Hirsch AT, Ringleb PA, Hacke W, Topol EJ. Amplified benefit of clopidogrel versus aspirin in patients with diabetes mellitus. Am J Cardiol 2002;90:625–8.
39. Ringleb PA, Bhatt DL, Hirsch AT, et al., for the CAPRIE Investigators. Benefit of clopidogrel over aspirin is amplified in patients with a history of ischemic events. Stroke 2004;35:528–32.

Key Words: clopidogrel ■ diabetes mellitus ■ platelet function ■ smoking.

IV. Clopidogrel pretreatment in primary percutaneous coronary intervention: Prevalence of high on-treatment platelet reactivity and impact on preprocedural patency of the infarct-related artery.

Ferreiro JL, Homs S, Berdejo J, Roura G, Gomez-Lara J, Romaguera R, Teruel L, Sánchez-Elvira G, Marcano AL, Gómez-Hospital JA, Angiolillo DJ, Cequier A.

Thromb Haemost. 2013;110:110-7.

Clopidogrel pretreatment in primary percutaneous coronary intervention: Prevalence of high on-treatment platelet reactivity and impact on preprocedural patency of the infarct-related artery

José Luis Ferreiro[1]*; Sílvia Homs[1]*; Javier Berdejo[1]; Gerard Roura[1]; Josep Gómez-Lara[1]; Rafael Romaguera[1]; Luis Teruel[1]; Guillermo Sánchez-Elvira[1]; Ana Lucrecia Marcano[1]; Joan Antoni Gómez-Hospital[1]; Dominick J. Angiolillo[2]; Ángel Cequier[1]

[1]Heart Diseases Institute, Bellvitge University Hospital – IDIBELL, University of Barcelona, L'Hospitalet de Llobregat, Barcelona, Spain; [2]University of Florida College of Medicine-Jacksonville, Jacksonville, Florida, USA

Summary

To date, there is limited data on levels of platelet inhibition achieved in patients with ST-elevation myocardial infarction (STEMI) who are loaded with clopidogrel and aspirin (ASA) prior to undergoing primary percutaneous coronary intervention (P-PCI). The aim of this investigation was to evaluate the percentage of STEMI patients with high on-treatment platelet reactivity (HPR) to clopidogrel at the time of initiating P-PCI and its association with the initial patency of the infarct-related artery (IRA). This prospective pharmacodynamic study included 50 STEMI patients, previously naïve to oral antiplatelet agents, who received 500-mg ASA and 600-mg clopidogrel loading doses prior to P-PCI. Platelet function assessment was performed at the beginning of the procedure using various assays, including VerifyNow™ system (primary endpoint), light transmission aggregometry and multiple electrode aggregometry. The percentage of patients with suboptimal response to clopidogrel and ASA assessed with the VerifyNow™ system was 88.0% and 28.6%, respectively. Similar results were obtained with the other assays used. A higher percentage of patients with initial patency of the IRA was observed among those patients without HPR compared with those with HPR to clopidogrel (66.7% vs 15.9%; p=0.013), while no differences were observed regarding postprocedural angiographic or electrocardiographic outcomes. In conclusion, this study shows that a high percentage of STEMI patients have inadequate levels of clopidogrel-induced and, to a lesser extent, aspirin-mediated platelet inhibition when starting a P-PCI procedure, and suggests that a poor response to clopidogrel might be associated with impaired initial TIMI flow in the IRA.

Keywords

Clopidogrel responsiveness, ST-elevation myocardial infarction, antiplatelet therapy

Correspondence to:
José Luis Ferreiro, MD
Director of Cardiovascular Research Lab, Heart Diseases Institute
Bellvitge University Hospital – IDIBELL
Feixa Llarga s/n. CP 08907
L'Hospitalet de Llobregat, Barcelona, Spain
Tel.: +34 93 260 74 95, Fax: +34 93 260 75 41
E-mail: jlferreiro@bellvitgehospital.cat

Financial support:
Silvia Homs is recipient of a Grant of the Interventional Cardiology Section of the Spanish Society of Cardiology for Post-Residency Training in Research.

Received: January 23, 2013
Accepted after major revision: April 8, 2013
Prepublished online: April 25, 2013

doi:10.1160/TH13-01-0057
Thromb Haemost 2013; 110: 110–117

* JLF and SH contributed equally as first authors to this manuscript.

Introduction

Dual antiplatelet therapy with aspirin (ASA) and a $P2Y_{12}$ receptor blocker is currently the oral antiplatelet treatment of choice in patients suffering an acute coronary syndrome (ACS), including those with ST-elevation myocardial infarction (STEMI) undergoing primary percutaneous coronary intervention (P-PCI) (1). Despite the introduction of novel and more potent $P2Y_{12}$ receptor antagonists such as prasugrel and ticagrelor, clopidogrel is still broadly used in daily clinical practice including in the setting of P-PCI. Further, a considerable proportion of these patients are pretreated with a loading dose of clopidogrel at first medical contact with the emergency medical system (2). Importantly, clopidogrel pretreatment has shown to be of benefit in patients undergoing P-PCI (3-6). However, the pharmacodynamic (PD) efficacy of clopidogrel is subject to broad inter-individual variability (7) and a considerable proportion of patients, particularly in the setting of STEMI, present with high on-treatment platelet reactivity (HPR) (8, 9) which is associated with an increased risk of cardiovascular events (10).

To date, there is limited PD data in STEMI patients who have been pretreated with a loading dose (LD) of clopidogrel before undergoing P-PCI. The present study aimed to evaluate the percentage of STEMI patients with HPR at the very moment of initiating the procedure after receiving a LD of clopidogrel at the moment of diagnosis and its association with the initial patency of the infarct-related artery (IRA) in patients undergoing P-PCI as a reperfusion strategy.

Materials and methods

Subject population and study design

This is a prospective observational pharmacodynamics (PD) study that included consecutive patients admitted to a tertiary center with diagnosis of STEMI who received a 600-mg clopidogrel LD at the moment of diagnosis and prior to undergoing P-PCI. Patients could be first admitted at the emergency room of the tertiary hospital or quickly transferred by the emergency medical system from home or primary care centres (2). All patients were previously naïve to aspirin (acetylsalicylic acid, ASA) and clopidogrel. All patients were also treated with 500-mg ASA administered orally at the moment of diagnosis. Exclusion criteria were prior treatment with any antiplatelet agent, known allergies to aspirin or clopidogrel, cardiogenic shock, any active bleeding or malignancy, platelet count $<100x10^6/\mu l$, severe chronic kidney disease (creatinine clearance <30 ml/minute) and pregnant females. Technical procedures and drugs administration in the catheterisation lab were left at operator's criteria according to standard clinical practice. Operators were unaware of the results of the platelet function assays. All patients received heparin (100 U/kg) at time of presentation per institution protocol, measuring activated-clotting time (ACT) when catheterisation is started, having an ACT target of 250-350 seconds (sec) and 200-250 sec in those receiving glycoprotein IIb/IIIa inhibitors (GPIs).

The study complied with the Declaration of Helsinki and was approved by the Ethics Committee of the Bellvitge University Hospital. All subjects provided written informed consent to the study.

Endpoints, assessments of outcomes, and definitions

The primary endpoint was the evaluation of the association between clopidogrel HPR and patency of the IRA at the beginning of the procedure, which was evaluated with the Thrombolysis in Myocardial Infarction (TIMI) flow grade dichotomised into two arbitrary categories: poor flow (TIMI 0-1) vs good flow (TIMI 2-3). This categorisation was used because TIMI grade 2-3 flow allows complete visualisation of the distal area of the lesion, facilitating the procedure (11). Secondary endpoints were the post-procedural frequencies of a TIMI flow grade of 3, myocardial blush grade of 0 or 1, and complete resolution of ST-segment elevation.

TIMI flow grades were assessed as previously described (12): 0: no perfusion (no antegrade flow beyond the point of occlusion); 1: penetration without perfusion (the contrast material passes beyond the area of obstruction but "hangs up" and fails to opacify the entire coronary bed distal to the obstruction for the duration of the cine run); 2: partial reperfusion (the contrast material passes across the obstruction and opacifies the coronary bed distal to the obstruction; however, the rate of entry of contrast into the vessel distal to the obstruction and/or its rate of clearance from the distal bed is perceptibly slower than its entry into and/or clearance from comparable areas not perfused by the culprit vessel, e.g. the opposite coronary artery or coronary bed proximal to the obstruction); and 3: complete perfusion (antegrade flow into the bed distal to the obstruction occurs as promptly as into the bed proximal to the obstruction and clearance of contrast material from the involved bed is as rapid as from an uninvolved bed in the same vessel or the opposite artery).

Myocardial blush grades were assigned as follows (13): 0: no myocardial blush; 1: minimal myocardial blush or contrast density; 2: moderate myocardial blush or contrast density but less than that obtained during angiography of a contralateral or ipsilateral non-infarct-related coronary artery; and 3: normal myocardial blush or contrast density, similar to that obtained during angiography of a contralateral or ipsilateral non-infarct-related coronary artery.

A 12-lead electrocardiogram (ECG) was acquired at presentation and 60 to 90 minutes (min) after PCI, and the ST-segments of the postprocedural ECG were compared with those of the ECG at presentation. The degree of resolution of ST-segment elevation was categorized as complete (>70%), partial (30 to 70%), or none (<30%) (14).

Sample collection and platelet function assays

Blood samples for platelet function analyses were collected when arterial sheath to perform catheterisation was placed. All procedures were performed via radial artery access. The first 2-4 ml of blood were discarded to avoid spontaneous platelet activation. Samples were processed by trained laboratory personnel within 2 hours (h) after blood drawing. Platelet function assays included VerifyNow™ system, light transmission aggregometry and multiple electrode aggregometry (MEA).

VerifyNow assay

The VerifyNow (VN) assay is a rapid whole blood point-of-care device and was utilised according to the instructions of the manufacturer (Accumetrics, Inc., San Diego, CA, USA) as previously described (15). In brief, VN-$P2Y_{12}$ assay mimics turbidometric aggregation and utilises disposable cartridges containing 20 μM adenosin diphosphate (ADP) and 22 nM PGE1. Aggregation testing using ADP as a sole agonist activates $P2Y_1$ and $P2Y_{12}$ purinergic signalling, while adding PGE_1 increases the specificity of the test for $P2Y_{12}$ signalling (16). In a separate channel of the cartridge in which iso-TRAP is used as an agonist, a baseline value for platelet function is obtained, enabling assessment of platelet inhibition without having to wean the patient off antiplatelet treatment. The VN-$P2Y_{12}$ assay reports the results as $P2Y_{12}$ reaction units (PRU) and percent inhibition of platelet aggregation (%IPA), which is calculated as [(baseline - PRU) / baseline] × 100. In contrast to IPA values, which increase with decreasing platelet function, PRU values decrease with decreasing platelet function. A cut-off point of >240 PRUs was used to define clopidogrel HPR. Similarly, VN-ASA assay utilises disposable cartridges containing arachidonic acid (AA) and reports the results as Aspirin reaction units (ARU). ARU values decrease with enhanced aspirin-induced platelet inhibition. A cut-off value of >550 ARUs was used to define aspirin HPR.

Light transmission aggregometry (LTA)

LTA was performed according to standard protocols as previously described (17). Briefly, blood-citrate tubes were centrifuged at 100 g for 10 min to recover platelet-rich plasma (PRP) and further centrifuged at 2,400 g for 15 min to recover platelet poor plasma (PPP). Platelet aggregation was assessed using PRP and PPP by the turbidometric method in a two-channel aggregometer (Chrono-Log 490 Model, Chrono-Log Corp., Havertown, PA, USA). Light transmission was adjusted to 0% for PRP and to 100% for PPP for each measurement. Maximal platelet aggregation (MPA) was measured following stimuli with AA (1 mmol/l), and ADP (5 µmol/l). The cut-off values used to define HPR were MPA ≥20% for ASA, and >46% MPA using 5 µmol/l ADP for clopidogrel.

Multiple electrode aggregometry (MEA)

Blood was collected in hirudin-treated tubes. MEA was assessed in whole blood with the Multiplate™ analyzer (Dynabyte Medical, Munich, Germany) as previously described (18, 19). This instrument can perform up to five parallel aggregometry measurements assessing the change in impedance caused by the adhesion of platelets onto sensor units formed by silver-covered electrodes. Curves were recorded for 6 min and platelet aggregation was determined as area under the curve of arbitrary aggregation units (AU*min). In the present investigation, 6.4 µmol/l ADP was used as agonist. A cut-off value of >468 AU*min was used to define clopidogrel HPR.

Table 1: Clinical, angiographic and procedural characteristics.

	Overall (n=50)	HPR (n=44)	No HPR (n=6)	P-value
Age (years), mean ± SD	59.7±11.2	59.7±11.7	59.7±6.7	0.998
Male gender, n (%)	38 (76.0)	32 (72.7)	6 (100)	0.314
Body mass index (kg/m^2), median [IQT]	27.3 [25.3–29.5]	26.9 [25.3–28.9]	30.1 [26.9–32.5]	0.190
Hypertension, n (%)	22 (44.0)	19 (43.2)	3 (50.0)	~1
Diabetes mellitus, n (%)	6 (12.0)	6 (13.6)	0 (0)	~1
Hyperlipidaemia, n (%)	20 (40.0)	17 (38.6)	3 (50.0)	0.672
Active smokers, n (%)	15 (30.0)	13 (29.5)	2 (33.3)	~1
Family history of CAD, n (%)	6 (12.0)	6 (13.6)	0 (0)	~1
Time (min) from LD to P-PCI, median [IQT]	85.0 [60.0–121.3]	85.0 [65.0–120.0]	80.0 [38.8–131.3]	0.626
Total ischaemic time (min); median [IQT]	192.0 [133.8–305.0]	192.0 [141.3–300.0]	181.0 [82.5–463.8]	0.570
Origin				0.568
Emergency room	12 (24)	10 (22.7)	2 (33.3)	
Transferred by emergency system	38 (76)	34 (77.3)	4 (66.6)	
Infarct-related artery, n (%)				0.731
Left anterior descending	14 (28.0%)	12 (27.3)	2 (33.3)	
Left circumflex	6 (12.0%)	5 (11.4)	1 (16.7)	
Right coronary artery	30 (60.0%)	27 (61.3)	3 (50.0)	
Number of diseased vessels, n (%)				0.798
One	27 (54.0)	23 (52.3)	4 (66.7)	
Two	18 (36.0)	16 (36.4)	2 (33.3)	
Three	5 (10.0)	5 (11.3)	0 (0)	
Thrombus aspiration, n (%)	31 (62)	29 (65.9)	2 (33.3)	0.184
Periprocedural abciximab, n (%)	13 (26.0)	12 (27.3)	1 (16.7)	~1
Number of stents per patient, mean ± SD	1.2±0.6	1.2±0.6	1.0±0.0	0.438
Bare metal stents / total stents, n/n (%)	46/54 (85.2%)	40/48 (83.3)	6/6 (100)	~1

LD: loading dose; P-PCI: primary percutaneous coronary intervention.

Sample size

The primary endpoint of this study was the comparison of the initial TIMI flow of 2 to 3 in patients with and without HPR, as defined by the VN-P2Y12 assay. Assuming that 50% of patients were to present with HPR (8), a total of 46 patients would be needed to detect an absolute difference of 40% in the percentage of patients with poor initial TIMI flow, with 80% power and two-sided significance level of 0.05. Considering an approximate 8% dropout rate, inclusion of 50 patients was allowed to ensure that PD data from 46 patients was available.

Statistical analysis

Continuous variables were summarised by mean ± standard deviation (SD) or by median and interquartile range (IQR) if a normal distribution could be assumed or not, respectively. The Kolmogorov-Smirnov normality test was used to test such assumption. Categorical variables were expressed as frequencies and percentages and tested by means of the Chi-square test or Fisher's exact test if application conditions were not fulfilled. A p-value <0.05 was considered statistically significant for all comparisons.

Logistic regression models (backward stepwise method) were used to evaluate the association between clopidogrel HPR and pre- and post-procedural endpoints. Each analysis included the variable of interest (initial TIMI flow dichotomised, final TIMI flow, final blush, and ST resolution) as the dependent variable and clopidogrel HPR status as the independent variable, adjusting by variables considered clinically relevant (age, body mass index [BMI], diabetes mellitus, smoking habit, time from LD to start of P-PCI, and time from onset of symptoms to start of P-PCI for all analyses, adding GPIs use during the procedure and thrombus aspiration for post-procedural endpoints) and baseline characteristics unbalanced between the two groups (p<0.20). Exploratory analyses of HPR to ASA and its association with the above mentioned endpoints were also performed.

Results

A total of 82 consecutive STEMI patients admitted to a tertiary centre with a diagnosis of STEMI who underwent P-PCI were prospectively screened, of whom 32 were excluded because of not fulfilling inclusion and exclusion criteria. Therefore, a total of 50 patients were included in the present analysis. Baseline demographics, procedural and angiographic characteristics of the overall population and according to HPR status are summarised in ►Table 1. The median time of clopidogrel pretreatment (time from LD administration to the beginning of the procedure) was 85 min [IQR 60.0 to 121.3]. There were no differences in time between patients with and without HPR (85.0 [65.0-120.0] vs. 80.0 [38.8-131.3]; p=0.626).

The percentage of patients with suboptimal response to clopidogrel assessed with the VN-P2Y12 was 88.0% (95% confidence interval [CI]: 76.2% to 94.4%). Distribution of clopidogrel-induced platelet reactivity is shown in ►Figure 1A. Consistent rates were obtained with the other platelet function assays used: 81.8% (95% CI: 68.0% to 90.5%) and 91.3% (95% CI: 79.7% to 96.6%) when evaluated with LTA and MEA, respectively. Although non-statistically significant differences in any clinical, angiographic or procedural variables were found according to HPR status, a numerical trend towards a greater use of thrombus aspiration and administration of abciximab during the procedure was observed in patients with HPR to clopidogrel defined by VN-P2Y12 (►Table 1).

A higher percentage of patients with good initial TIMI flow in the IRA was observed among patients without HPR compared

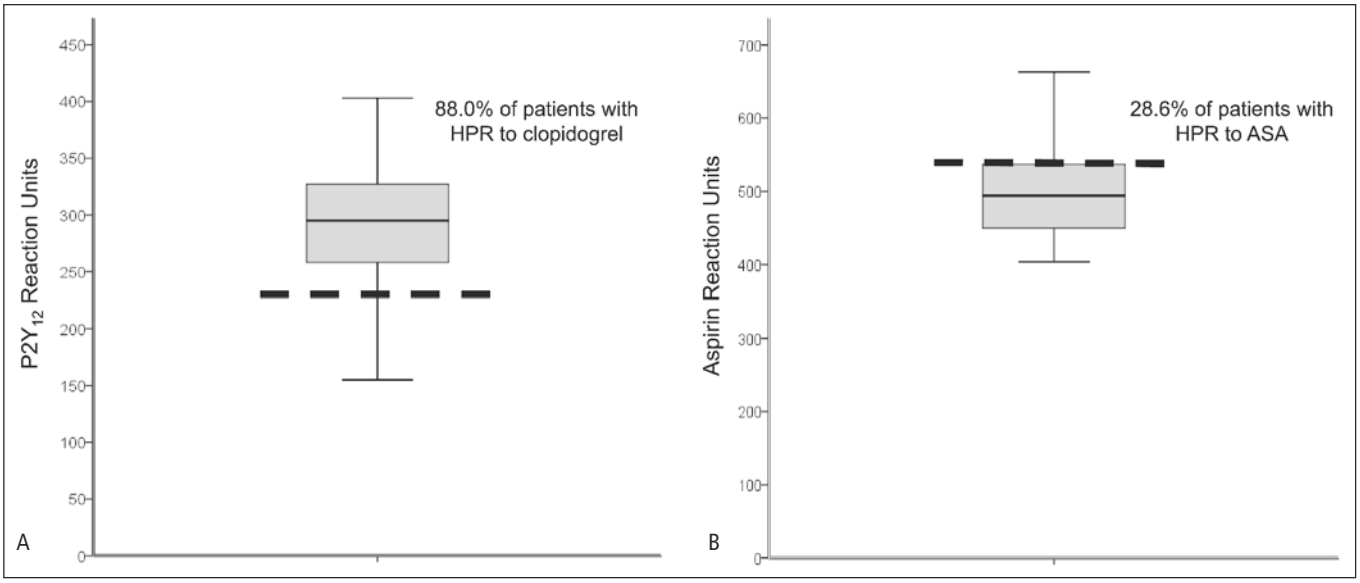


Figure 1: Distribution of clopidogrel and aspirin responsiveness measured by the VerifyNow system. ASA: Aspirin; HPR: high on-treatment platelet reactivity.

with those with HPR (66.7% vs 15.9%). HPR was the only variable statistically significant in the multivariate analysis (p=0.013). No significant differences were seen in the post-procedural frequencies of final TIMI flow grade of 3 (83.3% vs 72.7%; p=0.578), myocardial blush grade of 0 or 1 (33.3% vs 63.6%, p=0.328), and complete resolution of ST-segment elevation (66.7% vs 43.2%; p=0.279) (►Figure 2).

The percentage of patients with HPR to ASA was 28.6% (95% CI: 17.8% to 42.4%) and 38.1% (95% CI: 25.0% to 53.2%), assessed by VN-ASA (►Figure 1B) and LTA, respectively. No significant differences regarding initial TIMI flow, final TIMI flow, myocardial blush grade and ST resolution were observed between patients with and without HPR to ASA (data not shown).

Discussion

The findings of the present investigation performed in STEMI patients undergoing P-PCI pretreated with a 600-mg LD of clopidogrel and 500-mg of ASA showed that: 1) a high percentage of STEMI patients have inadequate levels of clopidogrel-induced and, to a lesser degree, aspirin-mediated platelet inhibition, at the moment of starting the P-PCI procedure; and 2) inadequate levels of clopidogrel-induced platelet inhibition are associated with impaired initial TIMI flow in the IRA.

Clopidogrel pretreatment is associated with a lower risk of adverse ischaemic events in STEMI patients undergoing P-PCI (3-6). However, clopidogrel efficacy is well-known to be hampered by a broad variability in response that leads to a relatively high percentage of patients with suboptimal response or HPR, which is associated with worse clinical outcomes (20). One of the main limitations of clopidogrel is its delayed onset of action, even after a

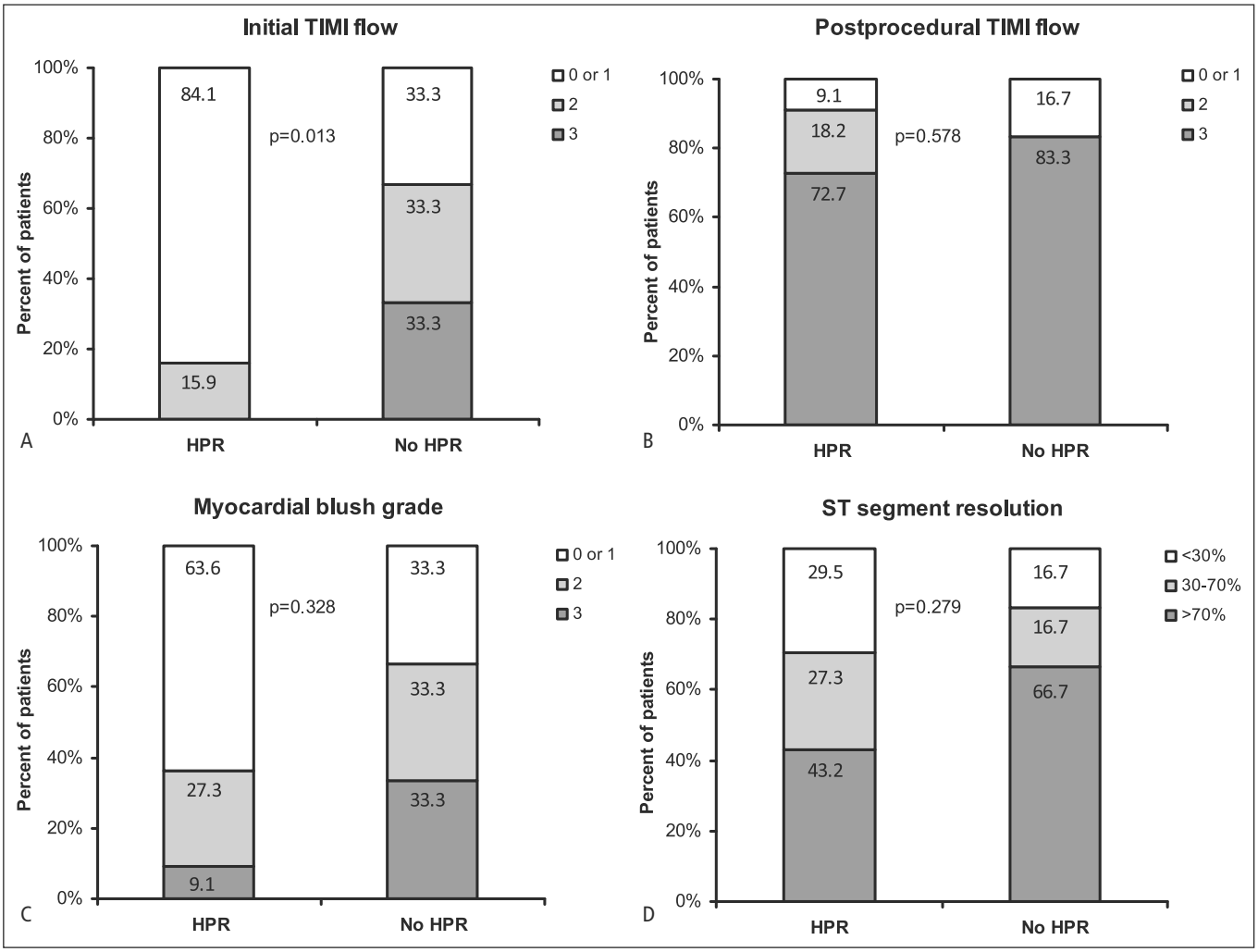


Figure 2: Preprocedural angiographic and postprocedural angiographic and electrogradiographic endpoints, according to high on-treatment platelet reactivity to clopidogrel status. The percentages of patients are shown according to initial TIMI flow grade (A), final TIMI flow grade (B), myocardial blush grade (C) and the degree of resolution of ST-segment elevation (D). HPR: high on-treatment platelet reactivity; TIMI: thrombolysis in myocardial infarction.

600-mg LD (21-23), which is of particular relevance in the setting of P-PCI given the need to minimise time delays between clinical presentation and mechanical reperfusion. In addition, prior investigations have shown that ACS is a predictor of diminished response to clopidogrel (24, 25). Notably, STEMI patients have higher rates of HPR than those with the other forms of ACS, as shown in a recent study by Bonello et al. (8). In line with this, Biscaglia et al. have recently reported that 90% of STEMI patients have negligible levels of platelet inhibition after pre-hospital administration of 600-mg clopidogrel, which confirm the results of the present investigation (26). These PD findings may also be attributed to impaired pharmacokinetics as a result of delayed intestinal absorption, which characterises STEMI patients, leading to impaired bioavailability of clopidogrel (27). This may occur due to selective shunting of blood to vital organs which may decrease gastrointestinal perfusion, or the elevated venous pressure and vasoconstriction of peripheral arteries during STEMI that stimulates the release of atrial natriuretic peptide, which in turn inhibits permeability and intestinal motility (28, 29). Of note, inter-individual variability of intestinal absorption has been previously associated with variability in clopidogrel-induced platelet inhibition (22, 30).

An important and novel finding of the present investigation is the observed association between suboptimal response to clopidogrel and worse rates of IRA patency, which may have consequences in PCI procedures (e.g. higher use of bail-out GPIs or thrombectomy devices) and clinical outcomes. Although the initial patency of the culprit vessel has been reported to be higher in those patients that have received clopidogrel pretreatment prior to P-PCI (4), this is the first investigation, to the best of our knowledge, to observe an association between HPR at the beginning of the procedure and lower rates of initial patency of the IRA. Further, although non-statistically significant differences were found, a numerical trend towards better post-procedural angiographic (final TIMI flow and myocardial blush) and electrocardiographic (ST-segment resolution) outcomes was observed in the subset of patients without clopidogrel HPR, despite a higher use of thrombus aspiration devices and peri-procedural abciximab administration in the group of patient with HPR.

Overall, these results support the idea that an antithrombotic strategy with more potent antiplatelet efficacy than that achieved with standard clopidogrel therapy may be a better option in STEMI patients undergoing P-PCI (1, 7, 31, 32). In particular, two strategies must be considered appealing options in order to obtain greater antiplatelet effects and, thus, better outcomes than standard clopidogrel therapy in the STEMI scenario: 1) the use of the newer and more potent oral $P2Y_{12}$ antagonists (prasugrel and ticagrelor); and 2) the use of intravenous agents, such as GPIs or cangrelor.

Newer and more potent oral antiplatelet agents with more rapid onset of action, such as prasugrel or ticagrelor, have demonstrated an important clinical benefit over clopidogrel in STEMI patients (33, 34). In line with this, a recent study by Nührenberg et al. performed in STEMI patients undergoing P-PCI found that the majority of subjects presented HPR to a 600-mg LD of clopidogrel 12-24 h after PCI, which corroborates the findings of the present investigation, and that this clopidogrel LD did not affected the PD efficacy of a 60-mg LD of prasugrel given afterwards (35). However, it is also important to note that studies evaluating the PD efficacy of prasugrel and ticagrelor in the setting of STEMI have also observed greater rates of HPR in the early hours post P-PCI than those reported in studies performed in non-STEMI patients (31, 36, 37). Remarkably, in a randomised PD study comparing ticagrelor vs prasugrel in STEMI patients undergoing P-PCI, both agents showed an important delay of action with HPR rates at 2 h after LD of 46.2% and 34.6% for ticagrelor and prasugrel, respectively (37). Further insights of the role of more potent $P2Y_{12}$ inhibitors in STEMI will be provided with the ongoing ATLANTIC (A 30 Day Study to Evaluate Efficacy and Safety of Pre-hospital vs. In-hospital Initiation of Ticagrelor Therapy in STEMI Patients Planned for Percutaneous Coronary Intervention) trial, which is evaluating the efficacy and safety of pre-hospital compared to in-hospital administration of ticagrelor in addition to aspirin in STEMI patients with planned P-PCI (NCT01347580).

The delayed onset of action of oral antiplatelet agents in STEMI patients may explain the observed clustered events like stent thrombosis in the first hours post P-PCI (38), and reflects the need for more potent and quicker antithrombotic strategies, such as the use of intravenous agents, in this setting. In line with this observation, Valgimigli et al. observed that a significant number of STEMI patients undergoing P-PCI had suboptimal platelet inhibition after prasugrel administration for at least 2 h, which was reverted with simultaneous administration of high-dose bolus of tirofiban (39). The use of cangrelor, a very potent intravenous $P2Y_{12}$ receptor blocker with a very short onset and offset of action (40, 41), might also be an attractive option in STEMI patients to achieve an early and strong platelet inhibition. The results of the recently presented phase III clinical trial CHAMPION (Cangrelor versus standard tHerapy to Achieve optimal Management of Platelet InhibitiON) – PHOENIX have shown a superior efficacy of cangrelor compared to clopidogrel in patients undergoing PCI, reducing ischaemic events at 48 h, and this effect was sustained through 30 days. Importantly, the benefit of cangrelor was consistent across the whole spectrum of PCI, including the subgroup of STEMI patients (42).

We acknowledge the inherent limitations of this investigation due to its observational design. Further, the small sample size of

What is known about this topic?

- Clopidogrel has a wide inter-individual variability in response.
- Clopidogrel has a limited efficacy on STEMI patients undergoing P-PCI due to its delayed onset of action and impaired bioavailability.

What does this paper add?

- A high percentage of STEMI patients have inadequate levels of clopidogrel-induced and, to a lesser degree, aspirin-mediated platelet inhibition at the moment of starting a P-PCI.
- A suboptimal response to clopidogrel may be associated with impaired initial patency of the infarct-related artery.

the study and, in particular, the very low number of patients without clopidogrel HPR at the beginning of the procedure makes it difficult to draw definitive conclusions regarding post-procedural outcomes and, therefore, these findings must be considered merely hypotheses-generating. In addition, the study was not powered to draw any conclusion on clinical outcomes during follow-up because of the small sample size. In fact, no patients in our study presented any ischaemic or bleeding events at 30-day follow-up. However, prior investigations have shown an association between clopidogrel responsiveness and adverse clinical outcomes in the setting of STEMI (10). Other limitations to be acknowledged are the lack of data on the novel platelet inhibitors prasugrel and ticagrelor, as well as having a single measurement of platelet function after loading. Indeed, having a second reassessment of platelet reactivity at a later time point would have been useful to prove that delayed absorption is the limiting step causing impaired clopidogrel efficacy. Finally, larger scale studies are warranted to define the clinical benefit of a tailored treatment strategy in STEMI patients based on the results of platelet function assays.

Conflicts of interest

José Luis Ferreiro (corresponding author) reports honoraria for lectures from Eli Lilly Co; Daiichi Sankyo, Inc.; Astra Zeneca. Dominick J. Angiolillo reports receiving: honoraria for lectures from Bristol Myers Squibb; Sanofi-Aventis; Eli Lilly Co; Daiichi Sankyo, Inc; Astra Zeneca; consulting fees from Bristol Myers Squibb; Sanofi-Aventis; Eli Lilly Co; Daiichi Sankyo, Inc.; The Medicines Company; Portola; Novartis; Astra Zeneca; Merck; Evolva; Abbott Vascular; research grants from Bristol Myers Squibb; Sanofi-Aventis; GlaxoSmithKline; Otsuka; Eli Lilly Co; Daiichi Sankyo, Inc., The Medicines Company; Portola; AstraZeneca. None of the other authors have conflicts of interest to report.

References

1. Ferreiro JL, Angiolillo DJ. New directions in antiplatelet therapy. Circ Cardiovasc Interv 2012; 5: 433-445.
2. Gómez-Hospital JA, Dallaglio PD, Sánchez-Salado JC, et al. Impact on Delay Times and Characteristics of Patients Undergoing Primary Percutaneous Coronary Intervention in the Southern Metropolitan Area of Barcelona After Implementation of the Infarction Code Program. Rev Esp Cardiol 2012; 65: 911-918.
3. Bellemain-Appaix A, O'Connor SA, Silvain J, et al; for the ACTION group. Association of Clopidogrel Pretreatment With Mortality, Cardiovascular Events, and Major Bleeding Among Patients Undergoing Percutaneous Coronary Intervention: A Systematic Review and Meta-analysis. J Am Med Assoc 2012; 308: 2507-2516.
4. Vlaar PJ, Svilaas T, Damman K, et al. Impact of pretreatment with clopidogrel on initial patency and outcome in patients treated with primary percutaneous coronary intervention for ST-segment elevation myocardial infarction: a systematic review. Circulation 2008; 118: 1828-1836.
5. Dörler J, Edlinger M, Alber HF, et al; Austrian Acute PCI Investigators. Clopidogrel pre-treatment is associated with reduced in-hospital mortality in primary percutaneous coronary intervention for acute ST-elevation myocardial infarction. Eur Heart J 2011; 32: 2954-2961.
6. Fefer P, Hod H, Hammerman H, et al. Usefulness of pretreatment with high-dose clopidogrel in patients undergoing primary angioplasty for ST-elevation myocardial infarction. Am J Cardiol 2009; 104: 514-518.
7. Ferreiro JL, Angiolillo DJ. Clopidogrel response variability: current status and future directions. Thromb Haemost 2009; 102: 7-14.
8. Bonello L, Berbis J, Laine M, et al. Biological efficacy of a 600 mg loading dose of clopidogrel in ST-elevation myocardial infarction. Thromb Haemost 2012; 108: 101-106.
9. Alexopoulos D, Theodoropoulos KC, Stavrou EF, et al. Prasugrel versus high dose clopidogrel to overcome early high on clopidogrel platelet reactivity in patients with ST elevation myocardial infarction. Cardiovasc Drugs Ther 2012; 26: 393-400.
10. Matetzky S, Shenkman B, Guetta V, et al. Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. Circulation 2004; 109: 3171-3175.
11. Ariza A, Ferreiro JL, Sánchez-Salado JC, et al. Early Anticoagulation May Improve Preprocedural Patency of the Infarct-related Artery in Primary Percutaneous Coronary Intervention. Rev Esp Cardiol 2012; Epub ahead of print.
12. TIMI Study Group. The Thrombolysis in Myocardial Infarction (TIMI) trial. Phase I findings. N Engl J Med 1985; 312: 932-936.
13. van 't Hof AW, Liem A, Suryapranata H, et al. Angiographic assessment of myocardial reperfusion in patients treated with primary angioplasty for acute myocardial infarction: myocardial blush grade. Zwolle Myocardial Infarction Study Group. Circulation 1998; 97: 2302-2306.
14. van 't Hof AW, Liem A, de Boer MJ, et al. Clinical value of 12-lead electrocardiogram after successful reperfusion therapy for acute myocardial infarction. Zwolle Myocardial infarction Study Group. Lancet 1997; 350: 615-619.
15. Malinin A, Pokov A, Spergling M, et al. Monitoring platelet inhibition after clopidogrel with the VerifyNow-P2Y12(R) rapid analyzer: the VERIfy Thrombosis risk ASsessment (VERITAS) study. Thromb Res 2007; 119: 277-284.
16. Fox SC, Behan MW, Heptinstall S. Inhibition of ADP-induced intracellular Ca2+ responses and platelet aggregation by the P2Y12 receptor antagonists AR-C69931MX and clopidogrel is enhanced by prostaglandin E1. Cell Calcium 2004; 35: 39-46.
17. Angiolillo DJ, Shoemaker SB, Desai B, et al. Randomized comparison of a high clopidogrel maintenance dose in patients with diabetes mellitus and coronary artery disease: results of the Optimizing Antiplatelet Therapy in Diabetes Mellitus (OPTIMUS) study. Circulation 2007; 115: 708-716.
18. Sibbing D, Braun S, Jawansky S, et al. Assessment of ADP-induced platelet aggregation with light transmission aggregometry and multiple electrode platelet aggregometry before and after clopidogrel treatment. Thromb Haemost 2008; 99: 121-126.
19. Ueno M, Ferreiro JL, Tomasello SD, et al. Impact of pentoxifylline on platelet function profiles in patients with type 2 diabetes mellitus and coronary artery disease on dual antiplatelet therapy with aspirin and clopidogrel. JACC Cardiovasc Interv 2011; 4: 905-912.
20. Ferreiro JL, Angiolillo DJ. Clopidogrel response variability: current status and future directions. Thromb Haemost 2009; 102: 7-14.
21. Montalescot G, Sideris G, Meuleman C, et al. A randomized comparison of high clopidogrel loading doses in patients with non-ST-segment elevation acute coronary syndromes: the ALBION (Assessment of the Best Loading Dose of Clopidogrel to Blunt Platelet Activation, Inflammation and Ongoing Necrosis) trial. J Am Coll Cardiol 2006; 48: 931-938.
22. von Beckerath N, Taubert D, Pogatsa-Murray G, et al. Absorption, metabolization, and antiplatelet effects of 300-, 600-, and 900-mg loading doses of clopidogrel: results of the ISAR-CHOICE (Intracoronary Stenting and Antithrombotic Regimen: Choose Between 3 High Oral Doses for Immediate Clopidogrel Effect) Trial. Circulation 2005; 112: 2946-2950.
23. Hochholzer W, Trenk D, Frundi D, et al. Time dependence of platelet inhibition after a 600-mg loading dose of clopidogrel in a large, unselected cohort of candidates for percutaneous coronary intervention. Circulation 2005; 111: 2560-2564.
24. Soffer D, Moussa I, Harjai KJ, et al. Impact of angina class on inhibition of platelet aggregation following clopidogrel loading in patients undergoing coronary intervention: do we need more aggressive dosing regimens in unstable angina? Catheter Cardiovasc Interv 2003; 59: 21-25.
25. Sibbing D, von Beckerath O, Schömig A, et al. Platelet function in clopidogrel-treated patients with acute coronary syndrome. Blood Coagul Fibrinolysis 2007; 18: 335-339.
26. Biscaglia S, Tebaldi M, Vranckx P, et al. Effects of pre-hospital clopidogrel administration on early and late residual platelet reactivity in ST-segment elev-

ation myocardial infarction patients undergoing primary intervention. J Thromb Haemost 2013; 11: 192-194.
27. Heestermans AA, van Werkum JW, Taubert D, et al. Impaired bioavailability of clopidogrel in patients with a ST-segment elevation myocardial infarction. Thromb Res 2008; 122: 776-781.
28. Bailey RW, Bulkley GB, Hamilton SR, et al. Protection of the small intestine from nonocclusivemesenteric ischemic injury due to cardiogenic shock. Am J Surg 1987; 153: 108-116.
29. Schrier RW, Abraham WT. Hormones and hemodynamics in heart failure. N Engl J Med 1999; 341: 577-585.
30. Taubert D, Kastrati A, Harlfinger S, et al. Pharmacokinetics of clopidogrel after administration of high loading dose. Thromb Haemost 2004; 92: 311-316.
31. Alexopoulos D, Theodoropoulos KC, Stavrou EF, et al. Prasugrel versus high dose clopidogrel to overcome early high on clopidogrel platelet reactivity in patients with ST elevation myocardial infarction. Cardiovasc Drugs Ther 2012; 26: 393-400.
32. Bliden KP, Tantry US, Storey RF, et al. The effect of ticagrelor versus clopidogrel on high on-treatment platelet reactivity: combined analysis of the ONSET/OFFSET and RESPOND studies. Am Heart J 2011; 162: 160-165.
33. Montalescot G, Wiviott SD, Braunwald E, et al.; TRITON-TIMI 38 investigators. Prasugrel compared with clopidogrel in patients undergoing percutaneous coronary intervention for ST-elevation myocardial infarction (TRITON-TIMI 38): double-blind, randomised controlled trial. Lancet 2009; 373: 723-731.
34. Steg PG, James S, Harrington RA, et al; PLATO Study Group. Ticagrelor versus clopidogrel in patients with ST-elevation acute coronary syndromes intended for reperfusion with primary percutaneous coronary intervention: A Platelet Inhibition and Patient Outcomes (PLATO) trial subgroup analysis. Circulation 2010; 122: 2131-2141.
35. Nührenberg TG, Trenk D, Leggewie S, et al. Clopidogrel pretreatment of patients with ST-elevation myocardial infarction does not affect platelet reactivity after subsequent prasugrel-loading: Platelet reactivity in an observational study. Platelets 2012; Epub ahead of print.
36. Alexopoulos D, Galati A, Xanthopoulou I, et al. Ticagrelor versus prasugrel in acute coronary syndrome patients with high on-clopidogrel platelet reactivity following percutaneous coronary intervention: a pharmacodynamic study. J Am Coll Cardiol 2012; 60: 193-199.
37. Alexopoulos D, Xanthopoulou I, Gkizas V, et al. Randomized Assessment of Ticagrelor Versus Prasugrel Antiplatelet Effects in Patients with ST-Segment-Elevation Myocardial Infarction. Circ Cardiovasc Interv 2012; 5: 797-804.
38. Heestermans AA, van Werkum JW, Zwart B, et al. Acute and subacute stent thrombosis after primary percutaneous coronary intervention for ST-segment elevation myocardial infarction: incidence, predictors and clinical outcome. J Thromb Haemost 2010; 8: 2385-2393.
39. Valgimigli M, Tebaldi M, Campo G, et al.; FABOLUS PRO Investigators. Prasugrel versus tirofiban bolus with or without short post-bolus infusion with or without concomitant prasugrel administration in patients with myocardial infarction undergoing coronary stenting: the FABOLUS PRO (Facilitation through Aggrastat By drOpping or shortening Infusion Line in patients with ST-segment elevation myocardial infarction compared to or on top of PRasugrel given at loading dOse) trial. JACC Cardiovasc Interv 2012; 5: 268-277.
40. Angiolillo DJ, Schneider DJ, Bhatt DL, et al. Pharmacodynamic effects of cangrelor and clopidogrel: the platelet function substudy from the cangrelor versus standard therapy to achieve optimal management of platelet inhibition (CHAMPION) trials. J Thromb Thrombolysis 2012; 34: 44-55.
41. Ferreiro JL, Ueno M, Tello-Montoliu A, et al. Effects of cangrelor in coronary artery disease patients with and without diabetes mellitus: an in vitro pharmacodynamic investigation. J Thromb Thrombolysis 2012; Epub ahead of print.
42. Bhatt DL, Stone GW, Mahaffey KW, et al. Effect of platelet inhibition with cangrelor during PCI on ischemic events. N Engl J Med 2013; Epub ahead of print.

V. Impact of mild hypothermia on platelet responsiveness to aspirin and clopidogrel: an in vitro pharmacodynamic investigation.

Ferreiro JL, Sánchez-Salado JC, Gracida M, Marcano AL, Roura G, Ariza A, Gómez-Lara J, Lorente V, Romaguera R, Homs S, Sánchez-Elvira G, Teruel L, Rivera K, Sosa SG, Gómez-Hospital JA, Angiolillo DJ, Cequier A.

J Cardiovasc Transl Res. 2014;7:39-46.

J. of Cardiovasc. Trans. Res. (2014) 7:39–46
DOI 10.1007/s12265-013-9533-5

Impact of Mild Hypothermia on Platelet Responsiveness to Aspirin and Clopidogrel: an In Vitro Pharmacodynamic Investigation

José Luis Ferreiro · José Carlos Sánchez-Salado · Montserrat Gracida · Ana Lucrecia Marcano · Gerard Roura · Albert Ariza · Josep Gómez-Lara · Victoria Lorente · Rafael Romaguera · Sílvia Homs · Guillermo Sánchez-Elvira · Luis Teruel · Kristian Rivera · Silvia Gabriela Sosa · Joan Antoni Gómez-Hospital · Dominick J. Angiolillo · Ángel Cequier

Received: 27 October 2013 / Accepted: 12 December 2013 / Published online: 21 December 2013

Abstract The combination of percutaneous coronary intervention (PCI) and therapeutic hypothermia in comatose patients after cardiac arrest due to an acute coronary syndrome has been reported to be safe and effective. However, recent investigations suggest that hypothermia may be associated with impaired response to clopidogrel and greater risk of thrombotic complications after PCI. This investigation aimed to evaluate the effect of hypothermia on the pharmacodynamic response of aspirin and clopidogrel in patients (n=20) with ST elevation myocardial infarction undergoing primary PCI. Higher platelet reactivity (ADP stimulus) was observed in samples incubated at 33 °C compared with those at 37 °C (multiple electrode aggregometry, 235.2±31.4 AU×min vs. 181.9±30.2 AU×min, p<0.001; VerifyNow P2Y12, 172.9±20.3 PRU vs. 151.0±19.3 PRU, p=0.004). Numerically greater rates of clopidogrel poor responsiveness were also observed at 33 °C. No differences were seen in aspirin responsiveness. In conclusion, mild hypothermia was associated with reduced clopidogrel-mediated platelet inhibition with no impact on aspirin effects.

Clinical relevance: Mild therapeutic hypothermia is associated with impaired response to clopidogrel therapy, which might contribute to increase the risk of thrombotic events in ACS comatose patients undergoing PCI.

Keywords Therapeutic hypothermia · Antiplatelet therapy · ST elevation myocardial infarction

In patients who remain comatose after return of spontaneous circulation, mild therapeutic hypothermia (cooling of 32 to 34 °C for 12 to 24 h) is recommended in practice guidelines for post-cardiac arrest care [1–3]. Overall, the most common cause of cardiac arrest is an acute coronary syndrome (ACS) [4, 5] in particular an ST elevation myocardial infarction (STEMI). In this setting, rapid reperfusion with primary percutaneous coronary intervention (PCI) and adequate antithrombotic therapy is recommended and should not be deferred in the presence of coma or in conjunction with therapeutic hypothermia [1]. Even though the combination of early reperfusion with PCI and therapeutic hypothermia has been reported to be safe and effective [6], the results of recent investigations have suggested that hypothermia might be associated with greater risk or acute atherothrombotic events in patients undergoing PCI [7, 8].

Dual antiplatelet therapy (DAPT) with aspirin and an adenosine diphosphate (ADP) $P2Y_{12}$ receptor antagonist is currently the standard of care of oral antiplatelet treatment in ACS patients, including those with STEMI and/or undergoing PCI [9]. Despite the introduction of more potent $P2Y_{12}$ receptor blockers such as prasugrel and ticagrelor, clopidogrel is still broadly used in daily clinical practice [10, 11]. Several

Associate Editor Emanuele Barbato oversaw the review of this article

José Luis Ferreiro and José Carlos Sánchez-Salado have contributed equally as first authors to this manuscript.

J. L. Ferreiro (✉) · J. C. Sánchez-Salado · M. Gracida · A. L. Marcano · G. Roura · A. Ariza · J. Gómez-Lara · V. Lorente · R. Romaguera · S. Homs · G. Sánchez-Elvira · L. Teruel · K. Rivera · S. G. Sosa · J. A. Gómez-Hospital · Á. Cequier
Heart Diseases Institute, Bellvitge University Hospital—IDIBELL, University of Barcelona, c/Feixa Llarga s/n. CP 08907 L'Hospitalet de Llobregat, Barcelona, Spain
e-mail: jlferreiro@bellvitgehospital.cat

D. J. Angiolillo
University of Florida College of Medicine-Jacksonville, Jacksonville, FL, USA

mechanistic studies have observed that therapeutic hypothermia may increase platelet activation and aggregation [12, 13], as well as impair response to clopidogrel [14, 15]. In line with these findings, recent reports of case series have observed higher than expected rates of stent thrombosis in patients undergoing PCI while treated with therapeutic hypothermia [7, 8], which has raised an important concern in the scientific community. However, the mechanisms of therapeutic hypothermia that can impact on the efficacy of oral antiplatelet agents is, to date, not fully elucidated. The aim of the present study was to evaluate the in vitro effect of mild hypothermia on the pharmacodynamic (PD) response to aspirin and clopidogrel in STEMI patients undergoing primary PCI.

Materials and Methods

Subject Population and Study Design

This was a prospective in vitro PD investigation conducted in consecutive STEMI patients who underwent primary PCI. All patients were between 18 and 75 years of age and received loading doses (LD) of clopidogrel (600 mg) and aspirin (250 mg) at the moment of diagnosis and prior to PCI. Exclusion criteria were known allergies to aspirin or clopidogrel, administration of glycoprotein IIb/IIIa inhibitors (GPIs) during primary PCI, cardiogenic shock, any active bleeding or malignancy, platelet count $<100\times10^6/\mu l$, severe chronic kidney disease (creatinine clearance <30 ml/min), and pregnant females. Blood samples for platelet function testing were collected in the morning of the next day after primary PCI, between 12 and 24 h after LDs, and before administering the first maintenance dose of aspirin and clopidogrel. Technical procedures and drug administration in the catheterization lab were left at the operator's criteria according to standard clinical practice. Patients could receive as anticoagulant therapy bivalirudin or weight-based unfractionated heparin (100 IU/kg). The study had a prospective design with paired data in which PD assessments were performed after in vitro incubation of samples at 33 and 37 °C, with the purpose of investigating the presence of a temperature-dependent effect on aspirin and clopidogrel.

Patients were screened at the Heart Diseases Institute of the Bellvitge University Hospital. The study complied with the Declaration of Helsinki and was approved by the Ethics Committee of the Bellvitge University Hospital. All subjects provided written informed consent to the study.

Sample Collection and Platelet Function Assays

Blood samples for platelet function analyses were collected from an antecubital vein, discarding the first 2–4 ml of blood to avoid spontaneous platelet activation. Tubes were immediately placed in two separate waterbaths, one of them previously warmed at 37 °C and the other at 33 °C, and incubated at such temperatures for 1 h. After incubation, samples were processed by trained laboratory personnel. Samples were processed within 2 h of blood drawing. Platelet function assays included multiple electrode aggregometry (MEA) and VerifyNow™ system.

MEA

Blood was collected in hirudin-treated tubes. MEA was assessed in whole blood with the Multiplate™ analyzer (Roche Diagnostics, Spain) as previously described [16]. This instrument can perform up to five parallel aggregometry measurements assessing the change in impedance caused by the adhesion of platelets onto sensor units formed by silver-covered electrodes. Curves were recorded for 6 min, and platelet aggregation was determined as area under the curve of arbitrary aggregation units (AU×min). Since the commercially available Multiplate™ analyzer can be set to different temperatures, the instrument was programmed at 33 or 37 °C as appropriate. In the present investigation, 6.4 μmol/L ADP and 0.5 mM arachidonic acid were used as agonists to evaluate clopidogrel and aspirin responsiveness, respectively. The cutoff values used to define high on-treatment platelet reactivity (HPR) were >468 AU×min for clopidogrel and >400 AU×min for aspirin [17].

VerifyNow Assay

The VerifyNow (VN) assay is a rapid whole blood point-of-care device and was utilized according to the instructions of the manufacturer (Accumetrics, Inc., San Diego, CA, USA) as previously described [18]. In brief, VN-$P2Y_{12}$ assay mimics turbidometric aggregation and utilizes disposable cartridges containing 20 μM ADP and 22 nM PGE1. Aggregation testing using ADP as a sole agonist activates $P2Y_1$ and $P2Y_{12}$ purinergic signaling, while adding PGE_1 increases the specificity of the test for $P2Y_{12}$ signaling [19]. In a separate channel of the cartridge in which iso-TRAP is used as an agonist, a baseline value for platelet function is obtained, enabling assessment of platelet inhibition without having to wean the patient of antiplatelet treatment. The VN-$P2Y_{12}$ assay reports the results as $P2Y_{12}$ reaction units (PRU) and percent inhibition of platelet aggregation (%IPA), which is calculated as [(baseline−PRU)/baseline]×100. In contrast to IPA values, which increase with decreasing platelet function, PRU values decrease with decreasing platelet function. Cutoff points of 240 PRU and ≤11%IPA were used to define clopidogrel HPR [17, 20]. Similarly, VN-aspirin assay utilizes disposable cartridges containing arachidonic acid (AA) and reports the results as aspirin reaction units (ARU). ARU values decrease with enhanced aspirin-induced platelet

inhibition. A cutoff value of >550 ARUs was used to define aspirin HPR.

Study Endpoints and Sample Size Calculation

The primary endpoint of this study was the comparison of clopidogrel-induced platelet aggregation determined by MEA (ADP stimulus) between measurements of samples at 33 and 37 °C. Assuming a standard deviation of 60 AU×min, we would be able to detect a difference (between aggregation at 33 and 37 °C) of 40 AU×min with 18 patients, with 80 % power and a two-tailed alpha value less than 0.05 for a paired data comparison. Considering an approximate 10 % dropout rate, inclusion of 20 patients was allowed to ensure that complete PD data from 18 patients was available for analysis. Secondary PD endpoints included comparison of values obtained at 33 and 37 °C of (a) clopidogrel-induced aggregation measured with VerifyNow system and (b) comparison of aspirin-induced aggregation measured both with MEA and VerifyNow. Exploratory analyses of the differences in HPR rates to clopidogrel and aspirin according to temperature values measured with MEA and VerifyNow assay were also performed.

Statistical Analysis

Conformity to the normal distribution was evaluated for continuous variables with the Kolmogorov-Smirnov test. For baseline characteristics, continuous variables and continuous variables were summarized by mean ± standard deviation or by median and interquartile range if a normal distribution could be assumed or not, respectively. A repeated measures ANOVA model was used to evaluate the primary endpoint and all other intragroup comparisons of aggregation values between temperatures (33 °C compared to 37 °C). Results are reported as least squares mean ± standard error of the mean for the above detailed analyses. Categorical variables were expressed as frequencies and percentages. Comparisons between HPR rates at 33 and 37 °C were performed with binomial exact test. A p value <0.05 was considered statistically significant for all comparisons. Statistical analysis was performed using SPSSv16.0 software (SPSS Inc., Chicago, IL).

Results

A total of 42 consecutive STEMI patients admitted to a tertiary center with a diagnosis of STEMI who underwent primary PCI were prospectively screened, of whom 22 were excluded because of not fulfilling inclusion and exclusion criteria (6 were older than 75 years old, 15 received GPIs during primary PCI, and 1 had cardiogenic shock). Therefore, a total of 20 patients were included in the present analysis. None of the subjects included had a late presentation STEMI. Baseline demographics and procedural and angiographic characteristics of the overall population are summarized in Table 1.

Effects of Mild Hypothermia on PD Response to Clopidogrel and Aspirin

Mild hypothermia generated in vitro was significantly associated with diminished clopidogrel-induced platelet inhibition with all measurements performed. Higher platelet reactivity (ADP stimulus) was observed in samples incubated at 33 °C (mild hypothermia range) compared with those at 37 °C measured both with MEA (235.2±31.4 AU×min vs. 181.9± 30.2 AU×min; p<0.001) and VerifyNow P2Y12 assay (172.9±20.3 PRU vs. 151.0±19.3 PRU; p=0.004) (Fig. 1). Consistently, a significant reduction in clopidogrel-mediated

Table 1 Clinical, angiographic, and procedural characteristics

	N=20
Age (years), mean ± SD	60.5±13.8
Male gender, *n* (%)	19 (95.0)
Body mass index (kg/m^2), median [IQT]	27.3 [26.3−30.2]
Hypertension, *n* (%)	9 (45.0)
Diabetes mellitus, *n* (%)	8 (40.0)
Hyperlipidemia, *n* (%)	11 (55.0)
Active smokers, *n* (%)	9 (45.0)
Family history of CAD, *n* (%)	1 (5.0)
Previous AMI, *n* (%)	5 (25)
Chronic kidney disease (GFR <60 ml/min/m^2), *n* (%)	1 (5.0)
Time (hours) from onset of symptoms to primary PCI, median [IQT]	3.7 [2.2−5.1]
Time (hours) from LD to blood draw for PFT, median [IQT]	14.5 [7.5−21.0]
Platelet count (×10^3/μl), mean ± SD	241.6±82.3
Infarct-related artery, *n* (%)	
Left anterior descending	10 (50.0)
Left circumflex	2 (10.0)
Right coronary artery	8 (40.0)
Number of diseased vessels, *n* (%)	
One	9 (45.0)
Two	6 (30.0)
Three	5 (25.0)
Thrombus aspiration, *n* (%)	18 (90)
Number of stents per patient, mean ± SD	1.5±0.7
Medications during PCI, *n* (%)	
Unfractionated heparin	17 (85)
Bivalirudin	3 (15)

LD loading dose, *PFT* platelet function testing, *PCI* percutaneous coronary intervention

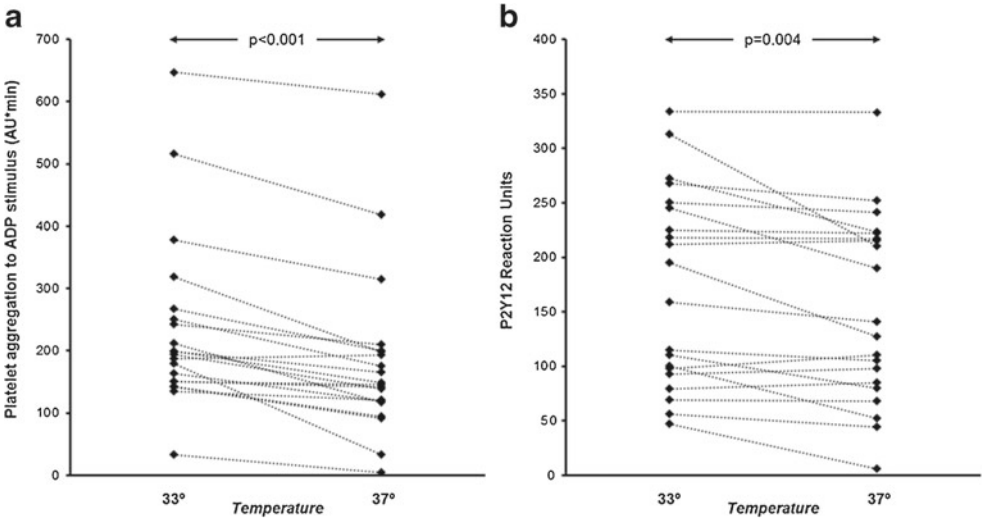


Fig. 1 Pharmacodynamic assessments of in vitro effect of mild hypothermia on clopidogrel-induced platelet reactivity. **a** Platelet aggregation measured by ADP-stimulated multiple electrode aggregometry. **b** P2Y12 reaction units measured by the VerifyNow P2Y12 assay. The *p* values indicate the difference of platelet reactivity among temperatures assessed by repeated measures ANOVA method. *ADP* adenosine diphosphate

platelet inhibition assessed by VerifyNow system was also seen (31.2±6.1 %IPA vs. 36.8±6.9 %IPA; $p<0.05$).

No differences were observed in platelet response to aspirin with any of the tests employed: MEA (122.2±13.1 AU×min vs. 129.4±17.4 AU×min; $p=0.608$) and VerifyNow Aspirin assay (473.1±19.2 ARU vs. 463.1±17.8 ARU; $p=0.499$) (Fig. 2).

Effects of Mild Hypothermia on HPR Rates

The percentage of HPR patients in samples incubated at 37 °C (body temperature) ranged from 5 to 25 % depending on the assay performed and the cutoff point used (MEA, 5 %; VerifyNow PRU, 15 %; VerifyNow %IPA, 25 %) (LTA ADP 5 μM, 9.4 %; LTA ADP 20 μM, 6.3 %). Although non-statistically significant values were obtained for all comparisons, samples incubated at 33 °C (mild hypothermia) had numerically greater HPR rates compared with samples at 37 °C using all assays (Table 2). No differences in HPR rates, even numerical, to aspirin were observed with any of the assays used.

Discussion

In the present investigation, we evaluated the effects of hypothermia at therapeutic range (33 °C) compared with normothermia (37 °C) on the PD response to aspirin and clopidogrel in blood samples from STEMI patients undergoing primary PCI. The findings of our in vitro study showed that mild hypothermia is associated with impaired clopidogrel-mediated platelet inhibition, with no effect on aspirin responsiveness. In particular, mild hypothermia was associated with increased platelet reactivity using various assays assessing $P2Y_{12}$-mediated signaling. Although there were no significant differences in HPR rates likely attributed to the sample size of the study which was powered to assess on-treatment platelet reactivity and not HPR, these were numerically higher using a

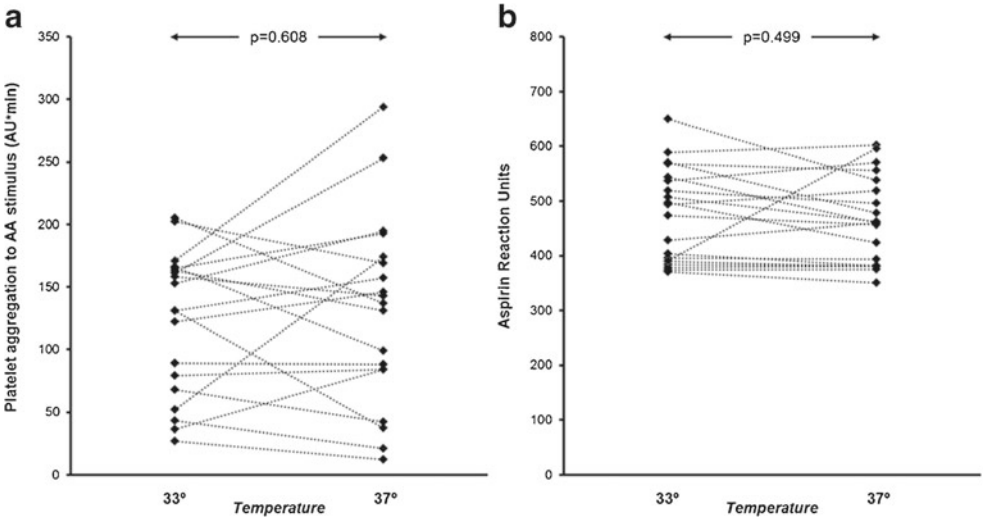


Fig. 2 Pharmacodynamic assessments of in vitro effect of mild hypothermia on aspirin-induced platelet reactivity. **a** Platelet aggregation measured by AA-stimulated multiple electrode aggregometry. **b** Aspirin reaction units measured by the VerifyNow Aspirin assay. The *s* values indicate the difference of platelet reactivity among temperatures assessed by repeated measures ANOVA method. *AA* arachidonic acid

Table 2 High on-treatment platelet reactivity rates to aspirin and clopidogrel measured with different platelet function assays at in vitro temperatures of 37 and 33 °C (mild hypothermia)

Antiplatelet agent	HPR definition	33 °C	37 °C
Clopidogrel	MEA ADP >468 AU×min	2 (10 %)	1 (5 %)
	VN-P2Y12 >240 PRU	6 (30 %)	3 (15 %)
	VN-IPA ≤11 % IPA	7 (35 %)	5 (25 %)
Aspirin	MEA AA >400 AU×min	0 (0 %)	0 (0 %)
	VN-Aspirin >550 ARU	4 (20 %)	4 (20 %)

AA arachidonic acid, *ADP* adenosine diphosphate, *ARU* aspirin reaction units, *HPR* high on-treatment platelet reactivity, *IPA* inhibition of platelet aggregation, *MEA* multiple electrode aggregometry, *PRU* $P2Y_{12}$ reaction units, *VN-Aspirin* VerifyNow aspirin assay, *VN-P2Y12* VerifyNow P2Y12 assay

variety of definitions. The consistent findings using a variety of assays are supportive of our study conclusions.

An optimal reperfusion strategy with PCI and appropriate periprocedural antithrombotic medication is mandatory in ACS patients [9, 21] and should not be deferred in the presence of coma or in conjunction with therapeutic hypothermia when indicated [1]. Of note, therapeutic hypothermia has been suggested to improve neurological recovery in comatose patients after cardiac arrest, which also may have a benefit in terms of mortality [2, 3], due to the fact that brain injury is a major determinant of survival after cardiac arrest [22]. These statements are currently under discussion, however, since a recently published clinical trial failed to show a benefit in mortality or neurological function of hypothermia at a targeted temperature of 33 °C compared to a targeted temperature of 36 °C in unconscious survivors of out-of-hospital cardiac arrest of presumed cardiac cause [23].

Despite newer and more potent $P2Y_{12}$ receptor blockers, such as prasugrel and ticagrelor, are currently available, clopidogrel is still extensively used in daily clinical practice [10]. However, the main downside of clopidogrel therapy is its broad variability in response, which leads to a considerable percentage of patients with suboptimal response or HPR, and is associated with increased risk of adverse ischemic outcomes [10, 11]. Of note, existence of an ACS, which is the leading cause of cardiac arrest, is per se a predictor of impaired response to clopidogrel [24, 25]. Among ACS patients, those with STEMI have higher rates of HPR than those with the other forms of ACS [26]. This is partially due to another important limitation of clopidogrel, a delayed onset of action even after a 600-mg LD [27–29], and to the impaired intestinal absorption that characterizes STEMI patients [30]. In fact, several studies have observed that the vast majority of STEMI patients have insufficient platelet inhibition after 600 mg LD of clopidogrel during primary PCI and the first hours after the procedure [26, 31, 32].

The combination of early reperfusion with PCI and therapeutic hypothermia has been reported to be safe and improve prognosis in patients suffering cardiac arrest [6] and, as such, is endorsed by current guidelines [1]. However, the findings of two recent studies have led to question the short-term safety of concomitant therapeutic hypothermia and PCI with stent placement [7, 8]. In both studies, much higher than expected rates of stent thrombosis in patients with hypothermia were observed [7, 8]. Notably, all patients were on oral DAPT with aspirin and clopidogrel, which raises questions about the optimal peri-interventional antithrombotic strategy in patients with therapeutic hypothermia in whom PCI is performed. However, these findings have not been confirmed in other studies [33].

The efficacy o f orally administered antiplatelet drugs in patients with cardiac arrest and therapeutic hypothermia is, to date, not fully elucidated. Patients with cardiac arrest have frequently reduced absorption of oral agents due to diminished gastrointestinal motility caused by hypothermia per se, opioids administration, and their acute critical illness [34]. In addition, hypothermia reduces the rate of metabolism and enzymatic activity [35], which is of relevance in the case on non-direct acting agents such as clopidogrel that needs to be converted in the liver into its active metabolite [10, 11]. In line with this, the only ex vivo study that have evaluated clopidogrel efficacy in 25 subjects with therapeutic hypothermia after cardiac arrest observed that all patients had suboptimal response to clopidogrel 24 h after beginning hypothermia and only 69 % of them continued with HPR at day 3 [15].

Despite initial observations that suggested a reduction in platelet reactivity with deep hypothermia conditions [36], more recent mechanistic investigations have observed that hypothermia at therapeutic range may increase platelet activation and aggregation [12, 13], preferentially through ADP-mediated signaling pathway [14]. This is in line with the results of our investigation in which we eliminated the effect of hypothermia on clopidogrel pharmacokinetics (intestinal absorption and metabolization) by reproducing hypothermic conditions with in vitro incubation of blood from STEMI patients treated with aspirin and clopidogrel. Since clopidogrel- but not aspirin-mediated platelet inhibition was impaired with mild hypothermia, our results suggest ADP signaling pathway as the major mediator of hypothermia-associated platelet activation. This is in agreement with prior studies that have observed increased platelet activation and aggregation with mild hypothermia when platelets are stimulated with ADP, but not with arachidonic acid or collagen [37, 38], which are agonists used to assess aspirin-induced antiplatelet effect. Several mechanisms have been suggested to contribute to the impact of hypothermia on platelet ADP signaling pathway: (a) reduction of the ADP metabolizing

enzyme CD39 (E-NTPDase1) activity, which leads to decreased ADP hydrolysis and, thus, increased plasma ADP concentration [39]; (b) enhanced fragility of red blood cells in hypothermic conditions, which may favor the release of ADP [12]; and (c) changes in platelet membrane fluidity [40], which has been reported to affect ADP-induced platelet aggregation in animal models [41]. However, further mechanistic studies are needed in order to expose the exact mechanisms by which hypothermia affects preferentially platelet ADP signaling pathway.

Overall, the results of the present investigation support the idea that the use of more potent oral $P2Y_{12}$ blockers with more rapid onset of action and less variability such as prasugrel or ticagrelor, which have demonstrated an clinical benefit over clopidogrel in ACS [42, 43], could represent a valid alternative to clopidogrel in ACS patients with hypothermia due to cardiac arrest. In fact, ticagrelor has been reported to overcome the decreased responsiveness to clopidogrel in mild hypothermia conditions in an in vitro experiment [14]. However, it is important to note that prasugrel and ticagrelor are oral agents, and their efficacy may be affected in patients with ACS, particularly those presenting with STEMI, and hypothermia mainly due to impaired pharmacokinetics [24–26, 33, 34]. In particular, PD studies have observed greater rates of HPR to prasugrel and ticagrelor in the early hours post-primary PCI than those reported in studies in non-STEMI patients [44, 45]. Therefore, the use of cangrelor, a very potent $P2Y_{12}$ receptor antagonist, might be an appealing option in patients with therapeutic hypothermia due to its pharmacologic properties [46, 47]. Cangrelor has a very short onset and offset of action and is administered intravenously [46], which can be of help in patients with impaired intestinal absorption and hepatic metabolism that could limit the pharmacological efficacy of oral agents. Interestingly, cangrelor administration was able to prevent platelet activation during extracorporeal circulation and hypothermia in an ex vivo investigation with an animal model [48]. Cangrelor is not yet available in clinical practice and, thus, the use of intravenous GPIs could be considered to avoid the limitations of oral agents in patients with hypothermia undergoing PCI. However, it is unclear if hypothermia could modify the efficacy of these agents (abciximab, tirofiban, and eptifibatide) in a differential manner [36]. Further, GPIs are the most potent antiplatelet agents, and the lack of clinical data regarding safety in patients with hypothermia makes it important to recommend caution about their use in this scenario. Therefore, further investigation is needed, and dedicated studies in the clinical setting are warranted to determine the potential usefulness of more potent antiplatelet agents than clopidogrel in patients with therapeutic hypothermia.

We acknowledge the inherent limitations of this investigation due to its in vitro design. Further, no pharmacokinetic assessments were performed, which could have provided a better understanding of the underlying mechanisms contributing to the observed diminished clopidogrel-mediated antiplatelet effect associated with hypothermia. However, the aim of this investigation was to evaluate the impact of mild hypothermia on the antiplatelet efficacy of aspirin and clopidogrel, independently of the impaired intestinal absorption and metabolism that is characteristic of patients with therapeutic hypothermia. Other limitation is the lack of data on the novel platelet inhibitors prasugrel and ticagrelor, but our study reflects the clinical practice of the emergency medical system at our geographic region at the time the study was performed.

Conflict of Interest José Luis Ferreiro (corresponding author) reports (a) honoraria for lectures from Eli Lilly Co., Daiichi Sankyo, Inc., and Astra Zeneca and (b) consulting fees from Astra Zeneca and Eli Lilly Co. Dominick J. Angiolillo received payment as an individual for (a) consulting fee or honorarium from Bristol Myers Squibb, Sanofi-Aventis, Eli Lilly, Daiichi Sankyo, The Medicines Company, AstraZeneca, Merck, Evolva, Abbott Vascular, and PLx Pharma and (b) participation in review activities from Johnson & Johnson, St. Jude, and Sunovion. Institutional payments for grants from Bristol Myers Squibb, Sanofi-Aventis, Glaxo Smith Kline, Otsuka, Eli Lilly, Daiichi Sankyo, The Medicines Company, AstraZeneca, and Evolva and has other financial relationships with Esther and King Biomedical Research Grant.

References

1. Peberdy, M. A., Callaway, C. W., Neumar, R. W., et al. (2010). Part 9: post-cardiac arrest care: 2010 American Heart Association Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. *Circulation, 122*(18 Suppl 3), S768–S786. doi:10.1161/CIRCULATIONAHA.110.971002.
2. Hypothermia after Cardiac Arrest Study Group. (2002). Mild therapeutic hypothermia to improve the neurologic outcome after cardiac arrest. *New England Journal of Medicine, 346*, 549–556. doi:10.1056/NEJMoa012689.
3. Bernard, S. A., Gray, T. W., Buist, M. D., et al. (2002). Treatment of comatose survivors of out-of-hospital cardiac arrest with induced hypothermia. *New England Journal of Medicine, 346*, 557–563.
4. Spaulding, C. M., Joly, L. M., Rosenberg, A., et al. (1997). Immediate coronary angiography in survivors of out-of-hospital cardiac arrest. *New England Journal of Medicine, 336*, 1629–1633. doi:10.1056/NEJM199706053362302.
5. Anyfantakis, Z. A., Baron, G., Aubry, P., et al. (2009). Acute coronary angiographic findings in survivors of out-of-hospital cardiac arrest. *American Heart Journal, 157*, 312–318. doi:10.1016/j.ahj.2008.09.016.
6. Dumas, F., White, L., Stubbs, B. A., Cariou, A., & Rea, T. D. (2012). Long-term prognosis following resuscitation from out of hospital cardiac arrest: role of percutaneous coronary intervention and therapeutic hypothermia. *Journal of the American College of Cardiology, 60*, 21–27. doi:10.1016/j.jacc.2012.03.036.
7. Ibrahim, K. (2011). Increased rate of stent thrombosis due to clopidogrel resistance in patients in therapeutic hypothermia after sudden cardiac death. *European Heart Journal, 32*(Suppl), 252 [abstract].
8. Penela, D., Magaldi, M., Fontanals, J., et al. (2013). Hypothermia in acute coronary syndrome: brain salvage versus stent thrombosis?

Journal of the American College of Cardiology, 61, 686–687. doi:10.1016/j.jacc.2012.10.029.

9. O'Gara, P. T., Kushner, F. G., Ascheim, D. D., et al. (2013). 2013 ACCF/AHA guideline for the management of ST-elevation myocardial infarction: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *Journal of the American College of Cardiology, 61*, e78–e140. doi:10.1016/j.jacc.2012.11.019.
10. Ferreiro, J. L., & Angiolillo, D. J. (2012). New directions in antiplatelet therapy. *Circulation Cardiovascular Interventions, 5*, 433–445. doi:10.1161/CIRCINTERVENTIONS.111.966176.
11. Angiolillo, D. J., & Ferreiro, J. L. (2010). Platelet adenosine diphosphate P2Y12 receptor antagonism: benefits and limitations of current treatment strategies and future directions. *Revista Española de Cardiología, 63*, 60–76. doi:10.1016/S1885-5857(10)70010-4.
12. Xavier, R. G., White, A. E., Fox, S. C., Wilcox, R. G., & Heptinstall, S. (2007). Enhanced platelet aggregation and activation under conditions of hypothermia. *Thrombosis and Haemostasis, 98*, 1266–1275. doi:10.1160/TH07-03-0189.
13. Scharbert, G., Kalb, M. L., Essmeister, R., & Kozek-Langenecker, S. A. (2010). Mild and moderate hypothermia increases platelet aggregation induced by various agonists: a whole blood in vitro study. *Platelets, 21*, 44–48. doi:10.3109/09537100903420269.
14. Högberg, C., Erlinge, D., & Braun, O. O. (2009). Mild hypothermia does not attenuate platelet aggregation and may even increase ADP-stimulated platelet aggregation after clopidogrel treatment. *Thrombosis Journal, 7*, 2. doi:10.1186/1477-9560-7-2.
15. Bjelland, T. W., Hjertner, Ø., Klepstad, P., Kaisen, K., Dale, O., & Haugen, B. O. (2011). Antiplatelet effect of clopidogrel is reduced in patients treated with therapeutic hypothermia after cardiac arrest. *Resuscitation, 81*, 1627–1631. doi:10.1016/j.resuscitation.2010.07.002.
16. Sibbing, D., Braun, S., Jawansky, S., et al. (2008). Assessment of ADP-induced platelet aggregation with light transmission aggregometry and multiple electrode platelet aggregometry before and after clopidogrel treatment. *Thrombosis and Haemostasis, 99*, 121–126. doi:10.1160/TH07-07-0478.
17. Bonello, L., Tantry, U. S., Marcucci, R., et al. (2010). Consensus and future directions on the definition of high on-treatment platelet reactivity to adenosine diphosphate. *Journal of the American College of Cardiology, 56*, 919–933. doi:10.1016/j.jacc.2010.04.047.
18. Malinin, A., Pokov, A., Spergling, M., et al. (2007). Monitoring platelet inhibition after clopidogrel with the VerifyNow-P2Y12(R) rapid analyzer: the VERIfy Thrombosis risk ASsessment (VERITAS) study. *Thrombosis Research, 119*, 277–284. doi:10.1016/j.thrombres.2006.01.019.
19. Fox, S. C., Behan, M. W., & Heptinstall, S. (2004). Inhibition of ADP-induced intracellular Ca2+ responses and platelet aggregation by the P2Y12 receptor antagonists AR-C69931MX and clopidogrel is enhanced by prostaglandin E1. *Cell Calcium, 35*, 39–46. doi:10.1016/s0146-4060(03)00170-2.
20. Stone, G. W., Witzenbichler, B., Weisz, G., et al. (2013). Platelet reactivity and clinical outcomes after coronary artery implantation of drug-eluting stents (ADAPT-DES): a prospective multicentre registry study. *Lancet, 382*, 614–623. doi:10.1016/S0140-6736(13)61170-8.
21. Anderson, J. L., Adams, C. D., Antman, E. M., et al. (2013). 2012 ACCF/AHA focused update incorporated into the ACCF/AHA 2007 guidelines for the management of patients with unstable angina/non-ST-elevation myocardial infarction: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *Journal of the American College of Cardiology, 61*, e179–e347. doi:10.1016/j.jacc.2013.01.014.
22. Laver, S., Farrow, C., Turner, D., & Nolan, J. (2004). Mode of death after admission to an intensive care unit following cardiac arrest. *Intensive Care Medicine, 30*, 2126–2128. doi:10.1007/s00134-004-2425-z.
23. Nielsen, N., Wetterslev, J., Cronberg, T., et al. (2013). Targeted temperature management at 33 °C versus 36 °C after cardiac arrest. *New England Journal of Medicine, 369*, 2197–2206. doi:10.1056/NEJMoa1310519.
24. Soffer, D., Moussa, I., Harjai, K. J., et al. (2003). Impact of angina class on inhibition of platelet aggregation following clopidogrel loading in patients undergoing coronary intervention: do we need more aggressive dosing regimens in unstable angina? *Catheterization and Cardiovascular Interventions, 59*, 21–25. doi:10.1002/ccd.10494.
25. Sibbing, D., von Beckerath, O., Schömig, A., Kastrati, A., & von Beckerath, N. (2007). Platelet function in clopidogrel-treated patients with acute coronary syndrome. *Blood Coagulation and Fibrinolysis, 18*, 335–339. doi:10.1097/MBC.0b013e3280d21aed.
26. Bonello, L., Berbis, J., Laine, M., et al. (2012). Biological efficacy of a 600 mg loading dose of clopidogrel in ST-elevation myocardial infarction. *Thrombosis and Haemostasis, 108*, 101–106. doi:10.1160/TH12-02-0125.
27. Montalescot, G., Sideris, G., Meuleman, C., et al. (2006). A randomized comparison of high clopidogrel loading doses in patients with non-ST-segment elevation acute coronary syndromes: the ALBION (Assessment of the Best Loading Dose of Clopidogrel to Blunt Platelet Activation, Inflammation and Ongoing Necrosis) trial. *Journal of the American College of Cardiology, 48*, 931–938. doi:10.1016/j.jacc.2006.04.090.
28. von Beckerath, N., Taubert, D., Pogatsa-Murray, G., Schömig, E., Kastrati, A., & Schömig, A. (2005). Absorption, metabolization, and antiplatelet effects of 300-, 600-, and 900-mg loading doses of clopidogrel: results of the ISAR-CHOICE (Intracoronary Stenting and Antithrombotic Regimen: Choose Between 3 High Oral Doses for Immediate Clopidogrel Effect) Trial. *Circulation, 112*, 2946–2950. doi:10.1161/CIRCULATIONAHA.105.559088.
29. Hochholzer, W., Trenk, D., Frundi, D., et al. (2005). Time dependence of platelet inhibition after a 600-mg loading dose of clopidogrel in a large, unselected cohort of candidates for percutaneous coronary intervention. *Circulation, 111*, 2560–2564. doi:10.1161/01.CIR.0000160869.75810.98.
30. Heestermans, A. A., van Werkum, J. W., Taubert, D., et al. (2008). Impaired bioavailability of clopidogrel in patients with a ST-segment elevation myocardial infarction. *Thrombosis Research, 122*, 776–781. doi:10.1016/j.thromres.2008.01.021.
31. Nührenberg, T. G., Trenk, D., Leggewie, S., et al. (2012). Clopidogrel pretreatment of patients with ST-elevation myocardial infarction does not affect platelet reactivity after subsequent prasugrel-loading: platelet reactivity in an observational study. *Platelets, 24*, 549–553. doi:10.3109/09537104.2012.736045.
32. Ferreiro, J. L., Homs, S., Berdejo, J., et al. (2013). Clopidogrel pretreatment in primary percutaneous coronary intervention: prevalence of high on-treatment platelet reactivity and impact on preprocedural patency of the infarct-related artery. *Thrombosis and Haemostasis, 110*, 110–117. doi:10.1160/TH13-01-0057.
33. Rosillo, S. O., Lopez-de-Sa, E., Iniesta, A. M., et al. (2013). Is therapeutic hypothermia a risk factor for stent thrombosis? *Journal of the American College of Cardiology*. doi:10.1016/j.jacc.2013.09.028.
34. Polderman, K. H. (2009). Mechanisms of action, physiological effects, and complications of hypothermia. *Critical Care Medicine, 37*(7 Suppl), S186–S202. doi:10.1097/CCM.0b013e3181aa5241.
35. Arpino, P. A., & Greer, D. M. (2008). Practical pharmacologic aspects of therapeutic hypothermia after cardiac arrest. *Pharmacotherapy, 28*, 102–111. doi:10.1592/phco.28.1.102.
36. Frelinger, A. L., 3rd, Furman, M. I., Barnard, M. R., Krueger, L. A., Dae, M. W., & Michelson, A. D. (2003). Combined effects of mild hypothermia and glycoprotein IIb/IIIa antagonists on platelet-platelet and leukocyte-platelet aggregation. *American Journal of Cardiology, 92*, 1099–1101. doi:10.1016/j.amjcard.2003.06.007.

37. Scharbert, G., Kalb, M., Marschalek, C., & Kozek-Langenecker, S. A. (2006). The effects of test temperature and storage temperature on platelet aggregation: a whole blood in vitro study. *Anesthesia and Analgesia, 102*, 1280–1284. doi:10.1213/01.ane.0000199399.04496.6d.
38. Faraday, N., & Rosenfeld, B. A. (1998). In vitro hypothermia enhances platelet GPIIb-IIIa activation and P-selectin expression. *Anesthesiology, 88*, 1579–1585. doi:10.1097/00000542-199806000-00022.
39. Straub, A., Krajewski, S., Hohmann, J. D., et al. (2011). Evidence of platelet activation at medically used hypothermia and mechanistic data indicating ADP as a key mediator and therapeutic target. *Arteriosclerosis, Thrombosis, and Vascular Biology, 31*, 1607–1616. doi:10.1161/ATVBAHA.111.226373.
40. Tablin, F., Oliver, A. E., Walker, N. J., Crowe, L. M., & Crowe, J. H. (1996). Membrane phase transition of intact human platelets: correlation with cold-induced activation. *Journal of Cellular Physiology, 168*, 305–313. doi:10.1002/(SICI)1097-4652(199608)168:2<305::AID-JCP9>3.0.CO;2-T.
41. Berlin, E., Shapiro, S. G., & Friedland, M. (1984). Platelet membrane fluidity and aggregation of rabbit platelets. *Atherosclerosis, 51*, 223–239. doi:10.1016/0021-9150(84)90170-9.
42. Wiviott, S. D., Braunwald, E., McCabe, C. H., et al. (2007). Prasugrel versus clopidogrel in patients with acute coronary syndromes. *New England Journal of Medicine, 357*, 2001–2015. doi:10.1056/NEJMoa0706482.
43. Wallentin, L., Becker, R. C., Budaj, A., et al. (2009). Ticagrelor versus clopidogrel in patients with acute coronary syndromes. *New England Journal of Medicine, 361*, 1045–1057. doi:10.1056/NEJMoa0904327.
44. Parodi, G., Valenti, R., Bellandi, B., et al. (2013). Comparison of prasugrel and ticagrelor loading doses in ST-segment elevation myocardial infarction patients: RAPID (Rapid Activity of Platelet Inhibitor Drugs) primary PCI study. *Journal of the American College of Cardiology, 61*, 1601–1606. doi:10.1016/j.jacc.2013.01.024.
45. Alexopoulos, D., Xanthopoulou, I., Gkizas, V., et al. (2012). Randomized assessment of ticagrelor versus prasugrel antiplatelet effects in patients with ST-segment-elevation myocardial infarction. *Circulation Cardiovascular Interventions, 5*, 797–804. doi:10.1161/CIRCINTERVENTIONS.112.972323.
46. Ueno, M., Ferreiro, J. L., & Angiolillo, D. J. (2010). Update on the clinical development of cangrelor. *Expert Review of Cardiovascular Therapy, 8*, 1069–1077. doi:10.1586/erc.10.90.
47. Angiolillo, D. J., Schneider, D. J., Bhatt, D. L., et al. (2012). Pharmacodynamic effects of cangrelor and clopidogrel: the platelet function substudy from the cangrelor versus standard therapy to achieve optimal management of platelet inhibition (CHAMPION) trials. *Journal of Thrombosis and Thrombolysis, 34*, 44–55. doi:10.1007/s11239-012-0737-3.
48. Krajewski, S., Kurz, J., Neumann, B., et al. (2012). Short-acting P2Y12 blockade to reduce platelet dysfunction and coagulopathy during experimental extracorporeal circulation and hypothermia. *British Journal of Anaesthesia, 108*, 912–921. doi:10.1093/bja/aer518.

4.2. Bloqueo potente del receptor $P2Y_{12}$ en pacientes con enfermedad coronaria

VI. Effects of cangrelor in coronary artery disease patients with and without diabetes mellitus: an in vitro pharmacodynamic investigation.

Ferreiro JL, Ueno M, Tello-Montoliu A, Tomasello SD, Capodanno D, Capranzano P, Dharmashankar K, Darlington A, Desai B, Rollini F, Guzman LA, Bass TA, Angiolillo DJ.

J Thromb Thrombolysis. 2013;35:155-64.

J Thromb Thrombolysis (2013) 35:155–164
DOI 10.1007/s11239-012-0846-z

Effects of cangrelor in coronary artery disease patients with and without diabetes mellitus: an in vitro pharmacodynamic investigation

José L. Ferreiro · Masafumi Ueno · Antonio Tello-Montoliu · Salvatore D. Tomasello · Davide Capodanno · Piera Capranzano · Kodlipet Dharmashankar · Andrew Darlington · Bhaloo Desai · Fabiana Rollini · Luis A. Guzman · Theodore A. Bass · Dominick J. Angiolillo

Published online: 25 November 2012

Abstract Platelets from patients with diabetes mellitus (DM) are hyper-reactive and whether cangrelor, a potent intravenous $P2Y_{12}$ receptor blocker, has differential pharmacodynamic (PD) effects according DM status is unknown. The aim of this investigation was to evaluate the in vitro PD effects of cangrelor in coronary artery disease (CAD) patients with and without DM. This prospective study enrolled 120 clopidogrel-naïve patients with CAD on aspirin therapy. PD assessments using cangrelor (500 nmol/l) in vitro included vasodilator-stimulated phosphoprotein assay to obtain the $P2Y_{12}$ reactivity index (PRI), and multiple electrode aggregometry (MEA). In a 20 patients subgroup, dose-dependent response was assessed following exposure to escalating concentrations (baseline, 5, 50, 500 and 5,000 nmol/l); thrombin generation processes were evaluated by thromboelastography (TEG). PD data were evaluable in 103 patients. No differences in baseline PD parameters were observed in DM ($n = 48$) and non-DM ($n = 45$) subjects. Cangrelor reduced PRI values irrespective of DM status ($p < 0.0001$), yielding no difference in patients with and without DM (16.1 ± 12.3 vs. 16.8 ± 11.3; $p = 0.346$). All MEA values were significantly reduced, although this was of greater magnitude with purinergic compared to non-purinergic agonists. A trend analysis showed a dose-dependent effect on platelet inhibition, with no interaction due to DM status, whereas no significant dose-dependent effect was observed for TEG-derived parameters. Therefore, in vitro cangrelor provides potent and dose-dependent blockade of the platelet $P2Y_{12}$ receptor, with no differential effect in DM and non-DM patients. In addition, in vitro cangrelor exerts moderate inhibitory effects on non-purinergic platelet signaling pathways, without modulating platelet-derived thrombin generation processes.

Keywords Cangrelor · Diabetes mellitus · Platelet inhibition · P2Y12 receptor · Antiplatelet agents

J. L. Ferreiro · M. Ueno · A. Tello-Montoliu · S. D. Tomasello · D. Capodanno · P. Capranzano · K. Dharmashankar · A. Darlington · B. Desai · F. Rollini · L. A. Guzman · T. A. Bass · D. J. Angiolillo (✉)
University of Florida College of Medicine-Jacksonville, 655 West 8th Street, Jacksonville, FL 32209, USA
e-mail: dominick.angiolillo@jax.ufl.edu

J. L. Ferreiro
Heart Diseases Institute, Hospital Universitari de Bellvitge-IDIBELL, University of Barcelona, L'Hospitalet de Llobregat, Barcelona, Spain

Diabetes mellitus (DM) has been shown to be associated with impaired response to antiplatelet therapies, particularly to the $P2Y_{12}$ receptor antagonist clopidogrel [1–3]. These pharmacodynamic (PD) findings may contribute to the increased rates of adverse atherothrombotic events observed in DM patients compared with non-DM subjects [4, 5]. Several metabolic and cellular abnormalities contribute to the hyper-reactive platelet phenotype observed in DM patients [6]. In particular, upregulation of $P2Y_{12}$ signaling has been postulated as a mechanism contributing to impaired clopidogrel response in DM patients [7]. Moreover, the functional status of the $P2Y_{12}$ signaling pathway has also been shown to be associated with platelet-derived thrombin generation [8–10], which is also increased in DM patients and thus contribute to their pro-thrombotic status [5, 11]. Overall, these findings underscore the need for more potent $P2Y_{12}$ receptor inhibiting strategies in patients with DM.

Cangrelor is a novel intravenous $P2Y_{12}$ receptor blocker under advanced clinical investigation characterized by a very rapid onset and offset of action (12). Cangrelor

directly, without need for metabolic biotransformation, and reversibly inhibits in a dose-dependent manner the $P2Y_{12}$ receptor, achieving very potent (>90 %) platelet inhibition [12–14]. However, the PD effects of cangrelor in DM and non-DM platelets remain unexplored. Further, if cangrelor can exert additional PD effects other than $P2Y_{12}$ blockade, such as modulating other platelet signaling pathways or thrombin generation processes, is unknown. The present manuscript describes the results of in vitro investigations aimed to provide these insights on the PD effects of cangrelor.

Methods

Subject population and study design

This was a prospective in vitro investigation conducted in patients with stable coronary artery disease. All patients were between 18 and 75 years of age, on maintenance aspirin therapy (81 mg daily), and naïve to treatment with $P2Y_{12}$ receptor inhibitors for at least 30 days prior to inclusion. Patients were classified as having type 2 DM according to criteria from the World Health Organization Report [15]. Patients on any anticoagulant or antiplatelet medication, other than aspirin, within the past 30 days were not eligible for the study. The study had a parallel design in which PD assessments to assess purinergic and non-purinergic mediated signaling were performed at baseline and after in vitro incubation with cangrelor. PD assessments included vasodilator-stimulated phosphoprotein (VASP) and multiple electrode aggregometry (MEA). Cangrelor at a final concentration of 500 nmol/l was chosen for in vitro incubation in line with prior investigations as it approximates that of the mean steady-state plasma concentration of 484 nmol/l at the infusion dose of 4 μg/kg/min, which is also the dose used in large-scale phase III clinical trial investigations [13, 16, 17]. PD assessments were performed in blood samples from 120 patients with and without DM. In a subgroup of patients ($n = 20$), an escalating concentration range of cangrelor (5, 50, 500 and 5,000 nmol/l) was used with the purpose of investigating the presence of a dose-dependent effect of cangrelor on purinergic and non-purinergic mediated platelet signaling; in addition to VASP and MEA, thrombin-generation processes assessed by thromboelastography (TEG) were also evaluated. This subgroup of patients enrolled to measure the dose-dependent effects of cangrelor represented the last 20 consecutive patients from the overall study cohort with analyzable blood samples.

Patients were screened at the Division of Cardiology of the Shands Jacksonville Hospital-University of Florida College of Medicine. The study complied with the Declaration of Helsinki and was approved by the Institutional Review Board of the University of Florida College of Medicine-Jacksonville. All subjects provided written informed consent.

Sample collection and platelet function assays

Blood samples were collected from an antecubital vein, discarding the first 2–4 ml of blood to avoid spontaneous platelet activation. Tubes were immediately incubated at 37 °C in a waterbath and cangrelor was added to the whole blood to reach the final concentrations desired and incubated for 5 min [14, 18]. The same procedure was followed with tubes used to perform baseline assessments, but without adding cangrelor. After incubation, samples were processed in parallel (all measurements of each assay at the same time) by trained laboratory personnel. Samples were processed within 2 h of blood drawing. PD assessments included flow cytometric analysis of the phosphorilation status of VASP, MEA and TEG.

VASP

The $P2Y_{12}$ reactivity index (PRI) was calculated as a measure of the functional status of the $P2Y_{12}$ signalling pathway. PRI was determined through assessment of phosphorylation status of vasodilator-stimulated phosphoprotein (VASP), a key and specific intraplatelet mediator of $P2Y_{12}$ signaling, according to standard protocols [19, 20]. In brief, VASP phosphorylation (VASP-P) was measured by quantitative flow cytometry using commercially available labelled monoclonal antibodies (Biocytex Inc., Marseille, France). The PRI was calculated after measuring the mean fluorescence intensity (MFI) of VASP-P levels following challenge with prostaglandin E_1 (PGE_1) and PGE_1 + adenosine diphosphate (ADP). PGE_1 increases VASP-P levels through stimulation of adenylate cyclase (AC); ADP binding to purinergic receptors leads to inhibition of AC; thus, the addition of ADP to PGE_1-stimulated platelets reduces levels of PGE_1-induced VASP-P. The PRI was calculated as follows: ([MFI PGE1] − [MFI PGE1 + ADP]/[MFI PGE1]) × 100 %. A reduced PRI is indicative of greater inhibition of the $P2Y_{12}$ signaling pathway. The relative decrease in platelet reactivity was defined as the percentage of inhibition of platelet aggregation and calculated as follows: (PRI value at baseline − PRI value after incubation with cangrelor 500nM) × 100/PRI value at baseline.

MEA

Blood was collected in hirudin-treated tubes. MEA was assessed in whole blood with the Multiplate analyzer (Dynabyte Medical, Munich, Germany) as previously described

[21, 22]. This instrument can perform up to five parallel aggregometry measurements assessing the change in impedance caused by the adhesion of platelets onto sensor units formed by silver-covered electrodes. Curves were recorded for 6 min and platelet aggregation was determined as area under the curve of arbitrary aggregation units (AU * min). The relative change in platelet aggregation was defined as the percentage of inhibition of platelet aggregation and calculated for each agonist as follows: (AU * min at baseline − AU * min after incubation with cangrelor 500 nM) × 100/AU * min at baseline. In the present investigation, the following 5 different agonists were used to assess for purinergic and non-purinergic mediated platelet signaling: (a) purinergic: 6.4 μmol/l ADP and 6.4 μmol/l ADP + 9.4 nmol/l PGE_1; and (b) non-purinergic: 0.5 mM arachidonic acid (AA), 32 μmol/l thrombin receptor activating peptide (TRAP), and 3.2 μg/ml collagen.

TEG

The Thrombelastograph® (TEG®) Hemostasis System (Haemoscope Corporation, Niles, IL, USA) equipped with automated software for the determination of the first derivative was used according to the manufacturer's instructions [8, 11]. Several parameters related to the rate of development of the tensile strength of the developing clot are derived from the first derivative of the waveform generated by the TEG system. In brief, TEG is a viscoelastic monitor that measures platelet–fibrin-mediated clot strength through a rotating sample cup with a stationary pin suspended by a torsion wire. The torque of the rotating cup is transmitted to the pin immersed in the blood sample and the movement of the pin, which depends of the contribution of platelets to the clot strength through platelet–fibrin binding, is transformed into an electrical signal generating a tracing. The reaction time (R), expressed in minutes, is a measure of time to initial thrombin induced platelet–fibrin clot formation and has been correlated with the velocity of thrombin generation [23]. The analytical software of the TEG system also allows use of the first derivative of the waveform generated by the system to determine the time to maximum rate of thrombin generation (TMRTG), also expressed in minutes. About 1 ml of heparinised blood is transferred to a vial containing kaolin and mixed by inversion. Afterwards, 500 μl of the activated blood is transferred to a vial containing heparinase and mixed to neutralize he heparin effect. The neutralised blood (360 μl) is immediately added to a heparinase-coated cup and assayed in the TEG analyser. Two TEG System devices were available, thus, up to four parallel measurements could be performed simultaneously.

Study endpoints and sample size calculation

The primary endpoint was the comparison of VASP-PRI values in DM and non-DM achieved after incubation with 500 nmol/l of cangrelor. Assuming that the standard deviation of the PRI is 10, we will be able to perform an equivalence analysis, being ± 6 % the limit of equivalence, with 80 % power and 2-sided alpha = 0.05 with 48 subjects per group. Considering an approximate dropout of 20 %, recruitment of up to 120 patients was allowed to ensure that complete data from 96 subjects was available for analysis. Other endpoints included the comparison of platelet function in DM versus non-DM patients with MEA using different stimuli, purinergic (ADP and ADP + PGE_1) and non-purinergic agonists (AA, TRAP, collagen). For the subgroup of 20 patients undergoing PD testing with escalating concentrations of cangrelor, the endpoints included: (a) evaluation of the dose-dependent effect achieved with escalating doses of cangrelor using VASP and MEA, investigating if DM status is an interaction factor; and (b) evaluation of the effect of escalating doses of cangrelor in platelet-derived thrombin generation processes measured with TEG.

Statistical analysis

For baseline characteristics, continuous variables are expressed as mean ± SD and categorical variables as frequencies and percentages. Normal distribution was evaluated for continuous variables with the Kolmogorov–Smirnov test. Comparisons of quantitative variables were made with non-paired Student's t test or Mann–Whitney's U test as appropriate, while qualitative variables were compared with Chi square test or Fisher's exact test (if expected value in any cell was fewer than 5). An ANCOVA method with a general linear model was used to evaluate the primary endpoint and all other between-groups comparisons, using as covariates the baseline value of the corresponding platelet function test, as well as unbalanced demographic or clinical variables ($p < 0.10$) in the univariate analysis. A repeated measures ANOVA model was used to evaluate intragroup comparisons, such as the comparison of functional assessments before and after cangrelor incubation, as well as the effect of escalating concentrations of cangrelor. In addition, p values for trend analyses to assess platelet reactivity with escalating doses of cangrelor were obtained using a polynomial contrast in the ANOVA method, considering concentration as a categorical variable with an ordinal scale. A two-tailed p value of less than 0.05 was considered to indicate a statistically significant difference for all the analyses performed. Results are reported as least squares mean (LSM) ± standard error of the mean (SEM) for the above detailed analyses. Statistical analysis was performed using SPSS version 16.0 software (SPSS Inc., Chicago, IL).

Results

Study population

A total of 470 patients were screened; of these, 218 refused to participate and 132 did not meet study inclusion criteria as they were not clopidogrel naïve or had been on other antithrombotic medications in the past 30 days. Therefore, a total of 120 patients were finally included in the study. A total of 17 samples were invalidated due to inability to measure platelet function for reasons including hemolysis, insufficient volume obtained or inaccurate processing of blood samples. Therefore, samples from a total of 103 patients (DM = 48; non-DM = 55) were available to assess the in vitro PD effects of a fixed concentration of cangrelor (500 nmol/l); in a subgroup of 20 patients (DM = 10; non-DM = 10) an escalating concentration range of cangrelor (5, 50, 500 and 5,000 nmol/l) was used. Baseline demographics and clinical characteristics of the overall study population are shown in Table 1. Among DM patients, HbA1c levels were 7.8 ± 2.2 and approximately half (n = 26; 54.2 %) were on insulin therapy. Baseline characteristics were overall well balanced between groups, with the exception of body mass index and creatinine concentration, which were higher among DM patients (Table 1) and were accordingly included in the statistical analyses as covariates.

Table 1 Baseline demographic data and clinical characteristics stratified according to diabetes mellitus status

	DM (n = 48)	Non-DM (n = 55)	*p* value
Age (years)	62.8 ± 9.4	62.5 ± 8.8	0.845
Male	28 (58.3 %)	40 (72.7 %)	0.124
BMI (kg/m^2)	33.3 ± 6.6	29.9 ± 6.2	0.012
Race			0.674
Caucasian	30 (62.5 %)	40 (72.7 %)	
Africanamerican	13 (27.1 %)	12 (21.8 %)	
Other	5 (10.4 %)	3 (6.5 %)	
Risk factors			
Current smoking	7 (14.5 %)	14 (27.3 %)	0.268
Hypertension	41 (91.1 %)	45 (81.8 %)	0.286
Dyslipidemia	41 (91.1 %)	45 (81.8 %)	0.286
Family history	28 (58.3 %)	31 (56.4 %)	0.793
Medical history			
Prior MI	24 (50.0 %)	30 (54.5 %)	0.680
Prior stroke	3 (6.25 %)	3 (5.5 %)	0.845
Prior PCI	28 (58.3 %)	28 (50.9 %)	0.293
Prior CABG	6 (12.5 %)	8 (14.5)	0.810
Symptomatic PAD	6 (12.5 %)	5 (9.1 %)	0.720
Multivessel CAD	31 (64.6 %)	31 (56.4 %)	0.462
Medical therapy			
Beta-blockers	39 (81.3 %)	40 (72.7 %)	0.420
ACEI/ARB	34 (70.8 %)	33 (60.0 %)	0.235
Nitrates	19 (39.6 %)	17 (30.9 %)	0.369
Calcium antagonists	18 (37.5 %)	16 (29.1 %)	0.269
Statins			0.699
CYP3A4 metabolism	37 (77.1 %)	39 (70.9 %)	
Non-CYP3A4 metabolism	5 (10.4 %)	8 (14.5 %)	
Proton-pump inhibitors			0.953
Omeprazole	8 (16.7 %)	10 (18.2 %)	
Other	15 (31.2 %)	19 (34.5 %)	
Oral antidiabetic agents	34 (70.8 %)	0 (0 %)	
Insulin	26 (54.2 %)	0 (0 %)	
Laboratory data			
Platelet count (10^3/mm^3)	225.0 ± 58.9	219.9 ± 59.3	0.432
Hematocrit (%)	40.2 ± 5.8	41.5 ± 4.4	0.145
Creatinine (g/dl)	1.3 ± 0.8	1.0 ± 0.3	<0.001

Values are expressed as mean ± SD or n (%)

ACEI/ARB angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, *BMI* body mass index, *CABG* coronary artery bypass graft, *CAD* coronary artery disease, *CYP* cythochrome P450, *DM* diabetes mellitus, *HbA1c* glycated hemoglobin A1c, *PAD* peripheral artery disease

In vitro PD effects of a fixed (500 nmol/l) cangrelor concentration

VASP-PRI

There were no statistical differences at baseline in PRI values between DM patients compared with non-DM subjects (84.3 ± 5.6 vs. 86.0 ± 3.8 %; $p = 0.072$). A significant reduction in VASP-PRI after in vitro incubation with 500 nmol/l of cangrelor was observed in the overall population, in whom there was a 80.6 ± 14.0 % relative reduction in PRI. This reduction was consistent in DM and non-DM patients ($p < 0.0001$ for both comparisons), with no difference in PRI values between groups (16.1 ± 12.3 vs. 16.8 ± 11.3; $p = 0.346$), as shown in Fig. 1a.

MEA

No differences in baseline values were found for all MEA measurements between DM and non-DM patients (Table 2). In the overall population, a marked decrease in platelet aggregation after in vitro incubation with 500 nmol/l of cangrelor was observed independently of the agonist used ($p < 0.0001$ for all comparisons, Table 2). When expressed as percentage of inhibition of platelet aggregation, the reduction of platelet reactivity was higher when using stimuli to assess purinergic mediated signaling (ADP and ADP + PGE_1) (Fig. 1b).

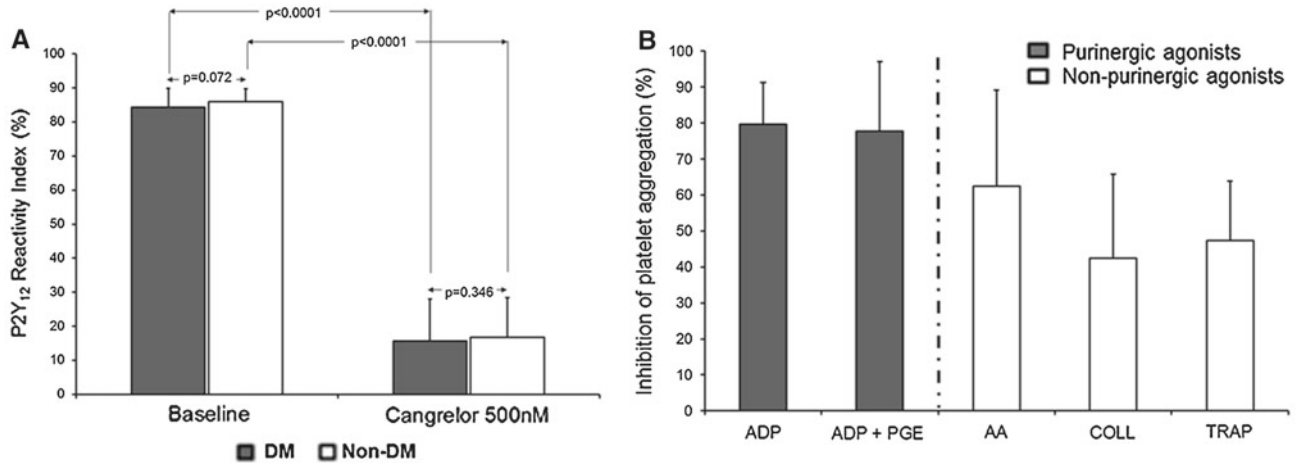


Fig. 1 Platelet function measurements at baseline and after in vitro incubation with cangrelor. **a** Platelet reactivity values according to DM status. **b** Relative reduction of platelet aggregation after in vitro incubation with cangrelor measured with multiple electrode aggregometry and using purinergic and non-purinergic stimuli. The percentage of inhibition of platelet aggregation, calculated as (AU * min at baseline—AU * min after incubation with cangrelor 500 nM) × 100/AU * min at baseline, is higher when using purinergic agonists that assess more specifically the $P2Y_{12}$ signalling pathway. *Values* are expressed as means and *error bars* indicate SD. *AA* arachidonic acid, *ADP* adenosine diphosphate, *COLL* collagen, *PGE* prostaglandin E_1, *TRAP* thrombin receptor activating peptide

Similarly to PRI, there were no significant differences in MEA measurements between DM and non-DM patients for all agonists (purinergic and non-purinergic) used (Table 2).

PD effects of escalating concentrations of cangrelor

VASP-PRI

Trend analysis showed a dose-dependent effect of escalating concentrations of cangrelor on PRI (expressed as LSM ± SEM): baseline: 86.1 ± 1.4 %; 5 nmol/l: 76.4 ± 2.5 %; 50 nmol/l: 48.7 ± 3.8 %; 500 nmol/l: 19.0 ± 3.2 %; 5,000 nmol/l: 9.5 ± 2.0 % (p for trend <0.0001). There was no interaction in this dose-dependent effect according to DM status (Fig. 2a).

MEA

PD results with MEA also showed a dose-dependent effect of cangrelor, irrespective of the agonist used (Fig. 2b). There was no interaction according to DM status for all MEA measurements (Table 3). In addition, no significant differences were observed at any cangrelor concentration between DM and non-DM patients, irrespective of agonists used to stimulate platelet aggregation (Table 3).

TEG

There were no significant differences in the R and TMRTG values at all concentrations of cangrelor ($p > 0.05$ for all between-concentrations comparisons). Accordingly, there was no significant trend for a dose-dependent effect

Table 2 Platelet reactivity values at baseline and after cangrelor incubation according to diabetes mellitus status measured by multiple electrode aggregometry using purinergic and non-purinergic agonists

Assay	Baseline			After cangrelor incubation		
	DM	Non-DM	p value	DM	Non-DM	p value
MEA ADP	633.6 ± 33.7	601.4 ± 31.5	0.976	116.9 ± 7.8	107.5 ± 7.3	0.408
MEA ADP + PGE	449.7 ± 30.7	416.3 ± 28.8	0.497	79.0 ± 8.0	76.5 ± 7.5	0.426
MEA AA	267.5 ± 39.3	269.2 ± 36.6	0.430	96.2 ± 13.7	76.6 ± 12.3	0.127
MEA TRAP	1,082.7 ± 42.9	1,070.0 ± 40.0	0.830	605.4 ± 36.9	544.2 ± 34.4	0.467
MEA COLL	477.2 ± 27.8	450.5 ± 26.0	0.484	251.3 ± 13.1	233.0 ± 12.3	0.365

MEA values are reported as area under the curve of arbitrary aggregation units (AU * min). Values are expressed as LSM ± SEM

AA arachidonic acid, *ADP* adenosine diphosphate, *COLL* collagen, *MEA* multiple electrode aggregometry, *PGE* prostaglandin E_1, *TRAP* thrombin receptor activating peptide, *DM* diabetes mellitus

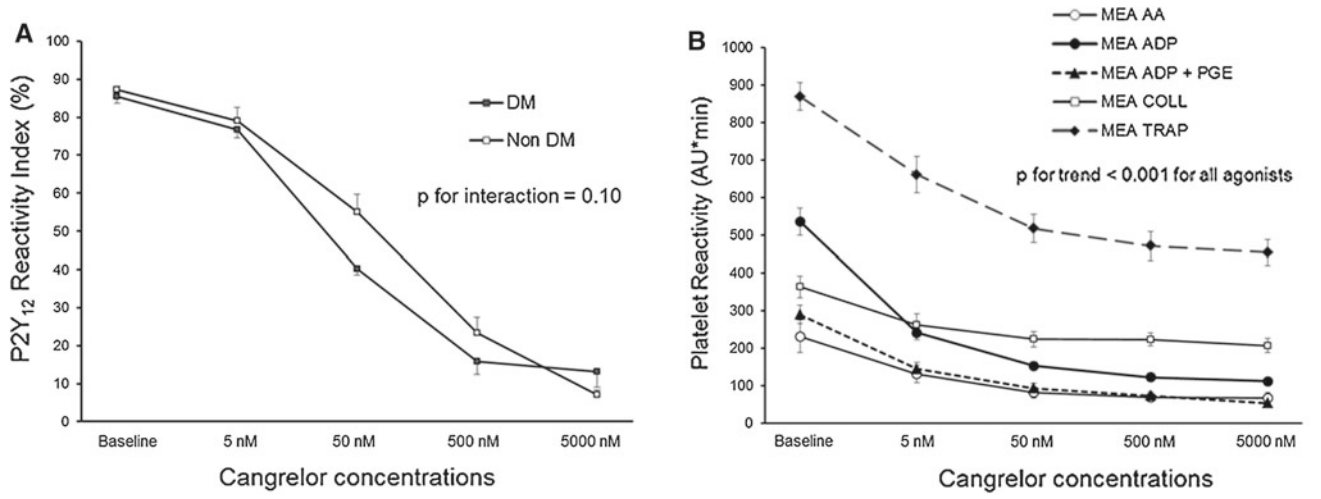


Fig. 2 Effects of escalating doses of cangrelor on: **a** Platelet reactivity index values according to DM status No interaction due to DM status was observed. *Values* are expressed as least standard means and *error bars* indicate SE of the mean. **b** Platelet reactivity measured by multiple electrode aggregometry using multiple agonists. *Values* are expressed as least standard means and *error bars* indicate SE of the mean. *AA* arachidonic acid, *ADP* adenosine diphosphate, *COLL* collagen, *MEA* multiple electrode aggregometry, *PGE* prostaglandin E_1, *TRAP* thrombin receptor activating peptide

Table 3 Platelet reactivity values achieved with increasing concentrations of cangrelor (in vitro incubation) according to diabetes mellitus status measured by multiple electrode aggregometry using purinergic and non-purinergic agonists

Assay	Baseline	Cangrelor 5 nM	Cangrelor 50 nM	Cangrelor 500 nM	Cangrelor 5,000 nM	*p* value for interaction
MEA AA						
DM	263.2 ± 77.8	144.1 ± 39.8	90.8 ± 23.9	74.0 ± 16.8	71.7 ± 24.9	0.509
Non-DM	199.4 ± 29.5	116.7 ± 21.7	71.0 ± 16.2	61.3 ± 13.6	61.3 ± 13.6	
p value	0.473	0.566	0.512	0.572	0.729	
MEA ADP						
DM	500.0 ± 51.1	216.9 ± 22.7	160.3 ± 14.6	135.7 ± 15.3	117.3 ± 13.0	0.645
Non-DM	571.9 ± 51.1	265.4 ± 30.1	144.8 ± 16.1	108.7 ± 17.0	104.7 ± 12.8	
p value	0.333	0.215	0.486	0.253	0.501	
MEA ADP + PGE						
DM	287.0 ± 35.3	134.7 ± 24.4	83.8 ± 18.4	62.8 ± 14.3	52.0 ± 15.6	0.610
Non-DM	292.2 ± 36.0	154.5 ± 22.2	102.1 ± 18.5	82.8 ± 15.9	54.5 ± 14.2	
p value	0.918	0.555	0.490	0.362	0.905	
MEA TRAP						
DM	891.4 ± 42.5	672.6 ± 52.5	530.1 ± 42.0	491.6 ± 48.2	459.8 ± 42.5	0.683
Non-DM	847.0 ± 61.0	648.8 ± 82.0	504.8 ± 61.2	450.7 ± 60.2	448.1 ± 52.7	
p value	0.558	0.809	0.737	0.603	0.865	
MEA COLL						
DM	386.9 ± 43.7	253.5 ± 28.7	219.2 ± 18.9	225.1 ± 19.2	205.4 ± 22.6	0.914
Non-DM	339.7 ± 38.0	270.7 ± 50.3	227.8 ± 37.0	221.5 ± 29.1	207.8 ± 29.0	
p value	0.425	0.770	0.837	0.919	0.948	

MEA values are reported as area under the curve of arbitrary aggregation units (AU * min). Values are expressed as LSM ± SEM

AA arachidonic acid, *ADP* adenosine diphosphate, *COLL* collagen, *MEA* multiple electrode aggregometry, *PGE* prostaglandin E_1, *TRAP* thrombin receptor activating peptide, *DM* diabetes mellitus

observed for any of these TEG-derived thrombin generation parameters. Similar results were obtained when evaluating DM and non-DM subjects separately (Table 4). Additionally, no significant differences were observed between DM and non-DM patients at any cangrelor concentration.

Discussion

Cangrelor is a novel intravenous $P2Y_{12}$ receptor inhibitor. In particular, it is an intravenous ATP analog, which reversibly and directly, thus, not needing any biotransformation, inhibits the $P2Y_{12}$ receptor [12]. It is able to achieve very potent (>90 %) platelet inhibition, with immediate onset of action and because of its ultra-short half-life (3–6 min), it has a very rapid offset of action with return to baseline platelet function within 30–60 min [13, 14]. In the present investigation we performed very comprehensive in vitro assessments to further elucidate the PD effects of cangrelor in patients with CAD, expanding upon prior studies by evaluating the impact of DM status on these findings. Our in vitro PD investigation showed that: (1) cangrelor potency is not affected by DM status; (2) cangrelor provides a potent and dose-dependent inhibition of the $P2Y_{12}$ receptor, as well as a moderate effect on other platelet signaling pathways; and (3) escalating concentrations of cangrelor do not modify platelet-derived thrombin generation processes.

Patients with DM have been shown to have impaired response to clopidogrel [1–3], the most commonly utilized $P2Y_{12}$ receptor inhibitor, which may contribute to their increased risk of ischemic recurrences, including stent thrombosis, compared with non-DM patients [4, 5]. This may in part be attributed to upregulation of $P2Y_{12}$ mediated signaling in these patients [7], underscoring the need for more potent $P2Y_{12}$ inhibiting strategies. The results of the present study showed that cangrelor achieves a great degree of platelet inhibition irrespective of DM status, which suggests that very potent $P2Y_{12}$ blockade may overcome the hyper-reactive platelet phenotype which characterizes DM patients [5]. This may contribute to the favorable outcomes in DM patients observed with the novel oral $P2Y_{12}$ receptor inhibitors, prasugrel and ticagrelor, which are characterized by more potent PD effects compared to clopidogrel [24–26]. In fact, although studies specifically assessing the PD effects in patients with DM have been conducted only with prasugrel [27], both ticagrelor and prasugrel have been associated with better ischemic outcomes compared with clopidogrel in patients with acute coronary syndromes (ACS) with DM [25, 26]. Indeed, cangrelor represents a potentially promising agent for clinical practice, and this underscores the need for a comprehensive understanding of the PD effects of this drug, particularly in high-risk patients, such as patients with DM. This is the first study evaluating the PD effects of a therapeutic concentration of cangrelor on several platelet signaling pathways other than the $P2Y_{12}$ receptor, the specific target of cangrelor. A marked decrease in platelet inhibition when using non-purinergic agonists to stimulate platelets was observed. Therefore, the findings of our study suggest that strong blockade of $P2Y_{12}$ mediated platelet activation may have an impact on other signaling pathways. This interplay between $P2Y_{12}$ receptor mediated signaling and other platelet activation signaling pathways has been reported previously [9, 10, 28–30]. In fact, our results are in line with those from a previous investigation that observed a reduction in platelet aggregation, in a concentration-dependent manner, after in vitro incubation with two potent $P2Y_{12}$ antagonists, ticagrelor and the active metabolite of prasugrel, using several platelet agonists other than ADP (including arachidonic acid, collagen and TRAP) [31, 32]. However, further studies are warranted to understand the clinical implications of these PD observations.

The functional status of the $P2Y_{12}$ signaling pathway has been associated with platelet-derived thrombin generation profiles. In particular, blockade of the $P2Y_{12}$ receptor with clopidogrel has been associated with a prolongation of the TEG parameters evaluated in this study [8, 11]. However, no effect of cangrelor on TEG parameters related with thrombin generation processes have been revealed in the present investigation. This is in contrast with other investigations, in which cangrelor did show to have an effect on thrombin generation, which however included a different methodological approach and a distinct study population [9]. Indeed, more studies are warranted to better understand the role of cangrelor on modulating procoagulant activities, which to date have been limited and conflicting. Recent observations suggest that cangrelor may exert differential actions from other $P2Y_{12}$ receptors inhibitors on thrombin generation processes due to its effects on intraplatelet signaling which can be mediated through activation of a G protein-coupled pathway separate from Gi, presumably involving Gs [30]. Similarly, the lack of modulating effects on thrombin generation processes has also been shown with other strategies that increase c-AMP

Table 4 Thrombin generation times, assessed by thromboelastography, observed with increasing concentrations of cangrelor (in vitro incubation) in the overall group and according to diabetes mellitus status

Assay	Baseline	Cangrelor 5 nM	Cangrelor 50 nM	Cangrelor 500 nM	*p* value for trend
R					
All	4.4 ± 0.4	4.3 ± 0.4	4.1 ± 0.4	4.2 ± 0.4	0.171
DM	4.3 ± 0.4	4.2 ± 0.3	3.9 ± 0.4	3.9 ± 0.3	0.097
Non-DM	4.4 ± 0.8	4.4 ± 0.8	4.3 ± 0.8	4.5 ± 0.7	0.844
TMRTG					
All	5.4 ± 0.5	5.2 ± 0.5	5.2 ± 0.5	5.2 ± 0.5	0.364
DM	5.4 ± 0.5	5.2 ± 0.4	5.0 ± 0.5	5.0 ± 0.4	0.186
Non-DM	5.5 ± 0.9	5.2 ± 0.9	5.3 ± 0.9	5.5 ± 0.9	0.706

R and TMRTG are expressed in minutes

R reaction time, *TMRTG* time to maximum rate of thrombin generation

levels which in turn are associated with enhanced inhibition of PD markers measuring the activity of the $P2Y_{12}$ pathway [33]. These findings have also been attributed to differential effects on intraplatelet signaling that way occur within the purinergic mediated pathways of platelet activation [34, 35]. These PD observations may explain why the rates of major bleeding and transfusions were not increased with cangrelor in a pooled analysis of the CHAMPION program [36].

The PD properties of cangrelor make this a potentially desirable antiplatelet agent for clinical practice. Cangrelor may have a role as a bridging strategy in the setting of patients requiring surgery but who may require treatment with a $P2Y_{12}$ inhibitor to prevent thrombotic complications, such as in ACS patients or those treated with coronary stents [37]. However, despite these promising findings, 2 large scale phase III clinical trials conducted in the setting of percutaneous coronary intervention (PCI) were both terminated before completion because of an interim analysis showing insufficient evidence of clinical effectiveness of cangrelor [16, 17]. A PD interaction between cangrelor and clopidogrel was deemed unlikely as a cause of these findings, and pitfalls in trial design, particularly with regards to the definition of myocardial infarction, may have been a potential explanation [38]. Notably, in a pooled analysis of the two CHAMPION trials ($n = 13{,}049$ patients), with the use of the universal myocardial infarction (MI) definition instead of the original definition used, cangrelor was associated with a significant 18 % relative risk reduction in the primary end point (death, myocardial infarction, or ischemia-driven revascularization at 48 h), which included a 66 % relative risk reduction in stent thrombosis [36]. Therefore, these observations have provided the rationale for the design of the ongoing large-scale phase III clinical trial CHAMPION-PHOENIX (NCT01156571), which evaluates the efficacy and safety of cangrelor compared to standard of care in patients undergoing PCI [39].

In conclusion, in vitro cangrelor provides a potent and dose-dependent blockade of the platelet $P2Y_{12}$ receptor, with no differential effect in patients with and without DM. In addition, in vitro cangrelor exerts moderate inhibitory effects on other non-purinergic platelet signaling pathways, without modulating platelet-derived thrombin generation processes. Ex vivo studies are warranted to confirm these in vitro findings.

Study limitations

The main limitation of the present investigation is derived from its very design, since in vitro conditions convert the results of this study in exploratory and ex vivo PD studies are warranted to confirm these findings. No significant differences in baseline platelet reactivity were found between DM and non-DM patients, although an upregulation of $P2Y_{12}$ signaling pathway has been reported in prior investigations [7]. This may be due to the fact that studies with a similar sample size to ours that have shown differences in platelet function profiles between patients with and without DM have usually included patients on dual antiplatelet therapy with aspirin and clopidogrel [29], while a larger sample size may be needed to find baseline differences in patients not taking a $P2Y_{12}$ inhibitor [3]. In addition, the effect of escalating concentrations of cangrelor was evaluated in a relatively small sample size, which may have played a role in the absence of interaction due to DM condition observed and in the lack of effects on TEG thrombin generation parameters found. Further, thrombin generation comprise a number of complex mechanisms that include cell interactions, thus, a cell-based model could have been potentially more fitting for the present investigation [40].

Acknowledgments Dominick J. Angiolillo (corresponding author) reports receiving: honoraria for lectures from Bristol Myers Squibb; Sanofi-Aventis; Eli Lilly Co; Daiichi Sankyo, Inc; Astra Zeneca; consulting fees from Bristol Myers Squibb; Sanofi-Aventis; Eli Lilly Co; Daiichi Sankyo, Inc.; The Medicines Company; Portola; Novartis; Medicure; Accumetrics; Arena Pharmaceuticals; Astra Zeneca; Merck; Evolva; Abbott Vascular; research grants from Bristol Myers Squibb; Sanofi-Aventis; GlaxoSmithKline; Otsuka; Eli Lilly Co; Daiichi Sankyo, Inc., The Medicines Company; Portola; Accumetrics; Schering-Plough; Astra-Zeneca; Eisai. José Luis Ferreiro reports honoraria for lectures from Eli Lilly Co; Daiichi Sankyo, Inc.; Astra Zeneca. Davide Capodanno reports honoraria for lectures from Eli Lilly Co; Daiichi Sankyo, Inc.; AstraZeneca; consulting fees from Eli Lilly Co; Daiichi Sankyo. Luis A. Guzman reports honoraria for lectures from AstraZeneca. This investigator-initiated study was funded by Institutional funding with no external financial support. The Medicines Company provided cangrelor for in vitro investigational use.

Conflict of Interest None of the other authors have conflict of interest to report.

References

1. Geisler T, Anders N, Paterok M et al (2007) Platelet response to clopidogrel is attenuated in diabetic patients undergoing coronary stent implantation. Diabetes Care 30:372–374
2. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E et al (2005) Platelet function profiles in patients with type 2 diabetes and coronary artery disease on combined aspirin and clopidogrel treatment. Diabetes 54:2430–2435
3. Serebruany V, Pokov I, Kuliczkowski W, Chesebro J, Badimon J (2008) Baseline platelet activity and response after clopidogrel in 257 diabetics among 822 patients with coronary artery disease. Thromb Haemost 100:76–82
4. Lüscher TF, Creager MA, Beckman JA, Cosentino F (2003) Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part II. Circulation 108:1655–1661
5. Ferreiro JL, Angiolillo DJ (2011) Diabetes and antiplatelet therapy in acute coronary syndrome. Circulation 123:798–813

6. Ferreiro JL, Cequier ÁR, Angiolillo DJ (2010) Antithrombotic therapy in patients with diabetes mellitus and coronary artery disease. Diab Vasc Dis Res 7:274–288
7. Ueno M, Ferreiro JL, Tomasello SD et al (2011) Functional profile of the platelet P2Y12 receptor signalling pathway in patients with type 2 diabetes mellitus and coronary artery disease. Thromb Haemost 105:730–732
8. Gurbel PA, Bliden KP, Guyer K, Aggarwal N, Tantry US (2007) Delayed thrombin-induced platelet-fibrin clot generation by clopidogrel: a new dose-related effect demonstrated by thrombelastography in patients undergoing coronary artery stenting. Thromb Res 119:563–570
9. Leon C, Ravanat C, Freund M, Cazenave JP, Gachet C (2003) Differential involvement of the P2Y1 and P2Y12 receptors in platelet procoagulant activity. Arterioscler Thromb Vasc Biol 23:1941–1947
10. van der Meijden PE, Feijge MA, Giesen PL, Huijberts M, van Raak LP, Heemskerk JW (2005) Platelet P2Y12 receptors enhance signalling towards procoagulant activity and thrombin generation. A study with healthy subjects and patients at thrombotic risk. Thromb Haemost 93:1128–1136
11. Angiolillo DJ, Capranzano P, Desai B et al (2009) Impact of P2Y(12) inhibitory effects induced by clopidogrel on platelet procoagulant activity in type 2 diabetes mellitus patients. Thromb Res 124:318–322
12. Ferreiro JL, Ueno M, Angiolillo DJ (2009) Cangrelor: a review on its mechanism of action and clinical development. Expert Rev Cardiovasc Ther 7:1195–1201
13. Storey RF, Oldroyd KG, Wilcox RG (2001) Open multicentre study of the P2T receptor antagonist AR-C69931MX assessing safety, tolerability and activity in patients with acute coronary syndromes. Thromb Haemost 85:401–407
14. Storey RF, Wilcox RG, Heptinstall S (2002) Comparison of the pharmacodynamic effects of the platelet ADP receptor antagonists clopidogrel and AR-C69931MX in patients with ischaemic heart disease. Platelets 13:407–413
15. Alberti KG, Zimmet PZ (1998) Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 15:539–553
16. Harrington RA, Stone GW, McNulty S et al (2009) Platelet inhibition with cangrelor in patients undergoing PCI. N Engl J Med 361:2318–2329
17. Bhatt DL, Lincoff AM, Gibson CM, CHAMPION PLATFORM Investigators et al (2009) Intravenous platelet blockade with cangrelor during PCI. N Engl J Med 361:2330–2341
18. Bouman HJ, van Werkum JW, Hackeng CM, Clappers N, Ten Berg JM (2009) Cangrelor increases the magnitude of platelet inhibition and reduces interindividual variability in clopidogrel-pretreated subjects. Neth Heart J 17:195–198
19. Aleil B, Ravanat C, Cazenave JP, Rochoux G, Heitz A, Gachet C (2005) Flow cytometric analysis of intraplatelet VASP phosphorylation for the detection of clopidogrel resistance in patients with ischemic cardiovascular diseases. J Thromb Haemost 3:85–92
20. Angiolillo DJ, Shoemaker SB, Desai B et al (2007) Randomized comparison of a high clopidogrel maintenance dose in patients with diabetes mellitus and coronary artery disease: results of the optimizing antiplatelet therapy in diabetes mellitus (OPTIMUS) study. Circulation 115:708–716
21. Sibbing D, Braun S, Jawansky S et al (2008) Assessment of ADP-induced platelet aggregation with light transmission aggregometry and multiple electrode platelet aggregometry before and after clopidogrel treatment. Thromb Haemost 99:121–126
22. Ueno M, Ferreiro JL, Tomasello SD et al (2011) Impact of pentoxifylline on platelet function profiles in patients with type 2 diabetes mellitus and coronary artery disease on dual antiplatelet therapy with aspirin and clopidogrel. JACC Cardiovasc Interv 4:905–912
23. Rivard GE, Brummel-Ziedins KE, Mann KG, Fan L, Hofer A, Cohen E (2005) Evaluation of the profile of thrombin generation during the process of whole blood clotting as assessed by thrombelastography. J Thromb Haemost 3:2039–2043
24. Ferreiro JL, Angiolillo DJ (2012) New directions in antiplatelet therapy. Circ Cardiovasc Interv 5:433–445
25. James S, Angiolillo DJ, Cornel JH, PLATO Study Group et al (2010) Ticagrelor vs. clopidogrel in patients with acute coronary syndromes and diabetes: a substudy from the PLATelet inhibition and patient outcomes (PLATO) trial. Eur Heart J 31:3006–3016
26. Wiviott SD, Braunwald E, Angiolillo DJ, TRITON-TIMI 38 Investigators et al (2008) Greater clinical benefit of more intensive oral antiplatelet therapy with prasugrel in patients with diabetes mellitus in the trial to assess improvement in therapeutic outcomes by optimizing platelet inhibition with prasugrel-Thrombolysis in Myocardial Infarction 38. Circulation 118:1626–1636
27. Angiolillo DJ, Badimon JJ, Saucedo JF et al (2011) A pharmacodynamic comparison of prasugrel vs. high-dose clopidogrel in patients with type 2 diabetes mellitus and coronary artery disease: results of the optimizing anti-platelet therapy in diabetes mellitus (OPTIMUS)-3 trial. Eur Heart J 32:838–846
28. Storey RF, Sanderson HM, White AE, May JA, Cameron KE, Heptinstall S (2000) The central role of the P(2T) receptor in amplification of human platelet activation, aggregation, secretion and procoagulant activity. Br J Haematol 110:925–934
29. Holinstat M, Voss B, Bilodeau ML, McLaughlin JN, Cleator J, Hamm HE (2006) PAR4, but not PAR1, signals human platelet aggregation via Ca2 + mobilization and synergistic P2Y12 receptor activation. J Biol Chem 281:26665–26674
30. Srinivasan S, Mir F, Huang JS, Khasawneh FT, Lam SC, Le Breton GC (2009) The P2Y12 antagonists, 2-methylthioadenosine 5′-monophosphate triethylammonium salt and cangrelor (ARC69931MX), can inhibit human platelet aggregation through a Gi-independent increase in cAMP levels. J Biol Chem 284:16108–16117
31. Kirkby NS, Leadbeater PD, Chan MV, Nylander S, Mitchell JA, Warner TD (2011) Antiplatelet effects of aspirin vary with level of P2Y12 receptor blockade supplied by either ticagrelor or prasugrel. J Thromb Haemost 9:2103–2105
32. Armstrong PC, Leadbeater PD, Chan MV et al (2011) In the presence of strong P2Y12 receptor blockade, aspirin provides little additional inhibition of platelet aggregation. J Thromb Haemost 9:552–561
33. Angiolillo DJ, Capranzano P, Ferreiro JL et al (2011) Impact of adjunctive cilostazol therapy on platelet function profiles in patients with and without diabetes mellitus on aspirin and clopidogrel therapy. Thromb Haemost 106:253–262
34. Hayashi H, Sudo T (2009) Effects of the cAMP-elevating agents cilostamide, cilostazol and forskolin on the phosphorylation of Akt and GSK-3beta in platelets. Thromb Haemost 102:327–335
35. Gachet C, Léon C, Hechler B (2006) The platelet P2 receptors in arterial thrombosis. Blood Cells Mol Dis 36:223–227
36. White H, Chew DP, Dauerman HL et al (2012) Reduced immediate ischemic events with cangrelor in PCI: a pooled analysis of the CHAMPION trials using the universal definition of myocardial infarction. Am Heart J 163:182–190
37. Angiolillo DJ, Firstenberg MS, Price MJ et al (2012) Bridging antiplatelet therapy with cangrelor in patients undergoing cardiac surgery: a randomized controlled trial. JAMA 307:265–274
38. Angiolillo DJ, Schneider DJ, Bhatt DL et al (2012) Pharmacodynamic effects of cangrelor and clopidogrel: the platelet function substudy from the cangrelor versus standard therapy to

achieve optimal management of platelet inhibition (CHAMPION) trials. J Thromb Thrombolysis 34:44–55
39. Leonardi S, Mahaffey KW, White HD et al (2012) Rationale and design of the Cangrelor versus standard therapy to acHieve optimal Management of Platelet InhibitiON PHOENIX trial. Am Heart J 163(768–776):e2
40. Roberts HR, Hoffman M, Monroe DM (2006) A cell-based model of thrombin generation. Semin Thromb Hemost 32(Suppl 1):32–38

4.3. Artículos de revisión

VII. Platelet adenosine diphosphate P2Y12 receptor antagonism: Benefits and limitations of current treatment strategies and future directions.

Angiolillo DJ, Ferreiro JL.

Rev Esp Cardiol. 2010:63:60-76.

REVIEW ARTICLE

Platelet Adenosine Diphosphate P2Y12 Receptor Antagonism: Benefits and Limitations of Current Treatment Strategies and Future Directions

Dominick J. Angiolillo and José Luis Ferreiro

University of Florida, College of Medicine-Jacksonville, Jacksonville, Florida, USA

Platelet P2Y12 receptor antagonism with clopidogrel has represented a major advancement in the pharmacological management of patients with atherothrombotic disease, in particular those with acute coronary syndromes and undergoing percutaneous coronary interventions. Despite the benefit associated with clopidogrel therapy in these high risk settings, laboratory and clinical experience have led to identify some of its caveats, among which its wide range of platelet inhibitory response is the most relevant. Genetic, cellular and clinical factors are implied in variability in response to clopidogrel. Importantly, pharmacodynamic findings have shown to have important prognostic implications, underscoring the need for more optimal antiplatelet treatment strategies. The aim of this manuscript is to provide an overview on the current status and future directions in $P2Y_{12}$ receptor antagonism, with particular emphasis on interindividual variability in response to clopidogrel and strategies, including novel antiplatelet agents, to improve platelet $P2Y_{12}$ inhibition.

Key words: *Platelet receptors; Thrombosis; Acute coronary syndrome; Clopidogrel.*

Inhibición del receptor plaquetario $P2Y_{12}$ de adenosina difosfato plaquetario: efectos beneficiosos y limitaciones de las estrategias terapéuticas actuales y perspectivas futuras

La inhibición del receptor plaquetario $P2Y_{12}$ con el empleo de clopidogrel ha representado un importante avance en el tratamiento farmacológico de los pacientes con enfermedad aterotrombótica, especialmente en los síndromes coronarios agudos y en el intervencionismo coronario percutáneo. A pesar de los efectos beneficiosos asociados al tratamiento con clopidogrel en estos contextos de alto riesgo, las experiencias clínicas y de laboratorio ha permitido identificar algunas de sus limitaciones, la más relevante de las cuales es la amplia variabilidad existente en la respuesta inhibitoria plaquetaria. En esta variabilidad de la respuesta al clopidogrel se han involucrado diferentes factores clínicos, genéticos y celulares.

Es importante señalar que los hallazgos farmacodinámicos han demostrado tener repercusiones pronósticas, lo cual subraya la necesidad de mejores estrategias de tratamiento antiagregante plaquetario. El objetivo de este artículo es aportar una visión general del estado actual y las perspectivas futuras sobre el antagonismo del receptor $P2Y_{12}$, con especial referencia a la variabilidad interindividual en la respuesta a clopidogrel y a las estrategias destinadas a mejorar la inhibición del receptor $P2Y_{12}$, incluidos los fármacos antiagregantes plaquetarios más recientes.

Palabras clave: *Receptores plaquetarios. Trombosis. Síndrome coronario agudo. Clopidogrel.*

INTRODUCTION

Atherosclerosis is the major underlying cause of ischemic coronary artery disease and platelets play a key role in atherothrombotic complications occurring in patients with acute coronary syndromes (ACS) and in those undergoing percutaneous coronary intervention (PCI).[1-3] Following atherosclerotic plaque rupture, platelet mediated thrombosis occurs through a 3-step process: adhesion, activation, and aggregation. Each of these phases represents a target for the development of antiplatelet agents. Inhibitors of platelet adhesion are still under investigation and not approved for clinical use. Inhibitors of platelet aggregation (ie, intravenous glycoprotein IIb/IIIa inhibitors) are reserved only for the acute phase treatment of high risk ACS patients undergoing PCI. Inhibitors of platelet activation processes represent

Disclosures: Dominick J. Angiolillo:
Honoraria/Lectures: Bristol Myers Squibb; Sanofi-aventis; Eli Lilly and Company; Daiichi Sankyo, Inc.
Honoraria/Advisory board: Bristol Myers Squibb; Sanofi-aventis; Eli Lilly and Company; Daiichi Sankyo, Inc.; Astra Zeneca; The Medicines Company; Portola Pharmaceuticals; Novartis; Arena Pharmaceuticals.
Research Grants: GlaxoSmithKline; Otsuka; Accumetrics; Eli Lilly and Company; Daiichi Sankyo, Inc.; The Medicines Company; AstraZeneca; Eisai; Portola Pharmaceutical; Schering-Plough; Johnson and Johnson.

Correspondence: D.J. Angiolillo, MD, PhD,
University of Florida College of Medicine-Jacksonville,
655 West 8th Street, Jacksonville, Florida, 32209, USA
E-mail: dominick.angiolillo@jax.ufl.edu

the mainstay treatment for the acute and long-term prevention of recurrent ischemic events in ACS and PCI patients.

Currently, 2 groups of platelet activation inhibitors, aspirin and thienopyridines, are clinically approved for prevention of recurrent ischemic events in ACS/PCI patients. Aspirin (acetylsalicylic acid) inhibits platelet activation through irreversible blockade of the cyclooxygenase (COX)-1, which in turn prevents production of thromboxane A_2. The benefit of aspirin therapy for short and long-term secondary prevention of thrombotic events has been extensively proven.[4,5] However, the elevated recurrence rate of ischemic events, particularly in high risk settings, sets the basis for the development of antiplatelet drugs that target other pivotal signaling pathways such as those mediated by adenosine diphosphate (ADP). Thienopyridines represents a class of antiplatelet agents that inhibit the $P2Y_{12}$ ADP receptor subtype and are now the cornerstone of treatment as an adjunct to aspirin in ACS/PCI patients. Clopidogrel is currently the thienopyridine of choice. Despite the clinical benefits observed with adjunctive clopidogrel treatment, shortcomings have also been identified with this drug.[6,7] The present manuscript provides an overview on the current status and future directions in $P2Y_{12}$ receptor antagonism, with particular emphasis on interindividual variability in response to clopidogrel and strategies, such as novel antiplatelet agents, to improve $P2Y_{12}$ inhibition.

PLATELET PURINERGIC RECEPTORS

Purinergic receptors expressed on platelets consist of $P2X_1$, $P2Y_1$, and $P2Y_{12}$. Adenosine triphosphate (ATP) is the physiological agonist of $P2X_1$. a ligand-gated cation channel. $P2X_1$ is involved in platelet shape change through extracellular calcium influx and helps to amplify platelet responses mediated by other agonists.[8] ADP is the physiological agonist and, thus, exerts its action on platelets through both G protein-coupled seven transmembrane domains purinergic receptors, $P2Y_1$ and $P2Y_{12}$.[9,10] Activation of the $P2Y_1$ receptor leads to a transient change in platelet shape, intracellular calcium mobilization, granule release of other mediators and finally initiates a weak and transient phase of platelet aggregation.[8,9] Although both P2Y receptors are needed for complete aggregation,[11] ADP-stimulated effects on platelets are upheld predominantly by the G_i-coupled $P2Y_{12}$ receptor signaling pathway. Activation of $P2Y_{12}$ receptors causes a series of intracellular events that result in calcium mobilization, granules release, thromboxane A_2 generation and activation of glycoprotein IIb/IIIa receptor, which leads to amplification of platelet aggregation and stabilization of the platelet aggregate.[10-12] Therefore, platelet $P2Y_{12}$ blockade is pivotal in order to inhibit platelet activation and aggregation, thus, preventing formation of platelet thrombus (Figure 1).

$P2Y_{12}$ Receptor Antagonism

Thienopyridines are non-direct and irreversible $P2Y_{12}$ receptor inhibitors, and represent the only $P2Y_{12}$ blockers currently approved for clinical use. Ticlopidine, a first-generation thienopyridine, in combination with aspirin was proven superior to aspirin alone or anticoagulation in combination with aspirin in the setting of PCI.[13-16] Due to safety concerns, mainly high rates of neutropenia, ticlopidine was soon widely replaced by clopidogrel, a second-generation thienopyridine with similar efficacy and a better safety profile.[17] In addition, clopidogrel achieves more rapid effects than ticlopidine through loading dose administration.[18] The stardom of clopidogrel in the clinical settings of PCI and ACS, including unstable angina, non-ST-segment elevation myocardial infarction (NSTEMI) and ST-segment elevation myocardial infarction (STEMI), has been undisputed up till now, given that several large-scale clinical trials have shown a clear benefit in terms of preventing recurrent ischemic events, including stent thrombosis, when clopidogrel is associated to aspirin.[19-23] In fact, dual antiplatelet therapy with aspirin and clopidogrel is currently accepted per guidelines as the antiplatelet treatment of choice for patients across the spectrum of ACS, including patients with unstable angina, NSTEMI[24,25] and STEMI,[26,27] as well as for patients undergoing PCI.[28,29] Despite these clinical benefits, a substantial number of patients may continue to have recurrent cardiovascular events. Accumulating observations have shown that variability in individual response profiles to clopidogrel has been proposed as one of the mechanisms involved in this limited efficacy.[6,7] This has led to investigations trying to identify the mechanisms associated with clopidogrel response variability as well as strategies to overcome the limitations associated with current treatment regimens.[30,31]

CLOPIDOGREL: INTERINDIVIDUAL VARIABILITY IN RESPONSE

Clopidogrel, like all thienopyridines, is a pro-drug that must undergo hepatic biotransformation to be converted to an active metabolite which will irreversibly bind and block $P2Y_{12}$ platelet receptor. Approximately 85% of the clopidogrel absorbed into the bloodstream from the intestine is hydrolyzed by esterases becoming inactive, whereas the remaining ≈15% is metabolized

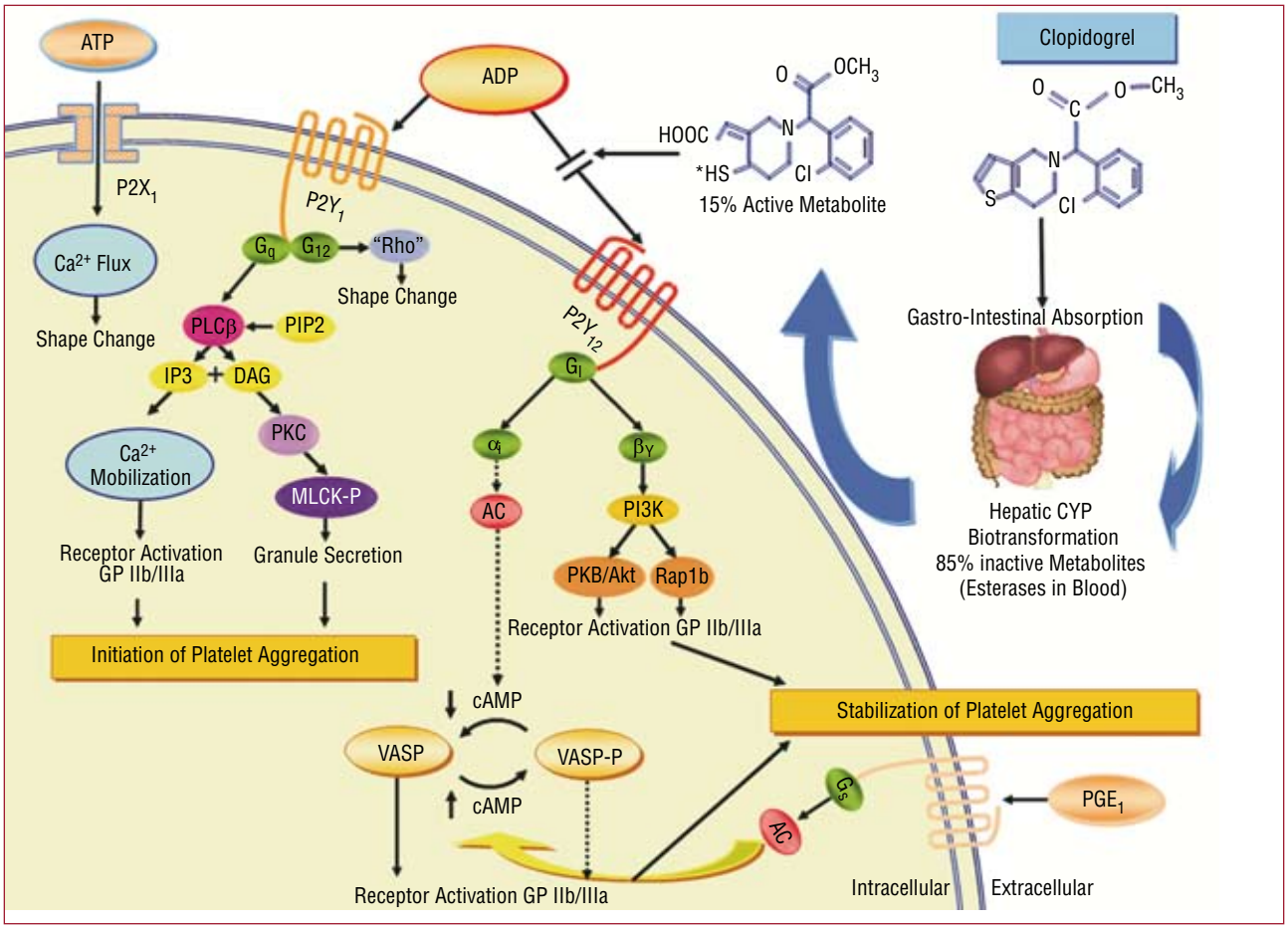


Figure 1. Purinergic receptors and mechanism of action of clopidogrel. Clopidogrel is a pro-drug of which approximately 85% is hydrolyzed by esterases in the blood to inactive metabolites and only 15% is metabolized by the cytochrome P450 (CYP) system in the liver into an active metabolite. The active metabolite irreversibly inhibits the adenosine diphosphate (ADP) $P2Y_{12}$ receptor. The $P2X_1$ receptor, which uses adenosine triphosphate (ATP) as an agonist, is involved in platelet shape change through extracellular calcium influx and helps to amplify platelet responses mediated by other agonists. Activation of the $P2Y_1$ receptor leads to alteration in shape and initiates a weak and transient phase of platelet aggregation. The binding of ADP to the G_q-coupled $P2Y_1$ receptor activates phospholipase C (PLC), which generates diacylglycerol (DAG) and inositol triphosphate (IP3) from phosphatidylinositol biphosphate (PIP2). Diacylglycerol activates protein kinase C (PKC) leading to phosphorylation of myosin light chain kinase (MLCK-P) and IP3 leads to mobilization of intracellular calcium. The $P2Y_1$ receptor is coupled to another G-protein, G_{12}, which activates the "Rho" protein and leads to the change in platelet shape. The binding of ADP to the G_i-coupled$P2Y_{12}$ receptor liberates the G_i protein subunits a_i and β_γ, resulting in stabilization of platelet aggregation. The α_i subunit inhibits adenylyl cyclase (AC) and, thus, reduces cyclic adenosine monophosphate (cAMP) levels, which diminishes cAMP-mediated phosphorylation of vasodilator-stimulated phosphoprotein (VASP-P). The status of VASP-P modulates glycoprotein (GP) IIb/IIIa receptor activation. The subunit β_γ activates the phosphatidylinositol 3-kinase (PI3K), which leads to GP IIb/IIIa receptor activation through activation of a serine-threonine protein kinase B (PKB/Akt) and of Rap1b GTP binding proteins. Prostaglandin E_1 (PGE_1) activates AC, which increases cAMP levels and status of VASP-P. Solid arrows indicate activation; dotted arrows indicate inhibition. With permission from Angiolillo DJ et al.[6]

in the liver through a double oxidation process mediated by several cytochrome P450 (CYP) isoforms to be converted to an active metabolite.[6,7] Due to the irreversible blockade of the $P2Y_{12}$ receptor by its active metabolite, clopidogrel effects last for the whole lifespan of the platelet (7-10 days).

The delayed onset of action of clopidogrel is one of its limitations. Thus, a loading dose must be administered when rapid inhibition is required, such as in the context of ACS or PCI.[18] Currently, the doses approved by regulatory authorities are a 300 mg loading dose and a 75 mg maintenance dose. Given the accumulating evidence of a more rapid and potent effect associated with a 600 mg loading as well as a better clinical benefit, this dosing regimen has now become the standard of care in clinical practice and is also endorsed by practice guidelines.[28,32-34] Clopidogrel's main caveat is its broad variability in

TABLE 1. **Inadequate Clopidogrel Response and Clinical Outcomes**

	Patients, No.	Clinical Setting	Test	Outcomes
Periprocedural events				
Gurbel et al[35]	120	Elective PCI	LTA	Myonecrosis/inflammation
Lev et al[36]	120	Elective PCI	LTA	Myonecrosis
Cuisset et al[37]	190	NSTEACS undergoing PCI	LTA	Periprocedural MI
Marcucci et al[38]	367	MI undergoing PCI	LTA	Myonecrosis
Short-term outcomes (≤30 days)				
Cuisset et al[39]	106	ACS undergoing PCI	LTA	Ischemic events (30 days)
Hochholzer et al[40]	802	Elective PCI	LTA	Ischemic events (30 days)
Frere et al[41]	195	NSTEACS undergoing PCI	VASP LTA	Ischemic events (30 days)
Patti et al[42]	160	PCI (not primary)	VN	MACE (30 days)
Long-term outcomes (>30 days)				
Matetzky et al[43]	60	STEMI (primary PCI)	LTA	Ischemic events (6 months)
Gurbel et al[44]	192	Nonemergent PCI	LTA	Ischemic events (6 months)
Geisler et al[45]	379	Stable and unstable angina undergoing PCI	LTA	MACE (3 months)
Bliden et al[46]	100	Nonemergent PCI (chronic clopidogrel therapy)	LTA	Ischemic events (12 months)
Bonello et al[47]	144	Stable angina and low-risk NSTEACS undergoing PCI	VASP	MACE (6 months)
Angiolillo et al[48]	173	DM patients with CAD on chronic clopidogrel therapy	LTA	MACE (2 years)
Price et al[49]	380	PCI with DES	VN	MACE and ST (6 months)
Marcucci et al[50]	683	ACS undergoing PCI	VN	MACE (12 months)
de Miguel et al[51]	179	NSTEACS undergoing coronary angiography	VN	MACE (12 months)
Stent thrombosis				
Barragan et al[52]	46	Subacute stent thrombosis	VASP	ST
Ajzenberg et al[53]	49	Subacute stent thrombosis	SIVA	ST
Gurbel et al[54]	120	Subacute stent thrombosis	VASP	ST
Blindt et al[55]	99	PCI with high risk for stent thrombosis	VASP	ST
Buonamici et al[56]	804	PCI with DES	LTA	ST
Sibbing et al[57]	1608	Elective PCI with DES	MEA	ST

ACS indicates acute coronary syndrome; DES, drug-eluting stents; LTA, light transmittance aggregometry; MACE, major adverse cardiovascular events; MEA, multiple electrode platelet aggregometry; MI, myocardial infarction; NSTEACS, non-ST elevation acute coronary syndrome; PCI, percutaneous coronary intervention; SIVA, shear-induced platelet aggregation; STEMI, ST elevation myocardial infarction; ST, stent thrombosis; VASP, vasodilator-stimulated phosphoprotein phosphorilation assay; VN, VerifyNow system.

response among treated individuals. A relatively high percentage of patients experience suboptimal effects; the rate of "low responders" or "resistant patients" ranges from 5% to 40%, depending on population characteristics as well as the platelet function assay and cut-off values used.[6,7] Variability in clopidogrel response is a well-known phenomenon the relevance of which is underscored by the fact that a multitude of studies have observed an association between low responsiveness and adverse cardiovascular outcomes.[6,7] These studies have been performed mainly in patients undergoing PCI (Table 1), where the use of clopidogrel is mandatory, but also in patients on chronic clopidogrel therapy.[35-57]

MECHANISMS INVOLVED IN CLOPIDOGREL RESPONSE VARIABILITY

Multiple mechanisms have been identified that play a role in clopidogrel response variability. These can be summarized into 3 broad categories: genetic, cellular, and clinical factors (Figure 2).

Genetic Factors

Pharmacogenetic studies have evaluated polymorphisms of different genes involved in the pharmacokinetic and pharmacodynamic effects of clopidogrel.[58] These include genes encoding for proteins and enzymes involved in clopidogrel's absorption and hepatic metabolism as well as genes encoding for platelet membrane receptors.

The gene ABCB1 codifies the intestinal P-glycoprotein MDR1 (multidrug resistance transporter), involved in clopidogrel absorption. Patients carrying two ABCB1 variant alleles may have reduced active metabolite generation after administration of a loading dose of clopidogrel.[59] Simon et al observed that the presence of these variant alleles was associated with a higher rate

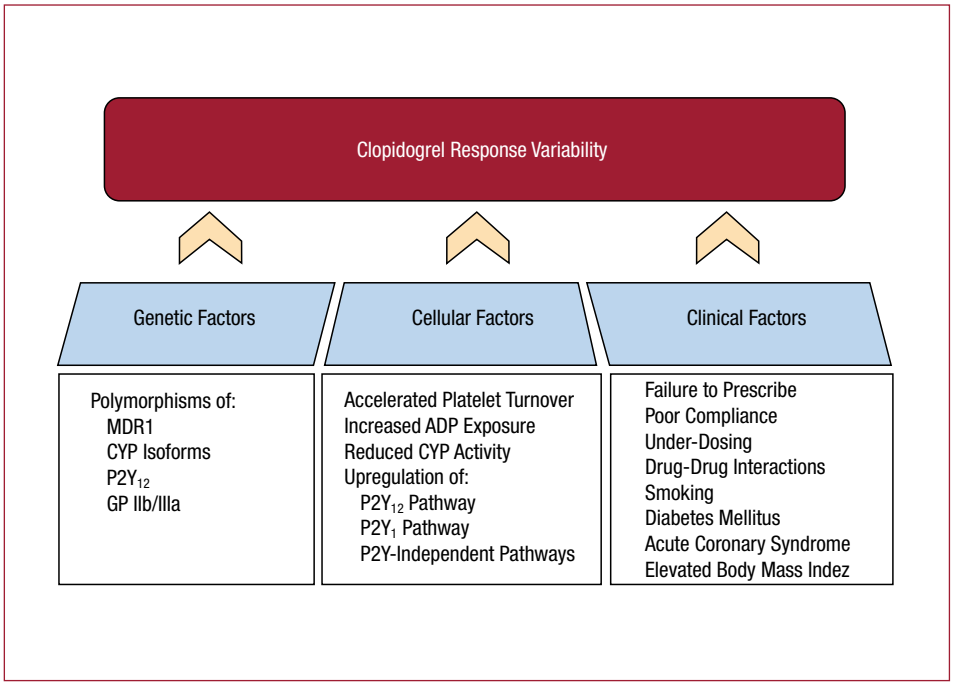


Figure 2. Mechanisms involved in clopidogrel response variability
Multiple mechanisms are involved in clopidogrel response variability, which can be grouped into three categories: genetic, cellular and clinical factors.
ADP indicates adenosine diphosphate; CYP, cytochrome P450; GP, glycoprotein; MDR, multidrug resistance transporter.

of cardiovascular events (death from any cause, nonfatal stroke and myocardial infarction) at 1 year of follow-up in a population of 2208 patients with an acute myocardial infarction receiving clopidogrel therapy.[60] However, the same ABCB1 polymorphism was not found to be associated with ADP-stimulated platelet aggregation after 1 week of clopidogrel therapy in a recently published genome-wide association study performed in an homogenous population (Amish) of healthy subjects.[61]

A number of CYP isoenzymes are involved in the hepatic oxidation steps that convert clopidogrel to its active metabolite. In particular, CYP3A4, CYP3A5, CYP2C9, and CYP1A2 are implicated in one step, while CYP2B6 and CYP2C19 are involved in both steps. Different experiences have reported polymorphisms in CYP3A4,[62] CYP3A5,[63] and CYP2C9[64] to be associated with clopidogrel responsiveness, although large-scale pharmacogenetic studies have failed to observe any association between these polymorphisms and clinical outcomes.[60,65] However, a number of recent large-scale studies have showed a strong association between CYP2C19 loss-of-function variant alleles (mainly CYP2C19*2) and impaired clinical outcomes.[60,61,65-68] This is in line with numerous studies showing the relation between CYP2C19 reduced-function alleles and decreased formation of active metabolite, lower platelet inhibition and impaired clinical outcomes.[64,69-71] In the study by Simon et al, acute myocardial infarction patients carrying any two CYP2C19 loss-of-function alleles (*2,*3,*4, or *5), especially those undergoing PCI, had a higher rate of cardiovascular events at 1 year of follow-up.[60] Consistently, a substudy of TRITON-TIMI 38 (Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel-Thrombolysis in Myocardial Infarction) showed that carriers of at least one CYP2C19 reduced-function allele had a higher rate of cardiovascular events among clopidogrel-treated subjects (n=1477).[65] In addition, the CYP2C19*2 variant has been observed to be an independent predictor of cardiovascular events in patients chronically treated with clopidogrel after a myocardial infarction[66] or undergoing PCI,[61] as well as to be significantly associated with an increased risk of stent thrombosis following coronary stent placement.[67,68]

Pharmacogenetic studies have also evaluated polymorphisms of genes encoding for platelet membrane receptors, such as the following: P2YR12 (ADP receptor $P2Y_{12}$), ITGB3 (platelet-fibrinogen receptor GP IIb/IIIa), ITGA2 (platelet-collagen receptor GP Ia), and PAR-1 (protease-activated receptor -1, a thrombin receptor). Some variants of these genes have been suggested to play a role in variability in clopidogrel response, although results have been inconsistent to date.[72-78]

Cellular Factors

Clopidogrel-induced antiplatelet effects also may be affected by several cellular factors. For

instance, an accelerated platelet turnover has been suggested to diminish clopidogrel responsiveness.[79] Platelet turnover is represented by the presence of reticulated (immature) platelets, which could have a greater reactivity and, therefore, result in impaired clopidogrel response. The association between a higher percentage of circulating reticulated platelets and a lower response to clopidogrel has been observed in patients with coronary artery disease, either high-risk[79] or stable patients.[80] Generation of active metabolite might be affected by cellular factors such as a different degree of baseline metabolic activity of the CYP system.[81] In addition, upregulation of both purinergic ($P2Y_{12}$ and $P2Y_1$) and P2Y-independent platelet signaling pathways have also been proposed to be implicated in clopidogrel variability in response, especially among patients with diabetes mellitus, which may have one or more of these cellular disorders.[82-84]

Clinical Factors

Multiple factors associated with inadequate clopidogrel response fall into this category. Compliance is the most important.[6,7] Clopidogrel dosing may also play a role; whether the currently approved loading and maintenance doses are the most optimal will be discussed later. Some clinical features are also involved in baseline platelet reactivity and response to clopidogrel. In particular, the presence of an acute coronary syndrome,[85,86] diabetes mellitus,[82,83,87,88] and obesity,[89,90] have been associated with lower clopidogrel effects, which may also contribute to higher atherothrombotic event rates.

The CYP system activates and metabolizes countless drugs and substances that could might interfere in hepatic formation of clopidogrel's active metabolite. Some frequently used drugs in cardiovascular therapy that have been suggested to impair clopidogrel-induced antiplatelet effects are lipophilic statins, calcium channel blockers (CCB) and proton pump inhibitors (PPI).

Initially, mechanistic studies observed a relation between the use of lipophilic statins (eg, simvastatin, lovastatin, atorvastatin), which are metabolized by CYP isoenzimes (mainly CYP3A4), and decreased clopidogrel-mediated inhibitory effects.[91,92] However, these findings were not corroborated in other functional studies and, importantly, post-hoc analysis of large-scale clinical trials or registries did not show any association with adverse clinical outcomes.[93-96] Calcium channel blockers (metabolized by CYP3A4), mainly dihydropyridines, have also been reported to decrease clopidogrel inhibitory effects on platelets and to impair clinical outcomes when both drugs are associated.[97,98]

A drug-drug interaction between PPIs and clopidogrel has been recently described and has raised an important concern due to the frequency with which these drugs are associated. The different PPIs available are metabolized by CYP isoforms (mainly CYP2C19 and CYP3A4), but with different specificities.[99] The most consistent results to date in functional studies have involved omeprazole, which is metabolized primarily by CYP2C19.[100,101] In a double-blind, randomized, placebo-controlled study, omeprazole significantly decreased clopidogrel antiplatelet effects in patients (n=124) receiving dual antiplatelet therapy and undergoing coronary artery stent implantation.[100] Other PPIs have also been evaluated in functional studies, which failed to show any effect of pantoprazole or esomeprazole on clopidogrel responsiveness,[102] while lansoprazole has been reported to reduce antiplatelet effects after a clopidogrel loading dose of 300 mg only in subjects with the higher response (upper tertile), but not in patients receiving a loading dose of prasugrel.[103] Data analyses of large clinical studies, mainly registries and post-hoc analysis of randomized clinical trials, have provided contradictory results when evaluating the effect of concomitant therapy with PPIs and clopidogrel on clinical outcomes. Ho et al observed that concurrent PPI and clopidogrel therapy was significantly associated with a 25% relative increase in long-term adverse outcomes (the composite endpoint of death and rehospitalization for ACS) in a cohort of 8205 patients taking clopidogrel after discharge for an ACS.[104] PPIs other than omeprazole were rarely used and, hence, the study was underpowered to determine their effects. Global use of PPIs was also found to be a predictor of reinfarction in a population-based case-control study in patients (n=2791) following discharge after treatment for a myocardial infarction. When PPIs were evaluated separately, pantoprazole (metabolized principally by CYP2C9) use was not associated with an increased risk of reinfarction.[105] Conversely, results of the Clopidogrel Medco Outcomes study presented during the Society for Cardiovascular Angiography and Interventions (SCAI) 2009 Annual Scientific Sessions (Las Vegas, NV, USA) suggested a class effect. In this large registry (n=16 690), PPIs were associated with increased risk (hazard ratio = 1.51) of cardiovascular events at 12 months of follow-up in patients on clopidogrel following coronary stenting. Each individual PPI (omeprazole, esomeprazole, pantoprazole, and lansoprazole) was associated with a greater risk (39%-61%) of cardiovascular events when compared with clopidogrel alone. However, a post-hoc analysis of the TRITON-

TIMI 38 trials failed to show any association of PPI use with clinical outcomes in patients on clopidogrel and those on prasugrel therapy, even though a post-hoc analysis of PRINCIPLE-TIMI 44 (Prasugrel in Comparison to clopidogrel for Inhibition of Platelet Activation and Aggregation-Thrombolysis in Myocardial Infarction 44) observed that platelet aggregation 6 hours after a 600 mg clopidogrel loading dose was lower for patients on a PPI, while a non significant difference was seen after a 60 mg loading dose of prasugrel.[106] These clinical findings are in line with the results of the Clopidogrel and the Optimization of Gastrointestinal Events (COGENT-1) trial, presented at the TCT 2009 meeting (San Francisco, CA, USA. COGENT-1 is the only prospective randomized double-blind placebo controlled trial to date comparing a PPI (omeprazole) with placebo in patients taking clopidogrel. The study enrolled 3627 patients in whom a requirement for clopidogrel therapy with concomitant aspirin was anticipated for at least 12 months. No difference was observed in the risk of cardiovascular events or myocardial infarction (hazard ratio = 1.02; 95% confidence interval, 0.70-1.51) in a median follow-up of 133 days, while a benefit in terms of reduced gastrointestinal effects, which was the primary outcome of the study, was seen in patients taking the PPI (hazard ratio = 0.55; $P<.007$).

Smoking is a major risk factor for atherothrombotic cardiovascular processes and smoking cessation is a class I recommendation for secondary prevention of ischemic events in patients with coronary artery disease.[24-29] Cigarette smoking is also a potent inducer of the CYP1A2 isoform[107] and, therefore, it may increase clopidogrel biotransformation. Some recent studies have reported that a heavy smoking habit enhances clopidogrel-induced inhibitory effects on platelets[108,109] and improves clinical outcomes in clopidogrel-treated patients.[110,111] However, a mechanistic study observed an association between cigarette smoking and a lower production of one of clopidogrel's metabolites.[112] Therefore, the role of smoking on clopidogrel effects warrants further investigation.

FUTURE DIRECTIONS

The prognostic implications associated with variability in clopidogrel-induced effects inevitably lead to questions on how to address and overcome this phenomenon. Essential first steps are to confirm patient compliance to antiplatelet treatment and rule out potential drug-drug interactions in the polymedicated patient. Three additional strategies have been suggested to overcome variability in response to clopidogrel[6,7]:

High Clopidogrel Dosing

A high clopidogrel loading dose of 600 mg achieves faster and greater platelet inhibition than the current standard of 300 mg,[32,113,114] while a 900 mg loading dose provides only a marginal increase in platelet inhibition when compared to a 600 mg loading dose.[113,114] This greater platelet inhibition with high clopidogrel loading regimens has been reflected in better clinical outcomes in patients undergoing PCI and has become common clinical practice despite the lower current standard..[33,34,115]

In a PCI setting, randomized experiences have observed a benefit of a high maintenance regimen (150 mg/day) of clopidogrel in terms of enhanced platelet inhibition when compared to the standard dose of 75 mg/day.[116-119] In a large observational study performed in a nonselected cohort of patients (n=2954) who underwent PCI with coronary stenting, Lemesle and colleagues compared the effect of a high loading dose followed by a high maintenance dose (600 mg and 150 mg/day, respectively) of clopidogrel with standard dosing during the first 15 days after PCI. In this registry, the high dosing regimen was significantly associated with a decrease in the composite end point of death, myocardial infarction and stent thrombosis (hazard ratio = 0.694) at 2 months without a significant increase in hemorrhagic complications.[120] These findings are in line with the results of the CURRENT/OASIS-7 (Clopidogrel optimal loading dose Usage to Reduce recurrent EveNTs/Optimal Antiplatelet Strategy for InterventionS; European Society of Cardiology Congress 2009, Barcelona, Spain). This multicenter, randomized, parallel-group trial enrolled 25 087 ACS patients scheduled to undergo angiography within 72 hours of hospital arrival who were randomized to high dose (600 mg of clopidogrel on the first day, then 150 mg once a day for 7 days, followed by 75 mg daily for the remainder of the month) or standard dose of clopidogrel for a month. This study had a 2×2 factorial design and patients were also randomized to receive high (300-325 mg daily) versus low (75-100 mg daily) dose of aspirin. Although the study did not find a statistical difference for the primary endpoint (the combined rate of cardiovascular death, myocardial infarction and stroke at 30 days) in the overall study population, the high clopidogrel dose regimen reduced the risk of stent thrombosis by 30% and the risk of myocardial infarction by 22% in the subgroup of patients undergoing PCI (n=17 232), while no benefit was observed in patients who did not undergo PCI. The benefit observed in the PCI subgroup

was, however, hampered by an increase in major bleeding in the high dose regimen group, although it was not significant for intracerebral or fatal bleeds. No significant difference in efficacy or bleeding between high and low-dose aspirin was observed, although a trend towards a higher rate of gastrointestinal bleeds in the high-dose group (0.38% vs 0.24%; *P*=.051) was found.

There has also been emerging interest in increasing clopidogrel dosing based on the degree of responsiveness of a given patient, which has been defined as "tailored" or "individualized" treatment. Bonello et al observed that additional 600 mg loading doses of clopidogrel (up to 2400 mg) administered to low-responders ("tailored" treatment) reduced the rates of adverse events, including stent thrombosis, compared to patients treated conventionally without increasing the bleeding risk.[121,122] The efficacy and safety of tailored treatment with high clopidogrel maintenance dose in low responders to standard clopidogrel dose is currently under evaluation in several ongoing clinical trials, such as GRAVITAS (Gauging Responsiveness With a VerifyNow Assay: Impact on Thrombosis and Safety; NCT00645918),[123] ARCTIC (Double Randomization of a Monitoring Adjusted Antiplatelet Treatment Versus a Common Antiplatelet Treatment for DES Implantation, and Interruption Versus Continuation of Double Antiplatelet Therapy; NCT00827411), and DANTE (Dual Antiplatelet Therapy Tailored on the Extent of Platelet Inhibition, NCT00774475).

Triple Antiplatelet Therapy

Adding a third antiplatelet drug may be considered as an option both in the acute and maintenance phases of treatment. Glycoprotein IIb/IIIa inhibitors may be used in the acute phase, as they markedly increase platelet inhibition when added on top of clopidogrel.[44] Studies evaluating tailored treatment according to the degree of responsiveness to standard antiplatelet therapy have obtained promising results. In a cohort of clopidogrel low responder patients (n=149) referred for elective PCI who were randomized to "conventional group" (standard dual antiplatelet therapy) or "active group" (addition of abciximab to dual antiplatelet therapy), Cuisset et al observed that patients in the active group had a significantly lower rate of cardiovascular events at 1 month (OR=2.8).[124] The recently published 3T/2R (Tailoring Treatment With Tirofiban in Patients Showing Resistance to Aspirin and/or Resistance to Clopidogrel) trial randomized stable or low-risk unstable angina patients undergoing elective PCI who were poor responders (n=263) to aspirin or clopidogrel to receive either tirofiban (n=132) or placebo (n=131) on top of standard aspirin and clopidogrel therapy. The rate of major adverse cardiovascular events within 30 days was reduced in the tirofiban group (3.8% vs 10.7%), without any increased risk in bleeding.[125]

In the maintenance phase of therapy, adjunctive use of cilostazol to standard dual antiplatelet therapy has been observed to increase the degree of platelet inhibition.[126] The enhanced platelet inhibition achieved with this triple therapy may contribute to the observed association with better clinical outcomes in patients undergoing PCI, including stent thrombosis rates.[127-129] Of note, this benefit seems not to be hampered by an increase in bleeding.[127] However, use of cilostazol is limited by the high frequency of side effects, mainly headache, palpitations, and gastrointestinal disturbances.[126]

New $P2Y_{12}$ Receptor Antagonists

The benefit achieved by blocking the $P2Y_{12}$ signaling pathway in patients with coronary artery disease for preventing recurrent events is indisputable. Thus, the search for new agents with higher inhibitory effects and less variability compared to clopidogrel is warranted (Figure 3). Currently, several novel $P2Y_{12}$ blockers are under different stages of clinical development[130,131] (Table 2). This section aims to provide an overview of these new agents.

Prasugrel

Prasugrel, a third-generation thienopyridine, is an orally administered pro-drug which needs hepatic biotransformation into its active metabolite to irreversibly block the $P2Y_{12}$ receptor.[132] The major pharmacokinetic difference with clopidogrel is that prasugrel is more effectively converted to its active metabolite, through a process involving hydrolysis by carboxyesterases, mainly in the intestine, followed by only a single hepatic CYP-dependent step. Since the active metabolites of clopidogrel and prasugrel are equipotent in terms of platelet inhibitions, the major production of active metabolites achieved by prasugrel provides greater platelet inhibition.[132] In addition, prasugrel has a more rapid onset of action and less interindividual response variability than clopidogrel even when used at high dosing regimens.[132,133]

The TRITON-TIMI 38 trial evaluated the clinical efficacy and safety of prasugrel (60 mg loading dose followed by a 10 mg maintenance dose), compared to standard clopidogrel loading and maintenance dose regimens in 13 608 patients

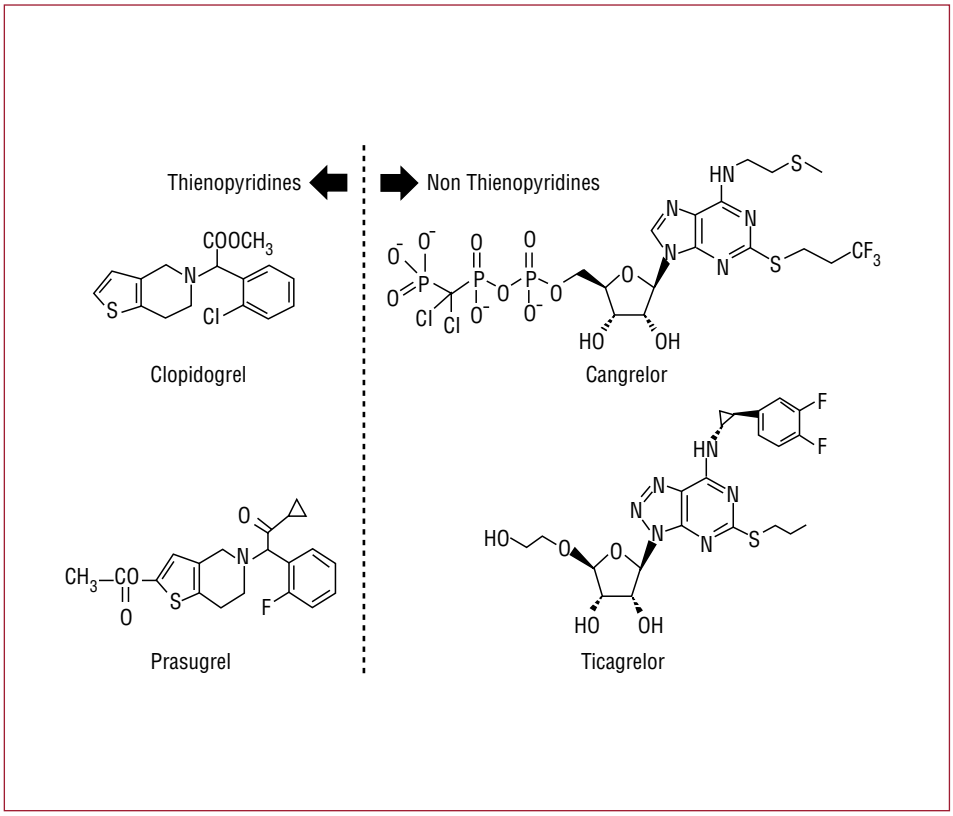


Figure 3. Chemical structure of $P2Y_{12}$ receptor antagonists.

with moderate to high-risk ACS undergoing PCI.[134] In this randomized, double-blind, parallel-group, phase III study, prasugrel obtained a significant 19% relative reduction (9.9% for prasugrel vs 12.1% for clopidogrel; hazard ratio = 0.81; *P*<.001) of the rates of the primary end point (composite of cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke), and a significant reduction of the rates of stent thrombosis (9.7% vs 11.9%; hazard ratio = 0.81; *P*=.0001),[135] over a follow-up period of 15 months. This occurred at the cost of an increased risk of TIMI major non-coronary artery bypass grafting (non-CABG) related bleeding (2.4% vs 1.8%; *P*=.03), mostly in the maintenance phase of prasugrel treatment.[134] An important feature of this trial is the performance of a net clinical benefit analysis (a composite of the efficacy and bleeding end points), in which prasugrel was still found superior despite the excess in bleeding (12.2% vs 13.9%; hazard ratio = 0.87; *P*=.004). The clinical benefit of prasugrel was largely driven by a marked reduction in non-fatal MI, while no differences were observed in death and stroke. Particular subgroups appeared to benefit more from the use of prasugrel, such as patients with diabetes mellitus[136] and patients with STEMI,[137] in which no increase in bleeding risk was observed. In contrast, the net analysis mentioned above showed no net benefit in the aged patients (≥75 years) and in those weighing less than 60 kg, and a net harm in patients with history of stroke or transient ischemic attack.[134] A landmark analysis of this trial showed a significant reduction in ischemic events in the prasugrel group by the third day and persisting throughout the follow-up period.[138] Importantly, this analysis suggests a continued clinical benefit of achieving greater platelet inhibition during the maintenance phase of therapy.

Prasugrel has been recently approved for clinical use by regulatory authorities, but only in the setting of ACS patients undergoing PCI. The clinical efficacy of prasugrel in medically managed patients with unstable angina/NSTEMI is currently being evaluated in the TRILOGY-ACS (Targeted Platelet Inhibition to Clarify the Optimal Strategy to Medically Manage Acute Coronary Syndromes) trial (NCT00699998).

Ticagrelor

Ticagrelor is an orally administered cyclopentyltriazolopyrimidine, which directly and reversibly inhibits the platelet $P2Y_{12}$ receptor.[132,139]

TABLE 2. **Platelet $P2Y_{12}$ Inhibitors**

	Clopidogrel	Prasugrel	Cangrelor	Ticagrelor	Elinogrel
Group	Thienopyridine	Thienopyridine	ATP analog	Cyclopentyltriazolopyridine	Quinazolinedione
Development status	Approved in 1997	Approved in 2009	Phase III completed in 2009	Phase III completed in 2009	Phase II ongoing
Administration	Oral	Oral	Parenteral	Oral	Oral and parenteral
Bioavailability	Prodrug	Prodrug	Direct-acting	Direct-acting	Direct-acting
Receptor inhibiton	Irreversible	Irreversible	Reversible	Reversible	Reversible
Frequency	Daily	Daily	Bolus and infusion	Twice daily	Twice daily

ATP indicates adenosine triphosphate.

Its pharmacokinetic and pharmacodynamic properties include: *a)* rapid absorption and onset of action; *b)* higher inhibition of platelet aggregation than clopidogrel; and *c)* rapid offset of action, as it has a half-life of 12 hours (requires twice daily dosing).[140,141] The recently published PLATO (Platelet Inhibition and Patient Outcomes) trial evaluated the benefit of ticagrelor (180 mg loading dose followed by 90 mg twice daily) compared to clopidogrel (300 to 600 mg loading dose followed by 75 mg daily) in preventing cardiovascular events in 18 624 patients with an acute coronary syndrome, with or without ST-segment elevation.[142] In this trial, ticagrelor therapy significantly reduced the rate of the primary endpoint (death from vascular causes, myocardial infarction or stroke) at 12 months (12.3% vs 10.2%; hazard ratio =0.84; *P*=.0001) and, remarkably, the rate of cardiovascular death (4.0% vs 5.1%; *P*=.001), death from any cause (4.5% vs 5.9%; *P*<.001) and definite or probable stent thrombosis (2.2% vs 2.9%; *P*=.02) in the subgroup of patients undergoing PCI. Although no increase in major bleeding was found using the protocol definition (11.6% vs 11.2%; *P*=.43), ticagrelor was associated with a higher rate of major bleeding not related to coronary-artery bypass grafting (4.5% vs 3.8%; *P*=.03). Under the TIMI major non-CABG related bleeding definition used in the TRITON trial, there was a similar increase in the rate of bleeding with ticagrelor (2.8% vs 2.2%; *P*=.03). In addition, non-bleeding safety concerns were noted. Dyspnea was more frequent in the ticagrelor group (13.8% vs 7.8%; *P*<.001), which led to a significant rate of treatment discontinuation compared to clopidogrel (0.9% vs 0.1%; *P*<.001). Also, patients in the ticagrelor group presented a significantly higher increase in creatinine and uric acid from baseline than those in clopidogrel group at 1 and 12 months (*P*<.001 for both), as well as a higher percentage of ventricular pauses (≥3 seconds) in the first week (*P*=.01), although no difference in bradycardia-related events was found. These non-bleeding side effects are likely attributed to off-target effects of ticagrelor or its metabolites.

Cangrelor

Cangrelor is an intravenous ATP analog which reversibly and directly, without any biotransformation, inhibits the $P2Y_{12}$ receptor.[132,139] The main pharmacokinetic and pharmacodynamic properties of cangrelor are: *a)* rapid onset of action, reaching steady-state concentrations within minutes; *b)* great degree of platelet inhibition (>90%); *c)* dose-dependent effects; and *d)* rapid offset of action, since it has an extremely short half-life (2-5 minutes) due to rapid deactivation by plasmatic ectonucleotidases.[143,144] In spite of the promising results obtained in phase II studies, which showed cangrelor to be a potent platelet inhibitor with a relatively safe profile,[143,144] these findings have not been corroborated in phase III studies. The CHAMPION (Cangrelor Versus Standard Therapy to Achieve Optimal Management of Platelet Inhibition) program included the recently published CHAMPION-PCI(n=8716)[145]andtheCHAMPION-PLATFORM[146] (n=5362) trials, which have been recently published. These studies aimed to evaluate the efficacy of cangrelor in patients, most with ACS, undergoing PCI. Cangrelor was not found superior for reducing the primary end point, a composite of death from any cause, MI, or ischemia-driven revascularization at 48 hours, when compared to clopidogrel in the CHAMPION-PCI study (7.5% vs 7.1% (OR=1.05 [0.88-1.24]; p=0.56) and compared to placebo in CHAMPION-PLATFORM (7.0% vs. 8.0%; OR=0.87 [0.71-1.07]; p=0.17). However, the pharmacological properties of cangrelor make this a promising drug in the setting of patients requiring surgery who need a bridging antiplatelet strategy. This is a current objective of the ongoing BRIDGE

(maintenance of platelet inihiBition with cangRelor after dIscontinuation of thienopyriDines in patients undergoing surGEry) trial (NCT 00767507).

Elinogrel

Elinogrel is a novel, direct-acting, and reversible $P2Y_{12}$ inhibitor which can be administered both orally and intravenously.[147] Elinogrel is currently in the preliminary stages of development, but phase I studies have shown interesting pharmacologic properties: *a)* rapid onset of action (almost immediate if administered intravenously); *b)* higher degree of platelet inhibition than clopidogrel; and *c)* rapid offset of action, being its half-life of 50 minutes and 12 hours for intravenously and oral administration, respectively.[147] Results from a pharmacodynamic study were presented at the American Heart Association Congress 2008 (New Orleans, LA, USA), showing that a single oral dose of elinogrel improved platelet inhibition in stable patients with coronary artery disease that were poor clopidogrel responders.[148] Currently, the ongoing INNOVATE (a Randomized, Double-Blind, Active-Controlled Trial to Evaluate Intravenous and Oral PRT060128, a Selective and Reversible P2Y12 Inhibitor, vs Clopidogrel, as a Novel Antiplatelet Therapy in Patients Undergoing Non-Urgent PCI) trial (NCT00751231) is evaluating clinical efficacy, biological activity, tolerability and safety of PRT060128 in patients undergoing non-urgent PCI, testing three doses of elinogrel (oral 50, 100, and 150 mg) twice daily, following an intravenous bolus.

CONCLUSIONS

Platelet $P2Y_{12}$ receptor antagonism with clopidogrel has represented a major advancement in the treatment of patients with atherothrombotic disease, in particular those with ACS and those undergoing PCI. Despite the clear clinical benefit associated with clopidogrel in these patients, laboratory and clinical experience have helped to identify some caveats, among which its broad platelet inhibitory response profile is the most relevant. Genetic, cellular and clinical factors are implicated in variability in response to clopidogrel, which has shown to be associated with adverse clinical outcomes. Therefore, the search for new strategies to optimize platelet inhibition is strongly warranted. Indeed, the development of new $P2Y_{12}$ receptor blockers with more favorable pharmacokinetic and pharmacodynamic profiles represent an important step forward in this field. Evaluation of recently reported large-scale trials and the upcoming results of ongoing clinical investigations will provide the bases for a future of individualized and more specific antiplatelet treatment regimens.

ACKNOWLEDGMENTS

We are grateful to Mariana Muñoz, MD, for her assistance in figure preparation.

REFERENCES

1. Fuster V, Moreno PR, Fayad ZA, Corti R, Badimon JJ. Atherothrombosis and high-risk plaque: part I: evolving concepts. J Am Coll Cardiol. 2005;46:937-54.
2. Davì G, Patrono C. Platelet activation and atherothrombosis. N Engl J Med. 2007;357:2482-94.
3. Badimón L, Vilahur G. Enfermedad aterotrombótica coronaria: avances en el tratamiento antiplaquetario. Rev Esp Cardiol. 2008;61:501-13.
4. Antithrombotic Trialists' Collaboration. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. BMJ. 2002;324:71-86.
5. Patrono C, García Rodríguez LA, Landolfi R, Baigent C. Low-dose aspirin for the prevention of atherothrombosis. N Engl J Med. 2005;353:2373-83.
6. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Alfonso F, Macaya C, Bass TA, et al. Variability in individual responsiveness to clopidogrel: clinical implications, management, and future perspectives. J Am Coll Cardiol. 2007;49:1505-16.
7. Ferreiro JL, Angiolillo DJ. Clopidogrel response variability: current status and future directions. Thromb Haemost. 2009;102:7-14.
8. Kahner BN, Shankar H, Murugappan S, Prasad GL, Kunapuli SP. Nucleotide receptor signaling in platelets. J Thromb Haemost. 2006;4:2317-26.
9. Storey RF, Newby LJ, Heptinstall S. Effects of P2Y(1) and P2Y(12) receptor antagonists on platelet aggregation induced by different agonists in human whole blood. Platelets. 2001;12:443-7.
10. Gachet C. ADP receptors of platelets and their inhibition. Thromb Haemost. 2001;86:222-32.
11. Turner NA, Moake JL, McIntire LV. Blockade of adenosine diphosphate receptors P2Y(12) and P2Y(1) is required to inhibit platelet aggregation in whole blood under flow. Blood. 2001;98:3340-5.

12. Shankar H, Garcia A, Prabhakar J, Kim S, Kunapuli SP. P2Y12 receptor-mediated potentiation of thrombin-induced thromboxane A2 generation in platelets occurs through regulation of Erk1/2 activation. J Thromb Haemost. 2006;4:638-47.
13. Schömig A, Neumann FJ, Kastrati A, Schühlen H, Blasini R, Hadamitzky M, et al. A randomized comparison of antiplatelet and anticoagulant therapy after the placement of coronary-artery stents. N Engl J Med. 1996;334:1084-9.
14. Leon MB, Baim DS, Popma JJ, Gordon PC, Cutlip DE, Ho KK, et al. A clinical trial comparing three antithrombotic-drug regimens after coronary-artery stenting. Stent Anticoagulation Restenosis Study Investigators. N Engl J Med. 1998;3391665-71.
15. Bertrand ME, Legrand V, Boland J, Fleck E, Bonnier J, Emmanuelson H, et al. Randomized multicenter comparison of conventional anticoagulation versus antiplatelet therapy in unplanned and elective coronary stenting. The full anticoagulation versus aspirin and ticlopidine (fantastic) study. Circulation. 1998;98:1597-603.
16. Urban P, Macaya C, Rupprecht HJ, Kiemeneij F, Emanuelsson H, Fontanelli A, et al. Randomized evaluation of anticoagulation versus antiplatelet therapy after coronary stent implantation in high-risk patients: the multicenter aspirin and ticlopidine trial after intracoronary stenting (MATTIS). Circulation. 1998;98:2126-32.
17. Bertrand ME, Rupprecht HJ, Urban P, Gershlick AH; CLASSICS Investigators. Double-blind study of the safety of clopidogrel with and without a loading dose in combination with aspirin compared with ticlopidine in combination with aspirin after coronary stenting: the clopidogrel aspirin stent international cooperative study (CLASSICS). Circulation. 2000;102:624-9.
18. Cadroy Y, Bossavy JP, Thalamas C, Sagnard L, Sakariassen K, Boneu B. Early potent antithrombotic effect with combined aspirin and a loading dose of clopidogrel on experimental arterial thrombogenesis in humans. Circulation. 2000;101:2823-8.
19. Yusuf S, Zhao F, Mehta SR, Chrolavicius S, Tognoni G, Fox KK; Clopidogrel in Unstable Angina to Prevent Recurrent Events Trial Investigators. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. N Engl J Med. 2001;345:494-502.
20. Mehta SR, Yusuf S, Peters RJ, Bertrand ME, Lewis BS, Natarajan MK, et al; Clopidogrel in Unstable angina to prevent Recurrent Events trial (CURE) Investigators. Effects of pretreatment with clopidogrel and aspirin followed by long-term therapy in patients undergoing percutaneous coronary intervention: the PCI-CURE study. Lancet. 2001;358: 527-33.
21. Sabatine MS, Cannon CP, Gibson CM, López-Sendón JL, Montalescot G, Theroux P, et al; CLARITY-TIMI 28 Investigators. Addition of clopidogrel to aspirin and fibrinolytic therapy for myocardial infarction with ST-segment elevation. N Engl J Med. 2005;352:1179-89.
22. Chen ZM, Jiang LX, Chen YP, Xie JX, Pan HC, Peto R, et al; COMMIT (ClOpidogrel and Metoprolol in Myocardial Infarction Trial) collaborative groupAddition of clopidogrel to aspirin in 45,852 patients with acute myocardial infarction: randomised placebo-controlled trial. Lancet. 2005;366: 1607-21.
23. Steinhubl SR, Berger PB, Mann JT 3rd, Fry ET, DeLago A, Wilmer C, et al; CREDO Investigators. Clopidogrel for the Reduction of Events During Observation. Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: a randomized controlled trial. JAMA. 2002;288:2411-20.
24. Anderson JL, Adams CD, Antman EM, Bridges CR, Califf RM, Casey DE Jr, et al; ACC/AHA 2007 guidelines for the management of patients with unstable angina/non-ST-Elevation myocardial infarction: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Revise the 2002 Guidelines for the Management of Patients With Unstable Angina/Non-ST-Elevation Myocardial Infarction) developed in collaboration with the American College of Emergency Physicians, the Society for Cardiovascular Angiography and Interventions, and the Society of Thoracic Surgeons endorsed by the American Association of Cardiovascular and Pulmonary Rehabilitation and the Society for Academic Emergency Medicine. J Am Coll Cardiol. 2007;50:e1-e157.
25. Bassand JP, Hamm CW, Ardissino D, Boersma E, Budaj A, Fernández-Avilés F, et al. Guía de Práctica Clínica para el diagnóstico y tratamiento del síndrome coronario agudo sin elevación del segmento ST. Rev Esp Cardiol. 2007; 60: 1070. e1-1070.e80.
26. Antman EM, Hand M, Armstrong PW, Bates ER, Green LA, Halasyamani LK, et al. 2007 focused update of the ACC/AHA 2004 guidelines for the management of patients with ST-elevation myocardial infarction: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol. 2008;51: 210-47.
27. van de Werf F, Bax J, Betriu A, Blomstrom-Lundqvist C, Crea F, Falk V, et al. Guía de práctica clínica de la Sociedad Europea de Cardiología (ESC). Manejo del infarto agudo de miocardio en pacientes con elevación persistente del segmento ST. Rev Esp Cardiol. 2009;62:e1-e47.
28. King SB 3rd, Smith SC Jr, Hirshfeld JW Jr, Jacobs AK, Morrison DA, Williams DO, et al. 2007 focused update of the ACC/AHA/SCAI 2005 guideline update for percutaneous coronary intervention: a report of the American College of Cardiology/American Heart Association Task Force on Practice guidelines. J Am Coll Cardiol. 2008;51:172-209.
29. Silber S, Albertsson P, Fernandez-Avilés F, Camici PG, Colombo A, Hamm C, et al. Guías de Práctica Clínica sobre intervencionismo coronario percutáneo. Rev Esp Cardiol. 2005;58:679-728.
30. Angiolillo DJ. Pruebas de función plaquetaria en la práctica clínica: ¿estamos preparados para que pasen a la primera línea? Rev Esp Cardiol. 2009;62:113-6.
31. Alfonso F, Angiolillo DJ. Platelet function assessment to predict outcomes after coronary interventions: hype or hope? J Am Coll Cardiol. 2006;48:1751-4.
32. Angiolillo DJ, Fernández-Ortiz A, Bernardo E, Ramírez C, Sabaté M, Bañuelos C, et al. High clopidogrel loading dose during coronary stenting: effects on drug response and interindividual variability. Eur Heart J. 2004;25:1903-10.
33. Patti G, Colonna G, Pasceri V, et al. Randomized trial of high loading dose of clopidogrel for reduction of periprocedural myocardial infarction in patients undergoing coronary intervention: results from the ARMYDA-2 (Antiplatelet therapy for Reduction of MYocardial Damage during Angioplasty) study. Circulation 2005;111:2099-106.
34. Cuisset T, Frere C, Quilici J, Morange PE, Nait-Saidi L, Carvajal J, et al. Benefit of a 600-mg loading dose of clopidogrel on platelet reactivity and clinical outcomes in patients with non-ST-segment elevation acute coronary syndrome undergoing coronary stenting. J Am Coll Cardiol. 2006;48:1339-45.
35. Gurbel PA, Bliden KP, Zaman KA, Yoho JA, Hayes KM, Tantry US. Clopidogrel loading with eptifibatide to arrest the

reactivity of platelets: results of the Clopidogrel Loading With Eptifibatide to Arrest the Reactivity of Platelets (CLEAR PLATELETS) study. Circulation. 2005;111:1153-9.

36. Lev EI, Patel RT, Maresh KJ, Guthikonda S, Granada J, DeLao T, et al. Aspirin and clopidogrel drug response in patients undergoing percutaneous coronary intervention: the role of dual drug resistance. J Am Coll Cardiol. 2006;47: 27-33.
37. Cuisset T, Frere C, Quilici J, Morange PE, Nait-Saidi L, Mielot C, et al. High post-treatment platelet reactivity is associated with a high incidence of myonecrosis after stenting for non-ST elevation acute coronary syndromes. Thromb Haemost. 2007;97:282-7.
38. Marcucci R, Paniccia R, Antonucci E, Poli S, Gori AM, Valente S, et al. Residual platelet reactivity is an independent predictor of myocardial injury in acute myocardial infarction patients on antiaggregant therapy. Thromb Haemost. 2007;98:844-51.
39. Cuisset T, Frere C, Quilici J, Barbou F, Morange PE, Hovasse T, et al. High post-treatment platelet reactivity identified low-responders to dual antiplatelet therapy at increased risk of recurrent cardiovascular events after stenting for acute coronary syndrome. J Thromb Haemost. 2006;4:542-9.
40. Hochholzer W, Trenk D, Bestehorn HP, Fischer B, Valina CM, Ferenc M, et al. Impact of the degree of peri-interventional platelet inhibition after loading with clopidogrel on early clinical outcome of elective coronary stent placement. J Am Coll Cardiol. 2006;48:1742-50.
41. Frere C, Cuisset T, Quilici J, Camoin L, Carvajal J, Morange, et al. ADP-induced platelet aggregation and platelet reactivity index VASP are good predictive markers for clinical outcomes in non-ST elevation acute coronary syndrome. Thromb Haemost. 2007;98:838-43.
42. Patti G, Nusca A, Mangiacapra F, Gatto L, D'Ambrosio A, Di Sciascio G. Point-of-care measurement of clopidogrel responsiveness predicts clinical outcome in patients undergoing percutaneous coronary intervention results of the ARMYDA-PRO (Antiplatelet therapy for Reduction of MYocardial Damage during Angioplasty-Platelet Reactivity Predicts Outcome) study. J Am Coll Cardiol. 2008;52: 1128-33.
43. Matetzky S, Shenkman B, Guetta V, Shechter M, Bienart R, Goldenberg I, et al. Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. Circulation. 2004;109:3171-5.
44. Gurbel PA, Bliden KP, Guyer K, Cho PW, Zaman KA, Kreutz RP, et al. Platelet reactivity in patients and recurrent events post-stenting: results of the PREPARE POST-STENTING Study. J Am Coll Cardiol. 2005;46:1820-6.
45. Geisler T, Langer H, Wydymus M, Göhring K, Zürn C, Bigalke B, et al. Low response to clopidogrel is associated with cardiovascular outcome after coronary stent implantation. Eur Heart J. 2006;27:2420-5.
46. Bliden KP, DiChiara J, Tantry US, Bassi AK, Chaganti SK, Gurbel PA. Increased risk in patients with high platelet aggregation receiving chronic clopidogrel therapy undergoing percutaneous coronary intervention: is the current antiplatelet therapy adequate? J Am Coll Cardiol. 2007;49:657-66.
47. Bonello L, Paganelli F, Arpin-Bornet M, Auquier P, Sampol J, Dignat-George F, et al. Vasodilator-stimulated phosphoprotein phosphorylation analysis prior to percutaneous coronary intervention for exclusion of postprocedural major adverse cardiovascular events. J Thromb Haemost. 2007;5:1630-6.
48. Angiolillo DJ, Bernardo E, Sabaté M, Jimenez-Quevedo P, Costa MA, Palazuelos J, et al. Impact of platelet reactivity on cardiovascular outcomes in patients with type 2 diabetes mellitus and coronary artery disease. J Am Coll Cardiol. 2007;50:1541-7.
49. Price MJ, Endemann S, Gollapudi RR, Valencia R, Stinis CT, Levisay JP, et al. Prognostic significance of post-clopidogrel platelet reactivity assessed by a point-of-care assay on thrombotic events after drug-eluting stent implantation. Eur Heart J. 2008;29:992-1000.
50. Marcucci R, Gori AM, Paniccia R, Giusti B, Valente S, Giglioli C, et al. Cardiovascular death and nonfatal myocardial infarction in acute coronary syndrome patients receiving coronary stenting are predicted by residual platelet reactivity to ADP detected by a point-of-care assay: a 12-month follow-up. Circulation. 2009;119:237-42.
51. de Miguel A, Cuellas C, Diego A, Samaniego B, Alonso D, Fernández F, et al. La reactividad plaquetaria post-tratamiento predice los eventos adversos a largo plazo mejor que la respuesta al clopidogrel en pacientes con síndrome coronario agudo sin elevación del ST. Rev Esp Cardiol. 2009;62:126-35.
52. Barragan P, Bouvier JL, Roquebert PO, Macaluso G, Commeau P, Comet B, et al. Resistance to thienopyridines: clinical detection of coronary stent thrombosis by monitoring of vasodilator-stimulated phosphoprotein phosphorylation. Catheter Cardiovasc Interv. 2003;59:295-302.
53. Ajzenberg N, Aubry P, Huisse MG, Cachier A, El Amara W, Feldman LJ, et al. Enhanced shear-induced platelet aggregation in patients who experience subacute stent thrombosis: a case-control study. J Am Coll Cardiol. 2005;45:1753-6.
54. Gurbel PA, Bliden KP, Samara W, Yoho JA, Hayes K, Fissha MZ, et al. Clopidogrel effect on platelet reactivity in patients with stent thrombosis: results of the CREST Study. J Am Coll Cardiol. 2005;46:1827-32.
55. Blindt R, Stellbrink K, de Taeye A, Müller R, Kiefer P, Yagmur E, et al. The significance of vasodilator-stimulated phosphoprotein for risk stratification of stent thrombosis. Thromb Haemost. 2007;98:1329-34.
56. Buonamici P, Marcucci R, Migliorini A, Gensini GF, Santini A, Paniccia R, et al. Impact of platelet reactivity after clopidogrel administration on drug-eluting stent thrombosis. J Am Coll Cardiol. 2007;49:2312-7.
57. Sibbing D, Braun S, Morath T, Mehilli J, Vogt W, Schömig A, et al. Platelet reactivity after clopidogrel treatment assessed with point-of-care analysis and early drug-eluting stent thrombosis. J Am Coll Cardiol. 2009;53:849-56.
58. Marín F, González-Conejero R, Capranzano P, Bass TA, Roldán V, Angiolillo DJ. Pharmacogenetics in cardiovascular antithrombotic therapy. J Am Coll Cardiol. 2009;54: 1041-57.
59. Taubert D, von Beckerath N, Grimberg G, Lazar A, Jung N, Goeser T, et al. Impact of P-glycoprotein on clopidogrel absorption. Clin Pharmacol Ther. 2006;80:486-501.
60. Simon T, Verstuyft C, Mary-Krause M, Quteineh L, Drouet E, Méneveau N, et al.; French Registry of Acute ST-Elevation and Non-ST-Elevation Myocardial Infarction (FAST-MI) Investigators. Genetic determinants of response to clopidogrel and cardiovascular events. N Engl J Med. 2009;360:363-75.
61. Shuldiner AR, O'Connell JR, Bliden KP, Gandhi A, Ryan K, Horenstein RB, et al. Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. JAMA. 2009;302:849-57.
62. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Ramírez C, Cavallari U, Trabetti E, et al. Contribution of gene sequence variations of the hepatic cytochrome P450 3A4 enzyme

to variability in individual responsiveness to clopidogrel. Arterioscler Thromb Vasc Biol. 2006;26:1895-900.

63. Suh JW, Koo BK, Zhang SY, Park KW, Cho JY, Jang IJ, et al. Increased risk of atherothrombotic events associated with cytochrome P450 3A5 polymorphism in patients taking clopidogrel. CMAJ. 2006;174:1715-22.
64. Brandt JT, Close SL, Iturria SJ, Payne CD, Farid NA, Ernest CS 2nd, et al. Common polymorphisms of CYP2C19 and CYP2C9 affect the pharmacokinetic and pharmacodynamic response to clopidogrel but not prasugrel. J Thromb Haemost. 2007;5:2429-36.
65. Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT, et al. Cytochrome p-450 polymorphisms and response to clopidogrel. N Engl J Med. 2009;360:354-62.
66. Collet JP, Hulot JS, Pena A, Villard E, Esteve JB, Silvain J, et al. Cytochrome P450 2C19 polymorphism in young patients treated with clopidogrel after myocardial infarction: a cohort study. Lancet 2009;373:309-17.
67. Sibbing D, Stegherr J, Latz W, Koch W, Mehilli J, Dörrler K, et al. Cytochrome P450 2C19 loss-of-function polymorphism and stent thrombosis following percutaneous coronary intervention. Eur Heart J. 2009;30:916-22.
68. Giusti B, Gori AM, Marcucci R, Saracini C, Sestini I, Paniccia R, et al. Relation of cytochrome P450 2C19 loss-of-function polymorphism to occurrence of drug-eluting coronary stent thrombosis. Am J Cardiol. 2009;103:806-11.
69. Varenhorst C, James S, Erlinge D, Brandt JT, Braun OO, Man M, et al. Genetic variation of CYP2C19 affects both pharmacokinetic and pharmacodynamic responses to clopidogrel but not prasugrel in aspirin-treated patients with coronary artery disease. Eur Heart J. 2009;30:1744-52.
70. Hulot JS, Bura A, Villard E, Azizi M, Remones V, Goyenvalle C, et al. Cytochrome P450 2C19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects. Blood. 2006;108:2244-7.
71. Trenk D, Hochholzer W, Fromm MF, Chialda LE, Pahl A, Valina CM, et al. Cytochrome P450 2C19 681G>A polymorphism and high on-clopidogrel platelet reactivity associated with adverse 1-year clinical outcome of elective percutaneous coronary intervention with drug-eluting or bare- metal stents. J Am Coll Cardiol. 2008;51:1925-34.
72. Fontana P, Dupont A, Gandrille S, Bachelot-Loza C, Reny JL, Aiach M, et al. Adenosine diphosphate-induced platelet aggregation is associated with P2Y12 gene sequence variations in healthy subjects. Circulation. 2003;108:989-95.
73. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Ramírez C, Cavallari U, Trabetti E, et al. Lack of association between the P2Y12 receptor gene polymorphism and platelet response to clopidogrel in patients with coronary artery disease. Thromb Res. 2005;116:491-7.
74. Angiolillo DJ, Fernández Ortiz A, Bernardo E, Ramírez C, Cavallari U, Trabetti E, et al. Variability in platelet aggregation following sustained aspirin and clopidogrel treatment in patients with coronary heart disease and influence of the 807C/T polymorphism of the glycoprotein Ia Gene. Am J Cardiol. 2005; 96: 1095-9.
75. Cuisset T, Frere C, Quilici J, Morange PE, Saut N, Romero-Barra M, et al. Lack of association between the 807 C/T polymorphism of glycoprotein Ia gene and post-treatment platelet reactivity after aspirin and clopidogrel in patients with acute coronary syndrome. Thromb Haemost. 2007; 97:212-7.
76. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Alfonso F, Sabaté M, Fernández C, et al. PlA polymorphism and platelet reactivity following clopidogrel loading dose in patients undergoing coronary stent implantation. Blood Coagul Fibrinolysis. 2004;15:89-93.
77. Lev EI, Patel RT, Guthikonda S, Lopez D, Bray PF, Kleiman NS. Genetic polymorphisms of the platelet receptors P2Y(12), P2Y(1) and GP IIIa and response to aspirin and clopidogrel. Thromb Res. 2007;119:355-60.
78. Smith SM, Judge HM, Peters G, Armstrong M, Dupont A, Gaussem P, et al. PAR-1 genotype influences platelet aggregation and procoagulant responses in patients with coronary artery disease prior to and during clopidogrel therapy. Platelets .2005; 16:340-5.
79. Cesari F, Marcucci R, Caporale R, Paniccia R, Romano E, Gensini GF, et al. Relationship between high platelet turnover and platelet function in high-risk patients with coronary artery disease on dual antiplatelet therapy. Thromb Haemost. 2008;99:930-5.
80. Guthikonda S, Alviar CL, Vaduganathan M, Arikan M, Tellez A, DeLao T, et al. Role of reticulated platelets and platelet size heterogeneity on platelet activity after dual antiplatelet therapy with aspirin and clopidogrel in patients with stable coronary artery disease. J Am Coll Cardiol. 2008;52:743-9.
81. Lau WC, Gurbel PA, Watkins PB, Neer CJ, Hopp AS, Carville DG, et al. Contribution of hepatic cytochrome P450 3A4 metabolic activity to the phenomenon of clopidogrel resistance. Circulation 2004;109:166-71.
82. Angiolillo DJ, Bernardo E, Ramírez C, Costa MA, Sabaté M, Jiménez-Quevedo P, et al. Insulin therapy is associated with platelet dysfunction in patients with type 2 diabetes mellitus on dual oral antiplatelet treatment. J Am Coll Cardiol. 2006;48:298-304.
83. Angiolillo DJ, Fernández-Ortiz A, Bernardo E, Ramírez C, Sabaté M, Jimenez-Quevedo P, et al. Platelet function profiles in patients with type 2 diabetes and coronary artery disease on combined aspirin and clopidogrel treatment. Diabetes. 2005;54:2430-5.
84. Capranzano P, Ferreiro JL, Kodlipet D, Alissa R, Darlington A, Charlton RK, et al. Comparative assessment of platelet P2Y12 signaling in type 2 diabetes mellitus versus non diabetic subjects [abstract]. Eur Heart J. 2009: 30 Suppl 1: P1309.
85. Soffer D, Moussa I, Harjai KJ, Boura JA, Dixon SR, Grines CL, et al. Impact of angina class on inhibition of platelet aggregation following clopidogrel loading in patients undergoing coronary intervention: do we need more aggressive dosing regimens in unstable angina? Catheter Cardiovasc Interv. 2003;59:21-5.
86. Sibbing D, von Beckerath O, Schömig A, Kastrati A, von Beckerath N. Platelet function in clopidogrel-treated patients with acute coronary syndrome. Blood Coagul Fibrinolysis. 2007;18:335-9.
87. Geisler T, Anders N, Paterok M, Langer H, Stellos K, Lindemann S, et al. Platelet response to clopidogrel is attenuated in diabetic patients undergoing coronary stent implantation. Diabetes Care. 2007;30:372-4.
88. Serebruany V, Pokov I, Kuliczkowski W, Chesebro J, Badimon J. Baseline platelet activity and response after clopidogrel in 257 diabetics among 822 patients with coronary artery disease. Thromb Haemost. 2008;100:76-82.
89. Angiolillo DJ, Fernández-Ortiz A, Bernardo E, Barrera Ramírez C, Sabaté M, Fernandez C, et al. Platelet aggregation according to body mass index in patients undergoing coronary stenting: should clopidogrel loading-dose be weight adjusted? J Invasive Cardiol. 2004;16:169-74.
90. Sibbing D, von Beckerath O, Schömig A, Kastrati A, von Beckerath N. Impact of body mass index on platelet aggregation after administration of a high loading dose of 600 mg of clopidogrel before percutaneous coronary intervention. Am J Cardiol. 2007;100:203-5.

91. Lau WC, Waskell LA, Watkins PB, Neer CJ, Horowitz K, Hopp AS, et al. Atorvastatin reduces the ability of clopidogrel to inhibit platelet aggregation: a new drug-drug interaction. Circulation. 2003;107:32-7.
92. Neubauer H, Günesdogan B, Hanefeld C, Spiecker M, Mügge A. Lipophilic statins interfere with the inhibitory effects of clopidogrel on platelet function--a flow cytometry study. Eur Heart J. 2003;24:1744-9.
93. Saw J, Steinhubl SR, Berger PB, Kereiakes DJ, Serebruany VL, Brennan D, et al; Clopidogrel for the Reduction of Events During Observation Investigators. Lack of adverse clopidogrel-atorvastatin clinical interaction from secondary analysis of a randomized, placebo-controlled clopidogrel trial. Circulation. 2003;108:921-4.
94. Saw J, Brennan DM, Steinhubl SR, Bhatt DL, Mak KH, Fox K, et al; CHARISMA Investigators. Lack of evidence of a clopidogrel-statin interaction in the CHARISMA trial. J Am Coll Cardiol. 2007;50:291-5.
95. Lim MJ, Spencer FA, Gore JM, Dabbous OH, Agnelli G, Kline-Rogers EM, et al.; GRACE Investigators. Impact of combined pharmacologic treatment with clopidogrel and a statin on outcomes of patients with non-ST-segment elevation acute coronary syndromes: perspectives from a large multinational registry. Eur Heart J. 2005;26:1063-9.
96. Angiolillo DJ, Alfonso F. Clopidogrel-statin interaction: myth or reality? J Am Coll Cardiol 2007;50:296-8.
97. Siller-Matula JM, Lang I, Christ G, Jilma B. Calcium-channel blockers reduce the antiplatelet effect of clopidogrel. J Am Coll Cardiol. 2008;52:1557-63.
98. Gremmel T, Steiner S, Seidinger D, Koppensteiner R, Panzer S, Kopp CW. Calcium-channel blockers decrease clopidogrel-mediated platelet inhibition. Heart. 2009 Aug 16. [Epub ahead of print].
99. Li XQ, Andersson TB, Ahlström M, Weidolf L. Comparison of inhibitory effects of the proton pump-inhibiting drugs omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole on human cytochrome P450 activities. Drug Metab Dispos. 2004;32:821-7.
100. Gilard M, Arnaud B, Cornily JC, Le Gal G, Lacut K, Le Calvez G, et al. Influence of omeprazole on the antiplatelet action of clopidogrel associated with aspirin: the randomized, double-blind OCLA (Omeprazole CLopidogrel Aspirin) study. J Am Coll Cardiol. 2008;51:256-60.
101. Sibbing D, Morath T, Stegherr J, Braun S, Vogt W, Hadamitzky M, et al. Impact of proton pump inhibitors on the antiplatelet effects of clopidogrel. Thromb Haemost. 2009;101:714-9.
102. Siller-Matula JM, Spiel AO, Lang IM, Kreiner G, Christ G, Jilma B. Effects of pantoprazole and esomeprazole on platelet inhibition by clopidogrel. Am Heart J. 2009;157:148.e1-5.
103. Small DS, Farid NA, Payne CD, Weerakkody GJ, Li YG, Brandt JT, et al. Effects of the proton pump inhibitor lansoprazole on the pharmacokinetics and pharmacodynamics of prasugrel and clopidogrel. J Clin Pharmacol. 2008;48: 475-84.
104. Ho PM, Maddox TM, Wang L, Fihn SD, Jesse RL, Peterson ED, et al. Risk of adverse outcomes associated with concomitant use of clopidogrel and proton pump inhibitors following acute coronary syndrome. JAMA. 2009;301:937-44.
105. Juurlink DN, Gomes T, Ko DT, Szmitko PE, Austin PC, Tu JV, et al. A population-based study of the drug interaction between proton pump inhibitors and clopidogrel. CMAJ. 2009;180:713-8.
106. O'Donoghue M, Braunwald E, Antman EM, Murphy SA, Bates ER, Rozenman Y, et al. Pharmacodynamic effect and clinical efficacy of clopidogrel and prasugrel with or without a proton-pump inhibitor: an analysis of two randomised trials. Lancet. 2009;374:989-97.
107. Faber MS, Fuhr U. Time response of cytochrome P450 1A2 activity on cessation of heavy smoking. Clin Pharmacol Ther. 2004;76:178-84.
108. Bliden KP, Dichiara J, Lawal L, Singla A, Antonino MJ, Baker BA, et al. The association of cigarette smoking with enhanced platelet inhibition by clopidogrel. J Am Coll Cardiol. 2008;52:531-3.
109. Gremmel T, Steiner S, Seidinger D, Koppensteiner R, Panzer S, Kopp CW. Smoking promotes clopidogrel-mediated platelet inhibition in patients receiving dual antiplatelet therapy. Thromb Res. 2009 [Epub ahead of print].
110. Desai NR, Mega JL, Jiang S, Cannon CP, Sabatine MS. Interaction between cigarette smoking and clinical benefit of clopidogrel. J Am Coll Cardiol. 2009;53:1273-8.
111. Saraff KY, Steinhubl SR, Hsu AP, Topol EJ. Smoking influences the effectiveness of dual antiplatelet therapy on long-term outcomes following percutaneous coronary intervention [abstract]. J Am Coll Cardiol. 2006;47:36B.
112. Yousef AM, Arafat T, Bulatova NR, Al-Zumyli R. Smoking behaviour modulates pharmacokinetics of orally administered clopidogrel. J Clin Pharm Ther. 2008;33:439-49.
113. Montalescot G, Sideris G, Meuleman C, Bal-dit-Sollier C, Lellouche N, Steg PG, et al.; ALBION Trial Investigators. A randomized comparison of high clopidogrel loading doses in patients with non-ST-segment elevation acute coronary syndromes: the ALBION (Assessment of the Best Loading Dose of Clopidogrel to Blunt Platelet Activation, Inflammation and Ongoing Necrosis) trial. J Am Coll Cardiol. 2006;48:931-8.
114. von Beckerath N, Taubert D, Pogatsa-Murray G, Schömig E, Kastrati A, Schömig A. Absorption, metabolization, and antiplatelet effects of 300-, 600-, and 900-mg loading doses of clopidogrel: results of the ISAR-CHOICE (Intracoronary Stenting and Antithrombotic Regimen: Choose Between 3 High Oral Doses for Immediate Clopidogrel Effect) Trial. Circulation. 2005;112:2946-50.
115. Lotrionte M, Biondi-Zoccai GG, Agostoni P, Abbate A, Angiolillo DJ, Valgimigli M, et al. Meta-analysis appraising high clopidogrel loading in patients undergoing percutaneous coronary intervention. Am J Cardiol. 2007;100:1199-206.
116. von Beckerath N, Kastrati A, Wieczorek A, Pogatsa-Murray G, Sibbing D, Graf I, et al. A double-blind, randomized study on platelet aggregation in patients treated with a daily dose of 150 or 75 mg of clopidogrel for 30 days. Eur Heart J. 2007;28:1814-9.
117. Angiolillo DJ, Shoemaker SB, Desai B, Yuan H, Charlton RK, Bernardo E, et al. Randomized comparison of a high clopidogrel maintenance dose in patients with diabetes mellitus and coronary artery disease: results of the Optimizing Antiplatelet Therapy in Diabetes Mellitus (OPTIMUS) study. Circulation. 2007;115:708-16.
118. Angiolillo DJ, Bernardo E, Palazuelos J, Desai B, Weisberg I, Alfonso F, et al. Functional impact of high clopidogrel maintenance dosing in patients undergoing elective percutaneous coronary interventions. Results of a randomized study. Thromb Haemost. 2008;99:161-8.
119. Angiolillo DJ, Costa MA, Shoemaker SB, Desai B, Bernardo E, Suzuki Y, et al. Functional effects of high clopidogrel maintenance dosing in patients with inadequate platelet inhibition on standard dose treatment. Am J Cardiol. 2008;101:440-5.
120. Lemesle G, Delhaye C, Sudre A, Broucqsault D, Rosey G, Bauters C, et al. Impact of high loading and maintenance

dose of clopidogrel within the first 15 days after percutaneous coronary intervention on patient outcome. Am Heart J. 2009;157:375-82.

121. Bonello L, Camoin-Jau L, Arques S, Boyer C, Panagides D, Wittenberg O, et al. Adjusted clopidogrel loading doses according to vasodilator-stimulated phosphoprotein phosphorylation index decrease rate of major adverse cardiovascular events in patients with clopidogrel resistance: a multicenter randomized prospective study. J Am Coll Cardiol. 2008;51:1404-11.
122. Bonello L, Camoin-Jau L, Armero S, Com O, Arques S, Burignat-Bonello C, et al. Tailored clopidogrel loading dose according to platelet reactivity monitoring to prevent acute and subacute stent thrombosis. Am J Cardiol. 2009;103: 5-10.
123. Price MJ, Berger PB, Angiolillo DJ, Teirstein PS, Tanguay JF, Kandzari DE, et al. Evaluation of individualized clopidogrel therapy after drug-eluting stent implantation in patients with high residual platelet reactivity: design and rationale of the GRAVITAS trial. Am Heart J. 2009;157:818-24.
124. Cuisset T, Frere C, Quilici J, Morange PE, Mouret JP, Bali L, et al. Glycoprotein IIb/IIIa inhibitors improve outcome after coronary stenting in clopidogrel nonresponders. JACC Cardiovasc Interv. 2008;1:649-53.
125. Valgimigli M, Campo G, de Cesare N, Meliga E, Vranckx P, Furgieri A, et al; Tailoring Treatment With Tirofiban in Patients Showing Resistance to Aspirin and/or Resistance to Clopidogrel (3T/2R) Investigators. Intensifying platelet inhibition with tirofiban in poor responders to aspirin, clopidogrel, or both agents undergoing elective coronary intervention: results from the double-blind, prospective, randomized Tailoring Treatment with Tirofiban in Patients Showing Resistance to Aspirin and/or Resistance to Clopidogrel study. Circulation. 2009;119:3215-22.
126. Angiolillo DJ, Capranzano P, Goto S, Aslam M, Desai B, Charlton RK, et al. A randomized study assessing the impact of cilostazol on platelet function profiles in patients with diabetes mellitus and coronary artery disease on dual antiplatelet therapy: results of the OPTIMUS-2 study. Eur Heart J. 2008;29:2202-11.
127. Douglas JS Jr, Holmes DR Jr, Kereiakes DJ, Grines CL, Block E, Ghazzal ZM, et al; Cilostazol for Restenosis Trial (CREST) Investigators. Coronary stent restenosis in patients treated with cilostazol. Circulation. 2005;112:2826-32.
128. Lee SW, Park SW, Hong MK, Kim YH, Lee BK, Song JM, et al. Triple versus dual antiplatelet therapy after coronary stenting: impact on stent thrombosis. J Am Coll Cardiol. 2005;46:1833-7.
129. Biondi-Zoccai GG, Lotrionte M, Anselmino M, Moretti C, Agostoni P, Testa L, et al. Systematic review and meta-analysis of randomized clinical trials appraising the impact of cilostazol after percutaneous coronary intervention. Am Heart J. 2008;155:1081-9.
130. Angiolillo DJ. ADP receptor antagonism: what's in the pipeline? Am J Cardiovasc Drugs. 2007;7:423-32.
131. Angiolillo DJ, Bhatt DL, Gurbel PA, Jennings LK. Advances in antiplatelet therapy: agents in clinical development. Am J Cardiol. 2009;103 Suppl 1:A40-51.
132. Angiolillo DJ, Capranzano P. Pharmacology of emerging novel platelet inhibitors. Am Heart J. 2008;156 Suppl :S10-5.
133. Wiviott SD, Trenk D, Frelinger AL, O'Donoghue M, Neumann FJ, Michelson AD, et al.; PRINCIPLE-TIMI 44 Investigators. Prasugrel compared with high loading- and maintenance-dose clopidogrel in patients with planned percutaneous coronary intervention: the Prasugrel in Comparison to Clopidogrel for Inhibition of Platelet Activation and Aggregation-Thrombolysis in Myocardial Infarction 44 trial. Circulation 2007;116:2923-32.
134. Wiviott SD, Braunwald E, McCabe CH, Montalescot G, Ruzyllo W, Gottlieb S, et al.; TRITON-TIMI 38 Investigators. Prasugrel versus clopidogrel in patients with acute coronary syndromes. N Engl J Med. 2007;357:2001-15.
135. Wiviott SD, Braunwald E, McCabe CH, Horvath I, Keltai M, Herrman JP, et al.; TRITON-TIMI 38 Investigators. Intensive oral antiplatelet therapy for reduction of ischaemic events including stent thrombosis in patients with acute coronary syndromes treated with percutaneous coronary intervention and stenting in the TRITON-TIMI 38 trial: a subanalysis of a randomised trial. Lancet. 2008;371:1353-63.
136. Wiviott SD, Braunwald E, Angiolillo DJ, Meisel S, Dalby AJ, Verheugt FW, et al; TRITON-TIMI 38 Investigators. Greater clinical benefit of more intensive oral antiplatelet therapy with prasugrel in patients with diabetes mellitus in the trial to assess improvement in therapeutic outcomes by optimizing platelet inhibition with prasugrel-Thrombolysis in Myocardial Infarction 38. Circulation 2008;118:1626-36.
137. Montalescot G, Wiviott SD, Braunwald E, Murphy SA, Gibson CM, McCabe CH, et al.; TRITON-TIMI 38 investigators. Prasugrel compared with clopidogrel in patients undergoing percutaneous coronary intervention for ST-elevation myocardial infarction (TRITON-TIMI 38): double-blind, randomised controlled trial. Lancet. 2009;373:723-31.
138. Antman EM, Wiviott SD, Murphy SA, Voitk J, Hasin Y, Widimsky P, et al. Early and late benefits of prasugrel in patients with acute coronary syndromes undergoing percutaneous coronary intervention: a TRITON-TIMI 38 (TRial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet InhibitioN with Prasugrel-Thrombolysis In Myocardial Infarction) analysis. J Am Coll Cardiol. 2008;51:2028-33.
139. Angiolillo DJ, Guzman LA. Clinical overview of promising nonthienopyridine antiplatelet agents. Am Heart J. 2008; 156:S23-8.
140. Husted S, Emanuelsson H, Heptinstall S, Sandset PM, Wickens M, Peters G. Pharmacodynamics, pharmacokinetics, and safety of the oral reversible P2Y12 antagonist AZD6140 with aspirin in patients with atherosclerosis: a double-blind comparison to clopidogrel with aspirin. Eur Heart J. 2006;27:1038-47.
141. Storey RF, Husted S, Harrington RA, Heptinstall S, Wilcox RG, Peters G, et al. Inhibition of platelet aggregation by AZD6140, a reversible oral P2Y12 receptor antagonist, compared with clopidogrel in patients with acute coronary syndromes. J Am Coll Cardiol. 2007;50:1852-6.
142. Wallentin L, Becker RC, Budaj A, Cannon CP, Emanuelsson H, Held C, et al. for the PLATO Investigators. Ticagrelor versus Clopidogrel in Patients with Acute Coronary Syndromes. N Engl J Med. 2009;361:1-13.
143. Storey RF, Oldroyd KG, Wilcox RG. Open multicentre study of the P2T receptor antagonist AR-C69931MX assessing safety, tolerability and activity in patients with acute coronary syndromes. Thromb Haemost. 2001;85:401-7.
144. Storey RF, Wilcox RG, Heptinstall S. Comparison of the pharmacodynamic effects of the platelet ADP receptor antagonists clopidogrel and AR-C69931MX in patients with ischaemic heart disease. Platelets. 2002;13:407-13.
145. Harrington RA, Stone GW, McNulty S, White HD, Lincoff AM, Gibson CM, et al. Platelet Inhibition with Cangrelor in Patients Undergoing PCI. N Engl J Med. 2009; 361:2318-29.
146. Bhatt DL, Lincoff AM, Gibson CM, Stone GW, McNulty S, Montalescot G, et al; the CHAMPION PLATFORM

Investigators. Intravenous Platelet Blockade with Cangrelor during PCI. N Engl J Med. 2009; 361:2330-41.

147. Gretler D, Conley P, Andre P, Jurek M, Pandey A, Romanko K, et al. "First In Human" Experience With PRT060128, A New Direct-acting, Reversible, P2Y12 Inhibitor For IV and Oral Use [abstract]. J Am Coll Cardiol. 2007;49:326A.

148. Gurbel P, Conley P, Andre P, Stephens G, Gretler DD, Jurek M, et al. Oral Dosing of PRT060128, a Novel Direct-acting, Reversible P2Y12 Antagonist Overcomes High Platelet Reactivity in Patients Non-responsive to Clopidogrel Therapy [abstract]. Circulation. 2008;118:S_972.

VIII. Diabetes and anti-platelet therapy in acute coronary syndrome.

Ferreiro JL, Angiolillo DJ.

Circulation. 2011;123:798-813.

Diabetes and Antiplatelet Therapy in Acute Coronary Syndrome

José Luis Ferreiro, MD; Dominick J. Angiolillo, MD, PhD

Cardiovascular disease, particularly coronary artery disease resulting from accelerated atherosclerosis, is the leading cause of morbidity and mortality in patients with diabetes mellitus (DM).[1] Of note, DM patients without a history of coronary artery disease have overall the same cardiac risk as non-DM patients with a history of myocardial infarction (MI).[2] Furthermore, patients with DM also have a higher risk of cardiovascular complications and recurrent atherothrombotic events than non-DM patients.[3] In fact, in the setting of acute coronary syndromes (ACS), the presence of DM is a strong independent predictor of short-term and long-term recurrent ischemic events, including mortality.[4,5] The concomitant presence of cardiovascular risk factors and comorbidities that negatively affect the outcomes of ACS is higher in DM patients.[6] The negative impact of DM on outcomes is maintained across the ACS spectrum, including unstable angina and non–ST-elevation MI (NSTEMI),[7] ST-elevation MI (STEMI) treated medically,[8] and ACS undergoing percutaneous coronary intervention (PCI).[9,10]

Platelets of DM patients are characterized by dysregulation of several signaling pathways, both receptor (eg, increased expression) and intracellular downstream signaling abnormalities, which leads to increased platelet reactivity.[11–15] This may play a role not only in the higher risk of developing ACS and the worse outcomes observed in DM, but also in the larger proportion of DM patients with inadequate response to antiplatelet agents compared with non-DM subjects,[13,16–18] which may also contribute to the impaired outcomes observed in DM patients despite compliance with recommended antiplatelet treatment regimens.

The aim of this article is to provide an overview of the current status of knowledge on platelet abnormalities that characterize DM patients, to analyze the benefits and limitations of currently available antiplatelet agents used in ACS, focusing on drawbacks of these therapies in DM patients, and to describe potential future directions to overcome these limitations, which include new agents and treatment strategies.

Platelet Dysfunction in DM: The "Diabetic" Platelet

Platelets play a pivotal role in atherogenesis and its thrombotic complications such as those occurring in patients with ACS,[19–22] which is a platelet-driven process. Platelets of DM patients have been proven to be hyperreactive with intensified adhesion, activation, and aggregation.[11–15] Multiple mechanisms have been proposed to contribute to increased platelet reactivity. Although many of them are closely interrelated, these mechanisms are caused by metabolic and cellular abnormalities that occur in DM patients, which can be grouped together into the following categories: hyperglycemia, insulin resistance, associated metabolic conditions, and other cellular abnormalities (Figure 1).

Hyperglycemia

Hyperglycemia, one of the most characteristic features of DM, may play an independent role in the abnormalities found in platelets of DM patients.[23] Induction of hyperglycemia has been shown to increase platelet P-selectin expression (a surface adhesion molecule) in patients with DM.[24] Correlation between levels of fasting glucose and P-selectin expression has also been reported.[25] Proposed mechanisms by which hyperglycemia may increase platelet reactivity are glycation of platelet surface proteins that decreases membrane fluidity, which may increase platelet adhesion[26,27]; osmotic effect of glucose,[28] and activation of protein kinase C, a mediator of platelet activation.[29]

In line with the laboratory findings, there are some clinical data supporting the idea that glucose-lowering therapy is beneficial in DM patients with ACS. The Diabetes Mellitus, Insulin Glucose Infusion in Acute Myocardial Infarction (DIGAMI) trial, which randomized patients with DM and acute MI to intensive glucose-lowering treatment (standard treatment plus insulin-glucose infusion for 24 hours followed by multidose insulin therapy) or standard treatment, observed a reduction in mortality in the intensive treatment group after 3.4 years of follow-up.[30] In the DIGAMI-2 trial, no differences in mortality or morbidity were observed among 3 different glucose-lowering strategies.[31] In this trial, the glucose-lowering levels were similar among the 3 groups, suggesting that the benefit of decreasing glucose levels is independent of the way this is achieved. However, the optimal blood glucose levels remain unknown. In fact, an excessive glucose lowering (targeting a glycohemoglobin level $<6.0\%$) was proven to be harmful in the Action to

From the University of Florida College of Medicine-Jacksonville, Jacksonville (J.L.F., D.J.A.); Heart Diseases Institute, Hospital Universitari de Bellvitge-IDIBELL, L'Hospitalet de Llobregat, Barcelona, Spain (J.L.F.).

Correspondence to Dominick J. Angiolillo, MD, PhD, University of Florida College of Medicine–Jacksonville, 655 W 8th St, Jacksonville, FL 32209. E-mail dominick.angiolillo@jax.ufl.edu

(*Circulation*. 2011;123:798-813.)

DOI: 10.1161/CIRCULATIONAHA.109.913376

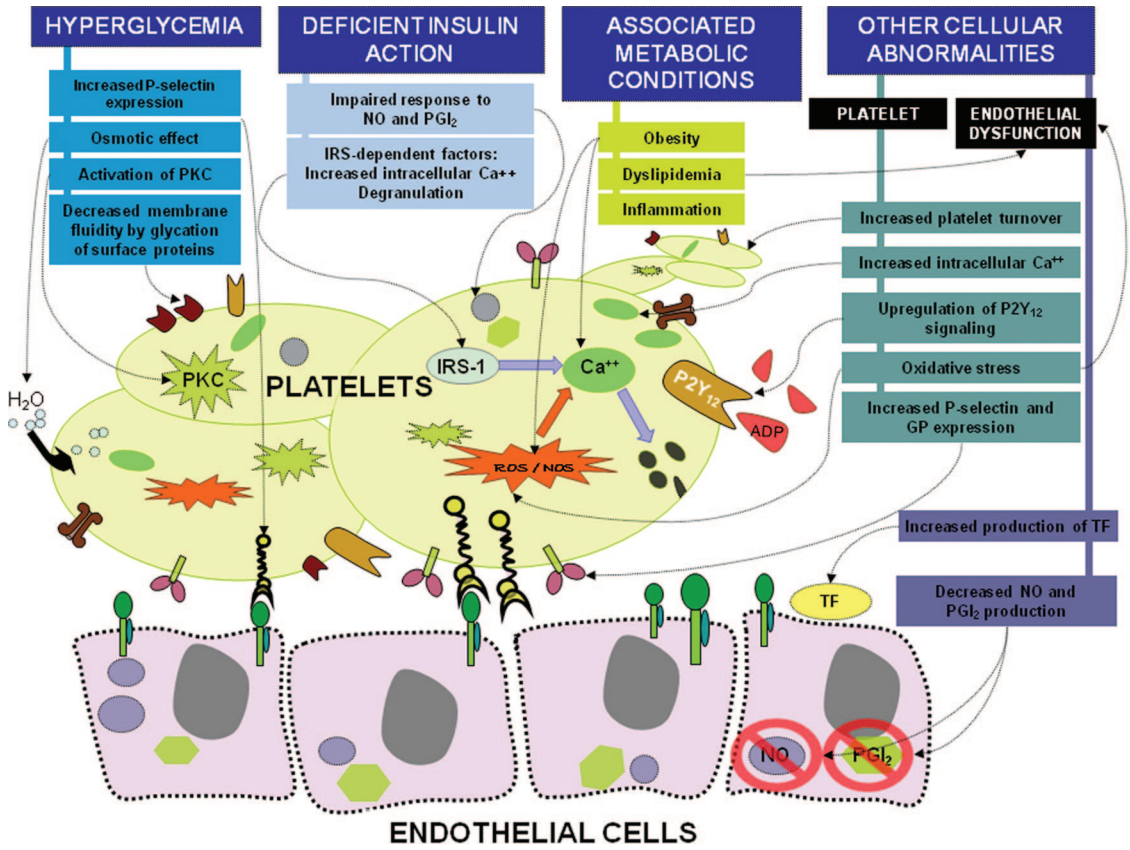


Figure 1. Mechanisms involved in platelet dysfunction in patients with DM. Several mechanisms contribute to platelet dysfunction in diabetes mellitus (DM) patients, including hyperglycemia, insulin deficiency, associated metabolic conditions, and other cellular abnormalities. Hyperglycemia may increase platelet reactivity by inducing P-selectin (a surface adhesion protein) expression, glycating platelet surface proteins (decreasing membrane fluidity and, thus, increasing platelet adhesion), and activating protein kinase C (PKC; a mediator of platelet activation) and as a result of the osmotic effect of glucose. Insulin deficiency also contributes to platelet dysfunction by different mechanisms. Some have been suggested to be IRS dependent such as the increase in intracellular calcium concentration, which leads to enhanced platelet degranulation and aggregation. Other factors associated with insulin resistance are not dependent on IRS, eg, the impaired response to NO and PGI_2, which enhances platelet reactivity. Some metabolic conditions frequently associated with DM may play a role in platelet hyperreactivity, including obesity, dyslipidemia, and enhanced systemic inflammation. In addition to being associated with insulin resistance, obesity contributes to platelet dysfunction, mainly in terms of adhesion and activation, with factors like augmented cytosolic calcium concentration and increased oxidative stress. Abnormalities of the lipid profile, especially hypertriglyceridemia, also affect platelet reactivity by different mechanisms, which include inducing endothelial dysfunction. The presence of endothelial dysfunction is another characteristic feature associated with DM, which enhances platelet reactivity by decreasing the production of NO and PGI_2 and contributes to a prothrombotic state through increased production of tissue factor (TF). Other platelet abnormalities present in DM patients can enhance platelet adhesion and activation, including increased expression of surface proteins (P-selectin and GP IIb/IIIa), augmented cytosolic calcium concentration, upregulation of certain pathways like $P2Y_{12}$ signaling, increased platelet turnover, and oxidative stress, which causes an impairment in platelet function as a result of overproduction of reactive oxygen (ROS) and nitrogen species (NOS).

Control Cardiovascular Risk in Diabetes (ACCORD) study, which randomized DM patients (n=10 251) to receive an intensive glucose-lowering regimen or a standard regimen, because the trial was interrupted after 3.5 years of follow-up as a result of an increased mortality in the intensive therapy group.[32]

Insulin Deficiency and Resistance

The majority of cases of DM fall into 2 etiopathogenetic categories. In type 1 DM, the underlying cause is an autoimmune destruction of the β cells of the pancreas, leading to an absolute deficiency of insulin secretion. In type 2 DM, which accounts for ≈90% to 95% of DM, the cause is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response, usually having relative (rather than absolute) insulin deficiency.[33] Deficient insulin action resulting from inadequate insulin secretion and/or diminished tissue responses is the cardinal factor for the development of DM and contributes to platelet dysfunction.[34] Platelets express both insulin receptors and insulin-like growth factor-1 (IGF-1) receptors.[35,36] Among other effects, the binding of insulin to platelets increases surface expression of adenylate cyclase–linked prostacyclin receptor.[37] However, insulin receptor expression is relatively low because the majority of its subunits heterodimerize with those of the IGF-1 receptor to form an insulin/IGF-1 hybrid receptor, which avidly binds IGF-1 but not insulin.[36] However, IGF-1 is present in the α granules of platelets, and its receptor is expressed on the platelet surface, which may contribute to the amplification of platelet responses and the pathogenesis of cardiovascular disease. The functional and signaling pathways involved in IGF-1 modulation of platelet function, however, are currently

not fully elucidated. IGF-1 stimulation of platelets results in dose-dependent phosphorylation of the IGF receptor. Furthermore, IGF-1 stimulates tyrosine phosphorylation of insulin receptor substrate (IRS)-1 and IRS-2 and their subsequent binding with the p85 subunit of phosphoinositide-3 kinase, leading to phosphorylation of protein kinase B, which is involved in several cellular responses to insulin and IGF-1, including modulation of platelet reactivity.[38]

Various abnormalities in insulin-mediated signaling have been proposed to be involved in the hampered or abolished platelet-inhibitory effect observed in patients with insulin resistance.[39] Among IRS-dependent factors, insulin resistance provokes an increase in intracellular calcium concentration, leading to enhanced platelet degranulation and aggregation.[40] However, the precise mechanism by which calcium concentration is increased is not yet fully elucidated.[41,42] IRS-independent pathways are also involved in platelet hyperreactivity caused by insulin resistance such as impairment in platelet sensitivity to nitric oxide (NO) and prostacyclin.[43,44] Both mediators are released by the endothelium and retard platelet activation. Therefore, impaired response to NO and prostacyclin is associated with enhanced platelet reactivity.

The importance of insulin resistance in platelet dysfunction among DM patients is underscored by recent studies with thiazolidinediones that have shown a beneficial effect of this group of insulin sensitizers on platelet function. Rosiglitazone improved sensitivity to NO in platelets and reduced P-selectin expression in DM and non-DM patients, respectively.[45,46] Clinical trials have also shown a benefit of insulin-sensitizer therapy over insulin-providing therapy in terms of atherosclerosis progression and cardiovascular outcomes.[47,48] The results of these studies emphasize the important role of insulin resistance in the development of atherothrombotic disease in DM patients.

Associated Metabolic Conditions

Type 2 DM is commonly associated with a number of metabolic conditions that may have an impact on platelet function, including obesity, dyslipidemia, and enhanced systemic inflammation.

Obesity is frequently associated with an insulin-resistant status. However, other factors present in obese subjects may contribute to platelet dysfunction: elevated platelet count and high mean platelet volume,[49] high blood leptin concentration,[50] increased cytosolic calcium concentration,[51] and increased oxidative stress.[52] These abnormalities result mostly in enhanced platelet adhesion and activation.[53,54] Likewise, response to antiplatelet drugs such as clopidogrel is also impaired in subjects with elevated body mass index.[55,56]

Abnormalities of the lipid profile commonly accompany DM. Hypertriglyceridemia, which induces higher platelet activation, is a typical manifestation.[57] This effect has been suggested to be mediated by the apolipoprotein E content of the very-low-density lipoprotein particles, which are rich in triglycerides.[58,59] Low levels of high-density lipoprotein have been associated with endothelial dysfunction, which may increase the atherothrombotic risk in DM patients.[60] Recently, Calkin et al[61] observed that administration of reconstituted high-density lipoprotein reduced platelet aggregation in DM subjects by promoting cholesterol efflux from platelets.

DM is also associated with systemic inflammation. In fact, DM patients show high levels of inflammatory and platelet activation markers.[62] In particular, an in vitro study showed that the platelet-activating factor released by leukocytes increased platelet activity. In addition, expression of platelet FcgammaRIIA receptor, which is enhanced in DM patients and involved in platelet activation, has been reported to be modulated by inflammation.[63,64] Therefore, systemic inflammation may contribute to increased platelet reactivity of DM subjects.

Other Cellular Abnormalities

Dysregulation of calcium metabolism is a major feature in DM platelets. To date, the exact mechanisms involved in calcium signaling abnormalities are not fully elucidated. Some of the proposed factors that may play a role are excessive influx of calcium through the sodium/calcium exchanger,[65] changes in the activity of calcium ATPases,[66] insulin resistance,[51] and augmented oxidative stress.[67] The result of this calcium dysregulation is an increase in cytosolic calcium concentration, which leads to enhanced platelet reactivity.[68]

DM is also associated with oxidative stress, in particular with an overproduction of reactive oxygen and nitrogen species, as well as reduced platelet antioxidant levels.[69,70] Alterations in the redox state of platelets may impair platelet function. The excessive generation of potent oxidants such as superoxide anions and hydrogen peroxide increases platelet activation.[69] An increase in reactive oxygen species enhances the production of advanced glycation end products.[71] These glycated proteins have been suggested to play a role in atherosclerosis by activation of the receptor for advanced glycation end products.[72] Furthermore, oxidative stress accompanying DM impairs endothelial function, which leads to increased platelet reactivity by decreasing the production of NO and prostacyclin.[73] In addition, platelets of DM patients have diminished sensitivity to the actions of NO and prostacyclin.[43,44] Endothelial dysfunction is another characteristic feature in DM patients that may result in a prothrombotic state through an increased production of tissue factor.[74]

An upregulation of platelet ADP $P2Y_{12}$ receptor signaling, which suppresses cAMP levels, and a lower responsiveness to insulin have been suggested in patients with type 2 DM, leading to increased adhesion, aggregation, and procoagulant activity.[75,75a] Another platelet abnormality observed in DM is an increased expression of surface proteins like P-selectin and glycoproteins (GPs) Ib and IIb/IIIa, which are integrins that mediate platelet adhesion.[53,76]

In addition to the above-mentioned mechanisms, DM patients have accelerated platelet turnover.[77] Platelet turnover is represented by the presence of a higher number of reticulated platelets, which are larger and more sensitive and thus result in platelet hyperreactivity and lower response to antiplatelet therapies like aspirin.[78] In line with these findings, Guthikonda et al[79] recently reported an association between a higher percentage of circulating reticulated platelets and a lower response to both aspirin and clopidogrel,

although only a small number of DM patients were included in this study.

Antiplatelet Therapies

Currently, 3 different classes of antiplatelet agents are approved for the treatment and/or prevention of recurrent events in the setting of ACS: cyclooxygenase-1 (COX-1) inhibitors (aspirin), ADP $P2Y_{12}$ receptor antagonists (thienopyridines), and GP IIb/IIIa inhibitors.[80,81] The following section provides an overview of the benefits and limitations of these drugs in DM patients.

Aspirin

Aspirin selectively acetylates the hydroxyl group of a serine residue at position 529 (Ser529) of the COX-1 enzyme, thereby blocking platelet formation of thromboxane A_2 (TXA_2) and thus diminishing platelet aggregation mediated by thromboxane and prostaglandin endoperoxide (TP) receptors pathway.[82] This effect is irreversible because platelets are enucleate and therefore unable to resynthesize COX-1. TXA_2 binds to TP receptors, which results in changes in platelet shape and enhancement of recruitment and aggregation of platelets. Although expert consensus statements recommend the use of aspirin for primary prevention in DM patients, its use in this setting has been controversial, and its description goes beyond the scope of this review, which focuses primarily on secondary prevention in the ACS setting.[83–89] Ongoing studies will provide further insights into the role of aspirin as a primary prevention measure in DM patients, including A Study of Cardiovascular Events in Diabetes (ASCEND; NCT00135226) and Aspirin and Simvastatin Combination for Cardiovascular Events Prevention Trial in Diabetes (ACCEPT-D; ISRCTN48110081).

Aspirin is still the antiplatelet drug of choice for secondary prevention of recurrent ischemic events in patients with atherothrombotic disease, including those with DM.[80–82,90,91] The benefit of aspirin therapy in the early management of ACS patients has been demonstrated repeatedly and consistently in earlier trials, including those evaluating unstable angina/NSTEMI[92–94] and STEMI.[95,96] Aspirin should be given as promptly as possible at an initial dose of 162 to 325 mg followed by a daily dose of 75 to 162 mg.[80,81] The recommended dose of aspirin for secondary prevention in DM patients with atherosclerotic disease is 75 to 162 mg daily.[90] The use of low-dose aspirin is supported mainly by 2 large meta-analyses of secondary prevention trials performed by the Antithrombotic Trialists' Collaboration that include 287 studies and involve 212 000 high-risk patients (with acute or previous vascular disease or some other predisposing condition implying an increased risk of occlusive vascular disease).[97,98] The results of these meta-analyses showed oral antiplatelet agents, mainly aspirin, to be protective for suffering vascular events in high-risk patients. In particular, the incidence of vascular events was reduced from 22.3% to 18.5% in the cohort of DM patients ($P<0.002$) and from 16.4% to 12.8% ($P<0.00001$) in non-DM patients. Although the overall incidence of vascular events was much higher in DM patients, the benefit of antiplatelet therapy was consistent regardless of DM status.[97] In these trials, aspirin was the most frequently evaluated antiplatelet agent at doses ranging from 75 to 325 mg daily. A low dose of aspirin (75 to 150 mg/d) was found to be at least as effective as higher daily doses, and importantly, bleeding complications were reduced with lower doses.[97,98]

The first large-scale prospective randomized study to compare high- and low-dose aspirin was the recently reported Clopidogrel Optimal Loading Dose Usage to Reduce Recurrent Events-Organization to Assess Strategies in Ischemic Syndromes (CURRENT/OASIS-7) trial, which randomized ACS patients scheduled to undergo angiography within 72 hours of hospital arrival.[99,99a] The study had a 2×2 factorial design, and patients were randomized in a double-blind fashion to high- or standard-dose clopidogrel for a month and in an open-label way to high-dose (300 to 325 mg daily) or low-dose (75 to 100 mg daily) aspirin. The trial did not show significant differences in efficacy between high- and low-dose aspirin. A trend toward a higher rate of gastrointestinal bleeds in the high-dose group (0.38% versus 0.24%; $P=0.051$) was observed.[99] No data regarding the DM subgroup of this study have been reported yet.

$P2Y_{12}$ receptor antagonists

Platelet ADP signaling pathways mediated by the $P2Y_1$ and $P2Y_{12}$ receptors play a central role in platelet activation and aggregation.[100,101] Although both receptors are needed for aggregation,[102] ADP-stimulated effects on platelets are mediated mainly by G_i-coupled $P2Y_{12}$ receptor activation, which leads to sustained platelet aggregation and stabilization of the platelet aggregate, whereas $P2Y_1$ is responsible for an initial weak, transient phase of platelet aggregation. Several families of $P2Y_{12}$ inhibitors have been developed. However, only thienopyridines (ticlopidine, clopidogrel, and prasugrel), which are nondirect, orally administered, irreversible $P2Y_{12}$ receptor inhibitors, are currently approved for clinical use. Ticlopidine was the first thienopyridine to be developed and was approved for clinical use in 1991. It showed its superiority in combination with aspirin compared with aspirin alone or anticoagulation in combination with aspirin in a number of trials for the prevention of recurrent ischemic events in patients undergoing PCI.[103–106] However, as a result of safety concerns (mainly high rates of neutropenia), ticlopidine has been largely replaced by clopidogrel (a second-generation thienopyridine) because of its better safety profile.[107]

Clopidogrel is currently the thienopyridine of choice because it has an efficacy similar to that of ticlopidine and a favorable safety profile.[107] In addition, clopidogrel has a faster onset of action through administration of a loading dose.[108] The Clopidogrel Versus Aspirin in Patients at Risk of Ischemic Events (CAPRIE) trial evaluated the efficacy of clopidogrel (75 mg daily) versus aspirin (325 mg daily) in reducing the risk of ischemic outcomes in patients (n=19 185) with a history of recent MI, recent ischemic stroke, or established peripheral artery disease. The global results showed a significantly lower annual rate of the composite end point (ischemic stroke, MI, or vascular death) with clopidogrel (5.32% versus 5.83%; $P=0.043$).[109] The benefit with clopidogrel therapy was higher in the DM subgroup (15.6% versus 17.7%; $P=0.042$), leading to 21

Table. Large-Scale Randomized Placebo-Controlled Clinical Trials Evaluating the Efficacy of Dual Antiplatelet Therapy With Aspirin and Clopidogrel Versus Aspirin Alone in ACS/PCI Patients in the Overall Study Population and in DM Patients

Study	n (Overall)	Scenario	Primary End Point	% of Events and Association Measure in the Overall Population	n (DM)	% of Events and Association Measure in DM
CURE[7]	12 562	UA/NSTEMI	Cardiovascular death, nonfatal MI or stroke at 1 y	9.3 vs 11.4	2840	14.2 vs 16.7
RR (95% CI)				0.80 (0.72–0.90)		0.84 (0.70–1.02)
PCI-CURE[111]	2658	CURE patients undergoing PCI	Cardiovascular death, MI, or urgent TVR at 30 d	4.5 vs 6.4	504	12.9 vs 16.5
RR (95% CI)				0.70 (0.50–0.97)		0.77 (0.48–1.22)
CREDO[112]	2116	Elective PCI	Death, MI, or stroke at 1 y	8.5 vs 11.5	560	NR
RRR (95% CI), %				26.9 (3.9–44.4)		11.2 (−46.8–46.2)
COMMIT[113]	45 852	Acute MI (93% STEMI)	Death, reinfarction, or stroke at discharge or 28 d	9.2 vs 10.1	NR	NR
OR (95% CI)				0.91 (0.86–0.97)		
CLARITY[114]	3491	STEMI with fibrinolysis	Occluded infarct-related artery on angiography or death or recurrent MI before angiography	15.0 vs 21.7	575	NR
OR (95% CI)				0.64 (0.53–0.76)		
PCI-CLARITY[115]	1863	CLARITY patients undergoing PCI	Cardiovascular death, recurrent MI, or stroke at 30 d	3.6 vs 6.2	282	6.0 vs 10.1
OR (95% CI)				0.54 (0.35–0.85)		0.61 (0.24–1.53)

CURE indicates Clopidogrel in Unstable Angina to Prevent Recurrent Events Trial; CREDO, Clopidogrel for the Reduction of Events During Observation; COMMIT, Clopidogrel and Metoprolol in Myocardial Infarction Trial; CLARITY, Clopidogrel as Adjunctive Reperfusion Therapy; UA, unstable angina; TVR, target vessel revascularization; NR, not reported; RR, relative risk; RRR, relative risk reduction; and OR, odds ratio.

vascular events prevented for every 1000 DM patients treated (38 among insulin-treated patients).[110] Of note, the reduction in the rates of the primary end point did not reach statistical significance in non-DM patients.

Currently, the American Diabetes Association recommends the use of clopidogrel in very high-risk DM patients or as an alternative therapy in patients intolerant to aspirin.[90] In line with this, current guidelines recommend dual antiplatelet therapy with aspirin and clopidogrel as the antiplatelet treatment of choice for patients with ACS, including patients with unstable angina or NSTEMI,[80] those with STEMI,[81] and patients undergoing PCI.[91] The recommended dose of clopidogrel is a 300-mg loading dose (up to 600 mg in the setting of PCI) followed by a maintenance dose of 75 mg daily. These recommendations have been made in light of the results of several large-scale clinical trials that have shown a clear benefit of clopidogrel in addition to aspirin in terms of preventing recurrent ischemic events, including stent thrombosis, compared aspirin alone.[7,111–115] The Table summarizes ACS/PCI trials comparing dual antiplatelet therapy with aspirin and clopidogrel versus aspirin alone, highlighting the relative benefits in the overall study population and in patients with DM.

The Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management, and Avoidance (CHARISMA) trial showed that in high-risk but non-ACS patients (n=15 603) with either clinically evident cardiovascular disease or multiple risk factors, clopidogrel and aspirin were not significantly more effective than aspirin alone in reducing the rate of cardiovascular death, MI, or stroke (6.8% versus 7.3%; $P=0.22$).[116] Being a high-risk feature, DM was an important inclusion criterion for this study and represented 42% (n=6555) of the population. Consistent with the results in the overall population, no benefit of combined therapy was observed in the DM subgroup. Therefore, long-term dual antiplatelet therapy with aspirin and clopidogrel should not be advocated, not even in DM patients, outside the ACS/PCI setting.

The CURRENT/OASIS-7 trial, which compared the efficacy of high-dose (600-mg loading dose and then 150 mg once a day for 7 days followed by 75 mg daily) or standard-dose (300-mg loading dose followed by 75 mg daily) clopidogrel for 1 month in ACS patients (n=25 087) scheduled to undergo angiography within 72 hours of hospital arrival, failed to find a statistical difference for the primary end point (cardiovascular death, MI, or stroke at 30 days) in the overall study population.[99] However, in the subgroup of patients undergoing PCI (n=17 232), the high-dose clopidogrel regimen significantly reduced the rates of the primary efficacy end point (3.9% versus 4.5%; hazard ratio [HR]=0.85; $P=0.036$), as well as the risk of stent thrombosis, but at the expense of an increase in study-defined major bleedings.[99a] No differences in efficacy were observed among DM patients undergoing PCI (4.9% versus 5.6%; HR=0.87; 95% confidence interval [CI], 0.66 to 1.15).[99]

Prasugrel is a third-generation thienopyridine that was recently approved for clinical use in ACS patients undergoing PCI. It is orally administered and, like all thienopyridines, is a prodrug that requires hepatic metabolism to give origin to its active metabolite that irreversibly inhibits the $P2Y_{12}$ receptor.[117] Prasugrel has a more rapid onset of action than clopidogrel and provides greater platelet inhibition because of

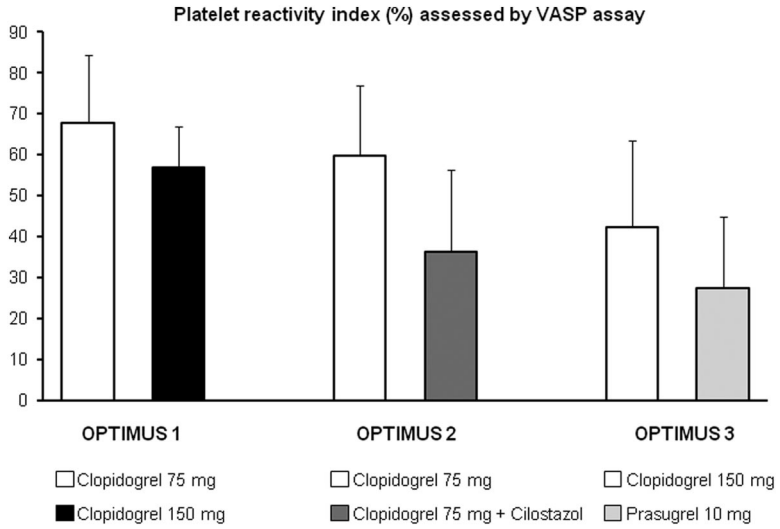


Figure 2. Antiplatelet effects of different treatment strategies to optimize platelet inhibition in diabetes mellitus (DM). The OPTIMUS studies were performed in patients with DM and coronary artery disease and evaluated platelet inhibition achieved by different antiplatelet treatment strategies using multiple pharmacodynamic measures. The platelet reactivity index (PRI), which is obtained by the flow cytometric analysis of the phosphorylation status of vasodilator-stimulated phosphoprotein (VASP) and is a specific measure of the degree of blockade of the $P2Y_{12}$ receptor signaling pathway, is illustrated. The OPTIMUS-1[124] study compared the effect of a high maintenance dose of clopidogrel (150 mg daily) and standard dosing at 30 days among suboptimal responders while on standard doses of dual antiplatelet therapy. The OPTIMUS-2[125] study compared the effect of adding cilostazol (100 mg BID) vs placebo at 2 weeks in patients on standard doses of dual antiplatelet therapy. The OPTIMUS-3[123] study compared the efficacy of prasugrel (60-mg loading dose and 10-mg daily maintenance dose) vs high-dose clopidogrel (600-mg loading dose and 150-mg daily maintenance dose) up to 1 week in patients on long-term aspirin therapy.

its more effective conversion into its active metabolite.[118] The Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition With Prasugrel–Thrombolysis in Myocardial Infarction 38 (TRITON-TIMI 38) examined the efficacy and safety of prasugrel (60-mg loading dose followed by 10 mg daily) versus standard clopidogrel therapy (300-mg loading dose followed by 75-mg/d maintenance dose) in patients (n=13 608) with moderate- to high-risk ACS undergoing PCI.[119] A significant reduction in the rates of the primary end point (composite of cardiovascular death, nonfatal MI, or nonfatal stroke) favoring prasugrel (9.9% versus 12.1%; HR=0.81; $P<0.001$) was found, as well as a reduction in the rates of stent thrombosis,[120]over a follow-up period of 15 months at the expense of an increased risk of major bleeding in the prasugrel group. Of note, no net clinical benefit was observed in the aged patients (≥75 years of age) and in those weighing <60 kg; in fact, a net harm was found in patients with history of stroke or transient ischemic attack.[119] However, particular subgroups appeared to have a higher benefit with prasugrel therapy such as patients with STEMI[121] and, importantly, DM patients.[122] The primary end point was reduced significantly with prasugrel in subjects with DM (12.2% versus 17.0%; HR=0.70; $P<0.001$). This benefit was consistent in patients with (14.3% versus 22.2%; HR=0.63; $P=0.009$) and without (11.5% versus 15.3%; HR=0.74; $P=0.009$) insulin treatment. Importantly, although major bleeding was higher overall in DM patients, which is consistent with the fact that DM per se is a risk factor for bleeding, there were no differences in major bleeding among DM patients treated with prasugrel compared with clopidogrel (2.6% versus 2.5%; HR=1.06; $P=0.81$). Prasugrel also improved the risk of stent thrombosis in the DM subgroup (overall DM cohort: 2.0% versus 3.6%; HR=0.52; $P=0.007$; insulin-dependent patients: 1.8% versus 5.7%; HR=0.31; $P=0.008$). Recently, the Optimizing Antiplatelet Therapy in Diabetes Mellitus (OPTIMUS)-3 study showed that prasugrel (60-mg loading dose followed by 10-mg maintenance dose daily for 1 week) achieved significantly greater platelet inhibition compared with double-dose clopidogrel (600-mg loading dose followed by 150-mg maintenance dose) in DM patients with coronary artery disease on long-term aspirin treatment using multiple pharmacodynamic measures (Figure 2).[123–125] These observations overall suggest that greater clinical benefit is derived by achieving higher platelet inhibition in DM patients. The clinical efficacy of prasugrel in medically managed patients with unstable angina/NSTEMI is being evaluated in the ongoing Targeted Platelet Inhibition to Clarify the Optimal Strategy to Medically Manage Acute Coronary Syndromes (TRILOGY-ACS; NCT00699998) trial.

GP IIb/IIIa Inhibitors

Currently, 3 different GP IIb/IIIa inhibitors (abciximab, eptifibatide, and tirofiban) are approved for clinical use, all of them administered intravenously. The efficacy of these agents correlates directly with the severity and the risk of the ACS, being questionable in low- to moderate-risk patients or in those in whom a conservative approach is chosen.[126] These agents can be administered only intravenously; thus, despite their potent inhibitory effects on platelets, their utility is limited to the acute phase of treatment.

A meta-analysis of 6 large trials evaluating the effect of GP IIb/IIIa inhibitors in ACS patients observed a 22% reduction of mortality at 30 days in DM patients (n=6458) associated

with the use of GP IIb/IIIa blockers compared with those not receiving these agents (4.6% versus 6.2%; $P=0.007$), whereas non-DM patients (n=23 072) had no benefit in survival.[5] Of note, the benefit among DM patients was greater in those patients (n=1279) who underwent PCI during the index hospitalization (1.2% versus 4%; $P=0.002$). However, the fact that these trials did not use regimens of high clopidogrel loading dose, which are associated with more potent antiplatelet effects and have become the standard of care in clinical practice, but instead used ticlopidine or standard-dose clopidogrel has led to questions about validity of these data in today's practice. In fact, a more recent study, the Intracoronary Stenting and Antithrombotic Regimen: Is Abciximab a Superior Way to Eliminate Elevated Thrombotic Risk in Diabetics (ISAR-SWEET) trial did not show a benefit of abciximab over placebo on the 1-year risk of death and MI in DM patients (n=701) undergoing elective PCI after pretreatment with a 600-mg loading dose of clopidogrel at least 2 hours before the procedure.[127] Therefore, these results do not support the routine use of GP IIb/IIIa inhibitors in elective PCI. Conversely, the Intracoronary Stenting and Antithrombotic: Regimen Rapid Early Action for Coronary Treatment 2 (ISAR-REACT 2) trial showed a significant reduction in the risk of adverse events with abciximab treatment compared with placebo in patients with high-risk ACS undergoing PCI after pretreatment with 600 mg clopidogrel.[128] This benefit, however, was restricted to patients with elevated troponin levels and was observed across all subgroups, including DM patients. These results support the use of GP IIb/IIIa receptor antagonists in high-risk ACS patients, in particular those with DM, as recommended in current guidelines.[80]

Few studies have evaluated the use of GP IIb/IIIa inhibitors in DM patients with STEMI undergoing PCI. In a small-scale study performed before the clopidogrel era, abciximab was associated with lower mortality and reinfarction rates across the DM subgroup (n=54) compared with placebo.[129] The Controlled Abciximab and Device Investigation to Lower Late Angioplasty Combinations (CADILLAC) trial did not find a benefit in terms of death, reinfarction, or stroke with the use of abciximab in low-risk DM patients (n=346) with acute MI treated with balloon angioplasty or stenting.[10] However, a recent meta-regression of randomized trials evaluating the effect of GP IIb/IIIa inhibitors in STEMI patients treated with primary PCI showed a benefit in terms of death, but not reinfarction, associated with the use of these agents in high-risk patients, including those with DM.[130]

The major limitation associated with GP IIb/IIIa inhibitors is the increased risk of bleeding. Of note, bleeding has an important impact on prognosis after an ACS, including mortality.[131,132] Bivalirudin, a direct thrombin inhibitor, may be a valid alternative because it has been shown to provide similar protection from ischemic events with lower major bleeding rates compared with GP IIb/IIIa inhibitors, as observed in the Acute Catheterization and Urgent Intervention Triage Strategy (ACUITY) trial.[133] In a subgroup analysis performed in the DM cohort (n=3852), bivalirudin monotherapy was associated with a similar rate of composite ischemia (death, MI, or unplanned ischemic revascularization) compared with GP IIb/IIIa plus heparin (7.9% versus 8.9%; $P=0.39$) and a lower rate of major bleedings (3.7% versus 7.1%; $P<0.001$), resulting in fewer net adverse clinical outcomes (10.9% versus 13.8%; $P=0.02$).[134] This reduction of ischemic risk is of special importance because DM is a predictor of bleeding complications in patients with ACS and/or PCI.[135]

Limitations of Current Treatment Strategies: Antiplatelet Drug Resistance and DM

Numerous reports have described a possible relationship between variability in response to antiplatelet therapy and clinical outcomes, thus suggesting that "resistance" to oral antiplatelet drugs may play a role in the risk of adverse cardiovascular events.[136–138] Because the risk of recurrent ischemic events is elevated in DM patients, there has been particular interest in understanding antiplatelet drug response in these high-risk subjects. In "resistant" patients, the antiplatelet drug fails to block its specific platelet target (eg, aspirin to block the COX-1 enzyme and clopidogrel to block the $P2Y_{12}$ receptor).[136] Therefore, it is a laboratory finding and should not be confused with "treatment failure," which means the recurrence of ischemic events despite treatment.[137,138]

Several clinical studies have shown an association between aspirin resistance and a higher risk of recurrent ischemic events.[139,140] However, the prevalence of aspirin resistance is widely variable among reported studies. These disparate findings are due mainly to differences in test used, definition of resistance, aspirin dose, and patient population considered. When COX-1–specific tests (eg, determination of serum or urine thromboxane and assays with arachidonic acid as agonist) are used, aspirin resistance is an infrequent phenomenon (<5% of patients).[141,142] The fact that the prevalence of aspirin resistance is higher when assays that are not specific to COX-1 signaling are used suggests that these results not only are derived from COX-1 degree of inhibition but also reflect aspirin-induced COX-1–independent effects.[136] The main cause of aspirin resistance, when assessed by COX-1–specific tests, is poor patient compliance.[137] Population selection is another factor that contributes to inadequate aspirin effects. DM patients have very high rates of inadequate response to aspirin when assessed by non–COX-1–specific methods[13,143]; in these patients, increasing aspirin dose has been suggested to overcome resistance.[144] This is in line with findings from a subanalysis of the Aspirin-Induced Platelet Effect (ASPECT) study, which compared the pharmacodynamic effect of different doses of aspirin in patients with and without DM and showed a higher percentage of aspirin resistance in the DM subgroup with the lower dose (81 mg daily). Interestingly, increasing aspirin dose (162 and 325 mg daily) significantly reduced platelet reactivity in patients with DM, resulting in similar rates of aspirin resistance in both groups.[142]

To date, there are no published studies specifically designed to assess the clinical efficacy of aspirin and the implications of aspirin resistance in DM patients with ACS. In addition, few studies have investigated the mechanisms of aspirin resistance that are inherent in patients with DM. Hyperglycemia has been proposed to play a role because an

interaction between glycation and acetylation has consistently been observed.[145] In addition, TXA_2 synthesis is increased in DM patients, and tight metabolic control may lead to a reduction in TXA_2 concentrations.[146] This may be related to the reduced response to aspirin observed in DM patients with poor metabolic control.[147] Elevated TXA_2 synthesis may also be attributed to increased platelet turnover in DM; thus, although aspirin may irreversibly inhibit COX-1, the introduction into the systemic circulation of newly generated platelets not exposed to aspirin continues to generate TXA_2, which may allow TP receptor activation despite COX-1 inhibition.[77] TP receptor activation has led to interest in developing pharmacological agents that can also block TP receptors. Picotamide is an inhibitor of both TXA_2 synthase and TP receptors, being able to block the effect of TXA_2 generated through COX-1 escape mechanisms, which may represent a pathway involved in inadequate aspirin-induced effect in DM patients. The Drug Evaluation in Atherosclerotic Vascular Disease in Diabetics (DAVID) trial randomized DM patients with peripheral artery disease (n=1209) to receive either picotamide (600 mg twice daily) or aspirin (320 mg once daily plus placebo once daily) for 24 months. In this trial, the cumulative incidence of the 2-year overall mortality (primary end point) was significantly lower among patients treated with picotamide compared with those receiving aspirin (3.0% versus 5.5%; $P=0.0474$). No statistical difference was observed in the secondary combined end point of mortality and morbidity (death and nonfatal vascular events, including MI, ischemic stroke, and major amputation).[148] Other novel agents targeting the TXA_2 pathway, including ridogrel (a combined TXA_2 synthase inhibitor and TP receptor blocker), ramatroban (a TP receptor inhibitor), NCX 4016 (an NO-releasing aspirin derivative), and Si8886/terutroban (a TP receptor inhibitor), have been evaluated. Some of them have been compared with aspirin in different settings with variable success and might be of future interest for specifically targeting DM platelets.[149–152]

Clopidogrel therapy, in addition to aspirin, has shown an undisputed clinical benefit in patients with ACS/PCI (the Table). However, a substantial number of recurrent cardiovascular events continue to occur. Accumulating evidence shows that variability in individual response is involved in this limited efficacy, even among DM patients.[138,153,154] The prevalence of clopidogrel low responsiveness reported in the literature varies considerably and is related to differences in definitions, type of test used, dose of clopidogrel, and patient population studied. Genetic, cellular, and clinical mechanisms have been observed to contribute to inadequate clopidogrel responsiveness.[138,153] The presence of DM is an important clinical factor that contributes to decreased clopidogrel-induced effects; a lower response to clopidogrel has repeatedly been shown in DM patients compared with non-DM patients in both the immediate and maintenance phases of therapy.[13,16,17] Among patients with DM, those at the most advanced stage who require insulin therapy have the highest degree of platelet reactivity while on dual antiplatelet therapy.[155] DM is also a risk factor for developing chronic kidney disease, which may affect platelet function and response to antiplatelet agents. The presence of moderate or severe chronic kidney disease is associated with impaired response to clopidogrel among DM patients on maintenance dual antiplatelet therapy.[156] This is in line with the findings of a recently reported posthoc analysis of the CHARISMA trial suggesting that clopidogrel use might be harmful in patients with diabetic nephropathy.[157] Overall, these findings contribute to an explanation of why DM is associated with a higher risk of recurrent ischemic events in patients with ACS[7] and is a strong predictor of stent thrombosis.[158–160]

Numerous mechanisms may play a role in the inadequate clopidogrel response observed in DM patients. Several small-scale in vitro or ex vivo studies have reported the following factors as possible causes of the impaired clopidogrel response present in DM patients: lack of response to insulin in platelets,[75] alterations in calcium metabolism,[42,65] upregulation of $P2Y_{12}$ receptor signaling,[75] increased exposure to ADP,[161] and increased platelet turnover.[79]

Future Directions

The persistence of high platelet reactivity in DM patients despite the use of standard recommended antiplatelet treatment regimens has raised interest in identifying strategies able to optimize platelet inhibitory effects in these high-risk subjects (Figure 2). The OPTIMUS study evaluated the effect of a 150-mg maintenance dose of clopidogrel versus standard dose of clopidogrel (75 mg) in a cohort of type 2 DM patients with coronary artery disease and high platelet reactivity while in their maintenance phase of clopidogrel therapy. Use of the high maintenance dose was associated with a marked improvement in platelet inhibition, although a significant number of patients remained with elevated platelet reactivity.[124] The efficacy and safety of tailored treatment with high clopidogrel maintenance dose in patients with inadequate response to standard clopidogrel dose are being evaluated in the ongoing Gauging Responsiveness With a VerifyNow Assay: Impact on Thrombosis and Safety (GRAVITAS; NCT00645918) trial, which will comprise a considerable number of DM patients.

Although modifying doses of currently approved drugs represents an option to optimize platelet inhibition in DM patients, the future will likely include newer agents, many of which are currently under clinical development. They may include agents that block multiple pathways involved in platelet adhesion, activation, and aggregation (Figure 3).[162] Among these agents, encouraging results have emerged from clinical trials evaluating novel and more potent $P2Y_{12}$ receptor inhibitors, which represent attractive treatment alternatives in high-risk patients such as those with DM (Figure 4).

Ticagrelor, a cyclopentyltriazolopyrimidine, is an orally administered, direct, reversible $P2Y_{12}$ inhibitor that has recently completed phase III clinical testing.[164] Ticagrelor is a direct-acting drug with no need for hepatic biotransformation into an active metabolite, which is an advantage over thienopyridines. In addition, ticagrelor achieves higher inhibition of platelet aggregation than clopidogrel in ACS patients.[165] The Platelet Inhibition and Patient Outcomes (PLATO) trial evaluated the benefit of ticagrelor (180-mg loading dose followed by 90 mg twice daily) compared with clopidogrel (300- to 600-mg loading dose followed by 75 mg daily) in

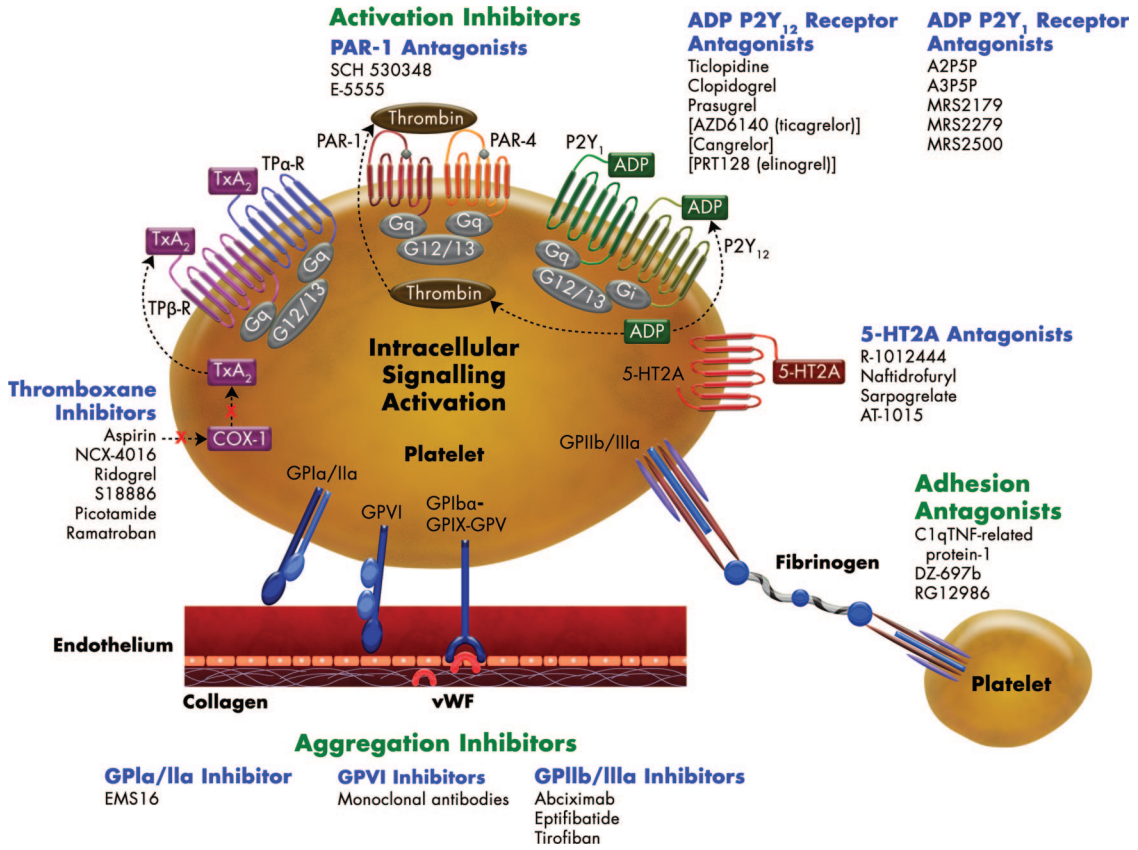


Figure 3. Currently available and novel antiplatelet agents under development. Platelet adhesion to the endothelium occurs at sites of vascular injury through the binding of GP receptors to exposed extracellular matrix proteins (collagen and von Willebrand factor [vWF]). Platelet activation occurs via intracellular signaling processes and causes the production and release of multiple agonists, including TXA_2 and ADP, and local production of thrombin. These factors bind to their respective G protein–coupled receptors, mediating paracrine and autocrine mechanisms. In addition, they potentiate each other's actions (eg, $P2Y_{12}$ signaling modulates thrombin generation). The major platelet integrin GP IIb/IIIa mediates the final common step of platelet activation by undergoing a conformational shape change and binding fibrinogen and von Willebrand factor, leading to platelet aggregation. The net result of these interactions is thrombus formation, resulting platelet/platelet interactions with fibrin. Current and emerging therapies inhibiting platelet receptors, integrins, and proteins involved in this process include thromboxane inhibitors, ADP receptor antagonists, GP IIb/IIIa inhibitors, and the novel protease-activated receptor (PAR) antagonists and adhesion antagonists. Reversible-acting agents are indicated by brackets. Reproduced with permission from Angiolillo DJ, Capodanno D, Goto S. Platelet thrombin receptor antagonism and atherothrombosis. *Eur Heart J*. 2010;31:17–28.[162] 5-HT2A indicates 5-hydroxytryptamine 2A receptor.

preventing cardiovascular events in ACS patients (n=18 624) with or without ST-segment elevation. The rate of the primary end point (death resulting from vascular causes, MI, or stroke) at 12 months was significantly decreased in the ticagrelor arm (10.2% versus 12.3%; HR=0.84; P=0.0001), as were the rates of cardiovascular death and stent thrombosis in the subgroup of PCI patients. Importantly, ticagrelor was not associated with an increase in protocol-defined major bleeding, although a higher rate of major bleeding not related to coronary artery bypass grafting was observed (4.5% versus 3.8%; HR=1.19; P=0.03). Side effects occurring more frequently with ticagrelor included dyspnea, ventricular pauses, and an increase in creatinine and uric acid levels.[163] In patients with DM (n=4662), the reduction in the primary composite endpoint (HR=0.88; 95% CI, 0.76 to 1.03), all-cause mortality (HR=0.82; 95% CI, 0.66 to 1.01), and stent thrombosis (HR=0.65; 95% CI, 0.36 to 1.17) with no increase in major bleeding (HR=0.95; 95% CI, 0.81 to 1.12) with ticagrelor was consistent with the overall cohort and without significant diabetes status-by-treatment interactions.[163a] There was no heterogeneity between patients with or without insulin therapy. Further, ticagrelor reduced the primary endpoint (HR=0.80; 95% CI, 0.70 to 0.91), all-cause mortality (HR=0.78; 95% CI, 0.65 to 0.93), and stent thrombosis (HR=0.62; 95% CI, 0.39 to 1.00) in patients with HbA1c above the median with similar bleeding rates (HR=0.98; 95% CI, 0.86 to 1.12). Ticagrelor has been recently approved in Europe, but it is not yet approved for clinical use by the FDA.

Cangrelor, an intravenous ATP analog, is a direct-acting and reversible $P2Y_{12}$ receptor inhibitor.[164] Phase II trials showed cangrelor to be a potent antiplatelet agent; it achieves a great degree of platelet inhibition (>90%) with extremely rapid onset and offset of action and has a relatively safe profile.[166] The results from the Cangrelor Versus Standard Therapy to Achieve Optimal Management of Platelet Inhibition (CHAMPION) program, which included CHAMPION-PCI (which randomized 8716 ACS patients to receive cangrelor or 600 mg of clopidogrel administered before PCI) and the CHAMPION-PLATFORM (which randomized 5362 patients not treated with clopidogrel to receive either cangrelor or

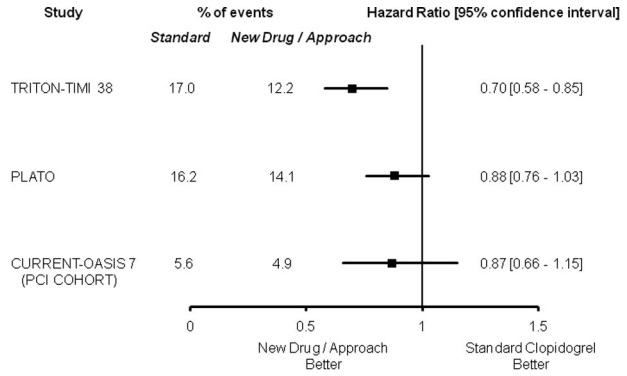


Figure 4. Efficacy in reducing adverse outcomes of new drugs and approaches tested in large-scale clinical trials in diabetes mellitus (DM) patients. Novel strategies to enhance platelet inhibition with the aim of improving outcomes (composite of cardiovascular death, myocardial infarction [MI], or stroke) include the use of prasugrel, ticagrelor, and high-dose clopidogrel. The data presented represent the composite of cardiovascular death, MI, or stroke in the DM cohort of these studies. The TRITON-TIMI 38 study[122] compared prasugrel (60-mg loading dose followed by a 10-mg maintenance dose) with standard clopidogrel therapy (300-mg loading dose followed by 75-mg daily maintenance dose) in patients with moderate- to high-risk acute coronary syndromes undergoing percutaneous coronary intervention (PCI) with up to 15 months of follow-up. The PLATO[163a] trial compared ticagrelor (180-mg loading dose followed by 90 mg twice daily) with clopidogrel (300- to 600-mg loading dose followed by 75 mg daily) with up to 12 months of follow-up. The CURRENT-OASIS 7[99a] trial evaluated 30-day outcomes comparing high (600-mg loading dose and then 150 mg once a day for 7 followed by 75 mg daily) and standard (300-mg loading dose followed by 75 mg daily) clopidogrel dosing in acute coronary syndromes ACS patients scheduled to undergo angiography within 72 hours of hospital arrival (results were obtained in the cohort of patients undergoing PCI).

placebo at the time of PCI, followed by 600 mg of clopidogrel) trials, have been recently published.[167,168] Both trials failed to show superiority in reducing the primary end point (composite of death from any cause, MI, or ischemia-driven revascularization at 48 hours) of cangrelor over clopidogrel (7.5% versus 7.1%; odds ratio=1.05; 95% CI, 0.88 to 1.24; P=0.56) in CHAMPION-PCI and over placebo (7.0% versus 8.0%; odds ratio=0.87; 95% CI, 0.71 to 1.07; P=0.17) in CHAMPION-PLATFORM. A subgroup analysis (n=2702) performed in CHAMPION-PCI showed that results were consistent among the cohort of DM patients (odds ratio=1.08; 95% CI, 0.80 to 1.46).

Despite the use of dual antiplatelet therapy with aspirin and a $P2Y_{12}$ blocker in the ACS setting as described previously, patients, particularly those with DM, may continue to have recurrent events. The reason may be that only 2 signaling pathways, COX-1 and $P2Y_{12}$, are blocked, leaving multiple other signaling pathways, many known to be upregulated in DM patients, uninhibited. Therefore, future strategies may include the use of antiplatelet agents that block pathways other than COX-1 and $P2Y_{12}$. Several drugs have been suggested for use as an adjunctive treatment to aspirin and $P2Y_{12}$ inhibitors. Agents that have the potential to be part of such "triple therapy" strategies include cilostazol, protease-activated receptor-1 antagonists, and new oral anticoagulants.

Cilostazol, a phosphodiesterase III inhibitor that increases intraplatelet cAMP concentration, in addition to standard dual antiplatelet therapy may be considered in the maintenance phase of therapy. The benefit of this triple antiplatelet treatment regimen has consistently been observed in patients undergoing PCI, mainly as a reduction in the rates of target lesion revascularization and even in stent thrombosis.[119,170] This benefit in ischemic outcomes, which is not accompanied by an increased risk of bleeding, is greater in patients with DM.[171,172] The latter is in line with the findings of the OPTIMUS-2 study, in which adjunctive treatment with cilostazol markedly increased the inhibition of platelet $P2Y_{12}$ signaling in DM patients on dual antiplatelet therapy.[125] Recently, the efficacy of cilostazol in the setting of ACS was evaluated in a clinical trial that randomized ACS patients (n=1212) to either standard dual-antiplatelet treatment with aspirin and clopidogrel or triple antiplatelet therapy with the addition of cilostazol for 6 months after successful PCI. In this study, triple antiplatelet treatment was associated with a significantly lower incidence (10.3% versus 15.1%; HR=0.65; P=0.011) of the primary end point (composite of cardiac death, nonfatal MI, stroke, or target vessel revascularization at 1 year after randomization), and importantly, no significant differences were found in the risks for major and minor bleeding.[173] In this study, the DM subgroup (n=263) had a particular benefit with triple therapy (9.9% versus 18.9%; HR=0.47; 95% CI, 0.23 to 0.96). The use of cilostazol, however, is limited by the high frequency of side effects (eg, headache, palpitations, and gastrointestinal disturbances) that often lead to withdrawal.

Thrombin is the link between plasmatic and cellular components of the thrombotic process because it plays a role in the coagulation cascade and is a potent agonist of platelet aggregation. Of note, thrombin generation processes are enhanced in patients with DM.[174] To date, 2 oral thrombin receptor antagonists that block the platelet protease-activated receptor-1 subtype, Vorapaxar (SCH530348) and atopaxar (E5555), are under advanced clinical development.[162] Atopaxar is still in an early stage of development; vorapaxar was recently compared with placebo in a large phase II safety and dose-ranging trial performed in patients (n=1030) undergoing nonurgent PCI or coronary angiography with planned PCI. Importantly, vorapaxar showed an excellent safety profile; concomitant administration with aspirin and clopidogrel was not associated with any significant increase in bleeding across all doses tested.[175] Currently, 2 large-scale phase III trials are evaluating the efficacy and safety of vorapaxar: the Trial to Assess the Effects of vorapaxar in Preventing Heart Attack and Stroke in Patients With Atherosclerosis (TRA 2°P; NCT00526474) in patients with atherosclerosis and the Trial to Assess the Effects of vorapaxar in Preventing Heart Attack and Stroke in Patients With Acute Coronary Syndrome (TRACER; NCT00527943) in ACS patients. Results from these trials in which DM patients will be highly represented will provide important insights into the future utility of these new agents.

Atherothrombotic complications are the result not only of platelet reactivity but also of dysregulation of coagulation processes. Importantly, DM patients are also characterized by several coagulation abnormalities, including increased plasma coagulation factors (eg, factor VII and thrombin) and

lesion-based coagulants (eg, tissue factor), decreased endogenous anticoagulants (eg, protein C and thrombomodulin), and increased production of plasminogen activator inhibitor-1, a fibrinolysis inhibitor.[11] These procoagulant abnormalities, coupled with the platelet hyperreactivity discussed previously, enhance the thrombotic risk of DM patients. Several new oral anticoagulants, including anti–factor IIa (eg, dabigatran) and anti–factor Xa (eg, rivaroxaban, apixaban), are currently in different stages of clinical development.[176] In addition to being studied as an alternative to warfarin in settings such as atrial fibrillation or venous thrombosis disorders,[177] many of these newer oral anticoagulant agents are currently being tested for long-term use in ACS populations as an adjunct to dual antiplatelet therapy, in which DM patients represent a cohort of particular interest.

Conclusions

DM patients have an increased atherothrombotic risk and elevated rates of recurrent ischemic events. This may be attributed in part to the abnormalities in platelet function that characterize this patient population and result in increased platelet reactivity. These findings underscore the importance of platelet-inhibiting drugs in DM patients. Although currently approved antiplatelet treatment strategies have proven successful in improving outcomes in ACS, DM patients continue to experience high rates of adverse cardiovascular events. The high prevalence among DM patients of suboptimal response to currently used oral antiplatelet agents may contribute to these impaired outcomes. Therefore, more potent antithrombotic treatment strategies are warranted in DM patients. The large number of novel antithrombotic agents, including antiplatelet and anticoagulant drugs, that are currently under advanced clinical development may represent important treatment alternatives in the near future to tackle the thrombotic burden of patients with DM.

Acknowledgments

We are grateful to Mariana Muñoz, MD, for her assistance with figure preparation.

Disclosures

Dr Angiolillo reports receiving honoraria for lectures from Bristol Myers Squibb, Sanofi-Aventis, Eli Lilly Co, and Daiichi Sankyo, Inc; consulting fees from Bristol Myers Squibb, Sanofi-Aventis, Eli Lilly Co, Daiichi Sankyo, Inc, The Medicines Company, Portola, Novartis, Medicure, Accumetrics, Arena Pharmaceuticals, and AstraZeneca; and research grants from Bristol Myers Squibb, Sanofi-Aventis, GlaxoSmithKline, Otsuka, Eli Lilly Co, Daiichi Sankyo, Inc, The Medicines Company, Portola, Accumetrics, Schering-Plough, AstraZeneca, and Eisai. Dr Ferreiro reports no conflicts.

References

1. Webster MW, Scott RS. What cardiologists need to know about diabetes. *Lancet.* 1997;350(suppl 1):SI23–SI28.
2. Haffner SM, Lehto S, Rönnemaa T, Pyörälä K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med*. 1998;339:229–234.
3. Lüscher TF, Creager MA, Beckman JA, Cosentino F. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part II. *Circulation*. 2003;108:1655–1661.
4. Malmberg K, Yusuf S, Gerstein HC, Brown J, Zhao F, Hunt D, Piegas L, Calvin J, Keltai M, Budaj A. Impact of diabetes on long-term prognosis in patients with unstable angina and non-Q-wave myocardial infarction: results of the OASIS (Organization to Assess Strategies for Ischemic Syndromes) Registry. *Circulation*. 2000;102:1014–1019.
5. Roffi M, Chew DP, Mukherjee D, Bhatt DL, White JA, Heeschen C, Hamm CW, Moliterno DJ, Califf RM, White HD, Kleiman NS, Théroux P, Topol EJ. Platelet glycoprotein IIb/IIIa inhibitors reduce mortality in diabetic patients with non-ST-segment-elevation acute coronary syndromes. *Circulation*. 2001;104:2767–2771.
6. Brogan GX Jr, Peterson ED, Mulgund J, Bhatt DL, Ohman EM, Gibler WB, Pollack CV Jr, Farkouh ME, Roe MT. Treatment disparities in the care of patients with and without diabetes presenting with non-ST-segment elevation acute coronary syndromes. *Diabetes Care*. 2006; 29:9–14.
7. Yusuf S, Zhao F, Mehta SR, Chrolavicius S, Tognoni G, Fox KK; Clopidogrel in Unstable Angina to Prevent Recurrent Events Trial Investigators. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. *N Engl J Med.* 2001;345:494–502.
8. Mak KH, Moliterno DJ, Granger CB, Miller DP, White HD, Wilcox RG, Califf RM, Topol EJ. Influence of diabetes mellitus on clinical outcome in the thrombolytic era of acute myocardial infarction: GUSTO-I Investigators: Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries. *J Am Coll Cardiol*. 1997;30: 171–179.
9. Roffi M, Topol EJ. Percutaneous coronary intervention in diabetic patients with non-ST-segment elevation acute coronary syndromes. *Eur Heart J*. 2004;25:190–198.
10. Stuckey TD, Stone GW, Cox DA, Tcheng JE, Garcia E, Carroll J, Guagliumi G, Rutherford BD, Griffin JJ, Turco M, Lansky AJ, Mehran R, Fahy M, Brodie BR, Grines CL; CADILLAC Investigators. Impact of stenting and abciximab in patients with diabetes mellitus undergoing primary angioplasty in acute myocardial infarction (the CADILLAC trial). *Am J Cardiol.* 2005;95:1–7.
11. Creager MA, Lüscher TF, Cosentino F, Beckman JA. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part I. *Circulation*. 2003;108:1527–1532.
12. Stratmann B, Tschoepe D. Pathobiology and cell interactions of platelets in diabetes. *Diab Vasc Dis Res*. 2005;2:16–23.
13. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Ramírez C, Sabaté M, Jimenez-Quevedo P, Hernández R, Moreno R, Escaned J, Alfonso F, Bañuelos C, Costa MA, Bass TA, Macaya C. Platelet function profiles in patients with type 2 diabetes and coronary artery disease on combined aspirin and clopidogrel treatment. *Diabetes*. 2005;54:2430–2435.
14. Vinik AI, Erbas T, Park TS, Nolan R, Pittenger GL. Platelet dysfunction in type 2 diabetes. *Diabetes Care*. 2001;24:1476–1485.
15. Ferroni P, Basili S, Falco A, Davì G. Platelet activation in type 2 diabetes mellitus. *J Thromb Haemost*. 2004;2:1282–1291.
16. Geisler T, Anders N, Paterok M, Langer H, Stellos K, Lindemann S, Herdeg C, May AE, Gawaz M. Platelet response to clopidogrel is attenuated in diabetic patients undergoing coronary stent implantation. *Diabetes Care*. 2007;30:372–374.
17. Serebruany V, Pokov I, Kuliczkowski W, Chesebro J, Badimon J. Baseline platelet activity and response after clopidogrel in 257 diabetics among 822 patients with coronary artery disease. *Thromb Haemost*. 2008;100:76–82.
18. Angiolillo DJ. Antiplatelet therapy in diabetes: efficacy and limitations of current treatment strategies and future directions. *Diabetes Care*. 2009;32:531–540.
19. Fuster V, Moreno PR, Fayad ZA, Corti R, Badimon JJ. Atherothrombosis and high-risk plaque, part I: evolving concepts. *J Am Coll Cardiol*. 2005;46:937–954.
20. Davì G, Patrono C. Platelet activation and atherothrombosis. *N Engl J Med*. 2007;357:2482–2494.
21. Jennings LK. Role of platelets in atherothrombosis. *Am J Cardiol.* 2009;103(suppl):4A–10A.
22. Langer HF, Gawaz M. Platelet-vessel wall interactions in atherosclerotic disease. *Thromb Haemost*. 2008;99:480–486.
23. Schneider DJ. Factors contributing to increased platelet reactivity in people with diabetes. *Diabetes Care*. 2009;32:525–527.
24. Vaidyula VR, Boden G, Rao AK. Platelet and monocyte activation by hyperglycemia and hyperinsulinemia in healthy subjects. *Platelets*. 2006;17:577–585.
25. Yngen M, Norhammar A, Hjemdahl P, Wallén NH. Effects of improved metabolic control on platelet reactivity in patients with type 2 diabetes

mellitus following coronary angioplasty. *Diab Vasc Dis Res*. 2006;3: 52–56.
26. Winocour PD, Watala C, Perry DW, Kinlough-Rathbone RL. Decreased platelet membrane fluidity due to glycation or acetylation of membrane proteins. *Thromb Haemost*. 1992;68:577–582.
27. Watala C, Golański J, Boncler MA, Pietrucha T, Gwoździński K. Membrane lipid fluidity of blood platelets: a common denominator that underlies the opposing actions of various agents that affect platelet activation in whole blood. *Platelets*. 1998;9:315–327.
28. Keating FK, Sobel BE, Schneider DJ. Effects of increased concentrations of glucose on platelet reactivity in healthy subjects and in patients with and without diabetes mellitus. *Am J Cardiol*. 2003;92: 1362–1365.
29. Assert R, Scherk G, Bumbure A, Pirags V, Schatz H, Pfeiffer AF. Regulation of protein kinase C by short term hyperglycaemia in human platelets in vivo and in vitro. *Diabetologia*. 2001;44:188–195.
30. Malmberg K. Prospective randomised study of intensive insulin treatment on long term survival after acute myocardial infarction in patients with diabetes mellitus: DIGAMI (Diabetes Mellitus, Insulin Glucose Infusion in Acute Myocardial Infarction) Study Group. *BMJ*. 1997;314:1512–1515.
31. Malmberg K, Rydén L, Wedel H, Birkeland K, Bootsma A, Dickstein K, Efendic S, Fisher M, Hamsten A, Herlitz J, Hildebrandt P, MacLeod K, Laakso M, Torp-Pedersen C, Waldenström A; DIGAMI 2 Investigators. Intense metabolic control by means of insulin in patients with diabetes mellitus and acute myocardial infarction (DIGAMI 2): effects on mortality and morbidity. *Eur Heart J*. 2005;26:650–661.
32. Gerstein HC, Miller ME, Byington RP, Goff DC Jr, Bigger JT, Buse JB, Cushman WC, Genuth S, Ismail-Beigi F, Grimm RH Jr, Probstfield JL, Simons-Morton DG, Friedewald WT. Effects of intensive glucose lowering in type 2 diabetes. *N Engl J Med*. 2008;358:2545–2559.
33. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2010;33:S62–S69.
34. Randriamboavonjy V, Fleming I. Insulin, insulin resistance, and platelet signaling in diabetes. *Diabetes Care*. 2009;32:528–530.
35. Hajek AS, Joist JH. Platelet insulin receptor. *Methods Enzymol*. 1992; 215:398–403.
36. Hunter RW, Hers I. Insulin/IGF-1 hybrid receptor expression on human platelets: consequences for the effect of insulin on platelet function. *J Thromb Haemost*. 2009;7:2123–2130.
37. Kahn NN. Insulin-induced expression of prostacyclin receptors on platelets is mediated through ADP-ribosylation of Gi alpha protein. *Life Sci*. 1998;63:2031–2038.
38. Hers I. Insulin-like growth factor-1 potentiates platelet activation via the IRS/PI3Kalpha pathway. *Blood*. 2007;110:4243–4252.
39. Westerbacka J, Yki-Järvinen H, Turpeinen A, Rissanen A, Vehkavaara S, Syrjälä M, Lassila R. Inhibition of platelet-collagen interaction: an in vivo action of insulin abolished by insulin resistance in obesity. *Arterioscler Thromb Vasc Biol*. 2002;22:167–172.
40. Ishida M, Ishida T, Ono N, Matsuura H, Watanabe M, Kajiyama G, Kambe M, Oshima T. Effects of insulin on calcium metabolism and platelet aggregation. *Hypertension*. 1996;28:209–212.
41. Algenstaedt P, Antonetti DA, Yaffe MB, Kahn CR. Insulin receptor substrate proteins create a link between the tyrosine phosphorylation cascade and the Ca^{2+}-ATPases in muscle and heart. *J Biol Chem*. 1997;272:23696–23702.
42. Ferreira IA, Eybrechts KL, Mocking AI, Kroner C, Akkerman JW. IRS-1 mediates inhibition of Ca^{2+} mobilization by insulin via the inhibitory G-protein Gi. *J Biol Chem*. 2004;279:3254–3264.
43. Betteridge DJ, El Tahir KE, Reckless JP, Williams KI. Platelets from diabetic subjects show diminished sensitivity to prostacyclin. *Eur J Clin Invest*. 1982;12:395–398.
44. Anfossi G, Mularoni EM, Burzacca S, Ponziani MC, Massucco P, Mattiello L, Cavalot F, Trovati M. Platelet resistance to nitrates in obesity and obese NIDDM, and normal platelet sensitivity to both insulin and nitrates in lean NIDDM. *Diabetes Care*. 1998;21:121–126.
45. Randriamboavonjy V, Pistrosch F, Bölck B, Schwinger RH, Dixit M, Badenhoop K, Cohen RA, Busse R, Fleming I. Platelet sarcoplasmic endoplasmic reticulum Ca^{2+}-ATPase and mu-calpain activity are altered in type 2 diabetes mellitus and restored by rosiglitazone. *Circulation*. 2008;117:52–60.
46. Sidhu JS, Cowan D, Tooze JA, Kaski JC. Peroxisome proliferator-activated receptor-gamma agonist rosiglitazone reduces circulating platelet activity in patients without diabetes mellitus who have coronary artery disease. *Am Heart J*. 2004;147:1032–1037.
47. Nissen SE, Nicholls SJ, Wolski K, Nesto R, Kupfer S, Perez A, Jure H, De Larochellière R, Staniloae CS, Mavromatis K, Saw J, Hu B, Lincoff AM, Tuzcu EM; PERISCOPE Investigators. Comparison of pioglitazone vs glimepiride on progression of coronary atherosclerosis in patients with type 2 diabetes: the PERISCOPE randomized controlled trial. *JAMA*. 2008;299:1561–1573.
48. McGuire DK, Newby LK, Bhapkar MV, Moliterno DJ, Hochman JS, Klein WW, Weaver WD, Pfisterer M, Corbalán R, Dellborg M, Granger CB, Van De Werf F, Topol EJ, Califf RM; SYMPHONY and II SYMPHONY Investigators. Association of diabetes mellitus and glycemic control strategies with clinical outcomes after acute coronary syndromes. *Am Heart J*. 2004;147:246–252.
49. Muscari A, De Pascalis S, Cenni A, Ludovico C, Castaldini N, Antonelli S, Bianchi G, Magalotti D, Zoli M. Determinants of mean platelet volume (MPV) in an elderly population: relevance of body fat, blood glucose and ischaemic electrocardiographic changes. *Thromb Haemost*. 2008;99:1079–1084.
50. Sugiyama C, Ishizawa M, Kajita K, Morita H, Uno Y, Matsubara K, Matsumoto M, Ikeda T, Ishizuka T. Platelet aggregation in obese and diabetic subjects: association with leptin level. *Platelets*. 2007;18: 128–134.
51. Scherrer U, Nussberger J, Torriani S, Waeber B, Darioli R, Hofstetter JR, Brunner HR. Effect of weight reduction in moderately overweight patients on recorded ambulatory blood pressure and free cytosolic platelet calcium. *Circulation*. 1991;83:552–558.
52. Anfossi G, Russo I, Trovati M. Platelet dysfunction in central obesity. *Nutr Metab Cardiovasc Dis*. 2009;19:440–449.
53. Schneider DJ, Hardison RM, Lopes N, Sobel BE, Brooks MM; Pro-Thrombosis Ancillary Study Group. Association between increased platelet P-selectin expression and obesity in patients with type 2 diabetes: a BARI 2D (Bypass Angioplasty Revascularization Investigation 2 Diabetes) substudy. *Diabetes Care*. 2009;32:944–949.
54. Murakami T, Horigome H, Tanaka K, Nakata Y, Ohkawara K, Katayama Y, Matsui A. Impact of weight reduction on production of platelet-derived microparticles and fibrinolytic parameters in obesity. *Thromb Res*. 2007;119:45–53.
55. Angiolillo DJ, Fernández-Ortiz A, Bernardo E, Barrera Ramírez C, Sabaté M, Fernandez C, Hernández-Antolín R, Escaned J, Alfonso F, Macaya C. Platelet aggregation according to body mass index in patients undergoing coronary stenting: should clopidogrel loading-dose be weight adjusted? *J Invasive Cardiol*. 2004;16:169–174.
56. Sibbing D, von Beckerath O, Schömig A, Kastrati A, von Beckerath N. Impact of body mass index on platelet aggregation after administration of a high loading dose of 600 mg of clopidogrel before percutaneous coronary intervention. *Am J Cardiol*. 2007;100:203–205.
57. de Man FH, Nieuwland R, van der Laarse A, Romijn F, Smelt AH, Gevers Leuven JA, Sturk A. Activated platelets in patients with severe hypertriglyceridemia: effects of triglyceride-lowering therapy. *Atherosclerosis*. 2000;152:407–414.
58. Pedreño J, Hurt-Camejo E, Wiklund O, Badimón L, Masana L. Platelet function in patients with familial hypertriglyceridemia: evidence that platelet reactivity is modulated by apolipoprotein E content of very-low-density lipoprotein particles. *Metabolism*. 2000;49:942–949.
59. Olufadi R, Byrne CD. Effects of VLDL and remnant particles on platelets. *Pathophysiol Haemost Thromb*. 2006;35:281–291.
60. Kuhn FE, Mohler ER, Satler LF, Reagan K, Lu DY, Rackley CE. Effects of high-density lipoprotein on acetylcholine-induced coronary vasoreactivity. *Am J Cardiol*. 1991;68:1425–1430.
61. Calkin AC, Drew BG, Ono A, Duffy SJ, Gordon MV, Schoenwaelder SM, Sviridov D, Cooper ME, Kingwell BA, Jackson SP. Reconstituted high-density lipoprotein attenuates platelet function in individuals with type 2 diabetes mellitus by promoting cholesterol efflux. *Circulation*. 2009;120:2095–2104.
62. Lim HS, Blann AD, Lip GY. Soluble CD40 ligand, soluble P-selectin, interleukin-6, and tissue factor in diabetes mellitus: relationships to cardiovascular disease and risk factor intervention. *Circulation*. 2004; 109:2524–2528.
63. Calverley DC, Hacker MR, Loda KA, Brass E, Buchanan TA, Tsao-Wei DD, Groshen S. Increased platelet Fc receptor expression as a potential contributing cause of platelet hypersensitivity to collagen in diabetes mellitus. *Br J Haematol*. 2003;121:139–142.
64. Belostocki K, Pricop L, Redecha PB, Aydin A, Leff L, Harrison MJ, Salmon JE. Infliximab treatment shifts the balance between stimulatory and inhibitory Fcgamma receptor type II isoforms on neutrophils in patients with rheumatoid arthritis. *Arthritis Rheum*. 2008;58:384–388.

65. Li Y, Woo V, Bose R. Platelet hyperactivity and abnormal Ca(2+) homeostasis in diabetes mellitus. *Am J Physiol Heart Circ Physiol*. 2001;280:H1480–H1489.
66. Dean WL, Chen D, Brandt PC, Vanaman TC. Regulation of platelet plasma membrane Ca^{2+}-ATPase by cAMP-dependent and tyrosine phosphorylation. *J Biol Chem*. 1997;272:15113–18119.
67. Schaeffer G, Wascher TC, Kostner GM, Graier WF. Alterations in platelet Ca^{2+} signalling in diabetic patients is due to increased formation of superoxide anions and reduced nitric oxide production. *Diabetologia*. 1999;42:167–176.
68. Ishii H, Umeda F, Hashimoto T, Nawata H. Increased intracellular calcium mobilization in platelets from patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia*. 1991;34:332–336.
69. Freedman JE. Oxidative stress and platelets. *Arterioscler Thromb Vasc Biol*. 2008;28:s11–s16.
70. Seghieri G, Di Simplicio P, Anichini R, Alviggi L, De Bellis A, Bennardini F, Franconi F. Platelet antioxidant enzymes in insulin-dependent diabetes mellitus. *Clin Chim Acta*. 2001;309:19–23.
71. Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, Giardino I, Brownlee M. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature*. 2000;404:787–790.
72. Schmidt AM, Yan SD, Wautier JL, Stern D. Activation of receptor for advanced glycation end products: a mechanism for chronic vascular dysfunction in diabetic vasculopathy and atherosclerosis. *Circ Res*. 1999;84:489–497.
73. Schäfer A, Bauersachs J. Endothelial dysfunction, impaired endogenous platelet inhibition and platelet activation in diabetes and atherosclerosis. *Curr Vasc Pharmacol*. 2008;6:52–60.
74. Kario K, Matsuo T, Kobayashi H, Matsuo M, Sakata T, Miyata T. Activation of tissue factor-induced coagulation and endothelial cell dysfunction in non-insulin-dependent diabetic patients with microalbuminuria. *Arterioscler Thromb Vasc Biol*. 1995;15:1114–1120.
75. Ferreira IA, Mocking AI, Feijge MA, Gorter G, van Haeften TW, Heemskerk JW, Akkerman JW. Platelet inhibition by insulin is absent in type 2 diabetes mellitus. *Arterioscler Thromb Vasc Biol*. 2006;26:417–422.
75a. Ueno M, Ferreiro JL, Tomasello SD, Capodanno D, Tello-Montoliu A, Kodali M, Seecheran N, Dharmashankar K, Alissa R, Capranzano P, Desai B, Charlton RK, Bass TA, Angiolillo DJ. Functional profile of the platelet P2Y12 receptor signalling pathway in patients with type 2 diabetes mellitus and coronary artery disease. *Thromb Haemost*. 2011 Jan 12;105(4). [Epub ahead of print].
76. Tschoepe D, Roesen P, Kaufmann L, Schauseil S, Kehrel B, Ostermann H, Gries FA. Evidence for abnormal platelet glycoprotein expression in diabetes mellitus. *Eur J Clin Invest*. 1990;20:166–170.
77. DiMinno G, Silver MJ, Cerbone AM, Murphy S. Trial of repeated low-dose aspirin in diabetic angiopathy. *Blood*. 1986;68:886–891.
78. Guthikonda S, Lev EI, Patel R, DeLao T, Bergeron AL, Dong JF, Kleiman NS. Reticulated platelets and uninhibited COX-1 and COX-2 decrease the antiplatelet effects of aspirin. *J Thromb Haemost*. 2007;5:490–496.
79. Guthikonda S, Alviar CL, Vaduganathan M, Arikan M, Tellez A, DeLao T, Granada JF, Dong JF, Kleiman NS, Lev EI. Role of reticulated platelets and platelet size heterogeneity on platelet activity after dual antiplatelet therapy with aspirin and clopidogrel in patients with stable coronary artery disease. *J Am Coll Cardiol*. 2008;52:743–749.
80. Anderson JL, Adams CD, Antman EM, Bridges CR, Califf RM, Casey DE Jr, Chavey WE II, Fesmire FM, Hochman JS, Levin TN, Lincoff AM, Peterson ED, Theroux P, Wenger NK, Wright RS, Smith SC Jr, Jacobs AK, Halperin JL, Hunt SA, Krumholz HM, Kushner FG, Lytle BW, Nishimura R, Ornato JP, Page RL, Riegel B. American College of Cardiology; American Heart Association Task Force on Practice Guidelines (Writing Committee to Revise the 2002 Guidelines for the Management of Patients With Unstable Angina/Non ST-Elevation Myocardial Infarction); American College of Emergency Physicians; Society for Cardiovascular Angiography and Interventions; Society of Thoracic Surgeons; American Association of Cardiovascular and Pulmonary Rehabilitation; Society for Academic Emergency Medicine. ACC/AHA 2007 guidelines for the management of patients with unstable angina/non ST-elevation myocardial infarction: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Revise the 2002 Guidelines for the Management of Patients With Unstable Angina/Non ST-Elevation Myocardial Infarction): developed in collaboration with the American College of Emergency Physicians, the Society for Cardiovascular Angiography and Interventions, and the Society of Thoracic Surgeons: endorsed by the American Association of Cardiovascular and Pulmonary Rehabilitation and the Society for Academic Emergency Medicine. *Circulation*. 2007;116:e148–e304.
81. Antman EM, Anbe DT, Armstrong PW, Bates ER, Green LA, Hand M, Hochman JS, Krumholz HM, Kushner FG, Lamas GA, Mullany CJ, Ornato JP, Pearle DL, Sloan MA, Smith SC Jr, Alpert JS, Anderson JL, Faxon DP, Fuster V, Gibbons RJ, Gregoratos G, Halperin JL, Hiratzka LF, Hunt SA, Jacobs AK; American College of Cardiology; American Heart Association Task Force on Practice Guidelines; Canadian Cardiovascular Society. ACC/AHA guidelines for the management of patients with ST-elevation myocardial infarction: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Revise the 1999 Guidelines for the Management of Patients with Acute Myocardial Infarction). *Circulation*. 2004;110:e82–e292.
82. Patrono C, García Rodríguez LA, Landolfi R, Baigent C. Low-dose aspirin for the prevention of atherothrombosis. *N Engl J Med*. 2005;353:2373–2383.
83. Final report on the aspirin component of the ongoing Physicians' Health Study. Steering Committee of the Physicians' Health Study Research Group. *N Engl J Med*. 1989;321:129–135.
84. Aspirin effects on mortality and morbidity in patients with diabetes mellitus: Early Treatment Diabetic Retinopathy Study report 14: ETDRS Investigators. *JAMA*. 1992;268:1292–300.
85. Hansson L, Zanchetti A, Carruthers SG, Dahlöf B, Elmfeldt D, Julius S, Ménard J, Rahn KH, Wedel H, Westerling S. Effects of intensive blood-pressure lowering and low-dose aspirin in patients with hypertension: principal results of the Hypertension Optimal Treatment (HOT) randomised trial; HOT Study Group. *Lancet*. 1998;351:1755–1762.
86. de Gaetano G; Collaborative Group of the Primary Prevention Project. Low-dose aspirin and vitamin E in people at cardiovascular risk: a randomised trial in general practice. *Lancet*. 2001;357:89–95.
87. Ogawa H, Nakayama M, Morimoto T, Uemura S, Kanauchi M, Doi N, Jinnouchi H, Sugiyama S, Saito Y; Japanese Primary Prevention of Atherosclerosis With Aspirin for Diabetes (JPAD) Trial Investigators. Low-dose aspirin for primary prevention of atherosclerotic events in patients with type 2 diabetes: a randomized controlled trial. *JAMA*. 2008;300:2134–2141.
88. De Berardis G, Sacco M, Strippoli GF, Pellegrini F, Graziano G, Tognoni G, Nicolucci A. Aspirin for primary prevention of cardiovascular events in people with diabetes: meta-analysis of randomised controlled trials. *BMJ*. 2009;339:1238.
89. Antithrombotic Trialists' (ATT) Collaboration, Baigent C, Blackwell L, Collins R, Emberson J, Godwin J, Peto R, Buring J, Hennekens C, Kearney P, Meade T, Patrono C, Roncaglioni MC, Zanchetti A. Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. *Lancet*. 2009;373:1849–1860.
90. Colwell JA; American Diabetes Association. Aspirin therapy in diabetes. *Diabetes Care*. 2004;27(suppl 1):S72–S73.
91. King SB III, Smith SC Jr, Hirshfeld JW Jr, Jacobs AK, Morrison DA, Williams DO; 2005 Writing Committee Members, Feldman TE, Kern MJ, O'Neill WW, Schaff HV, Whitlow PL, Adams CD, Anderson JL, Buller CE, Creager MA, Ettinger SM, Halperin JL, Hunt SA, Krumholz HM, Kushner FG, Lytle BW, Nishimura R, Page RL, Riegel B, Tarkington LG, Yancy CW. 2007 Focused update of the ACC/AHA/SCAI 2005 guideline update for percutaneous coronary intervention: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines: 2007 Writing Group to Review New Evidence and Update the ACC/AHA/SCAI 2005 Guideline Update for Percutaneous Coronary Intervention, writing on behalf of the 2005 Writing Committee. *Circulation*. 2008;117:261–925.
92. Lewis HD Jr, Davis JW, Archibald DG, Steinke WE, Smitherman TC, Doherty JE III, Schnaper HW, LeWinter MM, Linares E, Pouget JM, Sabharwal SC, Chesler E, DeMots H. Protective effects of aspirin against acute myocardial infarction and death in men with unstable angina: results of a Veterans Administration Cooperative Study. *N Engl J Med*. 1983;309:396–403.
93. Théroux P, Ouimet H, McCans J, Latour JG, Joly P, Lévy G, Pelletier E, Juneau M, Stasiak J, deGuise P. Aspirin, heparin, or both to treat acute unstable angina. *N Engl J Med*. 1988;319:1105–1111.

94. RISC Group. Risk of myocardial infarction and death during treatment with low dose aspirin and intravenous heparin in men with unstable coronary artery disease. *Lancet*. 1990;336:827–830.
95. ISIS-2 (Second International Study of Infarct Survival) Collaborative Group. Randomised trial of intravenous streptokinase, oral aspirin, both, or neither among 17,187 cases of suspected acute myocardial infarction: ISIS-2. *Lancet*. 1988;2:349–360.
96. Roux S, Christeller S, Lüdin E. Effects of aspirin on coronary reocclusion and recurrent ischemia after thrombolysis: a meta-analysis. *J Am Coll Cardiol*. 1992;19:671–677.
97. Antiplatelet Trialists' Collaboration. Collaborative overview of randomised trials of antiplatelet therapy, I: prevention of death, myocardial infarction, and stroke by prolonged antiplatelet therapy in various categories of patients. *BMJ*. 1994;308:81–106.
98. Antithrombotic Trialists' Collaboration. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. BMJ. 2002;324:71–86.
99. CURRENT-OASIS 7 Investigators, Mehta SR, Bassand JP, Chrolavicius S, Diaz R, Eikelboom JW, Fox KA, Granger CB, Jolly S, Joyner CD, Rupprecht HJ, Widimsky P, Afzal R, Pogue J, Yusuf S. Dose comparisons of clopidogrel and aspirin in acute coronary syndromes. *N Engl J Med*. 2010;363:930–942.

99a. Mehta SR, Tanguay JF, Eikelboom JW, Jolly SS, Joyner CD, Granger CB, Faxon DP, Rupprecht HJ, Budaj A, Avezum A, Widimsky P, Steg PG, Bassand JP, Montalescot G, Macaya C, Di Pasquale G, Niemela K, Ajani AE, White HD, Chrolavicius S, Gao P, Fox KA, Yusuf S; CURRENT-OASIS 7 trial investigators. Double-dose versus standard-dose clopidogrel and high-dose versus low-dose aspirin in individuals undergoing percutaneous coronary intervention for acute coronary syndromes (CURRENT-OASIS 7): a randomised factorial trial. *Lancet*. 2010;376:1233–1243.

100. Storey RF, Newby LJ, Heptinstall S. Effects of P2Y(1) and P2Y(12) receptor antagonists on platelet aggregation induced by different agonists in human whole blood. *Platelets*. 2001;12:443–447.
101. Gachet C. ADP receptors of platelets and their inhibition. *Thromb Haemost*. 2001;86:222–232.
102. Turner NA, Moake JL, McIntire LV. Blockade of adenosine diphosphate receptors P2Y(12) and P2Y(1) is required to inhibit platelet aggregation in whole blood under flow. *Blood*. 2001;98:3340–3345.
103. Schömig A, Neumann FJ, Kastrati A, Schühlen H, Blasini R, Hadamitzky M, Walter H, Zitzmann-Roth EM, Richardt G, Alt E, Schmitt C, Ulm K. A randomized comparison of antiplatelet and anticoagulant therapy after the placement of coronary-artery stents. *N Engl J Med*. 1996;334:1084–1089.
104. Leon MB, Baim DS, Popma JJ, Gordon PC, Cutlip DE, Ho KK, Giambartolomei A, Diver DJ, Lasorda DM, Williams DO, Pocock SJ, Kuntz RE. A clinical trial comparing three antithrombotic-drug regimens after coronary-artery stenting: Stent Anticoagulation Restenosis Study Investigators. *N Engl J Med*. 1998;3391665–1671.
105. Bertrand ME, Legrand V, Boland J, Fleck E, Bonnier J, Emmanuelson H, Vrolix M, Missault L, Chierchia S, Casaccia M, Niccoli L, Oto A, White C, Webb-Peploe M, Van Belle E, McFadden EP. Randomized multicenter comparison of conventional anticoagulation versus antiplatelet therapy in unplanned and elective coronary stenting; the Full Anticoagulation Versus Aspirin and Ticlopidine (FANTASTIC) study. *Circulation*. 1998;98:1597–1603.
106. Urban P, Macaya C, Rupprecht HJ, Kiemeneij F, Emanuelsson H, Fontanelli A, Pieper M, Wesseling T, Sagnard L. Randomized evaluation of anticoagulation versus antiplatelet therapy after coronary stent implantation in high-risk patients: the Multicenter Aspirin and Ticlopidine Trial After Intracoronary Stenting (MATTIS). *Circulation*. 1998;98:2126–2132.
107. Bertrand ME, Rupprecht HJ, Urban P, Gershlick AH; CLASSICS Investigators. Double-blind study of the safety of clopidogrel with and without a loading dose in combination with aspirin compared with ticlopidine in combination with aspirin after coronary stenting: the Clopidogrel Aspirin Stent International Cooperative Study (CLASSICS). *Circulation*. 2000;102:624–629.
108. Cadroy Y, Bossavy JP, Thalamas C, Sagnard L, Sakariassen K, Boneu B. Early potent antithrombotic effect with combined aspirin and a loading dose of clopidogrel on experimental arterial thrombogenesis in humans. *Circulation*. 2000;101:2823–2828.
109. CAPRIE Steering Committee. A randomised, blinded, trial of Clopidogrel Versus Aspirin in Patients at Risk of Ischaemic Events (CAPRIE). *Lancet*. 1996;348:1329–1339.
110. Bhatt DL, Marso SP, Hirsch AT, Ringleb PA, Hacke W, Topol EJ. Amplified benefit of clopidogrel versus aspirin in patients with diabetes mellitus. *Am J Cardiol*. 2002;90:625–628.
111. Mehta SR, Yusuf S, Peters RJ, Bertrand ME, Lewis BS, Natarajan MK, Malmberg K, Rupprecht H, Zhao F, Chrolavicius S, Copland I, Fox KA; Clopidogrel in Unstable Angina to Prevent Recurrent Events Trial (CURE) Investigators. Effects of pretreatment with clopidogrel and aspirin followed by long-term therapy in patients undergoing percutaneous coronary intervention: the PCI-CURE study. Lancet. 2001;358:527–533.
112. Steinhubl SR, Berger PB, Mann JT III, Fry ET, DeLago A, Wilmer C, Topol EJ; CREDO Investigators. Clopidogrel for the reduction of events during observation. early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: a randomized controlled trial. *JAMA*. 2002;288:2411–2420.
113. Chen ZM, Jiang LX, Chen YP, Xie JX, Pan HC, Peto R, Collins R, Liu LS; COMMIT (Clopidogrel and Metoprolol in Myocardial Infarction Trial) Collaborative Group. Addition of clopidogrel to aspirin in 45,852 patients with acute myocardial infarction: randomised placebo-controlled trial. *Lancet*. 2005;366:1607–1621.
114. Sabatine MS, Cannon CP, Gibson CM, López-Sendón JL, Montalescot G, Theroux P, Claeys MJ, Cools F, Hill KA, Skene AM, McCabe CH, Braunwald E; CLARITY-TIMI 28 Investigators. Addition of clopidogrel to aspirin and fibrinolytic therapy for myocardial infarction with ST-segment elevation. *N Engl J Med*. 2005;352:1179–1189.
115. Sabatine MS, Cannon CP, Gibson CM, López-Sendón JL, Montalescot G, Theroux P, Lewis BS, Murphy SA, McCabe CH, Braunwald E; Clopidogrel as Adjunctive Reperfusion Therapy (CLARITY)–Thrombolysis in Myocardial Infarction (TIMI) 28 Investigators. Effect of clopidogrel pretreatment before percutaneous coronary intervention in patients with ST-elevation myocardial infarction treated with fibrinolytics: the PCI-CLARITY study. *JAMA*. 2005;294:1224–1232.
116. Bhatt DL, Fox KA, Hacke W, Berger PB, Black HR, Boden WE, Cacoub P, Cohen EA, Creager MA, Easton JD, Flather MD, Haffner SM, Hamm CW, Hankey GJ, Johnston SC, Mak KH, Mas JL, Montalescot G, Pearson TA, Steg PG, Steinhubl SR, Weber MA, Brennan DM, Fabry-Ribaudo L, Booth J, Topol EJ; CHARISMA Investigators. Clopidogrel and aspirin versus aspirin alone for the prevention of atherothrombotic events. *N Engl J Med*. 2006;354:1706–1717.
117. Angiolillo DJ, Capranzano P. Pharmacology of emerging novel platelet inhibitors. *Am Heart J*. 2008;156(suppl):S10–S15.
118. Wiviott SD, Trenk D, Frelinger AL, O'Donoghue M, Neumann FJ, Michelson AD, Angiolillo DJ, Hod H, Montalescot G, Miller DL, Jakubowski JA, Cairns R, Murphy SA, McCabe CH, Antman EM, Braunwald E; PRINCIPLE-TIMI 44 Investigators. Prasugrel compared with high loading- and maintenance-dose clopidogrel in patients with planned percutaneous coronary intervention: the Prasugrel in Comparison to Clopidogrel for Inhibition of Platelet Activation and Aggregation–Thrombolysis in Myocardial Infarction 44 trial. *Circulation*. 2007;116:2923–1932.
119. Wiviott SD, Braunwald E, McCabe CH, Montalescot G, Ruzyllo W, Gottlieb S, Neumann FJ, Ardissino D, De Servi S, Murphy SA, Riesmeyer J, Weerakkody G, Gibson CM, Antman EM; TRITON-TIMI 38 Investigators. Prasugrel versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med*. 2007;357:2001–2015.
120. Wiviott SD, Braunwald E, McCabe CH, Horvath I, Keltai M, Herrman JP, Van de Werf F, Downey WE, Scirica BM, Murphy SA, Antman EM; TRITON-TIMI 38 Investigators. Intensive oral antiplatelet therapy for reduction of ischaemic events including stent thrombosis in patients with acute coronary syndromes treated with percutaneous coronary intervention and stenting in the TRITON-TIMI 38 trial: a subanalysis of a randomised trial. *Lancet*. 2008;371:1353–1363.
121. Montalescot G, Wiviott SD, Braunwald E, Murphy SA, Gibson CM, McCabe CH, Antman EM; TRITON-TIMI 38 Investigators. Prasugrel compared with clopidogrel in patients undergoing percutaneous coronary intervention for ST-elevation myocardial infarction (TRITON-TIMI 38): double-blind, randomised controlled trial. *Lancet*. 2009;373:723–731.
122. Wiviott SD, Braunwald E, Angiolillo DJ, Meisel S, Dalby AJ, Verheugt FW, Goodman SG, Corbalan R, Purdy DA, Murphy SA, McCabe CH, Antman EM; TRITON-TIMI 38 Investigators. Greater clinical benefit of more intensive oral antiplatelet therapy with prasugrel in patients with

diabetes mellitus in the Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition With Prasugrel–Thrombolysis in Myocardial Infarction 38. *Circulation*. 2008;118:1626–1636.
123. Angiolillo DJ, Badimon JJ, Saucedo JF, Frelinger AL, Michelson AD, Jakubowski JA, Zhu B, Ojeh CK, Baker BA, Effron MB. A pharmacodynamic comparison of prasugrel vs. high-dose clopidogrel in patients with type 2 diabetes mellitus and coronary artery disease: results of the Optimizing anti-Platelet Therapy In diabetes MellitUS (OPTIMUS)-3 Trial. *Eur Heart J*. 2011 Jan 20. [Epub ahead of print].
124. Angiolillo DJ, Shoemaker SB, Desai B, Yuan H, Charlton RK, Bernardo E, Zenni MM, Guzman LA, Bass TA, Costa MA. Randomized comparison of a high clopidogrel maintenance dose in patients with diabetes mellitus and coronary artery disease: results of the Optimizing Antiplatelet Therapy in Diabetes Mellitus (OPTIMUS) study. *Circulation*. 2007;115:708–716.
125. Angiolillo DJ, Capranzano P, Goto S, Aslam M, Desai B, Charlton RK, Suzuki Y, Box LC, Shoemaker SB, Zenni MM, Guzman LA, Bass TA. A randomized study assessing the impact of cilostazol on platelet function profiles in patients with diabetes mellitus and coronary artery disease on dual antiplatelet therapy: results of the OPTIMUS-2 study. *Eur Heart J*. 2008;29:2202–2211.
126. Roffi M, Chew DP, Mukherjee D, Bhatt DL, White JA, Moliterno DJ, Heeschen C, Hamm CW, Robbins MA, Kleiman NS, Théroux P, White HD, Topol EJ. Platelet glycoprotein IIb/IIIa inhibition in acute coronary syndromes: gradient of benefit related to the revascularization strategy. *Eur Heart J*. 2002;23:1441–1448.
127. Mehilli J, Kastrati A, Schühlen H, Dibra A, Dotzer F, von Beckerath N, Bollwein H, Pache J, Dirschinger J, Berger PP, Schömig A; Intracoronary Stenting and Antithrombotic Regimen: Is Abciximab a Superior Way to Eliminate Elevated Thrombotic Risk in Diabetics (ISAR-SWEET) Study Investigators. Randomized clinical trial of abciximab in diabetic patients undergoing elective percutaneous coronary interventions after treatment with a high loading dose of clopidogrel. *Circulation*. 2004;110:3627–3635.
128. Kastrati A, Mehilli J, Neumann FJ, Dotzer F, ten Berg J, Bollwein H, Graf I, Ibrahim M, Pache J, Seyfarth M, Schühlen H, Dirschinger J, Berger PB, Schömig A; Intracoronary Stenting and Antithrombotic: Regimen Rapid Early Action for Coronary Treatment 2 (ISAR-REACT 2) Trial Investigators. Abciximab in patients with acute coronary syndromes undergoing percutaneous coronary intervention after clopidogrel pretreatment: the ISAR-REACT 2 randomized trial. *JAMA*. 2006;295:1531–1538.
129. Montalescot G, Barragan P, Wittenberg O, Ecollan P, Elhadad S, Villain P, Boulenc JM, Morice MC, Maillard L, Pansiéri M, Choussat R, Pinton P; ADMIRAL Investigators. Platelet glycoprotein IIb/IIIa inhibition with coronary stenting for acute myocardial infarction: Abciximab Before Direct Angioplasty and Stenting in Myocardial Infarction Regarding Acute and Long-Term Follow-up. *N Engl J Med*. 2001;344:1895–1903.
130. De Luca G, Navarese E, Marino P. Risk profile and benefits from Gp IIb-IIIa inhibitors among patients with ST-segment elevation myocardial infarction treated with primary angioplasty: a meta-regression analysis of randomized trials. *Eur Heart J*. 2009;30:2705–2713.
131. Rao SV, Eikelboom JA, Granger CB, Harrington RA, Califf RM, Bassand JP. Bleeding and blood transfusion issues in patients with non-ST-segment elevation acute coronary syndromes. *Eur Heart J*. 2007;28:1193–1204.
132. Eikelboom JW, Mehta SR, Anand SS, Xie C, Fox KA, Yusuf S. Adverse impact of bleeding on prognosis in patients with acute coronary syndromes. *Circulation*. 2006;114:774–782.
133. Stone GW, McLaurin BT, Cox DA, Bertrand ME, Lincoff AM, Moses JW, White HD, Pocock SJ, Ware JH, Feit F, Colombo A, Aylward PE, Cequier AR, Darius H, Desmet W, Ebrahimi R, Hamon M, Rasmussen LH, Rupprecht HJ, Hoekstra J, Mehran R, Ohman EM; ACUITY Investigators. Bivalirudin for patients with acute coronary syndromes. *N Engl J Med*. 2006;355:2203–2216.
134. Feit F, Manoukian SV, Ebrahimi R, Pollack CV, Ohman EM, Attubato MJ, Mehran R, Stone GW. Safety and efficacy of bivalirudin monotherapy in patients with diabetes mellitus and acute coronary syndromes: a report from the ACUITY (Acute Catheterization and Urgent Intervention Triage Strategy) trial. *J Am Coll Cardiol*. 2008;51:1645–1652.
135. Manoukian SV. Predictors and impact of bleeding complications in percutaneous coronary intervention, acute coronary syndromes, and ST-segment elevation myocardial infarction. *Am J Cardiol*. 2009;104:9C–15C.
136. Angiolillo DJ. Variability in responsiveness to oral antiplatelet therapy. *Am J Cardiol*. 2009;103(suppl):27A–34A.
137. Michelson AD, Cattaneo M, Eikelboom JW, Gurbel P, Kottke-Marchant K, Kunicki TJ, Pulcinelli FM, Cerletti C, Rao AK; Platelet Physiology Subcommittee of the Scientific and Standardization Committee of the International Society on Thrombosis Haemostasis; Working Group on Aspirin Resistance. Aspirin resistance: position paper of the Working Group on Aspirin Resistance. *J Thromb Haemost*. 2005;3:1309–1311.
138. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Alfonso F, Macaya C, Bass TA, Costa MA. Variability in individual responsiveness to clopidogrel: clinical implications, management, and future perspectives. *J Am Coll Cardiol*. 2007;49:1505–1516.
139. Snoep JD, Hovens MM, Eikenboom JC, van der Bom JG, Huisman MV. Association of laboratory-defined aspirin resistance with a higher risk of recurrent cardiovascular events: a systematic review and meta-analysis. *Arch Intern Med*. 2007;167:1593–1599.
140. Krasopoulos G, Brister SJ, Beattie WS, Buchanan MR. Aspirin "resistance" and risk of cardiovascular morbidity: systematic review and meta-analysis. *BMJ*. 2008;336:195–198.
141. Gurbel PA, Bliden KP, DiChiara J, Newcomer J, Weng W, Neerchal NK, Gesheff T, Chaganti SK, Etherington A, Tantry US. Evaluation of dose-related effects of aspirin on platelet function: results from the Aspirin-Induced Platelet Effect (ASPECT) study. *Circulation*. 2007;115:3156–3164.
142. Frelinger AL III, Furman MI, Linden MD, Li Y, Fox ML, Barnard MR, Michelson AD. Residual arachidonic acid-induced platelet activation via an adenosine diphosphate-dependent but cyclooxygenase-1- and cyclooxygenase-2-independent pathway: a 700-patient study of aspirin resistance. *Circulation*. 2006;113:2888–2896.
143. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Ramírez C, Sabaté M, Jimenez-Quevedo P, Hernández R, Moreno R, Escaned J, Alfonso F, Bañuelos C, Costa MA, Bass TA, Macaya C. Influence of aspirin resistance on platelet function profiles in patients on long-term aspirin and clopidogrel after percutaneous coronary intervention. *Am J Cardiol*. 2006;97:38–43.
144. DiChiara J, Bliden KP, Tantry US, Hamed MS, Antonino MJ, Suarez TA, Bailon O, Singla A, Gurbel PA. The effect of aspirin dosing on platelet function in diabetic and nondiabetic patients: an analysis from the Aspirin-Induced Platelet Effect (ASPECT) study. *Diabetes*. 2007;56:3014–3019.
145. Watala C, Pluta J, Golanski J, Rozalski M, Czyz M, Trojanowski Z, Drzewoski J. Increased protein glycation in diabetes mellitus is associated with decreased aspirin-mediated protein acetylation and reduced sensitivity of blood platelets to aspirin. *J Mol Med*. 2005;83:148–158.
146. Davì G, Catalano I, Averna M, Notarbartolo A, Strano A, Ciabattoni G, Patrono C. Thromboxane biosynthesis and platelet function in type II diabetes mellitus. *N Engl J Med*. 1990;322:1769–1774.
147. Watala C, Golanski J, Pluta J, Boncler M, Rozalski M, Luzak B, Kropiwnicka A, Drzewoski J. Reduced sensitivity of platelets from type 2 diabetic patients to acetylsalicylic acid (aspirin): its relation to metabolic control. *Thromb Res*. 2004;113:101–113.
148. Neri Serneri GG, Coccheri S, Marubini E, Violi F; Drug Evaluation in Atherosclerotic Vascular Disease in Diabetics (DAVID) Study Group. Picotamide, a combined inhibitor of thromboxane A2 synthase and receptor, reduces 2-year mortality in diabetics with peripheral arterial disease: the DAVID study. *Eur Heart J*. 2004;25:1845–1852.
149. Randomized trial of ridogrel, a combined thromboxane A2 synthase inhibitor and thromboxane A2/prostaglandin endoperoxide receptor antagonist, versus aspirin as adjunct to thrombolysis in patients with acute myocardial infarction: the Ridogrel Versus Aspirin Patency Trial (RAPT). *Circulation*. 1994;89:588–595.
150. Gresele P, Migliacci R, Procacci A, De Monte P, Bonizzoni E. Prevention by NCX 4016, a nitric oxide-donating aspirin, but not by aspirin, of the acute endothelial dysfunction induced by exercise in patients with intermittent claudication. *Thromb Haemost*. 2007;97:444–450.
151. Kariyazono H, Nakamura K, Arima J, Ayukawa O, Onimaru S, Masuda H, Iguro Y, Majima HJ, Sakata R, Yamada K. Evaluation of anti-platelet aggregatory effects of aspirin, cilostazol and ramatroban on platelet-rich plasma and whole blood. *Blood Coagul Fibrinolysis*. 2004;15:157–167.
152. Chamorro A. TP receptor antagonism: a new concept in atherothrombosis and stroke prevention. *Cerebrovasc Dis*. 2009;27(suppl 3):20–27.
153. Ferreiro JL, Angiolillo DJ. Clopidogrel response variability: current status and future directions. *Thromb Haemost*. 2009;102:7–14.
154. Angiolillo DJ, Bernardo E, Sabaté M, Jimenez-Quevedo P, Costa MA, Palazuelos J, Hernández-Antolin R, Moreno R, Escaned J, Alfonso F,

Bañuelos C, Guzman LA, Bass TA, Macaya C, Fernandez-Ortiz A. Impact of platelet reactivity on cardiovascular outcomes in patients with type 2 diabetes mellitus and coronary artery disease. *J Am Coll Cardiol*. 2007;50:1541–1547.

155. Angiolillo DJ, Bernardo E, Ramírez C, Costa MA, Sabaté M, Jimenez-Quevedo P, Hernández R, Moreno R, Escaned J, Alfonso F, Bañuelos C, Bass TA, Macaya C, Fernandez-Ortiz A. Insulin therapy is associated with platelet dysfunction in patients with type 2 diabetes mellitus on dual oral antiplatelet treatment. *J Am Coll Cardiol*. 2006;48:298–304.
156. Angiolillo, DJ, Bernardo E, Capodanno D, Vivas D, Sabaté M, Ferreiro JL, Ueno M, Jimenez-Quevedo P, Alfonso F, Bass TA, Macaya C, Fernandez-Ortiz A. Impact of chronic kidney disease on platelet function profiles in diabetes mellitus patients with coronary artery disease on dual antiplatelet therapy. *J Am Coll Cardiol*. 2010;55: 1139–1146.
157. Dasgupta A, Steinhubl SR, Bhatt DL, Berger PB, Shao M, Mak KH, Fox KA, Montalescot G, Weber MA, Haffner SM, Dimas AP, Steg PG, Topol EJ; CHARISMA Investigators. Clinical outcomes of patients with diabetic nephropathy randomized to clopidogrel plus aspirin versus aspirin alone (a post hoc analysis of the Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management, and Avoidance [CHARISMA] trial). *Am J Cardiol*. 2009;103:1359–1363.
158. Iakovou I, Schmidt T, Bonizzoni E, Ge L, Sangiorgi GM, Stankovic G, Airoldi F, Chieffo A, Montorfano M, Carlino M, Michev I, Corvaja N, Briguori C, Gerckens U, Grube E, Colombo A. Incidence, predictors, and outcome of thrombosis after successful implantation of drug-eluting stents. *JAMA*. 2005;293:2126–2130.
159. Urban P, Gershlick AH, Guagliumi G, Guyon P, Lotan C, Schofer J, Seth A, Sousa JE, Wijns W, Berge C, Deme M, Stoll HP; e-Cypher Investigators. Safety of coronary sirolimus-eluting stents in daily clinical practice: one-year follow-up of the e-Cypher Registry. *Circulation*. 2006;113:1434–441.
160. Kuchulakanti PK, Chu WW, Torguson R, Ohlmann P, Rha SW, Clavijo LC, Kim SW, Bui A, Gevorkian N, Xue Z, Smith K, Fournadjieva J, Suddath WO, Satler LF, Pichard AD, Kent KM, Waksman R. Correlates and long-term outcomes of angiographically proven stent thrombosis with sirolimus- and paclitaxel-eluting stents. *Circulation*. 2006;113: 1108–1113.
161. Michno A, Bielarczyk H, Pawełczyk T, Jankowska-Kulawy A, Klimaszewska J, Szutowicz A. Alterations of adenine nucleotide metabolism and function of blood platelets in patients with diabetes. *Diabetes*. 2007;56:462–467.
162. Angiolillo DJ, Capodanno D, Goto S. Platelet thrombin receptor antagonism and atherothrombosis. *Eur Heart J*. 2010;31:17–28.
163. Wallentin L, Becker RC, Budaj A, Cannon CP, Emanuelsson H, Held C, Horrow J, Husted S, James S, Katus H, Mahaffey KW, Scirica BM, Skene A, Steg PG, Storey RF, Harrington RA; PLATO Investigators, Freij A, Thorsén M. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med*. 2009;361:1045–1057.

163a. James S, Angiolillo DJ, Cornel JH, Erlinge D, Husted S, Kontny F, Maya J, Nicolau JC, Spinar J, Storey RF, Stevens SR, Wallentin L; PLATO Study Group. Ticagrelor vs. clopidogrel in patients with acute coronary syndromes and diabetes: a substudy from the PLATelet inhibition and patient Outcomes (PLATO) trial. *Eur Heart J*. 2010;31: 3006–3016.

164. Angiolillo DJ, Guzman LA. Clinical overview of promising nonthienopyridine antiplatelet agents. *Am Heart J*. 2008;156:S23–S28.
165. Storey RF, Husted S, Harrington RA, Heptinstall S, Wilcox RG, Peters G, Wickens M, Emanuelsson H, Gurbel P, Grande P, Cannon CP. Inhibition of platelet aggregation by AZD6140, a reversible oral P2Y12 receptor antagonist, compared with clopidogrel in patients with acute coronary syndromes. *J Am Coll Cardiol*. 2007;50:1852–1856.
166. Storey RF, Wilcox RG, Heptinstall S. Comparison of the pharmacodynamic effects of the platelet ADP receptor antagonists clopidogrel and AR-C69931MX in patients with ischaemic heart disease. *Platelets*. 2002;13:407–413.
167. Harrington RA, Stone GW, McNulty S, White HD, Lincoff AM, Gibson CM, Pollack CV Jr, Montalescot G, Mahaffey KW, Kleiman NS, Goodman SG, Amine M, Angiolillo DJ, Becker RC, Chew DP, French WJ, Leisch F, Parikh KH, Skerjanec S, Bhatt DL. Platelet inhibition with cangrelor in patients undergoing PCI. *N Engl J Med*. 2009;361: 2318–2329.
168. Bhatt DL, Lincoff AM, Gibson CM, Stone GW, McNulty S, Montalescot G, Kleiman NS, Goodman SG, White HD, Mahaffey KW, Pollack CV Jr, Manoukian SV, Widimsky P, Chew DP, Cura F, Manukov I, Tousek F, Jafar MZ, Arneja J, Skerjanec S, Harrington RA; the CHAMPION PLATFORM Investigators. Intravenous platelet blockade with cangrelor during PCI. *N Engl J Med*. 2009;361: 2330–2341.
169. Douglas JS Jr, Holmes DR Jr, Kereiakes DJ, Grines CL, Block E, Ghazzal ZM, Morris DC, Liberman H, Parker K, Jurkovitz C, Murrah N, Foster J, Hyde P, Mancini GB, Weintraub WS; Cilostazol for Restenosis Trial (CREST) Investigators. Coronary stent restenosis in patients treated with cilostazol. *Circulation*. 2005;112:2826–2832.
170. Lee SW, Park SW, Hong MK, Kim YH, Lee BK, Song JM, Han KH, Lee CW, Kang DH, Song JK, Kim JJ, Park SJ. Triple versus dual antiplatelet therapy after coronary stenting: impact on stent thrombosis. *J Am Coll Cardiol*. 2005;46:1833–1837.
171. Biondi-Zoccai GG, Lotrionte M, Anselmino M, Moretti C, Agostoni P, Testa L, Abbate A, Cosgrave J, Laudito A, Trevi GP, Sheiban I. Systematic review and meta-analysis of randomized clinical trials appraising the impact of cilostazol after percutaneous coronary intervention. *Am Heart J*. 2008;155:1081–1089.
172. Lee SW, Park SW, Kim YH, Yun SC, Park DW, Lee CW, Hong MK, Kim HS, Ko JK, Park JH, Lee JH, Choi SW, Seong IW, Cho YH, Lee NH, Kim JH, Chun KJ, Park SJ. Drug-eluting stenting followed by cilostazol treatment reduces late restenosis in patients with diabetes mellitus: the DECLARE-DIABETES Trial (A Randomized Comparison of Triple Antiplatelet Therapy with Dual Antiplatelet Therapy After Drug-Eluting Stent Implantation in Diabetic Patients). *J Am Coll Cardiol*. 2008;51:1181–1187.
173. Han Y, Li Y, Wang S, Jing Q, Wang Z, Wang D, Shu Q, Tang X. Cilostazol in addition to aspirin and clopidogrel improves long-term outcomes after percutaneous coronary intervention in patients with acute coronary syndromes: a randomized, controlled study. *Am Heart J*. 2009; 157:733–739.
174. Angiolillo DJ, Capranzano P, Desai B, Shoemaker SB, Charlton R, Zenni MM, Guzman LA, Bass TA. Impact of P2Y(12) inhibitory effects induced by clopidogrel on platelet procoagulant activity in type 2 diabetes mellitus patients. *Thromb Res*. 2009;124:318–322.
175. Becker RC, Moliterno DJ, Jennings LK, Pieper KS, Pei J, Niederman A, Ziada KM, Berman G, Strony J, Joseph D, Mahaffey KW, Van de Werf F, Veltri E, Harrington RA; TRA-PCI Investigators. Safety and tolerability of SCH 530348 in patients undergoing non-urgent percutaneous coronary intervention: a randomised, double-blind, placebo-controlled phase II study. *Lancet*. 2009;373:919–928.
176. Wittkowsky AK. New oral anticoagulants: a practical guide for clinicians. *J Thromb Thrombolysis*. 2010;29:182–191.
177. Connolly SJ, Ezekowitz MD, Yusuf S, Eikelboom J, Oldgren J, Parekh A, Pogue J, Reilly PA, Themeles E, Varrone J, Wang S, Alings M, Xavier D, Zhu J, Diaz R, Lewis BS, Darius H, Diener HC, Joyner CD, Wallentin L; RE-LY Steering Committee and Investigators. Dabigatran versus warfarin in patients with atrial fibrillation. *N Engl J Med*. 2009;361:1139–1151.

KEY WORDS: diabetes mellitus ■ platelets ■ thrombosis

IX. New directions in antiplatelet therapy.

Ferreiro JL, Angiolillo DJ.

Circ Cardiovasc Interv. 2012;5:433-45.

New Directions in Antiplatelet Therapy

José Luis Ferreiro, MD; Dominick J. Angiolillo, MD, PhD

Atherosclerosis is a chronic inflammatory process that is known to be the underlying cause of coronary artery disease (CAD).[1] In addition to being the first step of primary hemostasis, platelets play a pivotal role in the thrombotic process that follows rupture, fissure, or erosion of an atherosclerotic plaque.[2] Because atherothrombotic events are essentially platelet-driven processes, this underscores the importance of antiplatelet agents, which represent the cornerstone of treatment, particularly in the settings of patients with acute coronary syndromes (ACS) and undergoing percutaneous coronary intervention (PCI).

Currently, there are 3 different classes of antiplatelet drugs that are approved for clinical use and recommended per guidelines for the treatment and prevention of ischemic events in the settings of ACS and PCI: (1) cycloxigenase-1 (COX-1) inhibitor: aspirin, (2) adenosine diphosphate (ADP) $P2Y_{12}$ receptor antagonists: ticlopidine, clopidogrel, prasugrel, and ticagrelor, and (3) glycoprotein IIb/IIIa inhibitors (GPI): abciximab, eptifibatide, and tirofiban.[3–6] GPIs currently are available only for parenteral administration, and therefore their use is limited only to the acute phase of treatment of ACS patients undergoing PCI. Oral antiplatelet agents, namely aspirin and $P2Y_{12}$ receptor inhibitors, are recommended for prevention of ischemic events in both the acute and long-term phases of treatment. For over a decade, dual antiplatelet therapy (DAPT) with aspirin and clopidogrel has been considered the standard of care in the setting of ACS and PCI. However, a considerable number of adverse ischemic events continue to occur with this DAPT regimen, which has led to the development of newer and more potent antiplatelet agents. The objective of the present manuscript is to provide an overview on the most recent advances of currently approved antiplatelet agents in the setting of ACS and PCI, as well as on emerging agents that are in clinical development (Figure 1). Other antiplatelet drugs that are available for clinical use, such as pentoxifylline, cilostazol, and dypirimidamole, but do not have an approved indication for patients with ACS or undergoing PCI, as well as advances in anticoagulant therapy, will not be discussed.

Currently Approved Agents

Aspirin

Aspirin exerts its action through an irreversible blockade of COX-1, the enzyme that catalyzes the synthesis of thromboxane A_2 (TXA_2) from arachidonic acid through selective acetylation of a serine residue at position 529 (Ser529). TXA_2 causes changes in platelet shape and enhances recruitment and aggregation of platelets through its binding to thromboxane and prostaglandin endoperoxide (TP) receptors. Therefore, aspirin decreases platelet activation and aggregation processes mediated by TP receptor pathways.[7]

Although the optimal dose of aspirin has been the subject of debate, the efficacy of low-dose aspirin is supported by the results of numerous studies.[8–10] In these investigations, a dose-dependent risk for bleeding, particularly upper gastrointestinal bleeding, with no increase in efficacy was observed. This is in line with the overall results of the CURRENT/OASIS-7 (Clopidogrel optimal loading dose Usage to Reduce Recurrent EveNTs-Organization to Assess Strategies in Ischemic Syndromes) trial, in which ACS patients (n=25 087) scheduled to undergo angiography were assigned to high or standard dose of clopidogrel for a month, including an open-label randomization to high (300–325 mg daily) versus low dose (75–100 mg daily) of aspirin. Although no significant differences between high and low dose aspirin were found in efficacy or bleeding, a trend toward a higher rate of gastrointestinal bleeds in the high dose aspirin group (0.38% versus 0.24%; $P=0.051$) at 30 days was observed.[10] Overall, these data suggest that after loading dose administration of aspirin, the use of a low maintenance dose regimen should be considered for secondary prevention of vascular events.

Several studies have observed an association between aspirin poor responsiveness and a higher risk of recurrent ischemic events.[11] The prevalence of aspirin resistance varies among studies, which can be attributed to differences in the definition of resistance, type of assay used, dose of aspirin, and population considered. In fact, when using COX-1 specific tests (eg, determination of serum thromboxane and assays using arachidonic acid as agonist), aspirin resistance is a sporadic phenomenon (less than 5% of patients).[11] Of note, poor patient compliance is the main cause of aspirin resistance, when assessed by COX-1 specific tests. Other possible causes that may play a role in a reduced response to aspirin include type of aspirin used (eg, enteric versus nonenteric coated), genetics (eg, COX-1 polymorphism), dosing regimen, and drug interactions (eg, ibuprofen).[12–16]

Received January 9, 2012; accepted April 19, 2012.

From the Heart Diseases Institute, Hospital Universitari de Bellvitge-IDIBELL, University of Barcelona, L'Hospitalet de Llobregat, Barcelona, Spain (J.L.F.); University of Florida College of Medicine-Jacksonville, Jacksonville, FL (J.L.F., D.J.A.).

Correspondence to Dr Dominick J. Angiolillo, University of Florida College of Medicine-Jacksonville, 655 West 8th Street, Jacksonville, Florida, 32209, E-mail dominick.angiolillo@jax.ufl.edu

(*Circ Cardiovasc Interv*. 2012;5:433-445.)

Circ Cardiovasc Interv is available at http://circinterventions.ahajournals.org DOI: 10.1161/CIRCINTERVENTIONS.111.966176

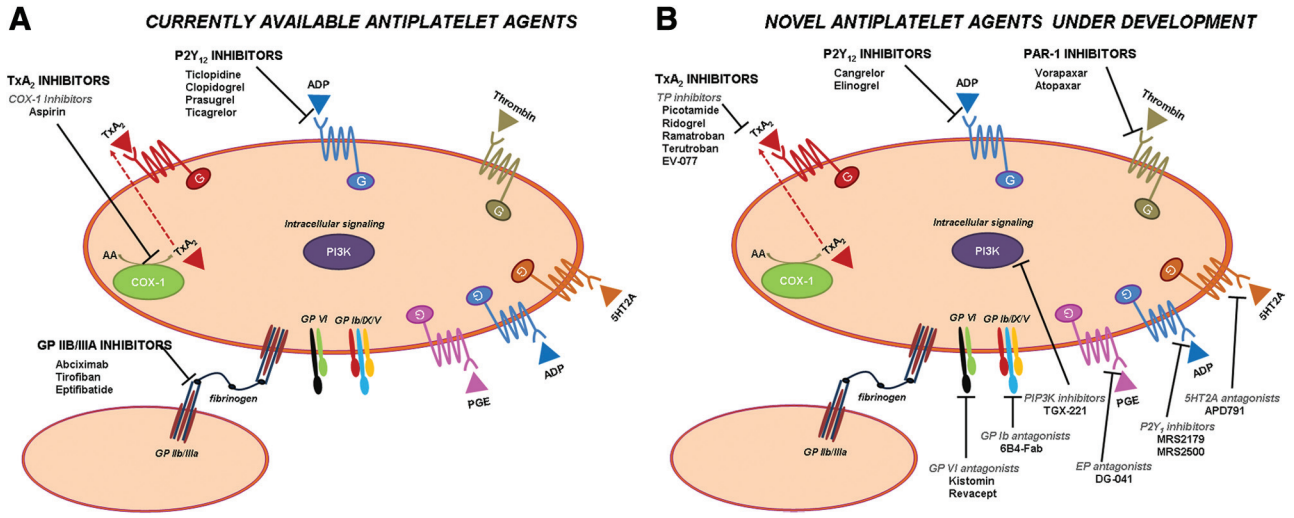


Figure 1. Sites of action of antiplatelet agents. **A**, Currently available agents for acute coronary syndromes or percutaneous coronary intervention. **B**, Novel antiplatelet agents under development. 5HT2A indicates serotonine; AA, arachidonic acid; ADP, adenosine diphosphate; COX-1, cyclooxygenase-1; EP, prostaglandin receptor; G, g-protein; GP, glycoprotein; PG, prostaglandin; PAR-1, platelet protease-activated receptor-1; PI3K, phosphatidylinositol 3-kinase; TP, thromboxane receptor; TxA_2, thromboxane A_2.

$P2Y_{12}$ Receptor Antagonists

Adenosine diphosphate exerts its effects on platelets via the $P2Y_1$ and $P2Y_{12}$ receptors. Although both receptors are needed for aggregation, activation of the $P2Y_{12}$ pathway plays the principal role, leading to sustained platelet aggregation and stabilization of the platelet aggregate.[17] $P2Y_{12}$ receptor inhibitors are recommended for prevention of ischemic events in both the acute and long-term phases of treatment, as summarized in Table 1 and described in details below.

Clopidogrel

Three generations of thienopyridines (ticlopidine, clopidogrel, and prasugrel), a family of nondirect, orally administered antiplatelet agents that irreversibly block the platelet ADP $P2Y_{12}$ receptor, are approved currently for clinical use. After its approval in 1997, clopidogrel soon replaced ticlopidine due to its more favorable safety profile.[18] Further, clopidogrel has a pharmacological advantage over ticlopidine, as it achieves a faster onset on action through administration of a loading dose.[19] Clopidogrel is a prodrug that requires metabolization in the liver through a double oxidation process mediated by several cytochrome P450 (CYP) isoforms, to be converted finally into its active metabolite, which irreversibly blocks the ADP $P2Y_{12}$ platelet receptor. Due to the irreversible blockade of the $P2Y_{12}$ receptor, clopidogrel effects last for the whole lifespan of the platelet (7–10 days).[20,21]

Dual antiplatelet therapy with aspirin and clopidogrel is recommended per guidelines for patients with ACS, including those with unstable angina (UA) or non-ST elevation acute coronary syndromes (NSTEACS), ST-elevation myocardial infarction (STEMI), and for patients undergoing PCI (Table 1).[3–6] This recommendation is based on the findings of several large-scale trials that have shown a clear benefit of adjunctive treatment with clopidogrel in addition to aspirin in preventing recurrent atherothrombotic events.[22–25] However, DAPT with aspirin and clopidogrel should not be recommended for primary prevention or in patients not presenting with an ACS or undergoing PCI, because it has not been proven superior to aspirin alone in this scenario.[26]

Despite the undisputed clinical benefit achieved with the combination of clopidogrel and aspirin in the setting of ACS or PCI, a considerable number of patients continue to experience recurrent ischemic events.[22–25] This is partially due to clopidogrel's main drawback, represented by its broad variability in platelet inhibitory effects, which includes a high percentage of patients with suboptimal antiplatelet effects. The percentage of "low responders" or "resistant" patients ranges from 5% to 40% across studies, depending on definitions, type of test used, dose of clopidogrel, and population characteristics. Genetic, cellular, and clinical mechanisms have been reported to play a role in inadequate clopidogrel responsiveness.[20,21] Some of these, such as poor clopidogrel metabolizer status due to the presence of loss-of-function alleles for the CYP2C19 enzyme and the use of proton pump inhibitors interfering with CYP2C19 activity (eg, omeprazole), have prompted the Food and Drug Administration and European Medicines Agency to issue box warnings.[27,28] Although the clinical relevance and the appropriateness of these warnings have been subject to controversies, the association between low responsiveness to clopidogrel and adverse ischemic outcomes, including stent thrombosis, is well established.[20,21] Overall, these results emphasize the need for finding new antiplatelet strategies to achieve more potent $P2Y_{12}$ receptor blockade with less variability in response (Figure 2),[29] especially in high risk subsets of patients, such as those suffering an ACS or undergoing PCI.

One of the strategies suggested to overcome nonresponsiveness is the use of a higher than currently approved loading and maintenance doses of clopidogrel, which have been observed to achieve greater platelet inhibitory effects.[20,21] The CURRENT/OASIS-7 trial, which assessed the efficacy of high (600 mg loading dose followed by 150 mg daily for 1 week and then 75 mg/daily until day 30) versus standard dose (300 mg loading followed by 75 mg daily until day 30) of clopidogrel for 1 month

Table 1. Guideline Recommendations for Available $P2Y_{12}$ Antagonists

	Clopidogrel	Prasugrel	Ticagrelor
2011 ACCF/AHA Focused Update of the Guidelines for the Management of Patients With Unstable Angina/Non–ST-Elevation Myocardial Infarction[3]	*Class I; Level of Evidence A* Clopidogrel 300 to 600 mg should be given as early as possible before or at the time of PCI, followed by 75 mg daily for at least 12 months: Class I; Level of Evidence B for duration	*Class I; Level of Evidence B* Prasugrel 60 mg should be given promptly and no later than 1 hour after PCI once coronary anatomy is defined and a decision is made to proceed with PCI, followed by 10 mg daily for at least 12 months: Class I; Level of Evidence B for duration	Not FDA approved or marketed at the time of writing of Guidelines
2011 ACCF/AHA/SCAI Guideline for Percutaneous Coronary Intervention[4]	*Class I; Level of Evidence B** Clopidogrel 600 mg (ACS and non-ACS patients) followed by 75 mg daily for at least 12 months	*Class I; Level of Evidence B** Prasugrel 60 mg (ACS patients) followed by 10 mg daily for at least 12 months	*Class I; Level of Evidence B** Ticagrelor 180 mg (ACS patients) followed by 90 mg twice daily for at least 12 months
2011 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation[5]	*Class I; Level of Evidence A* Clopidogrel (300-mg LD, 75-mg daily dose) is recommended for patients who cannot receive ticagrelor or prasugrel. A 600-mg LD (or a supplementary 300-mg dose at PCI following an initial 300-mg LD) is recommended for patients scheduled for an invasive strategy: Class I; Level of Evidence B. A higher MD of clopidogrel 150 mg daily should be considered for the first 7 days in patients managed with PCI and without increased risk of bleeding: Class IIa; Level of Evidence B	*Class I; Level of Evidence B* Prasugrel (60-mg LD, 10-mg daily dose) is recommended for $P2Y_{12}$-inhibitor-naïve patients (especially diabetics) in whom coronary anatomy is known and who are proceeding to PCI unless there is a high risk of life-threatening bleeding or other contraindications	*Class I; Level of Evidence B* Ticagrelor (180-mg LD, 90 mg twice daily) is recommended for all patients at moderate-to-high risk of ischaemic events (e.g. elevated troponins), regardless of initial treatment strategy and including those pre-treated with clopidogrel (which should be discontinued when ticagrelor is commenced).
2010 ESC/EACTS/EAPCI Guidelines on myocardial revascularization[6]	*Elective PCI: Class I; Level of Evidence A* *NSTE-ACS: Class I; Level of Evidence B* *STEMI: Class I; Level of Evidence C* Elective PCI: Pretreatment with 300 mg loading dose >6 h before PCI (or 600 mg >2 h before): Class I;Level of Evidence C NSTE-ACS: 600-mg LD as soon as possible: Class I; Level of Evidence C STEMI: 600-mg LD as soon as possible. Primarily if more efficient antiplatelet agents are contraindicated.	*NSTE-ACS: Class IIa; Level of Evidence B* *STEMI: Class I; Level of Evidence B* Prasugrel 60-mg LD followed by 10-mg daily dose Guidelines specify: "Depending on approval and availability. Direct comparison between prasugrel and ticagrelor is not available"	*NSTE-ACS: Class I; Level of Evidence B* *STEMI: Class I; Level of Evidence B* Ticagrelor 180-mg LD followed 90 mg twice daily) Guidelines specify: "Depending on approval and availability. Direct comparison between prasugrel and ticagrelor is not available"

*General recommendation: A loading dose of a $P2Y_{12}$ receptor inhibitor should be given to patients undergoing PCI with stenting: Level of Evidence A.

in ACS patients (n=25 087) scheduled to undergo angiography, included ACS patients (n=25 087) scheduled to undergo angiography within 72 hours of hospital arrival. In the overall study population, no benefit was derived from the high dose regimen.[10] However, in the subgroup of patients undergoing PCI (n=17 232), the high dose strategy was associated with a decrease in the rates of ischemic outcomes (3.9% versus 4.5%; hazards ration [HR], 0.85; $P=0.036$), and reduced the risk of stent thrombosis by 30%, at the expense, however, of a significant increase in study defined major bleedings.[30]

The concept of a "tailored treatment" by increasing clopidogrel dosing according to the degree of responsiveness of a given patient assessed by a platelet function assay was evaluated in the GRAVITAS (Gauging Responsiveness with a Verify Now Assay: Impact on Thrombosis And Safety) trial. In this investigation, the efficacy of high dose clopidogrel (600 mg initial dose and 150 mg daily thereafter for 6 months) versus standard dose clopidogrel (no additional loading dose and 75 mg daily) was compared in 2214 patients with high on-treatment reactivity, on the basis of Verify Now P2Y12 assay measurement, 12 to 24 hours after PCI with drug-eluting stents. No differences in the rates of ischemic (2.3% versus 2.3%; HR, 1.01 [0.58–1.76]; $P=0.97$) or bleeding outcomes (1.4% versus 2.3%; HR, 0.59 [0.31–1.11]; $P=0.10$) were found.[31] Thus, a benefit of a tailored strategy with clopidogrel therapy was not observed in this trial, which may be explained by the overall low percentage of events observed and the weak increase in platelet inhibition achieved with a high dose of clopidogrel compared with standard dosing. Indeed, other strategies (Figure 2) have shown to be associated with greater pharmacodynamic effects (ie, enhanced platelet inhibition), measured by different platelet function assays, than high dose clopidogrel among patients with high on-treatment platelet reactivity as well as poor clopidogrel metabolizers.[29] However, to date none of these strategies have shown to have an impact on clinical outcomes in large-scale studies. This includes using prasugrel among poor clopidogrel responders with stable coronary artery disease as shown in the TRIGGER-PCI (Testing platelet Reactivity In patients underGoing elective stent placement on clopidogrel to Guide alternative thErapy with pRasugrel) trial, in which despite the pharmacodynamic superiority of

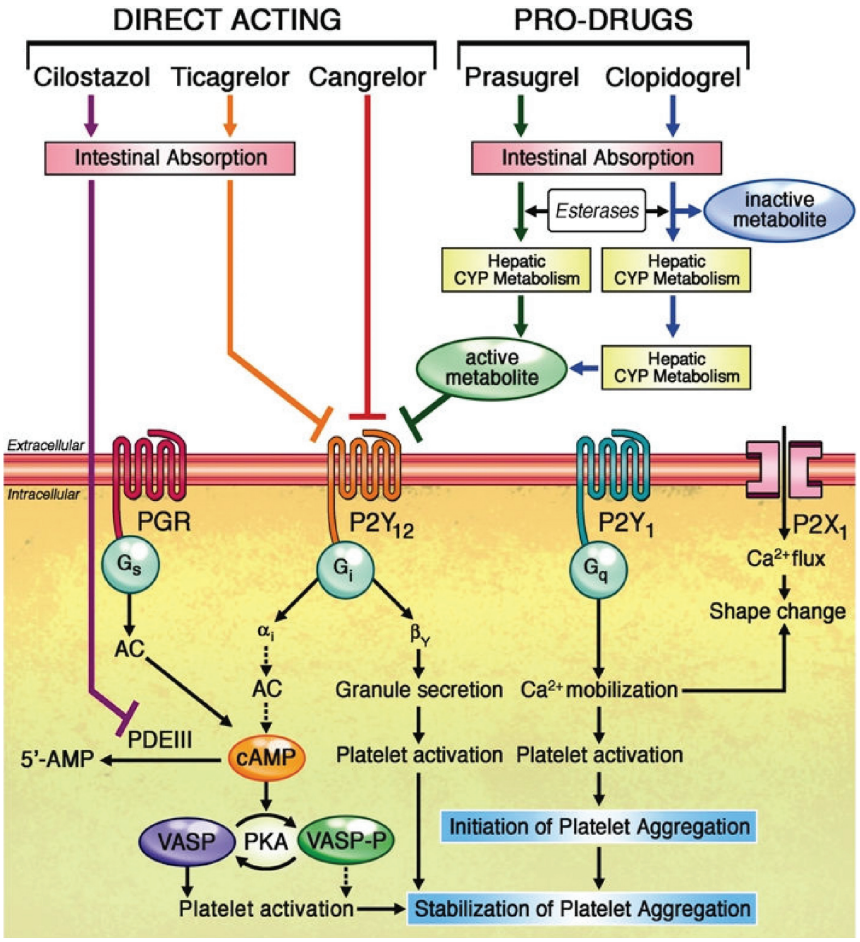


Figure 2. Schematic of different therapeutic options for inhibition of platelet $P2Y_{12}$ receptor. Clopidogrel is a prodrug, which, after intestinal absorption, undergoes metabolization in the liver through a double oxidation process mediated by several cytochrome P450 (CYP) isoforms to finally generate an active metabolite that inhibits platelet activation and aggregation processes through irreversible blockade of the $P2Y_{12}$ receptor. Approximately 85% of clopidogrel is hydrolyzed prehepatically by esterases into an inactive compound, thus, only 15% is available for hepatic metabolism. Prasugrel, like clopidogrel, is also an oral prodrug with a similar intestinal absorption process. However, in contrast to clopidogrel, esterases are part of prasugrel's activation pathway, and prasugrel is oxidized more efficiently to its active metabolite via a single CYP-dependent step. Direct-acting antiplatelet agents (cangrelor, ticagrelor, and cilostazol) have reversible effects and do not require hepatic metabolism for achieving pharmacodynamic activity. Ticagrelor and cilostazol are orally administered and, after intestinal absorption, inhibit platelet activation by direct blockade of the $P2Y_{12}$ receptor and PDE-III, respectively. Cangrelor is intravenously administered, and directly inhibits the $P2Y_{12}$ receptor, bypassing intestinal absorption. Genetic polymorphisms of target proteins/enzymes (intestine, liver, and platelet membrane) modulating clopidogrel-mediated platelet inhibition do not affect the pharmacodynamic activity of prasugrel, cilostazol, ticagrelor, and cangrelor, which ultimately inhibit platelet activation and aggregation processes by modulating intraplatelet levels of cAMP and VASP-P. Solid black arrows indicate activation. Dotted black arrows indicate inhibition. AC indicates adenylyl cyclase; ADP, adenosine diphosphate; ATP, adenosine triphosphate; PDE-III, phosphodiesterase III; PGE1, prostaglandin E1; PKA, protein kinases; VASP-P, phosphorylation of vasodilator-stimulated phosphoprotein. Reproduced with permission from Angiolillo DJ, Ueno M. Optimizing platelet inhibition in clopidogrel poor metabolizers: therapeutic options and practical considerations. *JACC Cardiovasc Interv*. 2011;4:411–414.

prasugrel, the trial was stopped prematurely for futility due to an event rate that was substantially lower than expected.[32]

Prasugrel

Prasugrel, a third generation thienopyridine, is an orally administered prodrug that needs hepatic biotransformation into its active metabolite to irreversibly block the $P2Y_{12}$ receptor.[33] Prasugrel has several pharmacological advantages over clopidogrel, because it is more effectively converted into its active metabolite and displays a faster onset of action and greater degree of platelet inhibition with less variability in response, even when compared with high dose clopidogrel.[34]

The TRITON-TIMI 38 (Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel-Thrombolysis In Myocardial Infarction 38) trial evaluated the clinical efficacy and safety of prasugrel (60 mg loading dose followed by a 10 mg maintenance dose), compared with standard clopidogrel (300 mg loading dose followed by 75 mg daily maintenance dose) therapy in 13 608 patients with moderate to high risk ACS undergoing PCI.[35] Patients pretreated with clopidogrel were not eligible for this study and patients were randomized only after coronary anatomy was established, with the exception of patients presenting with STEMI undergoing primary PCI in whom allocation to randomized treatment was allowed before coronary anatomy was known. The primary efficacy end point, which was the composite of death from cardiovascular causes, nonfatal myocardial infarction (MI), or nonfatal

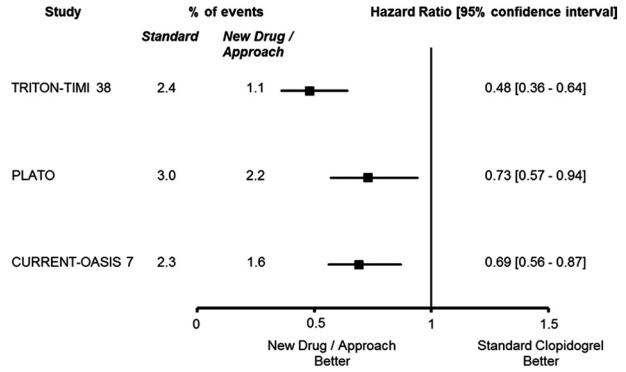


Figure 3. Efficacy in reducing the rates of definite and probable stent thrombosis of new drugs/approaches tested in large-scale clinical trials. The data presented represents the rates of definite and probable stent thrombosis in the cohort of patients undergoing stent placement in these studies. The TRITON-TIMI 38 trial compared prasugrel (60 mg loading dose followed by a 10 mg maintenance dose) versus standard clopidogrel therapy (300 mg loading dose followed by 75 mg daily maintenance dose) in patients with moderate to high risk acute coronary syndrome (ACS) undergoing percutaneous coronary intervention, with up to 15 months follow-up. The PLATO trial compared ticagrelor (180 mg loading dose followed by 90 mg twice daily) with clopidogrel (300 to 600 mg loading dose followed by 75 mg daily), with up to 12 months follow-up. The CURRENT-OASIS 7 trial evaluated 30 days outcomes comparing high (600 mg loading dose, then 150 mg once a day for 7 days, followed by 75 mg daily) versus standard (300 mg loading dose followed by 75 mg daily) clopidogrel dosing in ACS patients scheduled to undergo angiography within 72 hours of hospital arrival.

stroke over a follow-up period of 15 months, occurred in 9.9% of patients treated with prasugrel and in 12.1% of patients treated with clopidogrel, thus resulting in a significant 19% relative reduction with prasugrel (HR, 0.81 [0.73–0.90]; $P<0.001$). This benefit was hampered by an increased risk of TIMI major non-coronary artery bypass graft (CABG) related bleeding (2.4% versus 1.8%; $P=0.03$), including fatal bleeding (0.4% versus 0.1%; HR, 4.19 [1.58–11.11]; $P=0.002$), which occurred mostly in the maintenance phase of prasugrel treatment.[36] A prespecified net clinical benefit analysis (a composite of the rates of death from any cause, nonfatal MI, nonfatal stroke, and non–CABG- related TIMI major hemorrhage) was performed and a significant net clinical benefit was associated with prasugrel therapy despite the excess in bleeding (12.2% versus 13.9%; HR, 0.87 [0.79–0.95]; $P=0.004$). The clinical benefit of prasugrel was driven largely by a marked reduction in nonfatal MI, approximately 40% of which were periprocedural. In addition, a significant 52% reduction of the rates of definite or probable stent thrombosis was achieved with prasugrel compared with clopidogrel (1.13% versus 2.35%; HR, 0.48 [0.36–0.84]; $P<0.0001$).[37] A comparison of the efficacy of new antiplatelet strategies in the reduction of stent thrombosis is shown in Figure 3. Such benefit was both early (<30 days) and late (up to 15 months) and irrespective of stent type (bare metal or drug-eluting). Importantly, certain subgroups appeared to benefit the most from the use of prasugrel, such as patients with diabetes mellitus and those with STEMI, in whom there was a greater ischemic benefit without an increase in major bleeding complications.[38,39] In addition, in patients with an initial nonfatal event, recurrent events, including mortality, were significantly reduced with prasugrel compared with clopidogrel.[40] In contrast, no net benefit was observed in elderly patients (≥ 75 years) and in those weighing less than 60 kg due to an increase in bleeding complications. The Food and Drug Administration recommends using a 5 mg dose in low weight patients, although the safety of this dose, which derives from pharmacokinetic findings, has not been prospectively studied yet. In elderly patients, prasugrel is generally not recommended except in patients with diabetes or a prior MI, in whom the benefits outweighed the risks, supporting the use of prasugrel at standard dosing in the elderly with these characteristics. A net harm was found in patients with history of stroke or transient ischemic attack, and therefore prasugrel is contraindicated in these subjects. In addition, prasugrel is contraindicated in patients at high risk of bleeding. Patients who are treated with clopidogrel can switch to prasugrel without concerns of drug interactions and is associated with increased platelet inhibition.[41] Prasugrel effects have not shown to be modulated by aspirin dose or CYP interfering drugs, including proton pump inhibitors. A washout period of 7 days is warranted for prasugrel-treated patients requiring surgery. Prasugrel is only approved for clinical use in patients with ACS undergoing PCI, and the efficacy and safety of prasugrel in medically-managed patients (n=10 300) with UA/NSTEMI is currently being evaluated in the TRILOGY-ACS (TaRgeted platelet Inhibition to cLarify the Optimal strateGy to medicallY manage Acute Coronary Syndromes) trial (NCT00699998). Further, the benefits and risks associated with prasugrel pretreatment in ACS patients (n=4100) scheduled for an invasive strategy is being evaluated in the ACCOAST (A Comparison of Prasugrel at PCI or Time of Diagnosis of Non-ST Elevation Myocardial Infarction, NCT01015287) trial.

Ticagrelor

Ticagrelor is an orally administered cyclopentyltriazolopyrimidine, a new compound class, which directly and reversibly inhibits through allosteric modulation the platelet ADP $P2Y_{12}$ receptor.[42] Similarly to prasugrel, standard dose ticagrelor (180 mg loading dose/90 mg twice daily maintenance dose) has a faster onset of action and provides stronger and more consistent platelet inhibition than clopidogrel. Because ticagrelor has reversible binding effects and plasma half-life of 8 to 12 hours, twice daily dosing is required.[43] Approximately 30% to 40% of ticagrelor effects are attributed to metabolites generated by the hepatic CYP3A system, which also is involved in metabolism of the drug itself.

The PLATO (Platelet Inhibition and Patient Outcomes) trial evaluated the benefit of ticagrelor (180 mg loading dose followed by 90 mg twice daily) compared with clopidogrel (300 to 600 mg loading dose followed by 75 mg daily) in preventing cardiovascular events in 18 624 ACS patients.[44] PLATO is the latest of the pivotal large-scale clinical trials evaluating the efficacy of dual antiplatelet therapy with aspirin and an orally administered $P2Y_{12}$ receptor inhibitor in ACS patients (Table 2). In contrast to TRITON-TIMI 38, in PLATO patients pretreated with clopidogrel were eligible for enrollment, and randomization generally occurred before

Table 2. Pivotal Clinical Trials Evaluating the Efficacy of Dual Antiplatelet Therapy With Aspirin and an Orally Administered $P2Y_{12}$ Receptor Inhibitor

Study	N	Study Drugs	Setting	Primary End Point	Results*
CURE[22]	12 562	Aspirin+clopidogrel vs aspirin	UA/NSTEMI	Cardiovascular death, nonfatal MI, or stroke at 1 y	9.3% vs 11.4% RR = 0.80 [0.72–0.90]
CREDO[23]	2116	Aspirin+clopidogrel vs aspirin	Elective PCI	Death, MI, or stroke at 1 y	8.5% vs 11.5% RRR = 26.9% [3.9%–44.4%]
COMMIT[24]	45 852	Aspirin+clopidogrel vs aspirin	Acute MI (93% STEMI)	Death, reinfarction, or stroke at discharge or 28 d	9.2% vs 10.1% OR = 0.91 [0.86–0.97]
CLARITY[25]	3491	Aspirin+clopidogrel vs aspirin	STEMI with fibrinolysis	Occluded infarct-related artery on angiography or death or recurrent MI before angiography	15.0% vs 21.7% OR = 0.64 [0.53–0.76]
CURRENT OASIS-7[10]	25 086	Aspirin+clopidogrel (double dose for 1 wk) vs aspirin+clopidogrel (standard dose)	ACS patients referred for an invasive strategy	Cardiovascular death, MI, or stroke at 30 d	4.2% vs 4.4% HR = 0.94 [0.83–1.06]
TRITON-TIMI 38[35]	13 608	Aspirin+prasugrel vs aspirin+clopidogrel	ACS patients undergoing PCI	Cardiovascular death, nonfatal MI, or nonfatal stroke	9.9% vs 12.1% HR = 0.81 [0.7–0.90]
PLATO[44]	18 624	Aspirin+ticagrelor vs aspirin+clopidogrel	ACS patients	Death from vascular causes, MI, or stroke	10.2% vs 12.3% HR = 0.84 [0.77–0.92]

*Results are expressed as % of events and association measure [95% confidence interval].

UA indicates unstable angina; NSTEMI, non–ST-elevation myocardial infarction; MI, myocardial infarction; RR, relative risk; PCI, percutaneous coronary intervention; RRR, relative risk reduction; STEMI, ST-elevation myocardial infarction; OR, odds ratio; ACS, acute coronary syndromes; HR, hazard ratio.

defining coronary anatomy to reflect current practice patterns. In this trial, ticagrelor therapy significantly reduced the rate of the primary end point (death from vascular causes, nonfatal MI, or nonfatal stroke) at 12 months (9.8% versus 11.7%; HR, 0.84 [0.77–0.92]; $P=0.0001$). The outcomes were driven by a reduction of cardiovascular death (4.0% versus 5.1%; HR, 0.79; $P=0.001$) and MI (5.8% versus 6.9%; HR, 0.84 [0.75–0.95]; $P=0.005$). Ticagrelor-treated patients also experienced a reduction in definite or probable stent thrombosis (2.2% versus 3.0%; HR, 0.73 [0.57–0.94]; $P=0.014$; Figure 3). Although no differences in protocol-defined major bleeding was found (11.6% versus 11.2%; HR, 1.04; $P=0.43$), the rate of non-CABG major bleeding was increased significantly with ticagrelor when using both PLATO (4.5% versus 3.8%; $P=0.03$) and TIMI criteria (2.8% versus 2.2%; $P=0.03$).[44] In addition, although fatal intracranial bleeding was significantly more frequent in the ticagrelor arm (0.1% versus 0.01%; $P=0.02$), overall PLATO-defined fatal bleeding was not significantly different between arms (0.3% versus 0.3%; $P=0.66$). Of note, the benefit of ticagrelor was consistent across different subgroup analyses, such as patients with an initial conservative approach with noninvasive treatment strategy,[45] patients undergoing a planned invasive strategy,[46] and those undergoing CABG.[47] In addition, there weren't any specific subgroups that emerged to have higher bleeding potential with ticagrelor, including patients with prior transient ischemic/ischemic stroke. Several nonhematological safety end points, which have been associated with higher discontinuation rates, have been observed with ticagrelor. These include higher rates of dyspnea and ventricular pauses, and increased levels of creatinine and uric acid during treatment compared with clopidogrel. Although the mechanisms contributing to these effects have been attributed to off target effects of ticagrelor (eg, increased adenosine levels due to reduced erythrocyte uptake) or its metabolites, they remain elusive, and these side effect thus far have not been shown to have any significant clinical impact.[48,49]

Ticagrelor has been approved recently for clinical use and is indicated for the prevention of atherothrombotic events in patients with ACS, including patients managed medically and invasively. In addition to being contraindicated in patients at high risk of bleeding, ticagrelor is contraindicated in patients with prior hemorrhagic stroke and severe hepatic dysfunction. Ticagrelor-treated patients requiring surgery warrant a minimum of a 5 day washout period to minimize bleeding complications. Because ticagrelor is metabolized by CYP3A4/5 enzymes, the prescribing information for ticagrelor recommends that patients taking ticagrelor should avoid the use of strong inhibitors or inducers of CYP3A. In addition, patients taking ticagrelor should avoid simvastatin and lovastatin doses >40 mg and monitor digoxin levels with initiation of, or any change in, ticagrelor therapy. Furthermore, patients from North America participating in the PLATO trial had worse outcomes with ticagrelor compared with other geographic regions.[50] This result is believed to be related to the higher doses of long-term aspirin generally administered to patients with ACS in the United States, and the prescribing information for ticagrelor includes a warning to avoid aspirin doses >100 mg in patients receiving the drug.[50] The ongoing PEGASUS (Prevention of Cardiovascular Events in Patients With Prior Heart Attack Using Ticagrelor Compared to Placebo on a Background of Aspirin)-TIMI 54 trial is evaluating the efficacy and safety of ticagrelor in combination with aspirin (versus aspirin plus placebo) in patients (n=21 000) with a history of MI within 1 to 3 years (NCT01225562). The

ongoing ATLANTIC trial (A 30 Day Study to Evaluate Efficacy and Safety of Prehospital versus In-hospital Initiation of Ticagrelor Therapy in STEMI Patients Planned for Percutaneous Coronary Intervention, NCT01347580) is evaluating prehospital versus in hospital initiation of ticagrelor therapy in STEMI patients (n=1770) planned for PCI.

Glycoprotein IIb/IIIa Inhibitors

Three different GPIs are currently approved for clinical use: abciximab, eptifibatide, and tirofiban. These drugs are only available for intravenous use and have a rapid onset of action and a very potent inhibitory effect on platelets. However, their use is restricted to the acute phase of treatment. Importantly, the efficacy of these agents correlates directly with the severity and the risk of ACS, thus, its use is not generally recommended in low to moderate risk patients or in those in whom a conservative approach is chosen, whereas they reach their maximal benefit in high risk ACS patients undergoing PCI.[51] Of note, many trials evaluating GPIs' efficacy were performed before in the era in which regimens of clopidogrel that are currently being used (eg, pretreatment, high loading doses) were not part of the standard of care and the new $P2Y_{12}$ inhibiting agents prasugrel and ticagrelor were not available. Therefore, the role of GPIs role in today's clinical practice is diminished significantly.

The benefit of abciximab for reduction of ischemic events in ACS patients undergoing PCI after a clopidogrel 600 mg loading dose appears to be limited to high risk patients both in NSTEACS, such as a dose with elevated troponin levels, and STEMI.[52,53] However, the major limitation of GPIs is bleeding risk. Importantly, bleeding complications have shown to have important prognostic implications, including on short and long -term mortality, underscoring the need to identify safer antithrombotic treatment options.[54] Head-to-head comparisons between GPIs and bivalirudin, a direct thrombin inhibitor, have shown bivalirudin to be noninferior in terms of reducing ischemic events, but associated with better safety as indicated by the lower rates of major bleedings compared with GPIs. Such benefit has been demonstrated in a number of clinical settings of patients undergoing PCI, including in NSTEACS as demonstrated in the ACUITY (Acute Catheterization and Urgent Intervention Triage Strategy) and ISAR-REACT-4 (Intracoronary Stenting and Antithrombotic: Regimen Rapid Early Action for Coronary Treatment 4) trials,[55,56] as well as in STEMI undergoing primary PCI as demonstrated in the HORIZONS-AMI (Harmonizing Outcomes with Revascularization and Stents in Acute Myocardial Infarction) trials,[57] which also showed a mortality benefit.

Most recently 2 studies provided new insights on the use of intracoronary abciximab in patients with STEMI undergoing primary PCI. The prospective, randomized AIDA STEMI (Abciximab Intracoronary versus intravenous Drug Application in ST-Elevation Myocardial Infarction) trial showed that intracoronary as compared with intravenous abciximab did not result in a difference in the combined end point of death, reinfarction, or congestive heart failure in patients with STEMI (n=2065) undergoing primary PCI, although it did not raise any safety concerns and showed reduced rates of congestive heart failure with the intracoronary route. The INFUSE-AMI (Intracoronary Abciximab and Aspiration Thrombectomy in Patients With Large Anterior Myocardial Infarction) trial was a 2x2 factorial design study that showed that in patients with large anterior STEMI (n=452) presenting early after symptom onset (<4 hours) and undergoing primary PCI with bivalirudin as anticoagulant, infarct size at 30 days was significantly reduced by intracoronary bolus of abciximab delivered locally to the infarct lesion site but not by manual aspiration thrombectomy.[58]

Antiplatelet Agents Under Clinical Development

There are still drawbacks of currently approved antiplatelet agents, which include (1) no effective alternative to block TXA_2 pathway in patients with either severe allergy or inadequate response to aspirin, (2) a $P2Y_{12}$ inhibitor intravenously administered for patients in whom absorption of oral medications is compromised (eg, intubated patients), and (3) a $P2Y_{12}$ inhibitor with a very quick offset of action, which can be useful in patients with a bleeding event or as a bridging therapy to provide sufficient platelet inhibition in patients that need to undergo CABG. In this section, we provide an overview on several drugs under development that may play a future role if shown to be effective for these unmet needs.

Thromboxane A_2 Pathway Inhibitors

Because inhibition of TP receptors blocks the effect of TXA_2 on platelets as well as TP activation through other ligands, such as eicosanoids and endoperoxides, blockade of TP may have potential advantages over COX-1 inhibition achieved with aspirin. Further, many TXA_2 pathway inhibitors also exert inhibitory effects on TXA_2 synthase in addition to TP receptors, allowing more comprehensive blockade TXA_2 mediated signaling. Moreover, TPs are also expressed in inflammatory cells, the vascular wall, and in atherosclerotic plaques. Thus, TP antagonists may also exert some effect on these structures.

TXA_2 pathway inhibitors include picotamide (a combined TXA_2 synthase inhibitor and TP receptor blocker), ridogrel (a combined TXA_2 synthase inhibitor and TP receptor blocker), ramatroban (a TP receptor inhibitor), NCX 4016 (a nitric oxide-releasing aspirin derivative), Si8886/terutroban (a TP receptor inhibitor), and EV-077 (a combined TXA_2 synthase inhibitor and TP receptor blocker).[59,60] Some of these agents have been tested in clinical settings. In a randomized trial of patients with diabetes mellitus and peripheral artery disease (PAD), picotamide reduced long term overall mortality, but not major cardiovascular events, compared with aspirin.[61] Ridogrel failed to show any benefit over aspirin as adjunct therapy to thrombolysis in patients with acute MI.[62] Terutroban (S18886) is a novel oral, selective, and reversible TP antagonist, which has shown an excellent safety profile in patients with stable PAD.[63] However, terutroban failed to meet the primary end point of noninferiority compared with aspirin in a cohort of patients with cerebrovascular disease.[64] At the present time, none of the above mentioned agents appear to be suitable for replacing aspirin in patients with CAD.

$P2Y_{12}$ Inhibitors

Cangrelor is the $P2Y_{12}$ inhibitor at the most advanced stage of clinical development. Cangrelor is an intravenous adenosine

Table 3. Pharmacological Properties of Currently Approved and Investigational $P2Y_{12}$ Inhibitors

	Clopidogrel	Prasugrel	Ticagrelor	Cangrelor*	Elinogrel*
Group	Thienopyridine	Thienopyridine	CPTP	ATP analog	Quinazolinedione
Administration	Oral	Oral	Oral (bid)	IV	IV and oral
Receptor blockade	Irreversible	Irreversible	Reversible	Reversible	Reversible
Onset of action	2–8 h	30 min–4 h	30 min– 2 h	Seconds	Seconds
Offset of action	7–10 d	7–10 d	3–5 d	~60 min	50 min (IV) 12 h (oral)
CYP drug interactions	Yes	No	Yes	No	No

*Cangrelor and elinogrel are investigational agents and not approved for clinical use at the time of preparation of this manuscript.
CPTP indicates cyclopentyltriazolopyrimidine; ATP, adenosine triphosphate; IV, intravenous; CYP, cytochrome P450.

triphosphate analog, which reversibly and directly, thus, not needing any biotransformation, inhibits the $P2Y_{12}$ receptor.[65] Cangrelor has dose dependent and, thus, predictable, pharmacodynamics effects. It achieves very potent (>90%) platelet inhibition, with immediate onset of action, and because of its ultrashort half-life (3–6 minutes), it has a very rapid offset of action, with return to baseline platelet function within 30 to 60 minutes.[65]

Despite the promising results obtained in phase II studies, which showed cangrelor to be a very potent platelet inhibitor with a relatively safe profile, these findings were not corroborated in phase III studies. The CHAMPION (Cangrelor versus standard tHerapy to Achieve optimal Management of Platelet InhibitiON) program included the CHAMPION-PCI and the CHAMPION-PLATFORM trials, which evaluated mostly ACS patients undergoing PCI, and were terminated before completion because of an interim analysis showing insufficient evidence of clinical effectiveness of cangrelor (bolus 30 μg/kg plus infusion of 4μk/kg/min for the duration of the PCI procedure, with a minimum infusion duration of 2 hours and a maximum of 4 hours).[66,67] Pitfalls in trial design and definition of study end points may have contributed to failure to show superiority in terms of reduction of adverse ischemic outcomes of cangrelor over clopidogrel in CHAMPION-PCI (n=8716), and over placebo in CHAMPION-PLATFORM (n=5362) trials. In a pooled analysis of the 2 CHAMPION trials comprising a total of 13 049 patients, cangrelor had no effect on the primary end point with the original MI definition (P=0.646). However, with the use of the universal definition, the primary end point was decreased with cangrelor (odds ratio [OR], 0.82 [0.68–0.99]; P=0.037). Stent thrombosis was reduced from 0.4% to 0.2% (OR, 0.44 [0.22–0.87]; P=0.018). Major bleeding and transfusions were not increased with cangrelor.[68] Based on this evidence, another randomized large scale phase III clinical trial, the CHAMPION-PHOENIX (NCT01156571), is currently ongoing to evaluate efficacy and safety of cangrelor compared with standard of care patients undergoing PCI. Thus, the potential role of cangrelor in reducing ischemic events in PCI patients remains to be determined.

Cangrelor may still have a role, due to its pharmacological properties, as a bridging strategy in the setting of patients requiring surgery but who require treatment with a $P2Y_{12}$ inhibitor to prevent thrombotic complications, such as in ACS patients or those treated with drug-eluting stents. The BRIDGE (Maintenance of platelet inhiBition with cangreloR after dIscontinuation of thienopyriDines in patients undergoing surGEry) trial was a prospective, randomized double-blind, placebo-controlled, multicenter trial in patients (n=210) with an ACS or treated with a coronary stent on a thienopyridine awaiting CABG to receive either placebo or cangrelor at a dose (0.75 μg/kg/min) identified in dose-finding phase of the trial.[69] Therefore, cangrelor may represent a future option for bridging therapy in patients with ACS or treated with coronary stents who require surgery.

Elinogrel is a novel direct-acting agent that reversibly inhibits the $P2Y_{12}$ receptor and provides a high degree of platelet inhibition with rapid onset and offset of action.[70] Elinogrel has the important feature of having both oral and intravenous ways of administration. A comparison of pharmacological properties of $P2Y_{12}$ antagonists is provided in Table 3. The phase II INNOVATE-PCI (A Randomized, Double-Blind, Active-Controlled Trial to Evaluate Intravenous and Oral PRT060128, a Selective and Reversible P2Y12 Inhibitor, versus Clopidogrel, as a Novel Antiplatelet Therapy in Patients Undergoing Non-Urgent PCI) trial (NCT00751231) has evaluated clinical efficacy, biological activity, tolerability, and safety of elinogrel in patients undergoing nonurgent PCI, testing 3 different doses (oral 50, 100, and 150 mg twice daily for 120 days, following an intravenous bolus of 80 mg), compared with clopidogrel. This trial provided promising results of elinogrel in terms of platelet inhibition, as both intravenous and oral dosing achieved greater and more rapid platelet inhibition than clopidogrel, and safety, as no significant increase in major bleedings was found.[71,72] A safety concern was the presence of elevated liver enzymes in 4.0% and 4.8% of the elinogrel 100 mg and 150 mg twice daily arms, respectively, mostly within the first 60 days, compared with 1% in the clopidogrel group. Phase III clinical evaluation of elinogrel is still pending.

Protease-Activated Receptor-1 Inhibitors

Dual antiplatelet therapy with aspirin and a $P2Y_{12}$ receptor inhibitor represents the current standard of care for patients with ACS or undergoing PCI. However, aspirin and $P2Y_{12}$ inhibitors target the TXA_2 and ADP $P2Y_{12}$ platelet activation pathways and minimally affect other pathways, such as thrombin mediated platelet activation. Thrombin is an essential component of the coagulation cascade, and also a potent agonist for platelet activation.[73] This may help explain why patients continue to experience recurrent ischemic events despite receiving standard DAPT. A selective inhibition of

thrombin-mediated platelet activation, the most potent pathway for platelet aggregation, without other effects on hemostatic processes that involve thrombin therefore may represent an attractive strategy for patients with atherothrombotic diseases. Currently, 2 oral thrombin receptor antagonists, which selectively block the platelet protease-activated receptor-1 (PAR-1) receptor subtype, are under clinical development: vorapaxar (SCH530348) and atopaxar (E5555).[73] Vorapaxar is a selective and potent oral PAR-1 (the principal thrombin receptor in humans) antagonist, which has shown a good efficacy and safety profile in preclinical and phase I and II studies, in which addition of vorapaxar to DAPT with aspirin and clopidogrel, also known as triple antiplatelet therapy, was not associated with increased risk of bleeding.[74]

The phase III clinical development of vorapaxar includes 2 large-scale trials: TRACER (Trial to Assess the Effects of SCH 530348 in Preventing Heart Attack and Stroke in Patients With Acute Coronary Syndrome) and TRA 2°P (Trial to Assess the Effects of SCH 530348 in Preventing Heart Attack and Stroke in Patients With Atherosclerosis)-TIMI 50 Results of the TRACER trial, which randomized patients with NSTEACS (n=12 944) to receive vorapaxar or placebo on top of standard antiplatelet therapy (approximately 90% on DAPT with aspirin and clopidogrel), has been published recently.[75] Follow-up in the trial was stopped prematurely due to a safety review that observed an excess in the rates of moderate and severe bleeding in the vorapaxar arm compared with placebo (7.2% versus 5.2%; HR, 1.65 [1.16–1.58]; $P<0.001$), as well as in the rates of intracranial hemorrhage (1.1% versus 0.2%; HR, 3.39 [1.78–6.45]; $P<0.001$). The primary efficacy end point (composite of death from cardiovascular causes, MI, stroke, recurrent ischemia with rehospitalization, or urgent coronary revascularization) was numerically but not significantly reduced with the addition of vorapaxar to standard therapy (18.5% versus 19.9%; HR, 0.92 [0.85–1.01]; $P=0.07$).[75] In TRA 2°P-TIMI 50 trial, patients who had a history of MI, ischemic stroke, or PAD (n=26 449) were randomized to receive vorapaxar (2.5 mg daily) or placebo with a median follow-up of 30 months. Vorapaxar reduced the rates of the primary efficacy end point (composite of death from cardiovascular causes, MI, or stroke) compared with placebo (9.3% versus 10.5%; HR, 0.87 [0.80–0.94]; $P<0.001$), at the cost of increasing the risk of moderate or severe bleeding (4.2% versus 2.5%; HR, 1.66 [1.43–1.93]; $P<0.001$), including intracranial hemorrhage (1.0% versus 0.5%; $P<0.001$). Of note, vorapaxar treatment was discontinued in patients with a prior stroke due to the risk of intracranial hemorrhage.[76]

Atopaxar is in an earlier stage of development that has recently completed phase II testing. Two phase II studies, the LANCELOT-ACS (Lessons From Antagonizing the Cellular Effects of Thrombin-Acute Coronary Syndromes) and the LANCELOT-CAD (Lessons From Antagonizing the Cellular Effect of Thrombin-Coronary Artery Disease) recently have observed a good safety profile in terms of bleeding risk of atopaxar compared with placebo in patients with ACS and with CAD, respectively.[77,78] However, dose-dependent QTc prolongation without apparent complications and transient elevation in liver transaminases were observed with the highest doses of atopaxar.[77,78] Parallel findings were found in another phase II study performed in Japanese patients with ACS or high risk CAD.[79] Larger trials are warranted to establish the real clinical value of this new agent. However, phase III investigations are not being planned for atopaxar.

Other Antiplatelet Agents in Early Phase Clinical Development

Several other agents that target a number of platelet signaling pathways have been evaluated in preclinical or early phase clinical studies, including inhibitors of collagen-platelet interaction, such as glycoprotein VI antagonists (kistomin, revacept) or glycoprotein Ib antagonist (6B4-Fab monoclonal antibody), serotonin receptor inhibitors (APD791), prostaglandin E receptor 3 antagonists (DG-041), nitric oxide donors (LA846, LA419), and phosphatidylinositol 3-kinase inhibitors (TGX-221).[59,80] These agents need to undergo more advanced clinical testing before establishing its possible applications in clinical practice.

Future Perspectives and Conclusions

Dual antiplatelet therapy with aspirin and clopidogrel has been for many years the antiplatelet treatment of choice for patients with ACS and undergoing PCI. Despite the benefit of this combination, a substantial percentage of patients still present recurrent atherothrombotic events, leading to the development of newer and more potent antiplatelet agents, some of which have already been approved for clinical use, such as prasugrel and ticagrelor.[29] Both agents support the concept that in high-risk settings more potent platelet inhibition translates into reduced risk of ischemic events at the expense of increased bleeding risk.[35,44] However, because there is some overlapping in the recommendations of currently available guidelines,[3–6] the choice of a particular antiplatelet strategy for a given patient may be confusing. Until more evidence derived from large scale studies is presented (eg, head-to head comparisons between prasugrel and ticagrelor), subgroup analyses of available data might represent a reasonable option to determine the best niche for the use of each of the newer antiplatelet agents, as well as to define settings in which 1 or both of these drugs should not be used. However, clinicians must also be cautious when using subgroup data to guide therapy because these analyses are sometimes methodologically limited because they are underpowered to demonstrate a treatment effect, and the analysis is often not planned but performed post hoc. Indeed, costs remain a key decision factor for the patient on whether a novel $P2Y_{12}$ receptor inhibitor will be chosen over clopidogrel, which will soon be available in a generic and less expensive formulation in most countries. Similar cost-effectiveness considerations can be made with regards on how to implement other proposed antithrombotic approaches, such as adding the novel oral anticoagulant rivaroxiban to standard DAPT, a strategy that was associated with a reduction in ischemic events, including reduced cardiovascular mortality using a 2.5 mg twice daily dosing regimen, albeit at the expense of increased major bleeding and intracranial hemorrhage.[81]

Strategies of stratifying patients based on results of platelet function and genetic testing, which have been able to identify patients at increased risk of recurrent atherothrombotic events

Table 4. Guideline Recommendations on the Use of Platelet Function and Genetic Testing

	Platelet Function Testing	Genetic Testing
2011 ACCF/AHA Focused Update of the Guidelines for the Management of Patients With Unstable Angina/Non–ST-Elevation Myocardial Infarction[3]	*Class IIb; Level of Evidence B* Platelet function testing to determine platelet inhibitory response in patients with UA/NSTEMI (or, after ACS and PCI) on thienopyridine therapy may be considered if results of testing may alter management.	*Class IIb; Level of Evidence C* Genotyping for a CYP2C19 loss of function variant in patients with UA/NSTEMI (or, after ACS and with PCI) on clopidogrel therapy might be considered if results of testing may alter management.
2011 ACCF/AHA/SCAI Guideline for Percutaneous Coronary Intervention[4]	*Class IIb; Level of Evidence C* Platelet function testing may be considered in patients at high risk for poor clinical outcomes. In patients treated with clopidogrel with high platelet reactivity, alternative agents, such as prasugrel or ticagrelor, might be considered.	*Class IIb; Level of Evidence C* Genetic testing might be considered to identify whether a patient at high risk for poor clinical outcomes is predisposed to inadequate platelet inhibition with clopidogrel. When a patient predisposed to inadequate platelet inhibition with clopidogrel is identified by genetic testing, treatment with an alternate $P2Y_{12}$ inhibitor (e.g., prasugrel or ticagrelor) might be considered.
	Class III; Level of Evidence C The routine clinical use of platelet function testing to screen patients treated with clopidogrel who are undergoing PCI is not recommended.	*Class III; Level of Evidence C* The routine clinical use of genetic testing to screen patients treated with clopidogrel who are undergoing PCI is not recommended.
2011 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation[5]	*Class IIb; Level of Evidence B* Increasing the maintenance dose of clopidogrel based on platelet function testing is not advised as routine, but may be considered in selected cases.	*Class IIb; Level of Evidence B* Genotyping and/or platelet function testing may be considered in selected cases when clopidogrel is used.
2010 ESC/EACTS/EAPCI Guidelines on myocardial revascularization[6]	No recommendation	No recommendation

ACS: acute coronary syndrome; NSTEMI: non-ST-elevation myocardial infarction; PCI: percutaneous coronary intervention; UA: unstable angina.

despite compliance with clopidogrel therapy, have represented very important advancements in our field.[20,21] These strategies may set the basis for investigations to identify patients who can potentially benefit from antiplatelet treatment strategies tailored to the individual patient, with the goal of maximizing ischemic benefit and minimizing bleeding risk.[82,83] Defining a "therapeutic window" of levels of platelet reactivity associated with reduced risk of ischemic and bleeding events is indeed a promising area of research that, however, requires further investigation. However, to date, larger scale clinical studies have failed to show that modifying therapy translates into improved clinical outcomes and current guidelines do not support their routine use of platelet function and genetic testing (Table 4).[3–6] Ongoing clinical trials assessing novel antiplatelet agents or treatment strategies will indeed provide the safety and efficacy information to define the best combination of antiplatelet treatment strategies to treat patients with ACS or undergoing PCI.

Disclosures

Dominick J. Angiolillo (corresponding author) reports receiving honoraria for lectures from Bristol Myers Squibb, Sanofi-Aventis, Eli Lilly Co., Daiichi Sankyo, Inc., and Astra Zeneca, consulting fees from Bristol Myers Squibb, Sanofi-Aventis, Eli Lilly Co, Daiichi Sankyo, Inc., The Medicines Company, Portola, Novartis, Medicure, Accumetrics, Arena Pharmaceutical, Astra Zeneca, Merck, Evolva, and Abbott Vascular, and research grants from Bristol Myers Squibb, Sanofi-Aventis, GlaxoSmithKline, Otsuka, Eli Lilly Co., Daiichi Sankyo, Inc., The Medicines Company, Portola, Accumetrics, Schering-Plough, Astra-Zeneca, and Eisai.

José Luis Ferreiro reports honoraria for lectures from Eli Lilly Co., Daiichi Sankyo, Inc., and Astra Zeneca.

References

1. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med*. 2005;352:1685–1895.
2. Davì G, Patrono C. Platelet activation and atherothrombosis. *N Engl J Med*. 2007;357:2482–2494.
3. Wright RS, Anderson JL, Adams CD, Bridges CR, Casey DE Jr., Ettinger SM, Fesmire FM, Ganiats TG, Jneid H, Lincoff AM, Peterson ED, Philippides GJ, Theroux P, Wenger NK, Zidar JP, Jacobs AK. 2011 ACCF/AHA Focused Update of the Guidelines for the Management of Patients With Unstable Angina/ Non-ST-Elevation Myocardial Infarction (Updating the 2007 Guideline): a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *Circulation*. 2011;123:2022–2060.
4. Levine GN, Bates ER, Blankenship JC, Bailey SR, Bittl JA, Cercek B, Chambers CE, Ellis SG, Guyton RA, Hollenberg SM, Khot UN, Lange RA, Mauri L, Mehran R, Moussa ID, Mukherjee D, Nallamothu BK, Ting HH. 2011 ACCF/AHA/SCAI Guideline for Percutaneous Coronary Intervention: a Report of the American College of Cardiology Foundation/ American Heart Association Task Force on Practice Guidelines and the Society for Cardiovascular Angiography and Interventions. *Circulation*. 2011;124:e574–651.
5. Hamm CW, Bassand JP, Agewall S, Bax J, Boersma E, Bueno H, Caso P, Dudek D, Gielen S, Huber K, Ohman M, Petrie MC, Sonntag F, Uva MS, Storey RF, Wijns W, Zahger D. ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: the Task Force for the management of acute coronary syndromes (ACS) in patients presenting without persistent

ST-segment elevation of the European Society of Cardiology (ESC). *Eur Heart J*. 2011;32:2999–3054.
6. Wijns W, Kolh P, Danchin N, Di Mario C, Falk V, Folliguet T, Garg S, Huber K, James S, Knuuti J, Lopez-Sendon J, Marco J, Menicanti L, Ostojic M, Piepoli MF, Pirlet C, Pomar JL, Reifart N, Ribichini FL, Schalij MJ, Sergeant P, Serruys PW, Silber S, Sousa Uva M, Taggart D. Guidelines on myocardial revascularization. *Eur Heart J*. 2010;31: 2501–2555.
7. Patrono C, García Rodríguez LA, Landolfi R, Baigent C. Low-dose aspirin for the prevention of atherothrombosis. *N Engl J Med*. 2005;353: 2373–2383.
8. Antithrombotic Trialists' Collaboration. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. *BMJ*. 2002;324:71–86.
9. Peters RJ, Mehta SR, Fox KA, Zhao F, Lewis BS, Kopecky SL, Diaz R, Commerford PJ, Valentin V, Yusuf S: Clopidogrel in Unstable angina to prevent Recurrent Events (CURE) Trial Investigators. Effects of aspirin dose when used alone or in combination with clopidogrel in patients with acute coronary syndromes: observations from the Clopidogrel in Unstable angina to prevent Recurrent Events (CURE) study. *Circulation*. 2003; 108:1682–1687.
10. CURRENT-OASIS 7 Investigators, Mehta SR, Bassand JP, Chrolavicius S, Diaz R, Eikelboom JW, Fox KA, Granger CB, Jolly S, Joyner CD, Rupprecht HJ, Widimsky P, Afzal R, Pogue J, Yusuf S. Dose comparisons of clopidogrel and aspirin in acute coronary syndromes. *N Engl J Med*. 2010;363:930–942.
11. Krasopoulos G, Brister SJ, Beattie WS, Buchanan MR. Aspirin "resistance" and risk of cardiovascular morbidity: systematic review and meta-analysis. *BMJ*. 2008;336:195–198.
12. Gurbel PA, Bliden KP, DiChiara J, Newcomer J, Weng W, Neerchal NK, Gesheff T, Chaganti SK, Etherington A, Tantry US. Evaluation of dose-related effects of aspirin on platelet function: results from the Aspirin-Induced Platelet Effect (ASPECT) study. *Circulation*. 2007;115: 3156–3164.
13. Cox D, Maree AO, Dooley M, Conroy R, Byrne MF, Fitzgerald DJ. Effect of enteric coating on antiplatelet activity of low-dose aspirin in healthy volunteers. *Stroke*. 2006;37:2153–2158.
14. Maree AO, Curtin RJ, Chubb A, Dolan C, Cox D, O'Brien J, Crean P, Shields DC, Fitzgerald DJ. Cyclooxygenase-1 haplotype modulates platelet response to aspirin. *J Thromb Haemost*. 2005;3:2340–2345.
15. Capodanno D, Patel A, Dharmashankar K, Ferreiro JL, Ueno M, Kodali M, Tomasello SD, Capranzano P, Seecheran N, Darlington A, Tello-Montoliu A, Desai B, Bass TA, Angiolillo DJ. Pharmacodynamic effects of different aspirin dosing regimens in type 2 diabetes mellitus patients with coronary artery disease. *Circ Cardiovasc Interv*. 2011;4:180–187.
16. Catella-Lawson F, Reilly MP, Kapoor SC, Cucchiara AJ, DeMarco S, Tournier B, Vyas SN, FitzGerald GA. Cyclooxygenase inhibitors and the antiplatelet effects of aspirin. *N Engl J Med*. 2001;345:1809–1817.
17. Storey RF, Newby LJ, Heptinstall S. Effects of P2Y(1) and P2Y(12) receptor antagonists on platelet aggregation induced by different agonists in human whole blood. *Platelets*. 2001;12:443–447.
18. Bertrand ME, Rupprecht HJ, Urban P, Gershlick AH: CLASSICS Investigators. Double-blind study of the safety of clopidogrel with and without a loading dose in combination with aspirin compared with ticlopidine in combination with aspirin after coronary stenting: the clopidogrel aspirin stent international cooperative study (CLASSICS). *Circulation*. 2000; 102:624–629.
19. Cadroy Y, Bossavy JP, Thalamas C, Sagnard L, Sakariassen K, Boneu B. Early potent antithrombotic effect with combined aspirin and a loading dose of clopidogrel on experimental arterial thrombogenesis in humans. *Circulation*. 2000;101:2823–2828.
20. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Alfonso F, Macaya C, Bass TA, Costa MA. Variability in individual responsiveness to clopidogrel: clinical implications, management, and future perspectives. *J Am Coll Cardiol*. 2007;49:1505–1516.
21. Ferreiro JL, Angiolillo DJ. Clopidogrel response variability: current status and future directions. *Thromb Haemost*. 2009;102:7–14.
22. Yusuf S, Zhao F, Mehta SR, Chrolavicius S, Tognoni G, Fox KK: Clopidogrel in Unstable Angina to Prevent Recurrent Events Trial Investigators. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. *N Engl J Med*. 2001; 345:494–502.
23. Steinhubl SR, Berger PB, Mann JT III, Fry ET, DeLago A, Wilmer C, Topol EJ: CREDO Investigators. Clopidogrel for the Reduction of Events During Observation. Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: a randomized controlled trial. *JAMA*. 2002;288:2411–2420.
24. Chen ZM, Jiang LX, Chen YP, Xie JX, Pan HC, Peto R, Collins R, Liu LS: COMMIT (ClOpidogrel and Metoprolol in Myocardial Infarction Trial) collaborative group. Addition of clopidogrel to aspirin in 45 852 patients with acute myocardial infarction: randomised placebo-controlled trial. *Lancet*. 2005;366:1607–1621.
25. Sabatine MS, Cannon CP, Gibson CM, López-Sendón JL, Montalescot G, Theroux P, Claeys MJ, Cools F, Hill KA, Skene AM, McCabe CH, Braunwald E: CLARITY-TIMI 28 Investigators. Addition of clopidogrel to aspirin and fibrinolytic therapy for myocardial infarction with ST-segment elevation. *N Engl J Med*. 2005;352:1179–1189.
26. Bhatt DL, Fox KA, Hacke W, Berger PB, Black HR, Boden WE, Cacoub P, Cohen EA, Creager MA, Easton JD, Flather MD, Haffner SM, Hamm CW, Hankey GJ, Johnston SC, Mak KH, Mas JL, Montalescot G, Pearson TA, Steg PG, Steinhubl SR, Weber MA, Brennan DM, Fabry-Ribaudo L, Booth J, Topol EJ: CHARISMA Investigators. Clopidogrel and aspirin versus aspirin alone for the prevention of atherothrombotic events. *N Engl J Med*. 2006;354:1706–1717.
27. Angiolillo DJ, Gibson CM, Cheng S, Ollier C, Nicolas O, Bergougnan L, Perrin L, LaCreta FP, Hurbin F, Dubar M. Differential effects of omeprazole and pantoprazole on the pharmacodynamics and pharmacokinetics of clopidogrel in healthy subjects: randomized, placebo-controlled, crossover comparison studies. *Clin Pharmacol Ther*. 2011;89: 65–74.
28. Simon T, Bhatt DL, Bergougnan L, Farenc C, Pearson K, Perrin L, Vicaut E, Lacreta F, Hurbin F, Dubar M. Genetic polymorphisms and the impact of a higher clopidogrel dose regimen on active metabolite exposure and antiplatelet response in healthy subjects. *Clin Pharmacol Ther*. 2011;90: 287–295.
29. Angiolillo DJ, Ueno M. Optimizing platelet inhibition in clopidogrel poor metabolizers: therapeutic options and practical considerations. *J Am Coll Cardiol Cardiovasc Interv*. 2011;4:411–414.
30. Mehta SR, Tanguay JF, Eikelboom JW, Jolly SS, Joyner CD, Granger CB, Faxon DP, Rupprecht HJ, Budaj A, Avezum A, Widimsky P, Steg PG, Bassand JP, Montalescot G, Macaya C, Di Pasquale G, Niemela K, Ajani AE, White HD, Chrolavicius S, Gao P, Fox KA, Yusuf S: CURRENT-OASIS 7 trial investigators. Double-dose versus standard-dose clopidogrel and high-dose versus low-dose aspirin in individuals undergoing percutaneous coronary intervention for acute coronary syndromes (CURRENT-OASIS 7): a randomised factorial trial. *Lancet*. 2010;376:1233–1243.
31. Price MJ, Berger PB, Teirstein PS, Tanguay JF, Angiolillo DJ, Spriggs D, Puri S, Robbins M, Garratt KN, Bertrand OF, Stillabower ME, Aragon JR, Kandzari DE, Stinis CT, Lee MS, Manoukian SV, Cannon CP, Schork NJ, Topol EJ: GRAVITAS Investigators. Standard- vs high-dose clopidogrel based on platelet function testing after percutaneous coronary intervention: the GRAVITAS randomized trial. *JAMA*. 2011;305: 1097–1105.
32. Trenk D, Stone GW, Gawaz M, Kastrati A, Angiolillo DJ, Müller U, Richardt G, Jakubowski JA, Neumann FJ. A randomized trial of prasugrel versus clopidogrel in patients with high platelet reactivity on clopidogrel after elective percutaneous coronary intervention with implantation of drug-eluting stents: results of the TRIGGER-PCI study. *J Am Coll Cardiol*. 2012 April 9. [Epub ahead of print].
33. Angiolillo DJ, Capranzano P. Pharmacology of emerging novel platelet inhibitors. *Am Heart J*. 2008;156(2 Suppl):S10–S15.
34. Wiviott SD, Trenk D, Frelinger AL, O'Donoghue M, Neumann FJ, Michelson AD, Angiolillo DJ, Hod H, Montalescot G, Miller DL, Jakubowski JA, Cairns R, Murphy SA, McCabe CH, Antman EM, Braunwald E; PRINCIPLE-TIMI 44 Investigators. Prasugrel compared with high loading- and maintenance-dose clopidogrel in patients with planned percutaneous coronary intervention: the prasugrel in comparison to clopidogrel for inhibition of platelet activation and aggregation-thrombolysis in myocardial infarction 44 trial. *Circulation*. 2007;116: 2923–2932.
35. Wiviott SD, Braunwald E, McCabe CH, Montalescot G, Ruzyllo W, Gottlieb S, Neumann FJ, Ardissino D, De Servi S, Murphy SA, Riesmeyer J, Weerakkody G, Gibson CM, Antman EM: TRITON-TIMI 38 Investigators. Prasugrel versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med*. 2007;357:2001–2015.
36. Antman EM, Wiviott SD, Murphy SA, Voitk J, Hasin Y, Widimsky P, Chandna H, Macias W, McCabe CH, Braunwald E. Early and late benefits of prasugrel in patients with acute coronary syndromes undergoing percutaneous coronary intervention: a TRITON-TIMI 38

(TRial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet InhibitioN with Prasugrel-Thrombolysis In Myocardial Infarction) analysis. *J Am Coll Cardiol*. 2008;51:2028–2033.
37. Wiviott SD, Braunwald E, McCabe CH, Horvath I, Keltai M, Herrman JP, Van de Werf F, Downey WE, Scirica BM, Murphy SA, Antman EM: TRITON-TIMI 38 Investigators. Intensive oral antiplatelet therapy for reduction of ischaemic events including stent thrombosis in patients with acute coronary syndromes treated with percutaneous coronary intervention and stenting in the TRITON-TIMI 38 trial: a subanalysis of a randomised trial. *Lancet*. 2008;371:1353–1363.
38. Wiviott SD, Braunwald E, Angiolillo DJ, Meisel S, Dalby AJ, Verheugt FW, Goodman SG, Corbalan R, Purdy DA, Murphy SA, McCabe CH, Antman EM: TRITON-TIMI 38 Investigators. Greater clinical benefit of more intensive oral antiplatelet therapy with prasugrel in patients with diabetes mellitus in the trial to assess improvement in therapeutic outcomes by optimizing platelet inhibition with prasugrel-Thrombolysis in Myocardial Infarction 38. *Circulation*. 2008;118:1626–1636.
39. Montalescot G, Wiviott SD, Braunwald E, Murphy SA, Gibson CM, McCabe CH, Antman EM: TRITON-TIMI 38 investigators. Prasugrel compared with clopidogrel in patients undergoing percutaneous coronary intervention for ST-elevation myocardial infarction (TRITON-TIMI 38): double-blind, randomised controlled trial. *Lancet*. 2009;373:723–731.
40. Murphy SA, Antman EM, Wiviott SD, Weerakkody G, Morocutti G, Huber K, Lopez-Sendon J, McCabe CH, Braunwald E; TRITON-TIMI 38 Investigators. Reduction in recurrent cardiovascular events with prasugrel compared with clopidogrel in patients with acute coronary syndromes from the TRITON-TIMI 38 trial. *Eur Heart J*. 2008;29:2473–2479.
41. Angiolillo DJ, Saucedo JF, Deraad R, Frelinger AL, Gurbel PA, Costigan TM, Jakubowski JA, Ojeh CK, Effron MB: SWAP Investigators. Increased platelet inhibition after switching from maintenance clopidogrel to prasugrel in patients with acute coronary syndromes: results of the SWAP (SWitching Anti Platelet) study. *J Am Coll Cardiol*. 2010;56:1017–1023.
42. Angiolillo DJ, Guzman LA. Clinical overview of promising nonthienopyridine antiplatelet agents. *Am Heart J*. 2008;156:S23–28.
43. Gurbel PA, Bliden KP, Butler K, Tantry US, Gesheff T, Wei C, Teng R, Antonino MJ, Patil SB, Karunakaran A, Kereiakes DJ, Parris C, Purdy D, Wilson V, Ledley GS, Storey RF. Randomized double-blind assessment of the ONSET and OFFSET of the antiplatelet effects of ticagrelor versus clopidogrel in patients with stable coronary artery disease: the ONSET/OFFSET study. *Circulation*. 2009;120:2577–2585.
44. Wallentin L, Becker RC, Budaj A, Cannon CP, Emanuelsson H, Held C, Horrow J, Husted S, James S, Katus H, Mahaffey KW, Scirica BM, Skene A, Steg PG, Storey RF, Harrington RA: PLATO Investigators, Freij A, Thorsén M. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med*. 2009;361:1045–1057.
45. James SK, Roe MT, Cannon CP, Cornel JH, Horrow J, Husted S, Katus H, Morais J, Steg PG, Storey RF, Stevens S, Wallentin L, Harrington RA: PLATO Study Group. Ticagrelor versus clopidogrel in patients with acute coronary syndromes intended for non-invasive management: substudy from prospective randomised PLATelet inhibition and patient Outcomes (PLATO) trial. *BMJ*. 2011;342:d3527.
46. Cannon CP, Harrington RA, James S, Ardissino D, Becker RC, Emanuelsson H, Husted S, Katus H, Keltai M, Khurmi NS, Kontny F, Lewis BS, Steg PG, Storey RF, Wojdyla D, Wallentin L: PLATelet inhibition and patient Outcomes Investigators. Comparison of ticagrelor with clopidogrel in patients with a planned invasive strategy for acute coronary syndromes (PLATO): a randomised double-blind study. *Lancet*. 2010;375:283–293.
47. Held C, Asenblad N, Bassand JP, Becker RC, Cannon CP, Claeys MJ, Harrington RA, Horrow J, Husted S, James SK, Mahaffey KW, Nicolau JC, Scirica BM, Storey RF, Vintila M, Ycas J, Wallentin L. Ticagrelor versus clopidogrel in patients with acute coronary syndromes undergoing coronary artery bypass surgery: results from the PLATO (Platelet Inhibition and Patient Outcomes) trial. *J Am Coll Cardiol*. 2011;57:672–684.
48. Storey RF, Bliden KP, Patil SB, Karunakaran A, Ecob R, Butler K, Teng R, Wei C, Tantry US, Gurbel PA: ONSET/OFFSET investigators. Incidence of dyspnea and assessment of cardiac and pulmonary function in patients with stable coronary artery disease receiving ticagrelor, clopidogrel, or placebo in the ONSET/OFFSET study. *J Am Coll Cardiol*. 2010;56:185–193.
49. Scirica BM, Cannon CP, Emanuelsson H, Michelson EL, Harrington RA, Husted S, James S, Katus H, Pais P, Raev D, Spinar J, Steg PG, Storey RF, Wallentin L: PLATO Investigators. The incidence of bradyarrhythmias and clinical bradyarrhythmic events in patients with acute coronary syndromes treated with ticagrelor or clopidogrel in the PLATO (Platelet Inhibition and Patient Outcomes) trial: results of the continuous electrocardiographic assessment substudy. *J Am Coll Cardiol*. 2011;57:1908–1916.
50. Mahaffey KW, Wojdyla DM, Carroll K, Becker RC, Storey RF, Angiolillo DJ, Held C, Cannon CP, James S, Pieper KS, Horrow J, Harrington RA, Wallentin L: PLATO Investigators. Ticagrelor compared with clopidogrel by geographic region in the Platelet Inhibition and Patient Outcomes (PLATO) trial. *Circulation*. 2011;124:544–554.
51. Roffi M, Chew DP, Mukherjee D, Bhatt DL, White JA, Moliterno DJ, Heeschen C, Hamm CW, Robbins MA, Kleiman NS, Théroux P, White HD, Topol EJ. Platelet glycoprotein IIb/IIIa inhibition in acute coronary syndromes. Gradient of benefit related to the revascularization strategy. *Eur Heart J*. 2002;23:1441–1448.
52. Kastrati A, Mehilli J, Neumann FJ, Dotzer F, ten Berg J, Bollwein H, Graf I, Ibrahim M, Pache J, Seyfarth M, Schühlen H, Dirschinger J, Berger PB, Schömig A: Intracoronary Stenting and Antithrombotic: Regimen Rapid Early Action for Coronary Treatment 2 (ISAR-REACT 2) trial investigators. Abciximab in patients with acute coronary syndromes undergoing percutaneous coronary intervention after clopidogrel pretreatment: the ISAR-REACT 2 randomized trial. *JAMA*. 2006;295:1531–1538.
53. De Luca G, Navarese E, Marino P. Risk profile and benefits from GP IIb-IIIa inhibitors among patients with ST-segment elevation myocardial infarction treated with primary angioplasty: a meta-regression analysis of randomized trials. *Eur Heart J*. 2009;30:2705–2713.
54. Eikelboom JW, Mehta SR, Anand SS, Xie C, Fox KA, Yusuf S. Adverse impact of bleeding on prognosis in patients with acute coronary syndromes. *Circulation*. 2006;114:774–782.
55. Stone GW, McLaurin BT, Cox DA, Bertrand ME, Lincoff AM, Moses JW, White HD, Pocock SJ, Ware JH, Feit F, Colombo A, Aylward PE, Cequier AR, Darius H, Desmet W, Ebrahimi R, Hamon M, Rasmussen LH, Rupprecht HJ, Hoekstra J, Mehran R, Ohman EM: ACUITY investigators. Bivalirudin for patients with acute coronary syndromes. *N Engl J Med*. 2006;355:2203–2216.
56. Kastrati A, Neumann FJ, Schulz S, Massberg S, Byrne RA, Ferenc M, Laugwitz KL, Pache J, Ott I, Hausleiter J, Seyfarth M, Gick M, Antoniucci D, Schömig A, Berger PB, Mehilli J: ISAR-REACT 4 trial investigators. Abciximab and heparin versus bivalirudin for non-ST-elevation myocardial infarction. *N Engl J Med*. 2011;365:1980–1989.
57. Stone GW, Witzenbichler B, Guagliumi G, Peruga JZ, Brodie BR, Dudek D, Kornowski R, Hartmann F, Gersh BJ, Pocock SJ, Dangas G, Wong SC, Kirtane AJ, Parise H, Mehran R: HORIZONS-AMI trial investigators. Bivalirudin during primary PCI in acute myocardial infarction. *N Engl J Med*. 2008;358:2218–2230.
58. Stone GW, Maehara A, Witzenbichler B, Godlewski J, Parise H, Dambrink JH, Ochala A, Carlton TW, Cristea E, Wolff SD, Brener SJ, Chowdhary S, El-Omar M, Neunteufl T, Metzger DC, Karwoski T, Dizon JM, Mehran R, Gibson CM: for the INFUSE-AMI investigators. Intracoronary abciximab and aspiration thrombectomy in patients with large anterior myocardial infarction: the INFUSE-AMI randomized trial. *JAMA*. 2012 Mar 25. [Epub ahead of print].
59. Tello-Montoliu A, Jover E, Rivera J, Valdé M, Angiolillo DJ, Marín F. New perspectives in antiplatelet therapy. *Curr Med Chem*. 2012;19.406–427.
60. Chamorro A. TP receptor antagonism: a new concept in atherothrombosis and stroke prevention. *Cerebrovasc Dis*. 2009;27 Suppl 3:20–27.
61. Neri Serneri GG, Coccheri S, Marubini E, Violi F: Drug Evaluation in Atherosclerotic Vascular Disease in Diabetics (DAVID) study group. Picotamide, a combined inhibitor of thromboxane A2 synthase and receptor, reduces 2-year mortality in diabetics with peripheral arterial disease: the DAVID study. *Eur Heart J*. 2004;25:1845–1852.
62. van der Wieken LR, Simoons ML, Laarman GJ, Van den Brand M, Nijssen KM, Dellborg M, Hermens W, Vrolik W. Ridogrel as an adjunct to thrombolysis in acute myocardial infarction. *Int J Cardiol*. 1995;52:125–134.
63. Gaussem P, Reny JL, Thalamas C, Chatelain N, Kroumova M, Jude B, Boneu B, Fiessinger JN. The specific thromboxane receptor antagonist S18886: pharmacokinetic and pharmacodynamic studies. *J Thromb Haemost*. 2005;3:1437–1445.
64. Bousser MG, Amarenco P, Chamorro A, Fisher M, Ford I, Fox KM, Hennerici MG, Mattle HP, Rothwell PM, de Cordoüe A, Fratacci MD: PERFORM study investigators. Terutroban versus aspirin in patients with cerebral ischaemic events (PERFORM): a randomised, double-blind, parallel-group trial. *Lancet*. 2011;377:2013–2022.

65. Ferreiro JL, Ueno M, Angiolillo DJ. Cangrelor: a review on its mechanism of action and clinical development. *Expert Rev Cardiovasc Ther*. 2009;7:1195–1201.
66. Harrington RA, Stone GW, McNulty S, White HD, Lincoff AM, Gibson CM, Pollack CV Jr., Montalescot G, Mahaffey KW, Kleiman NS, Goodman SG, Amine M, Angiolillo DJ, Becker RC, Chew DP, French WJ, Leisch F, Parikh KH, Skerjanec S, Bhatt DL. Platelet inhibition with cangrelor in patients undergoing PCI. *N Engl J Med*. 2009;361: 2318–2329.
67. Bhatt DL, Lincoff AM, Gibson CM, Stone GW, McNulty S, Montalescot G, Kleiman NS, Goodman SG, White HD, Mahaffey KW, Pollack CV Jr., Manoukian SV, Widimsky P, Chew DP, Cura F, Manukov I, Tousek F, Jafar MZ, Arneja J, Skerjanec S, Harrington RA: CHAMPION PLATFORM investigators. Intravenous platelet blockade with cangrelor during PCI. *N Engl J Med*. 2009;361:2330–2341.
68. White H, Chew DP, Dauerman HL, Mahaffey KW, Gibson CM, Stone GW, Gruberg L, Harrington RA, Bhatt DL. Reduced immediate ischemic events with cangrelor in PCI: a pooled analysis of the CHAMPION trials using the universal definition of myocardial infarction. *Am Heart J*. 2012;163:182–190.e4.
69. Angiolillo DJ, Firstenberg MS, Price MJ, Tummala PE, Hutyra M, Welsby IJ, Voeltz MD, Chandna H, Ramaiah C, Brtko M, Cannon L, Dyke C, Liu T, Montalescot G, Manoukian SV, Prats J, Topol EJ. Bridging antiplatelet therapy with cangrelor in patients undergoing cardiac surgery: a randomized controlled trial. *JAMA*. 2012;307:265–274.
70. Ueno M, Rao SV, Angiolillo DJ. Elinogrel: pharmacological principles, preclinical and early phase clinical testing. *Future Cardiol*. 2010;6: 445–453.
71. Welsh RC, Rao SV, Zeymer U, Thompson VP, Huber K, Kochman J, McClure MW, Gretler DD, Bhatt DL, Gibson CM, Angiolillo DJ, Gurbel PA, Berdan LG, Paynter G, Leonardi S, Madan M, French WJ, Harrington RA; on behalf of the INNOVATE-PCI investigators. A randomized, double-blind, active-controlled phase 2 trial to evaluate a novel selective and reversible intravenous and oral $P2Y_{12}$ inhibitor elinogrel versus clopidogrel in patients undergoing nonurgent percutaneous coronary intervention: the INNOVATE-PCI Trial. *Circ Cardiovasc Interv*. 2012;5:336–346.
72. Angiolillo DJ, Welsh RC, Trenk D, Neumann FJ, Conley PB, McClure MW, Stephens G, Kochman J, Jennings LK, Gurbel PA, Wójcik J, Dabrowski M, Saucedo JF, Stumpf J, Buerke M, Broderick S, Harrington RA, Rao SV. Pharmacokinetic and pharmacodynamic effects of elinogrel: results of the platelet function substudy from the intravenous and oral administration of elinogrel to evaluate tolerability and efficacy in non-urgent percutaneous coronary intervention patients (INNOVATE-PCI) trial. *Circ Cardiovasc Interv*. 2012;5:347–356.
73. Angiolillo DJ, Capodanno D, Goto S. Platelet thrombin receptor antagonism and atherothrombosis. *Eur Heart J*. 2010;31:17–28.
74. Becker RC, Moliterno DJ, Jennings LK, Pieper KS, Pei J, Niederman A, Ziada KM, Berman G, Strony J, Joseph D, Mahaffey KW, Van de Werf F, Veltri E, Harrington RA; TRA-PCI investigators. Safety and tolerability of SCH 530348 in patients undergoing non-urgent percutaneous coronary intervention: a randomised, double-blind, placebo-controlled phase II study. *Lancet*. 2009;373:919–928.
75. Tricoci P, Huang Z, Held C, Moliterno DJ, Armstrong PW, Van de Werf F, White HD, Aylward PE, Wallentin L, Chen E, Lokhnygina Y, Pei J, Leonardi S, Rorick TL, Kilian AM, Jennings LH, Ambrosio G, Bode C, Cequier A, Cornel JH, Diaz R, Erkan A, Huber K, Hudson MP, Jiang L, Jukema JW, Lewis BS, Lincoff AM, Montalescot G, Nicolau JC, Ogawa H, Pfisterer M, Prieto JC, Ruzyllo W, Sinnaeve PR, Storey RF, Valgimigli M, Whellan DJ, Widimsky P, Strony J, Harrington RA, Mahaffey KW: the TRACER Investigators. Thrombin-receptor antagonist vorapaxar in acute coronary syndromes. *N Engl J Med*. 2012;366:20–33.
76. Morrow DA, Braunwald E, Bonaca MP, Ameriso SF, Dalby AJ, Fish MP, Fox KA, Lipka LJ, Liu X, Nicolau JC, Ophuis AJ, Paolasso E, Scirica BM, Spinar J, Theroux P, Wiviott SD, Strony J, Murphy SA: TRA 2P–TIMI 50 steering committee and investigators. Vorapaxar in the secondary prevention of atherothrombotic events. *N Engl J Med*. 2012; 366:1404–1413.
77. O'Donoghue ML, Bhatt DL, Wiviott SD, Goodman SG, Fitzgerald DJ, Angiolillo DJ, Goto S, Montalescot G, Zeymer U, Aylward PE, Guetta V, Dudek D, Ziecina R, Contant CF, Flather MD: LANCELOT-ACS investigators. Safety and tolerability of atopaxar in the treatment of patients with acute coronary syndromes: the lessons from antagonizing the cellular effects of thrombin–acute coronary syndromes trial. *Circulation*. 2011;123:1843–1853.
78. Wiviott SD, Flather MD, O'Donoghue ML, Goto S, Fitzgerald DJ, Cura F, Aylward P, Guetta V, Dudek D, Contant CF, Angiolillo DJ, Bhatt DL: LANCELOT-CAD investigators. Randomized trial of atopaxar in the treatment of patients with coronary artery disease: the lessons from antagonizing the cellular effect of thrombin–coronary artery disease trial. *Circulation*. 2011;123:1854–1863.
79. Goto S, Ogawa H, Takeuchi M, Flather MD, Bhatt DL; J-LANCELOT (Japanese-Lesson from Antagonizing the Cellular Effect of Thrombin) investigators. Double-blind, placebo-controlled phase II studies of the protease-activated receptor 1 antagonist E5555 (atopaxar) in Japanese patients with acute coronary syndrome or high-risk coronary artery disease. *Eur Heart J*. 2010;31:2601–2613.
80. Meadows TA, Bhatt DL. Clinical aspects of platelet inhibitors and thrombus formation. *Circ Res*. 2007;100:1261–1275.
81. Mega JL, Braunwald E, Wiviott SD, Bassand JP, Bhatt DL, Bode C, Burton P, Cohen M, Cook-Bruns N, Fox KA, Goto S, Murphy SA, Plotnikov AN, Schneider D, Sun X, Verheugt FW, Gibson CM: ATLAS ACS 2–TIMI 51 investigators. Rivaroxaban in patients with a recent acute coronary syndrome. *N Engl J Med*. 2012;366:9–19.
82. Angiolillo DJ. Applying platelet function testing in clinical practice: what are the unmet needs? *JAMA*. 2011;306:1260–1261.
83. Ferreiro JL, Sibbing D, Angiolillo DJ. Platelet function testing and risk of bleeding complications. *Thromb Haemost*. 2010;103:1128–1135.

KEY WORDS: acute coronary syndrome ■ antiplatelet therapy ■ percutaneous transluminal coronary angioplasty ■ pharmacology ■ platelets

5. RESUMEN DE RESULTADOS:

ARTÍCULOS ORIGINALES

Vale más hacer y arrepentirse, que no hacer y arrepentirse.

NICOLÁS MAQUIAVELO

5.1. Estudio I

Pharmacodynamic effects of concomitant versus staggered clopidogrel and omeprazole intake: results of a prospective randomized crossover study.

Ferreiro JL, Ueno M, Capodanno D, Desai B, Dharmashankar K, Darlington A, Charlton RK, Bass TA, Angiolillo DJ.

Circ Cardiovasc Interv. 2010;3:436-41.

Estudio prospectivo farmacodinámico con un diseño cruzado (2 secuencias y 3 periodos) realizado en voluntarios sanos entre 18 y 65 años. Se aleatorizó a 24 sujetos (20 completaron los 3 regímenes de tratamiento y fueron analizados finalmente) a recibir clopidogrel (dosis de carga de 600 mg + dosis de mantenimiento de 75 mg/día) y omeprazol 40 mg/día concomitantemente (régimen CONC, ambos fármacos al mismo tiempo por la mañana) o separada la administración de ambos fármacos entre 8 y 12 horas (régimen STAG, clopidogrel por la mañana y omeprazol por la noche) durante 1 semana y, tras un periodo de blanqueo o lavado de 2-4 semanas, se intercambiaron regímenes de tratamiento. Después de otro periodo de blanqueo, todos los sujetos recibieron únicamente clopidogrel durante 1 semana (régimen CLOP). Se evaluó la función plaquetar con el análisis de fosforilación de la *vasodilator-stimulated phosphoprotein* (VASP), agregometría óptica (LTA, *"light transmittance aggregometry"*) y el sistema VerifyNow en 3 momentos: basal, 24 horas y 1 semana. La variable de valoración principal fue

la comparación del *"$P2Y_{12}$ reactivity index"* (PRI) obtenido con VASP al cabo de una semana de tratamiento entre los regímenes CONC y STAG.

No se encontraron diferencias significativas en la reactividad plaquetar medida como PRI entre los regímenes CONC y STAG tras 1 semana de tratamiento (56,1±3,5% vs. 61,6±3,4%; p=0,08), mientras que los valores de PRI con el régimen CLOP (48,8±3,4%) fueron inferiores significativamente que los obtenidos con CONC y STAG (p=0,02 y p<0,001 respectivamente), es decir, la respuesta a clopidogrel se encontraba disminuida de forma estadísticamente significativa cuando se administraba también omeprazol, sin hallarse diferencias farmacodinámicas entre la administración de ambos fármacos al mismo tiempo o separados 8-12 horas.

No se hallaron diferencias en los valores de reactividad plaquetar basales o a las 24 horas (evaluando la dosis de carga) entre ninguno de los regímenes de tratamiento.

Se obtuvieron resultados similares al utilizar como pruebas de función plaquetar la LTA (usando como agonista ADP a concentraciones de 5 y 20 μM) y el sistema VerifyNow P2Y12.

5.2. Estudio II

Pharmacodynamic evaluation of pantoprazole therapy on clopidogrel-effects: results of a prospective randomized crossover study.

Ferreiro JL, Ueno M, Tomasello SD, Capodanno D, Desai B, Dharmashankar K, Seecheran N, Kodali MK, Darlington A, Pham, JP, Tello-Montoliu A, Charlton RK, Bass TA, Angiolillo DJ.

Circ Cardiovasc Interv. 2011;4:273-9.

Estudio prospectivo farmacodinámico con un diseño cruzado (2 secuencias y 3 periodos) realizado en voluntarios sanos entre 18 y 65 años. Se aleatorizó a 22 sujetos (20 completaron los 3 regímenes de tratamiento y fueron analizados finalmente) a recibir clopidogrel (dosis de carga de 600 mg + dosis de mantenimiento de 75 mg/día) y pantoprazol 80 mg/día concomitantemente (régimen CONC, ambos fármacos al mismo tiempo por la mañana) o separada la administración de ambos fármacos entre 8 y 12 horas (régimen STAG, clopidogrel por la mañana y pantoprazol por la noche) durante 1 semana y, tras un periodo de blanqueo o lavado de 2-4 semanas, se intercambiaron regímenes de tratamiento. Todos los sujetos recibieron únicamente clopidogrel durante 1 semana (régimen CLOP), seguido de un periodo de blanqueo, previamente a la aleatorización. Se evaluó la función plaquetar con el análisis de VASP, LTA y el sistema VerifyNow en 3 momentos: basal, 24 horas y 1 semana. La variable de valoración principal fue la

comparación del PRI obtenido con VASP al cabo de una semana de tratamiento entre los regímenes CONC y STAG.

No se encontraron diferencias significativas en la reactividad plaquetar medida como PRI entre los regímenes CONC y STAG tras 1 semana de tratamiento (56,0±3,9% vs. 56,1±3,9%; p=0,974), ni tampoco en la comparación con el régimen CLOP (61.0±3,9%; p=0,100 vs. CONC y p=0,107 vs. STAG), es decir, la respuesta a clopidogrel no se vio afectada por la administración de pantoprazol, sin importar el momento de administración de ambos fármacos.

No se hallaron diferencias en los valores de reactividad plaquetar basales o a las 24 horas (evaluando la dosis de carga) entre ninguno de los regímenes de tratamiento.

Se obtuvieron resultados similares al utilizar como pruebas de función plaquetar la LTA (usando como agonista ADP a concentraciones de 5 y 20 μM) y el sistema VerifyNow P2Y12.

5.3. Estudio III

Cigarette smoking is associated with a dose-response effect in clopidogrel-treated patients with diabetes mellitus and coronary artery disease: results of a pharmacodynamic study.

Ueno M, Ferreiro JL, Desai B, Tomasello SD, Tello-Montoliu A, Capodanno D, Capranzano P, Kodali M, Dharmashankar K, Charlton RK, Bass TA, Angiolillo DJ.

JACC Cardiovasc Interv. 2012;5:293-300.

Estudio farmacodinámico observacional transversal realizado en muestras de 134 pacientes con DM tipo II y cardiopatía isquémica estable en tratamiento de mantenimiento (al menos 1 mes) con AAS (81 mg/día) y clopidogrel (75 mg/día) tras haberse sometido a un ICP con implantación de stent. Se dividió a los pacientes en tres grupos según las concentraciones de cotinina sérica (reflejo del consumo de tabaco de los sujetos): <3 ng/ml (no fumadores, n=85), 3 - 199 ng/ml (fumadores leves, n=27) y >200 ng/ml (fumadores severos, n=22). La función plaquetar se evaluó con: a) LTA (usando como agonista ADP a concentraciones de 5 y 20 μM), informando los resultados como *"maximal platelet aggregation"* (MPA) y *"late platelet aggregation"* (LPA); b) el test VerifyNow P2Y12, informando los resultados como *"$P2Y_{12}$ reaction units"* (PRU) e inhibición de la agregación plaquetar (IPA); y c) el análisis de VASP, informando los resultados como PRI. Se definió la respuesta subóptima al tratamiento con clopidogrel (HTPR, *"high on-*

treatment platelet reactivity") con los siguientes puntos de corte: MPA-ADP (20 μmol/l) >50%, MPA-ADP (5 μmol/l) >46%, PRU >230, IPA <40% y PRI >50%.

Se evidenció una relación dosis-respuesta estadísticamente significativa entre el hábito tabáquico y la respuesta a clopidogrel con todas las pruebas de función plaquetar utilizadas. Las concentraciones séricas de cotinina se asociaron significativamente de manera inversa con los niveles de reactividad plaquetar obtenidos (p de tendencia: <0,0001 para MPA con 5 y 20 μmol/l, <0,0001 para PRU, 0,002 para IPA y 0,001 para PRI).

La prevalencia de respuesta subóptima a clopidogrel en el global de la población del estudio osciló entre el 39% y el 73% según el test empleado. Las concentraciones elevadas de cotinina se asociaron con menores tasas de HTPR de forma estadísticamente significativa con todas las pruebas farmacodinámicas utilizadas.

Un análisis multivariable de regresión logística (incluyendo como covariables edad, uso de insulina, índice de masa corporal, creatinina >1,5mg/dl, hemoglobina A_{1c}, uso de estatinas y tratamiento con IBP, además del grado de tabaquismo como variable independiente de interés, usando como referencia la categoría de no fumadores) mostró que tanto los fumadores severos como los leves tenían unas menores tasas de respuesta subóptima a clopidogrel comparados con los no fumadores. Se obtuvo una odds ratio ajustada (OR adj) de 0,24 (IC95% 0,074-0,76; p=0,015) para la comparación fumadores ligeros vs. no fumadores y una OR adj de 0,10 (IC95% 0,027-0,37;p=0,001) para la comparación fumadores severos vs. no fumadores,

usando LTA con ADP 20μM. Se apreciaron resultados similares al utilizar el resto de pruebas de función plaquetar.

5.4. Estudio IV

Clopidogrel pretreatment in primary percutaneous coronary intervention: Prevalence of high on-treatment platelet reactivity and impact on preprocedural patency of the infarct-related artery.

Ferreiro JL, Homs S, Berdejo J, Roura G, Gomez-Lara J, Romaguera R, Teruel L, Sánchez-Elvira G, Marcano AL, Gómez-Hospital JA, Angiolillo DJ, Cequier A.

Thromb Haemost. 2013;110:110-7.

Estudio prospectivo farmacodinámico observacional realizado en 50 pacientes con IAMCEST que recibieron dosis de carga de 600mg de clopidogrel y 500mg de AAS en el momento del diagnóstico, no estando previamente bajo tratamiento antiagregante, y antes de la realización de una angioplastia primaria. Las muestras sanguíneas para las pruebas de función plaquetar se extrajeron inmediatamente después de colocar el catéter arterial para iniciar el procedimiento. Las pruebas de función plaquetar empleadas y los puntos de corte utilizados para determinar una respuesta subóptima (HTPR) a los fármacos antiagregantes fueron: a) VerifyNow: PRU >240 para clopidogrel y *"Aspirin reaction units"* (ARU) >550 para AAS; b) Agregometría de electrodos múltiples (MEA, *"multiple electrode aggregometry"*): >468 AU*min para clopidogrel; y c) LTA: MPA >46% para clopidogrel (con 5μM de ADP como agonista) y MPA >20% para AAS (estímulo con ácido araquidónico 1mM). La variable de valoración principal fue la evaluación de la asociación entre HTPR a clopidogrel (medida con el VerifyNow) y la permeabilidad inicial (al principio del

procedimiento) de la arteria responsable del infarto (ARI), evaluada mediante el grado de flujo según la escala *"Thrombolysis in Myocardial Infarction"* (TIMI), dicotomizado en dos categorías: flujo pobre (TIMI 0-1) y buen flujo (TIMI 2-3). Variables secundarias fueron las frecuencias al final del procedimiento de un flujo TIMI 3, de un grado de *"blush"* miocárdico 0-1 y de una resolución completa del segmento ST.

El porcentaje de pacientes con HTPR a clopidogrel medida con el sistema VerifyNow fue del 88,0% (IC 95%: 76,2-94,4%), con porcentajes similares del 81,8% (IC 95%: 68,0-90,5%) usando LTA y del 91,3% (IC 95%: 79,7-96,6%) usando MEA. La mediana de tiempo desde la administración de la dosis de carga de clopidogrel hasta el inicio del procedimiento fue de 85 min [rango intercuartílico 60,0-121,3], sin diferencias entre pacientes con y sin HTPR a clopidogrel (85,0 [65,0-120,0] vs. 80,0 [38,8-131,3]).

Se observó un mayor porcentaje de pacientes con buen flujo inicial en la ARI en los pacientes sin HTPR a clopidogrel comparado con los pacientes con HTPR (66,7% vs. 15,9%; p=0,013), destacando que la HTPR fue la única variable asociada de manera estadísticamente significativa con la permeabilidad inicial de la ARI en el análisis multivariable. No se apreciaron diferencias significativas en las frecuencias postprocedimiento de flujo TIMI 3, de "blush" miocárdico 0-1 o en la resolución completa del segmento ST.

El porcentaje de pacientes con HTPR a AAS fue del 28,6% (IC 95%: 17,8-42,4%) medido con el Verify Now y del 38,1% (IC 95%: 25,0-53,2%) con LTA.

5.5. Estudio V

Impact of mild hypothermia on platelet responsiveness to aspirin and clopidogrel: an in vitro pharmacodynamic investigation.

Ferreiro JL, Sánchez-Salado JC, Gracida M, Marcano AL, Roura G, Ariza A, Gómez-Lara J, Lorente V, Romaguera R, Homs S, Sánchez-Elvira G, Teruel L, Rivera K, Sosa SG, Gómez-Hospital JA, Angiolillo DJ, Cequier A.

J Cardiovasc Transl Res. 2014;7:39-46.

Estudio *in vitro* prospectivo farmacodinámico con datos apareados, que se realizó en muestras de 20 pacientes con un IAMCEST y que recibieron dosis de carga de clopidogrel (600mg) y AAS (250mg) en el momento del diagnóstico antes de proceder a una angioplastia primaria. Las muestras sanguíneas se extrajeron la mañana del día siguiente al ICP, entre 12 y 24 horas tras las dosis de carga y antes de recibir la primera dosis de mantenimiento de AAS y clopidogrel. Inmediatamente tras la extracción, las muestras se incubaron durante 1 hora a 33ºC (rango de hipotermia leve terapéutica) y 37ºC, realizándose posteriormente las pruebas de función plaquetar, que incluyeron: a) MEA, usando como puntos de corte de HTPR >468 AU*min para clopidogrel y >400 AU*min para AAS; y b) VerifyNow, usando como puntos de corte >240 PRU y ≤11% IPA para clopidogrel y >550 ARU para AAS. La variable de valoración principal fue la comparación entre la inhibición plaquetar inducida por clopidogrel (medida con MEA) entre las muestras incubadas a 33ºC y 37ºC.

La hipotermia leve generada *in vitro* se asoció de manera estadísticamente significativa con una reducción de la inhibición plaquetar inducida por clopidogrel, medida con cualquiera de las pruebas de función plaquetar empleadas. En concreto, se observó una mayor reactividad plaquetar en las muestras incubadas a temperatura de 33ºC comparado con las de 37ºC, tanto medido con MEA (235,2±31,4 AU*min vs. 181,9±30,2 AU*min; p<0,001) como con el sistema VerifyNow, expresado como PRU (172,9±20,3 vs. 150,9±19,3; p=0,004) o como IPA (31,2±6,1% vs. 36,8±6,9%; p<0,05). Las tasas de HTPR a clopidogrel fueron numéricamente superiores, aunque sin alcanzar significación estadística, en las muestras a 33ºC comparado con las de las muestras a 37ºC.

No se observaron diferencias en la inhibición plaquetaria inducida por AAS, ni tampoco en las tasas de HTPR a AAS, con ninguno de los tests de función plaquetar empleados.

5.6. Estudio VI

Effects of cangrelor in coronary artery disease patients with and without diabetes mellitus: an in vitro pharmacodynamic investigation.

Ferreiro JL, Ueno M, Tello-Montoliu A, Tomasello SD, Capodanno D, Capranzano P, Dharmashankar K, Darlington A, Desai B, Rollini F, Guzman LA, Bass TA, Angiolillo DJ.

J Thromb Thrombolysis. 2013;35:155-64.

Estudio *in vitro* prospectivo farmacodinámico con datos apareados realizado en muestras de 120 pacientes (se eliminaron 17 muestras por imposibilidad de procesamiento, quedando 103 para el análisis final) con cardiopatía isquémica estable bajo tratamiento antiagregante de mantenimiento con AAS 81 mg/día y sin haber recibido ningún antagonista del receptor $P2Y_{12}$ al menos durante los 30 días previos a la inclusión. Los sujetos se estratificaron según si tenían DM (n=48) o no (n=55). Las muestras se analizaron basalmente y tras incubación *in vitro* a 37ºC con cangrelor 500 nmol/l, simulando la concentración plasmática obtenida con la perfusión empleada en los ensayos de fase III. Las pruebas de función plaquetar empleadas fueron: a) análisis de VASP, expresando los valores como PRI; y b) MEA, expresando los valores como AU*min y utilizando agonistas purinérgicos, ADP con y sin prostaglandina E_1 (PGE_1) y no purinérgicos como ácido araquidónico (AA), colágeno y péptido agonista del receptor de trombina (TRAP). La variable de valoración principal fue la comparación de la inhibición plaquetar, medida con el

PRI obtenido con VASP, entre los pacientes con y sin DM en las muestras incubadas con cangrelor 500 nmol/l. En un subgrupo de 20 pacientes se evaluó si existía un efecto dosis-dependiente de cangrelor, incubando las muestras con concentraciones crecientes del fármaco (5, 50, 500 y 5000 nmol/l) sobre la función plaquetar (evaluando vías purinérgicas y no purinérgicas) y sobre los procesos de generación de trombina evaluados con tromboelastografía (TEG).

Se observó una reducción muy importante en los valores de PRI tras la incubación *in vitro* con 500 nmol/l de cangrelor en el global de la población estudiada (reducción relativa de 80,6±10,4%). Asimismo, se apreció una marcada reducción en la reactividad plaquetar (comparando el valor basal con el obtenido tras la incubación con cangrelor) evaluada con MEA con todos los agonistas empleados, aunque esta reducción fue de mayor magnitud al usar los agonistas purinérgicos (ADP y ADP+PGE_1) que los que evalúan otras vía de señalización plaquetar (AA, colágeno y TRAP).

No se observaron diferencias en los parámetros farmacodinámicos basales entre las muestras de pacientes con y sin DM. La inhibición plaquetar conseguida al incubar las muestras con cangrelor fue similar independientemente de la presencia o no de DM, sin encontrarse diferencias significativas en los valores de PRI (16,1±12,3 en diabéticos vs. 16,8±11.3 en no diabéticos; p=0,346). De igual modo, no se hallaron diferencias entre los pacientes con y sin DM en los valores de función plaquetar obtenidos con MEA (con todos los agonistas empleados) tras la incubación con cangrelor.

Al evaluar la eficacia farmacodinámica de las concentraciones crecientes del fármaco, los análisis de tendencia mostraron un efecto dosis-dependiente

de cangrelor sobre la inhibición plaquetar con todas las pruebas de función plaquetar utilizadas. Además, no se evidenció interacción debida a la existencia de DM en los análisis de tendencia, ni tampoco se apreciaron diferencias entre los valores de los pacientes con y sin DM a ninguna de las concentraciones de cangrelor evaluadas, independientemente de los agonistas utilizados.

No se encontraron diferencias en los parámetros de generación de trombina obtenidos por TEG con ninguna de las concentraciones de cangrelor utilizadas, sin apreciarse tampoco diferencias entre muestras de sujetos con y sin DM.

6. DISCUSIÓN CONJUNTA

Y es que en el mundo traidor
nada hay verdad ni mentira:
todo es según el color
del cristal con que se mira.

RAMÓN DE CAMPOAMOR

Una inhibición plaquetaria correcta es un pilar fundamental en el tratamiento de los pacientes con un SCA o en los que se realiza un ICP, siendo actualmente de elección en este contexto la DAP con AAS y un inhibidor del receptor $P2Y_{12}$. Pese al desarrollo en los últimos años de nuevos antagonistas del receptor $P2Y_{12}$ más potentes y clínicamente más eficaces en los pacientes con SCA [89,90], el clopidogrel es todavía el fármaco de este grupo más usado en nuestro medio. El principal problema de clopidogrel es su gran variabilidad interindividual de respuesta que se traduce en un porcentaje importante de pacientes que presentan una respuesta subóptima al fármaco, lo que se asocia claramente con un mayor riesgo de presentar eventos cardiovasculares isquémicos y, por tanto, con una peor evolución clínica [33]. Los mecanismos identificados que contribuyen a la variabilidad de respuesta del clopidogrel se han agrupado como factores genéticos, celulares o clínicos. Son de especial interés estos últimos, los factores clínicos, porque es a este nivel donde es más factible poder realizar acciones terapéuticas que mitiguen su impacto deletéreo.

La presente tesis doctoral se ha centrado en dos aspectos: el primero de ellos, al que se ha dedicado la mayor parte del trabajo, ha sido profundizar en el conocimiento de diferentes factores clínicos potencialmente asociados con una hiperreactividad plaquetar y una respuesta subóptima a clopidogrel (artículos I al V), y el segundo ha sido evaluar si el uso *in vitro* de cangrelor, el antagonista más potente del receptor $P2Y_{12}$, puede conseguir un nivel de inhibición plaquetar adecuado en pacientes con un elevado riesgo de presentar respuesta subóptima a clopidogrel, como son los sujetos con DM (artículo VI).

6.1. Mecanismos implicados en la variabilidad de respuesta a clopidogrel

6.1.1. Interacción entre inhibidores de la bomba de protones y clopidogrel

La posible interacción farmacológica entre los IBPs y el clopidogrel, que provocaría un empeoramiento de la eficacia antiplaquetaria de este último, motivó inicialmente una importante preocupación en la comunidad médica a causa de la frecuencia con que se combinan ambos tipos de fármacos en pacientes con un SCA o sometidos a ICP y, por tanto, por la potencial repercusión clínica de esta interacción. Los resultados de estudios farmacodinámicos más consistentes a la hora de mostrar un empeoramiento en el efecto antiagregante de clopidogrel al asociar un IBP fueron los obtenidos cuando se asociaba omeprazol, el IBP más utilizado [75,106]. El mecanismo subyacente sugerido para explicar esta interacción es una inhibición competitiva a nivel de la isoenzima CYP2C19, que es la isoforma principal encargada de metabolizar el omeprazol y además está implicada en los dos pasos de oxidación hepática del clopidogrel.

Dado que omeprazol y clopidogrel tienen una vida plasmática corta, se planteó la hipótesis de que al separar el momento de la administración de ambos fármacos, se conseguiría evitar la interacción farmacológica. El estudio I de esta tesis fue diseñado específicamente para evaluar esta hipótesis. Los resultados obtenidos permitieron confirmar que la administración de omeprazol se asocia con un empeoramiento de la inhibición plaquetar inducida por clopidogrel en la fase de mantenimiento. Sin embargo, no se encontraron

diferencias (con ninguna de las pruebas de función plaquetar empleadas) si se administraban ambos fármacos al mismo tiempo o separados 8-12 horas. Es de señalar que en este estudio la interacción se produjo únicamente en la fase de mantenimiento, pero no tras la administración de la dosis de carga, lo que se puede explicar por el uso de una dosis de carga elevada (600 mg) de clopidogrel que sería capaz de superar dicha interacción, mitigando o eliminando su impacto farmacodinámico. Este efecto dosis-dependiente sería consistente con la hipótesis de una inhibición competitiva a nivel de la isoforma del CYP implicada, habiéndose observado un fenómeno similar con la interacción farmacodinámica entre clopidogrel y atorvastatina (a nivel del CYP3A4 en este caso) [50].

Los diferentes IBPs comercializados son metabolizados por isoformas del CYP (principalmente CYP2C19 y CYP3A4), pero con diferentes especificidades, lo que plantea la cuestión de si esta interacción farmacodinámica es un efecto de clase (se produce con todos los IBPs) o se produce específicamente con los fármacos de este grupo metabolizados mayoritariamente por CYP2C19 (p.ej. omeprazol). El estudio II de esta tesis se diseñó específicamente para evaluar si el uso de pantoprazol (metabolizado principalmente por la isoenzima CYP2C9 y con poco potencial para inhibir el CYP2C19) afecta la inhibición plaquetaria mediada por clopidogrel y si este impacto se modifica según el momento de administración de ambos fármacos. En este estudio, la respuesta a clopidogrel no se vio afectada por la administración de pantoprazol (datos consistentes en todas las pruebas de

función plaquetar empleadas), sin importar el momento de administración de ambos fármacos.

Los resultados de estos dos estudios han sido confirmados posteriormente por otras investigaciones farmacodinámicas [107], sugiriendo globalmente que la interacción entre IBPs y clopidogrel no es un efecto de clase y sería específica de los IBPs con un mayor potencial de afectación del CYP2C19, lo que justifica que exista interacción farmacodinámica con omeprazol y no se observe con pantoprazol. Es de resaltar que los estudios incluidos en esta tesis fueron los primeros en evaluar y demostrar que separar el momento de administración de los IBPs y el clopidogrel no tenía un impacto en la inhibición plaquetar, lo que también se corroboró posteriormente en otras investigaciones [108].

La relevancia clínica, sin embargo, de la interacción entre clopidogrel y los IBPs (fundamentalmente omeprazol) no está clara. Los análisis de datos de estudios clínicos han aportado resultados contradictorios al evaluar el impacto clínico de esta interacción en pacientes con SCA o en los que se realizaba ICP. Algunos estudios observacionales han mostrado un aumento del riesgo de eventos adversos en aquellos pacientes en los que se asociaba un IBP al tratamiento con clopidogrel comparado con los que recibían únicamente clopidogrel [73,74,109-111]. Sin embargo, los resultados de otros estudios observacionales, de análisis post-hoc de ensayos clínicos aleatorizados y del único estudio aleatorizado que ha evaluado esta interacción (a pesar de haberse interrumpido prematuramente por falta de financiación), el COGENT (Clopidogrel and the Optimization of Gastrointestinal Events Trial), no han

objetivado generalmente ningún indicio de un aumento del riesgo cardiovascular debido a la coadministración de ambos fármacos [112-115]. Globalmente, los resultados de todas estas investigaciones evidencian que los pacientes que reciben IBPs son de mayor edad y tienen más comorbilidades, lo que podría suponer un factor de confusión difícil de controlar a la hora de analizar estudios observacionales, al ser prescritos en pacientes de más alto riesgo, pudiendo explicar parcialmente este sesgo los resultados obtenidos en alguno de los estudios mencionados. Por otra parte, el empeoramiento en la reactividad plaquetar con esta interacción es relativamente pequeño (aproximadamente un 10-15%), por lo que se ha sugerido que tendría el potencial de afectar únicamente a pacientes de alto riesgo con una inhibición plaquetar inducida por clopidogrel “en el límite” (cercana al umbral de la respuesta subóptima) y no al global de la población [116].

6.1.2. Tabaquismo

La asociación entre tabaquismo y variabilidad de respuesta a clopidogrel vendría explicada por el hecho de que el consumo de cigarrillos es un potente inductor de CYP1A2 (la isoforma con mayor implicación en el primer paso de oxidación hepática de clopidogrel) [76], con lo que aumentaría la generación del metabolito activo de clopidogrel y, por tanto, su efecto antiagregante. De hecho, estudios farmacodinámicos y clínicos han mostrado que, entre los pacientes en tratamiento con clopidogrel, los fumadores presentan una inhibición plaquetar superior y una mayor eficacia clínica del fármaco, al compararlos con los no fumadores [77-79,108]. Este aumento relativo del

beneficio clínico observado entre los pacientes tratados con clopidogrel se ha denominado “paradoja de los fumadores”. Sin embargo, los estudios mencionados presentan una importante limitación, ya que el consumo de tabaco se registró según el hábito declarado por el paciente, lo que no es una medida cuantitativa objetiva de la exposición a la nicotina en cada paciente, que además puede depender de otros factores (tipo y marca de cigarrillos, manera de inhalar...). Por tanto, este punto imposibilitaba determinar de forma fehaciente en esos estudios previos la existencia o no de una relación dosis-respuesta entre el consumo de tabaco y la eficacia farmacodinámica de clopidogrel.

El estudio III de esta tesis fue diseñado específicamente para evaluar si existe una relación dosis-respuesta en el impacto del consumo de tabaco medido según los niveles de cotinina sérica, el principal producto de degradación estable de la nicotina, sobre la inhibición plaquetar mediada por clopidogrel en una cohorte de pacientes con DM (con mayor riesgo de presentar respuesta subóptima a clopidogrel que la población general). Los hallazgos de esta investigación demostraron la existencia de una relación dosis-respuesta entre el hábito tabáquico y un aumento del efecto antiagregante inducido por clopidogrel, lo que se reflejó también en un menor porcentaje de pacientes con respuesta subóptima al fármaco entre los fumadores, obteniendo resultados consistentes con todas las pruebas de función plaquetar utilizadas. Cabe valorar que, de los estudios farmacodinámicos que han evaluado la asociación entre reactividad plaquetar y consumo de tabaco, éste fue el primero en cuantificar objetivamente dicho

consumo mediante la determinación de las concentraciones plasmáticas de cotinina.

Los resultados de esta investigación, consistentes con los de otros estudios farmacodinámicos [77,117-118], sugieren que la mayor magnitud del beneficio obtenido con clopidogrel en pacientes fumadores comparados con los que no tienen hábito tabáquico (“paradoja de los fumadores”) que se ha observado en algunos estudios clínicos (análisis *post hoc* de ensayos clínicos, en su mayoría) [78,79,119] sería atribuible a una mayor eficacia de la inhibición plaquetaria en este grupo de pacientes. El mecanismo principal responsable de este efecto sería el incremento de actividad del CYP1A2 inducido por el consumo de cigarrillos, que aumentaría la conversión de clopidogrel en su metabolito activo y, por tanto, su efecto antiagregante. Sin embargo, existen otros factores que podrían contribuir a explicar los resultados del presente estudio. Entre ellos, destaca el hecho de que este estudio se condujo selectivamente en pacientes con DM, que presentan una actividad metabólica reducida del sistema CYP [120], lo que acrecentaría las posibilidades de encontrar un efecto dosis-respuesta al evaluar el impacto de un inductor de alguna de las isoenzimas CYP. Esta idea se sustentaría también en los hallazgos de un estudio farmacogenético que concluyó que el impacto del consumo de tabaco sobre la eficacia antiagregante de clopidogrel se limitaría únicamente a los pacientes con un polimorfismo particular del CYP1A2 [121].

El aumento en el beneficio clínico del clopidogrel en los pacientes fumadores se ha observado en subanálisis de estudios que han evaluado la eficacia de la DAP con AAS y clopidogrel [78,79,119] y también en un análisis

post hoc del ensayo CAPRIE (Clopidogrel Versus Aspirin in Patients at Risk of Ischemic Events), el único que ha comparado AAS frente a clopidogrel en monoterapia en el contexto de prevención secundaria [122]. Sin embargo, es importante no sobreestimar o interpretar erróneamente la importancia clínica de la "paradoja de los fumadores" y concluir que clopidogrel no es eficaz en los pacientes no fumadores o que no es relevante insistir en el abandono del hábito tabáquico en los pacientes en tratamiento con clopidogrel. De hecho, en los estudios comentados también se ha evidenciado que los pacientes no fumadores obtenían un beneficio del tratamiento con clopidogrel (aunque inferior al de los fumadores en términos relativos) y que los pacientes fumadores presentan globalmente un riesgo incrementado de eventos isquémicos en el seguimiento [78,79,122]. Por todo ello, cabe recordar que el tabaquismo es un factor de riesgo sobradamente establecido de eventos aterotrombóticos y dejar de fumar es una recomendación de clase I como prevención secundaria en pacientes con enfermedad coronaria.

6.1.3. Presencia de un síndrome coronario agudo tipo infarto agudo de miocardio con elevación del segmento ST

La presencia de un SCA es uno de los factores clínicos claramente asociados con una mayor agregabilidad plaquetar y una peor respuesta inicial a los fármacos antiplaquetarios [57,58]. Esto se muestra de forma todavía más acusada en los pacientes con un IAMCEST, lo que puede contribuir a las tasas más elevadas de episodios aterotrombóticos que presentan estos pacientes en su evolución inicial [123]. Un empeoramiento en la farmacocinética de

clopidogrel en el contexto del IAMCEST, fundamentalmente debido a una menor absorción, que resultaría en una menor biodisponibilidad del fármaco es uno de los mecanismos propuestos como causantes de la elevada frecuencia de pacientes con respuesta subóptima inicial a clopidogrel en este escenario [124]. Además, el clopidogrel presenta un inicio de acción lento, lo que podría verse agravado en este contexto por la reducción del tiempo entre el primer contacto médico con el paciente y la reperfusión, que se consigue con los programas de angioplastia primaria. Existe poca evidencia en la literatura sobre la respuesta farmacodinámica a clopidogrel, administrado con dosis de carga en el momento del primer contacto médico como recomiendan las guías de práctica clínica, en pacientes con un IAMCEST en los que se realiza angioplastia primaria.

El estudio IV presentado en esta tesis fue diseñado particularmente para evaluar el porcentaje de pacientes con respuesta inadecuada a clopidogrel en el momento justo de iniciar el procedimiento de angioplastia primaria, tras haber recibido una dosis de carga del fármaco en el momento del diagnóstico, y analizar su asociación con la permeabilidad inicial de la ARI. Los resultados de este estudio señalaron unos pobres niveles de antiagregación mediada por clopidogrel en el contexto del IAMCEST en el momento de iniciarse el cateterismo coronario, evidenciándose un porcentaje de pacientes muy elevado (cercano al 90%) con respuesta subóptima al fármaco. Se mostró también la existencia de una proporción de sujetos, aunque mucho menor, que no presentaba una respuesta adecuada a AAS. Además, esta investigación deparó un hallazgo muy relevante al ser la primera que mostró una asociación

entre una respuesta subóptima al clopidogrel y un empeoramiento del flujo en la ARI al empezar el procedimiento.

El pretratamiento con clopidogrel se asocia con un menor riesgo de eventos isquémicos en pacientes con IAMCEST, comparado con administrar el fármaco tras realizar el cateterismo coronario [125]. Sin embargo, el lento inicio de acción del fármaco juega un papel relevante en el alto porcentaje de pacientes que presentan una respuesta subóptima a clopidogrel, pese a la administración de una dosis de carga de 600mg, en el contexto de la angioplastia primaria, donde es de capital importancia reducir los retrasos y minimizar el tiempo entre la presentación clínica y la reperfusión. Los resultados de otros estudios farmacodinámicos han mostrado unas tasas muy elevadas (similares a las del presente estudio) de respuesta inadecuada a clopidogrel en los pacientes con IAMCEST [126], lo que confirmaría los hallazgos de la presente investigación. Además, la frecuencia de pobre respuesta a clopidogrel es superior en el IAMCEST que en las otras formas de SCA [127]. Entre las causas responsables de esta peor eficacia farmacodinámica se encuentra una absorción disminuida de clopidogrel, siendo característica de los pacientes con IAMCEST una peor absorción de los fármacos orales, que conllevaría una menor biodisponibilidad del mismo [124].

Un hallazgo relevante y novedoso de este estudio es la asociación observada entre una respuesta subóptima al clopidogrel y la permeabilidad inicial de la ARI, lo que puede tener consecuencias en ciertos aspectos del intervencionismo coronario al visualizar la arteria distal a la lesión (p.ej. realización de trombectomía o predilatación...) y en eventos clínicos. Aunque

en algún estudio previo se ha observado que la permeabilidad inicial de la ARI es mejor en pacientes que han recibido pretratamiento con clopidogrel respecto a los que no [128], ésta es la primera investigación en la que se aprecia que la respuesta al fármaco podría ser determinante.

Globalmente, estos resultados refuerzan la idea de que, en el IAMCEST en que se realiza angioplastia primaria, lograr una mayor eficacia antiagregante que la conseguida con clopidogrel es de gran importancia para disminuir el número de eventos adversos en este escenario. De hecho, el alto porcentaje de pacientes con una pobre respuesta a clopidogrel explicaría en parte los mejores resultados obtenidos por prasugrel y ticagrelor en los pacientes con IAMCEST, al ser fármacos más potentes y con un inicio de acción más rápido [92,129].

6.1.4. Hipotermia leve en rango terapéutico

La hipotermia leve se emplea en los pacientes supervivientes a una parada cardiaca que persisten en situación de coma. Dado que la causa más frecuente de paro cardiaco es un SCA, la combinación de hipotermia terapéutica e ICP es frecuente y generalmente se ha definido como eficaz y segura [80]. Sin embargo, investigaciones recientes sugieren que la hipotermia terapéutica podría inducir un aumento en la reactividad plaquetar y una reducción de la respuesta a los fármacos antiagregantes orales, fundamentalmente a clopidogrel [81,82]. Adicionalmente, esta pobre respuesta podría causar un aumento de eventos aterotrombóticos, ya que se ha descrito

en algunas series de casos un aumento del riesgo de trombosis del stent en pacientes con hipotermia terapéutica tras un ICP, a pesar del tratamiento con DAP [83]. No obstante, si existe realmente un impacto de la hipotermia terapéutica sobre la reactividad plaquetar y la respuesta a los fármacos antiagregantes orales es todavía objeto de debate.

El estudio V de esta tesis fue diseñado para analizar el efecto *in vitro* de la hipotermia leve en rango terapéutico sobre la respuesta farmacodinámica a clopidogrel y AAS en muestras de pacientes con IAMCEST en los que se realizó angioplastia primaria. En esta investigación, se observó una reducción significativa de la inhibición plaquetar mediada por clopidogrel en las muestras en las que se generó *in vitro* una hipotermia en rango terapéutico, siendo los resultados consistentes con todas las pruebas de función plaquetar utilizadas. La temperatura en el rango de la hipotermia terapéutica no afectó, sin embargo, la inhibición plaquetar inducida por AAS.

La combinación de hipotermia terapéutica y una reperfusión temprana mediante ICP ha sido calificada clásicamente como eficaz y segura en pacientes que han sufrido un paro cardiaco y, como tal, ha sido recomendada en guías de práctica clínica [80]. Sin embargo, este concepto ha sido puesto en duda a raíz de la comunicación en los últimos años de algunas series de casos en las que pacientes con hipotermia terapéutica tenían un riesgo aumentado de sufrir una trombosis del stent pese a recibir DAP con AAS y clopidogrel [83,130], aunque es cierto asimismo que otras investigaciones no han observado los mismos hallazgos [131]. De hecho, la eficacia de los fármacos

antiagregantes orales en pacientes con hipotermia terapéutica tras un paro cardiaco recuperado no está en la actualidad completamente dilucidada.

Hay varios factores que pueden contribuir a una menor eficacia de clopidogrel en este escenario clínico, como serían una menor absorción de los agentes orales (debido a la hipotermia, a la administración de derivados opioides y a la condición crítica de los pacientes), una reducción del metabolismo y la actividad enzimática debido a la hipotermia (lo que afectaría a fármacos de acción indirecta que necesitan transformarse en un metabolito activo), y la presencia de un SCA, la principal causa de parada cardiaca, que es *per se* un predictor de respuesta subóptima a clopidogrel. En esta línea, cabe señalar los resultados de un estudio *in vivo* realizado en sujetos con hipotermia terapéutica tras un paro cardiaco, en el que todos los pacientes presentaron respuesta subóptima a clopidogrel 24 horas después de iniciada la hipotermia, persistiendo en un 69% de ellos a los 3 días [81].

Diversos estudios mecanísticos han observado que la hipotermia en rango terapéutico produce un aumento de la activación y agregación plaquetar, preferencialmente a través de la vía de señales mediada por ADP [82,132]. Estos hallazgos estarían en consonancia con los resultados de nuestra investigación, que sugieren la vía de señalización plaquetaria estimulada por ADP como el mediador principal de la activación plaquetaria asociada a la hipotermia. Un aspecto novedoso del presente estudio es que se eliminó el posible efecto de la temperatura en la farmacocinética de clopidogrel al reproducir las condiciones de hipotermia *in vitro* incubando la sangre de pacientes con IAMCEST tratados con AAS y clopidogrel. Entre los mecanismos

que se ha sugerido que pueden afectar la vía plaquetaria del ADP como consecuencia de la hipotermia se encuentran una reducción de la hidrólisis del ADP, cambios en la fluidez de la membrana y una mayor fragilidad de los eritrocitos que aumentaría la liberación de ADP [133,134].

En general, los resultados comentados sugieren que la eficacia de clopidogrel está disminuida en los pacientes con hipotermia terapéutica tras un paro cardiaco, por lo que sería interesante investigar en este contexto clínico el empleo de fármacos orales más potentes como prasugrel o ticagrelor, o el uso de un antagonista $P2Y_{12}$ endovenoso potente como cangrelor, con el que podrían evitarse los problemas de los fármacos orales en este escenario.

6.2. Bloqueo potente del receptor $P2Y_{12}$ en pacientes con enfermedad coronaria

La presencia de DM se asocia a una serie de alteraciones metabólicas y celulares que conducen a un estado de hiperreactividad plaquetar que juega, a su vez, un papel importante en la aterosclerosis acelerada y el alto riesgo de complicaciones aterotrombóticas que presentan estos pacientes [59,63]. Asimismo, este fenotipo plaquetar hiperreactivo induce una menor respuesta farmacodinámica a los fármacos antiagregantes orales, entre ellos fundamentalmente a clopidogrel, un antagonista del receptor $P2Y_{12}$ [59,65]. En concreto, esta vía de señalización plaquetar iniciada en el receptor $P2Y_{12}$ se encuentra regulada al alza en los pacientes diabéticos [49], lo que podría contribuir a un efecto diferencial de los fármacos antiagregantes que bloquean

esta vía según la existencia o no de DM. Globalmente, los aspectos mencionados contribuyen al riesgo aumentado de eventos isquémicos que presentan los pacientes con DM y al menor beneficio relativo que obtienen de las terapias antiagregantes orales en comparación con los sujetos no diabéticos [65]. El cangrelor, un análogo de ATP, es un fármaco endovenoso que antagoniza el receptor $P2Y_{12}$ de manera potente, reversible y directa (sin necesidad de metabolito activo), además de tener un inicio y un fin de acción muy rápidos y un efecto dosis-dependiente [99]. Dado que cangrelor logra un bloqueo muy potente de la vía de señalización del receptor $P2Y_{12}$ (>90%), se ha planteado si el uso de este fármaco puede conseguir una inhibición plaquetar similar en pacientes con y sin DM y, por tanto, superar el efecto de los diversos mecanismos que contribuyen a la hiperreactividad plaquetar característica de los pacientes diabéticos.

El estudio VI presentado en esta tesis fue diseñado específicamente para comparar la eficacia farmacodinámica *in vitro* de cangrelor en muestras de pacientes con y sin DM, además de investigar si un bloqueo potente del receptor $P2Y_{12}$ con cangrelor puede afectar otras vías de señalización plaquetar o de procesos de generación de trombina. Los resultados de esta investigación objetivaron que la administración de cangrelor *in vitro* produce una inhibición plaquetar muy potente y dosis-dependiente de la vía del receptor $P2Y_{12}$, sin diferencias en su eficacia entre pacientes con y sin DM. Además, se observaron unos moderados efectos inhibitorios en vías de señalización plaquetar no purinérgicas con la adición *in vitro* de cangrelor a las muestras, sin

apreciarse cambios en los procesos de generación de trombina dependientes de plaquetas.

Dentro de la cardiopatía isquémica, los pacientes con DM tienen un mayor riesgo de eventos isquémicos en el seguimiento que los sujetos sin DM. A este hecho puede contribuir en parte que la DM es una patología que aglutina varios de los mecanismos que condicionan una mayor reactividad plaquetar y una peor respuesta a los fármacos antiagregantes [59,65]. Los hallazgos de nuestra investigación sugieren que un bloqueo muy potente de la vía iniciada en el receptor $P2Y_{12}$, como la conseguida con cangrelor, puede superar la disfunción plaquetar característica de los pacientes con DM, lo que podría tener relevancia clínica. En la línea de este argumento se encontrarían los resultados favorables obtenidos con prasugrel y ticagrelor, antagonistas orales más potentes que clopidogrel, en el subgrupo de pacientes diabéticos de sus respectivos estudios pivotales [93,135].

La inhibición del receptor $P2Y_{12}$ mediada por clopidogrel se ha asociado en algunos estudios con una prolongación de los parámetros tromboelastográficos que reflejan los procesos de generación de trombina dependientes de plaquetas [136,137], involucrados en la coagulación. No está claro si esta asociación puede producirse también con otros antagonistas del receptor $P2Y_{12}$. En el caso de cangrelor, existe una escasa evidencia que apoye un posible efecto del fármaco en dichos procesos [138], mientras que, por el contrario, los resultados de otras investigaciones sugieren que cangrelor podría no tener ningún impacto modulador en los mismos, lo que estaría en consonancia con los hallazgos de nuestro estudio. La justificación vendría por

la existencia de unos mecanismos de señalización intracelular ligeramente diferentes a los de otros antagonistas del receptor $P2Y_{12}$, que conducirían a un aumento del adenosín monofosfato cíclico [139], que también se produce con otras estrategias de tratamiento antiagregante que no modifican los procesos de generación de trombina [140].

Un aspecto novedoso del presente estudio es que fue el primero en evaluar los efectos farmacodinámicos de una concentración terapéutica de cangrelor en vías de señalización plaquetar (no purinérgicas) diferentes a la del receptor de ADP $P2Y_{12}$, la diana específica del fármaco. En concreto, se observó que la adición *in vitro* de cangrelor produjo una marcada disminución de la agregabilidad plaquetar al usar agonistas no purinérgicos, lo que es relevante al plantear que un bloqueo potente del receptor $P2Y_{12}$ tendría un impacto en otras vías de señalización. Estos resultados son consistentes con los de otros estudios en los que también se ha apreciado que un antagonismo potente del receptor $P2Y_{12}$, conseguido con incubaciones *in vitro* de ticagrelor y el metabolito activo de prasugrel, tiene un impacto sobre vías no purinérgicas [141,142]. Sin embargo, la posible relevancia clínica de estos hallazgos farmacodinámicos que apuntan a una interacción entre vías de señalización plaquetar no está determinada y será posiblemente objeto de futuras investigaciones.

En el momento de la redacción de esta tesis, cangrelor ha sido recientemente aprobado para uso clínico en pacientes en los que se realiza ICP [104], aunque todavía no se encuentra disponible en España. El aspecto más destacable de los resultados de nuestro estudio es que no se apreciaron

diferencias en el potente efecto del fármaco en sujetos con y sin DM, lo que podría sugerir que cangrelor sería una opción terapéutica atractiva en aquellos subgrupos de pacientes con una mayor probabilidad de presentar una respuesta subóptima o más lenta a los antagonistas orales del receptor $P2Y_{12}$ usados actualmente. Evidentemente, cualquier hipótesis acerca de un subgrupo de pacientes que podría beneficiarse en mayor medida del tratamiento con cangrelor debe confirmarse en estudios clínicos diseñados específicamente a tal efecto.

7. CONCLUSIONES

"Fere libenter homines, id quod volunt, credunt."
(La gente casi siempre cree de buena gana lo que quiere.)

JULIO CÉSAR

- La administración de omeprazol reduce el efecto antiplaquetario del clopidogrel en la fase de mantenimiento, independientemente del momento de administración de ambos fármacos (al mismo tiempo o separados entre 8 y 12 horas).
- El uso de pantoprazol a dosis altas no se asocia con una modulación de la eficacia antiagregante del clopidogrel, sin importar el momento de administración de ambos fármacos.
- Los dos puntos anteriores sugieren que la interacción farmacodinámica entre clopidogrel y los inhibidores de la bomba de protones no es un efecto de clase y sería específica de determinados fármacos de este grupo (con un mayor potencial de afectación del CYP2C19).
- El hábito tabáquico se asocia con un aumento del efecto antiagregante mediado por el clopidogrel, presentando una relación dosis-respuesta, y con unas menores tasas de respuesta subóptima al fármaco en los pacientes con diabetes mellitus.
- Un porcentaje elevado de pacientes con IAMCEST presentan una respuesta subóptima a clopidogrel y, en menor grado, a AAS al iniciar el procedimiento de angioplastia primaria. Además, la pobre respuesta a clopidogrel podría estar asociada con una menor permeabilidad inicial de la arteria responsable del infarto.
- Las temperaturas en el rango de la hipotermia terapéutica producen un empeoramiento *in vitro* de la respuesta a clopidogrel, sin afectar la inhibición plaquetar inducida por AAS.

- La administración de cangrelor *in vitro* produce un bloqueo muy potente del receptor $P2Y_{12}$, sin diferencias en su eficacia entre pacientes con y sin diabetes mellitus. Además, el cangrelor *in vitro* consigue unos moderados efectos inhibitorios en vías de señalización plaquetar no purinérgicas, sin afectar los procesos de generación de trombina dependientes de plaquetas.

8. SUMMARY IN ENGLISH

Pharmacodynamic Variability in Response to Clopidogrel: Mechanisms Involved and Use of More Potent Platelet $P2Y_{12}$ Inhibitors in Patients with Coronary Artery Disease

Insanity: doing the same thing over and over again and expecting different results.

ALBERT EINSTEIN

INTRODUCTION

Atherosclerosis is the main underlying cause of coronary artery disease, being a chronic inflammatory process that causes a progressive narrowing of the coronary arteries. The disruption (rupture or superficial erosion) of an atherosclerotic plaque triggers a series of mechanisms that initiate the process of thrombus formation, a phenomenon in which platelets play a key role [3,4]. Of note, plaque disruption may be spontaneous or iatrogenic, such as in an acute coronary syndrome (ACS) or during a percutaneous coronary intervention (PCI), respectively.

The first step of primary haemostasis as well as of the thrombotic complications of atherosclerosis is the contact between platelets and the thrombogenic matrix following disruption of an atherosclerotic plaque, which leads to a three-phase process of platelet adhesion, activation and, finally, aggregation [3,4]. In brief, after exposure or release of thrombogenic substances (collagen, tissue factor and von Willebrand factor play a relevant role), platelets are recruited, roll and adhere at plaque or endothelial injury sites, which is followed by platelet activation and aggregation [5]. Noteworthy, platelets are the major and most relevant component at the initial phase of thrombus formation [4,5]. Therefore, since atherothrombotic events are essentially platelet-driven processes, this underscores the importance of using antiplatelet agents in patients suffering an ACS and/or undergoing PCI, which represents the keystone of treatment in these scenarios.

Each of the three phases (adhesion, activation and aggregation) involved in platelet-mediated thrombotic processes represent a potential target for the

development of antithrombotic drugs. Inhibitors of platelet adhesion are still under investigation and none of them are approved for clinical use at the present time [6]. Glycoprotein (GP) IIb/IIIa inhibitors block the final common pathway of platelet aggregation (binding of GP IIb/IIIa receptor to fibrinogen, von Willebrand factor, fibronectin, and prothrombin) and are available only for intravenous use, thus, being restricted for the acute phase of treatment of high risk ACS patients undergoing PCI, particularly in cases of great thrombus burden or in “bail-out” situations [7]. Therefore, inhibitors of platelet activation processes represent the keystone of treatment and prevention of recurrent ischemic events in ACS patients, including those with unstable angina, non-ST-elevation acute coronary syndrome (NSTE-ACS) or ST elevation myocardial infarction (STEMI), and/or in patients undergoing PCI [7-9]

There are currently two groups of platelet activation inhibitors available for clinical use for treatment and prevention of recurrent events in the ACS or PCI setting: a) Thromboxane A_2 (TxA_2) pathway antagonists: aspirin (ASA: acetylsalicylic acid), a cycloxigenase-1 irreversible inhibitor through selective acetylation of a serine residue at position 529 (Ser529) that prevents formation of TxA_2 [10], which is the only available agent of this group and whose benefit in coronary artery disease has been extensively proven [11,12]; and b) adenosine diphosphate (ADP) $P2Y_{12}$ receptor antagonists: ticlopidine, clopidogrel, prasugrel, ticagrelor. Dual antiplatelet therapy (DAPT) with ASA and a $P2Y_{12}$ receptor antagonist is currently the antiplatelet treatment of choice for the whole spectrum of patients with ACS and/or undergoing PCI [7-9].

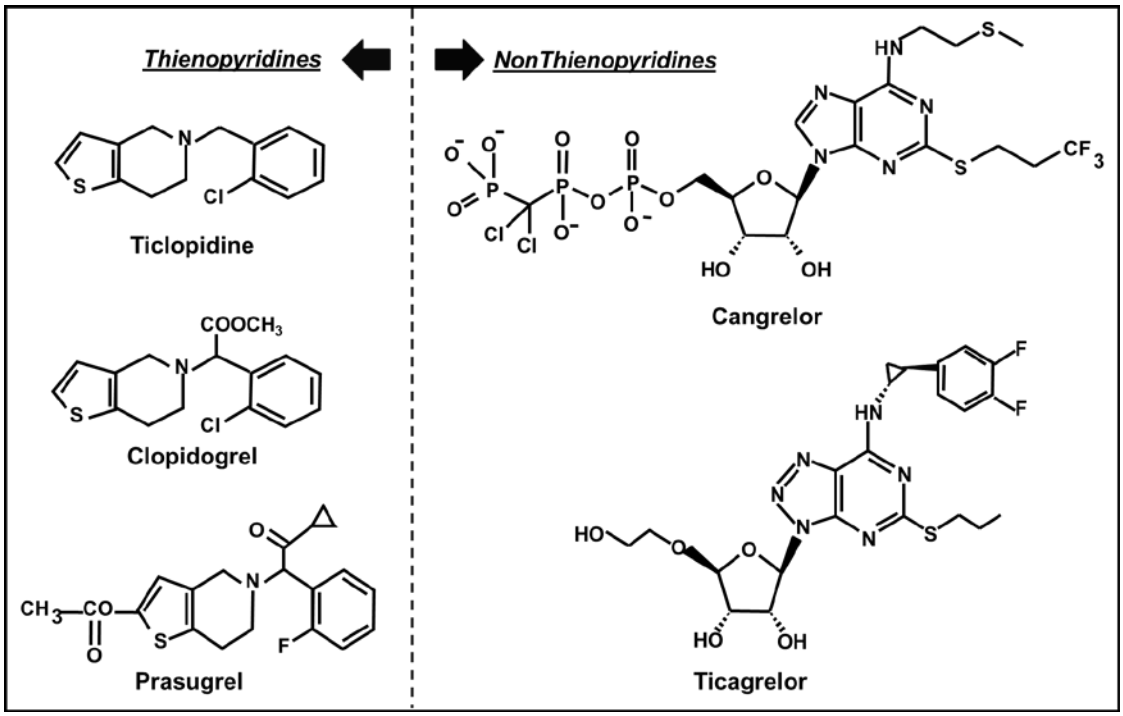
Platelet $P2Y_{12}$ purinergic receptor

Purinergic receptors expressed on platelets are the following: $P2X_1$, $P2Y_1$ and $P2Y_{12}$. $P2X_1$ is a ligand-gated cation channel, which has adenosine triphosphate (ATP) as its physiological agonist and is involved in platelet shape change through extracellular calcium influx, in addition to help amplifying platelet responses mediated by other agonists [13]. ADP is the physiological agonist and, consequently, exerts its action on platelets through $P2Y_1$ and $P2Y_{12}$, two G protein-coupled seven transmembrane domains purinergic receptors [14]. Activation of the $P2Y_1$ receptor generates a transient change in platelet shape, granule release of other mediators, intracellular calcium mobilization and, finally, starts a weak and transient phase of platelet aggregation [14]. Even though both P2Y receptors are necessary to produce a complete aggregation [15], ADP-mediated effects on platelets are upheld predominantly by the $P2Y_{12}$ receptor signaling pathway. In short, activation of $P2Y_{12}$ pathway provokes a series of intracellular events that result in calcium mobilization, granules release, TxA_2 generation and activation of GP IIb/IIIa receptor, which results in amplification of platelet aggregation and stabilization of the platelet aggregate [12,15,16]. Therefore, $P2Y_{12}$ blockade is crucial in order to inhibit platelet activation and aggregation with the purpose of preventing formation of platelet thrombus.

The $P2Y_{12}$ receptor antagonists that are currently available are orally administered and can be grouped as follows: a) ticlopidine, clopidogrel and prasugrel, three generations of thienopyridines, which are non-direct antagonists (prodrugs that require hepatic metabolism to be converted into an

active metabolite) that irreversibly block the $P2Y_{12}$ receptor; and b) ticagrelor, a cyclopentyltriazolopyrimidine, which directly (without needing conversion into an active metabolite and reversibly inhibits the $P2Y_{12}$ receptor (Figure 1). Blockade of the $P2Y_{12}$ pathway is an established therapeutic target in patients with coronary artery disease, whose importance was soon confirmed by the clinical benefit demonstrated, in association with aspirin, in the initial studies evaluating ticlopidine (the first $P2Y_{12}$ blocker available) [17]. Ticlopidine, a first-generation thienopyridine, in combination with aspirin was proven superior to aspirin alone or anticoagulation plus aspirin in terms of reducing ischemic events in the PCI setting [18-21]. Due to certain safety concerns, mainly high rates of neutropenia, ticlopidine was soon extensively replaced by clopidogrel, a thienopyridine with similar efficacy and a better safety profile [22].

Figure 1. Chemical structure of $P2Y_{12}$ platelet receptor antagonists



Clopidogrel: Variability in response

Clopidogrel, a second-generation thienopyridine, is a prodrug that must undergo hepatic biotransformation to be converted into an active metabolite that will irreversibly bind and block the $P2Y_{12}$ platelet receptor. Approximately 15% of the clopidogrel absorbed into the bloodstream from the intestine (the remaining 85%is inactivated by esterases) is metabolized in the liver through a double oxidation process mediated by several cytochrome P450 (CYP) isoforms to obtain its active metabolite [23]. Due to the irreversible inhibition achieved of the $P2Y_{12}$ receptor, clopidogrel effects last for the whole lifespan of the platelet (7-10 days). Since clopidogrel has a delayed onset of action, it needs a loading dose (usually 300 or 600mg) to shorten it when rapid inhibition is required, such as in the context of ACS or PCI, followed by a 75mg maintenance dose. Due to a more rapid and potent effect [24-26], the use of a 600mg loading dose has been widespread in clinical practice and is also endorsed by guidelines [7-9].

Clopidogrel soon replaced ticlopidine after its approval in 1997 due to a better safety profile, particularly regarding hematologic toxicity [22], in addition to having the advantage over ticlopidine of achieving a faster onset on action through administration of a loading dose [27]. Until the appearance of the newer and more potent $P2Y_{12}$ inhibitors that will be discussed later, the prominence of clopidogrel for more than a decade in the clinical settings of ACS and PCI was undisputed. In fact, DAPT with ASA and clopidogrel was considered the standard of care in these scenarios during that period, which is based on the findings of several large-scale clinical trials that observed a clear benefit of this combination in preventing recurrent ischemic events, including stent thrombosis

[28-32]. Despite these benefits, a considerable number of patients continue to experience recurrent ischemic events, which has been partially attributed to the phenomenon known as variability in response to clopidogrel.

The main downside of clopidogrel is its broad variability in response, which leads to a relatively high percentage of patients (ranging from 5 to 40% and depending on population characteristics, platelet function assay and cutoff values used) with diminished or suboptimal response, also named occasionally “resistance” [33]. The relevance of this variability in response is underscored by the fact that a multitude of studies have demonstrated an association between low responsiveness to clopidogrel and adverse cardiovascular outcomes [33].

Mechanisms of clopidogrel response variability

Multiple mechanisms have been identified to contribute to clopidogrel response variability, which can be classified into 3 main categories: genetic, cellular, and clinical factors (Figure 2).

Several pharmacogenetic studies have evaluated a number of polymorphisms of different genes involved in pharmacokinetics and pharmacodynamics of clopidogrel. The ABCB1 gene codes for intestinal P-glycoprotein MDR1 (multidrug resistance transporter), which is involved in clopidogrel absorption. It has been observed that homozygous patients (carriers of two variant alleles) for an ABCB1 polymorphism had a higher risk of cardiovascular events in a cohort of patients with an acute myocardial infarction receiving clopidogrel therapy [34]. In line with this, it has been suggested that

subjects carrying two ABCB1 variant alleles may have reduced active metabolite generation after administration of a loading dose of clopidogrel [35]; however, its association with the pharmacodynamic response to clopidogrel has not been proven [36]. Several CYP isoforms are involved in the hepatic oxidation steps that convert clopidogrel into its active metabolite: CYP3A4, CYP3A5, CYP2C9, and CYP1A2 are implicated in one step, while CYP2B6 and CYP2C19 contribute to both steps [23]. Polymorphisms in CYP3A4, CYP3A5, CYP2C9 and CYP2C19 [37-41] have been reported in mechanistic studies as possible determinants of clopidogrel variability in response, although large-scale pharmacogenetic studies have only consistently observed an association with clinical outcomes of certain CYP2C19 polymorphisms. In fact, several investigations have demonstrated an intense association between CYP2C19 loss-of-function variant alleles (mainly CYP2C19*2) and decreased formation of active metabolite, which leads to lower platelet inhibition and, finally, to a higher risk of ischemic events [36,42-44]. Conversely, the presence of the CYP2C19*17 gain-of-function variant allele has been associated with increased formation of active metabolite, greater clopidogrel-mediated platelet inhibition and higher bleeding risk [45]. Other small pharmacogenetic studies have suggested that allelic variant of genes encoding for platelet membrane receptors might be involved clopidogrel variability in response. These include polymorphisms of P2YR12 ($P2Y_{12}$ receptor), ITGB3 (platelet-fibrinogen receptor GP IIb/IIIa), ITGA2 (platelet-collagen receptor GP Ia), and PAR-1 (protease-activated receptor -1, a thrombin receptor) genes; however, evidence of their impact has not been consistent.

Several cellular factors have also been proposed to affect clopidogrel-induced antiplatelet effects. An accelerated platelet turnover, which is typical of patients with diabetes mellitus (DM), is represented by the presence of higher number immature reticulated platelets. Some studies have observed an association between a higher percentage of circulating reticulated platelets, which have a greater reactivity, and a lower response to clopidogrel [47,48]. Another cellular factor that may affect clopidogrel efficacy is an upregulation of platelet signaling pathways, in particular the one initiated in the $P2Y_{12}$ receptor, which is also present in DM patients [49]. As a final point, the baseline degree of metabolic activity of the CYP system is a cellular factor that may condition clopidogrel conversion into its active metabolite and, thus, its efficacy.

Multiple clinical factors have been associated with higher platelet reactivity and suboptimal response to clopidogrel. Currently, it is not possible to modify or act on the genetic factors and very difficult on the cellular factors described above. However, it is feasible to undergo therapeutic actions to diminish the impact of some clinical factors that affect clopidogrel efficacy, which underscores the great relevance of deepen our knowledge of these mechanisms. Among them, compliance is the most important [51], and a correct dosing also plays a role in clopidogrel efficacy [33]. There are also some clinical features that affect platelet reactivity and clopidogrel responsiveness, such as obesity [52,53], DM [54-56] and the presence of an ACS [57,58]. The last two are especially noteworthy due to their great prognostic impact, since these two features are strongly associated with higher platelet reactivity and impaired response to antiplatelet agents [54-58]. The presence of an ACS is *per se* a

predictor of reduced response to clopidogrel and, notably, STEMI patients have higher rates of suboptimal response than those with the other types of ACS [57]. This issue is of interest in the setting of community programs to implement primary PCI, which can reduce time delays between administration of antiplatelet agents at clinical presentation and reperfusion, but at the same time may impair clopidogrel efficacy during the peri-interventional period because of its delayed onset of action [33]. With regards to DM, numerous metabolic and cellular abnormalities that occur in this disease result in platelet hyperreactivity, which is one of the determinants of the prothrombotic state which characterizes DM patients and plays an essential role in the accelerated atherosclerosis and higher risk of atherothrombotic complications in this population [59]. The mechanisms that contribute to the important platelet dysfunction of patients with DM (the “diabetic” platelet) can be grouped in four etiopathogenic categories: a) hyperglycemia, b) insulin deficiency of action, c) associated metabolic conditions, and d) other cellular abnormalities [60-63]. In brief, the hyperreactive platelet phenotype causes a suboptimal response to antiplatelet agents, particularly to clopidogrel [64,65], which contributes to an augmented risk of ischemic events in patients with DM and to a lower relative benefit obtained with antiplatelet drugs when comparing with subjects without DM [65].

Hepatic biotransformation by the CYP system is a critical step to achieve clopidogrel antiplatelet effects. Therefore, drugs that are activated or metabolized by CYP isoforms involved in clopidogrel metabolism can potentially interfere in its active metabolite generation and, thus, in its antiplatelet effects. Some pharmacodynamic studies have suggested potential drug interactions

with agents commonly used in cardiovascular therapy that might reduce clopidogrel efficacy: a) lipophilic statins, with discordant results among studies and without clear evidence of having an impact on outcomes from large-scale studies [66-70]; b) calcium channel blockers, mainly dihydropyridines (metabolized by CYP3A4) [71,72]; and c) proton-pump inhibitors (PPIs).

A possible drug interaction between PPIs and clopidogrel is of relevance due to the frequency with which both drugs are associated, since PPIs are routinely prescribed in patients on DAPT to prevent gastrointestinal haemorrhages. In fact, the first studies reporting that concomitant use of PPIs and clopidogrel was associated with an increased risk of cardiovascular events after an ACS when compared with patients not taking PPIs [73,74] raised an important concern in the scientific community. A competitive inhibition at the level of CYP2C19 isoenzyme is the postulated mechanism to explain this interaction. In fact, the most consistent results to date have been obtained with omeprazole, which is metabolized primarily by CYP2C19. In particular, omeprazole administration has been reported in pharmacodynamic studies to reduce clopidogrel-induced antiplatelet effects [75], and the first large-scale registries and *post hoc* analysis of trials observed that omeprazole use could be associated with worse clinical outcomes in ACS patients receiving clopidogrel [73,74]. Conversely, results of other mechanistic studies evaluating other PPIs such as pantoprazole (mainly metabolized by CYP2C9) do not allow drawing definitive conclusions about this interaction being a class effect or drug specific. Interestingly, it has been hypothesized that staggering administration of

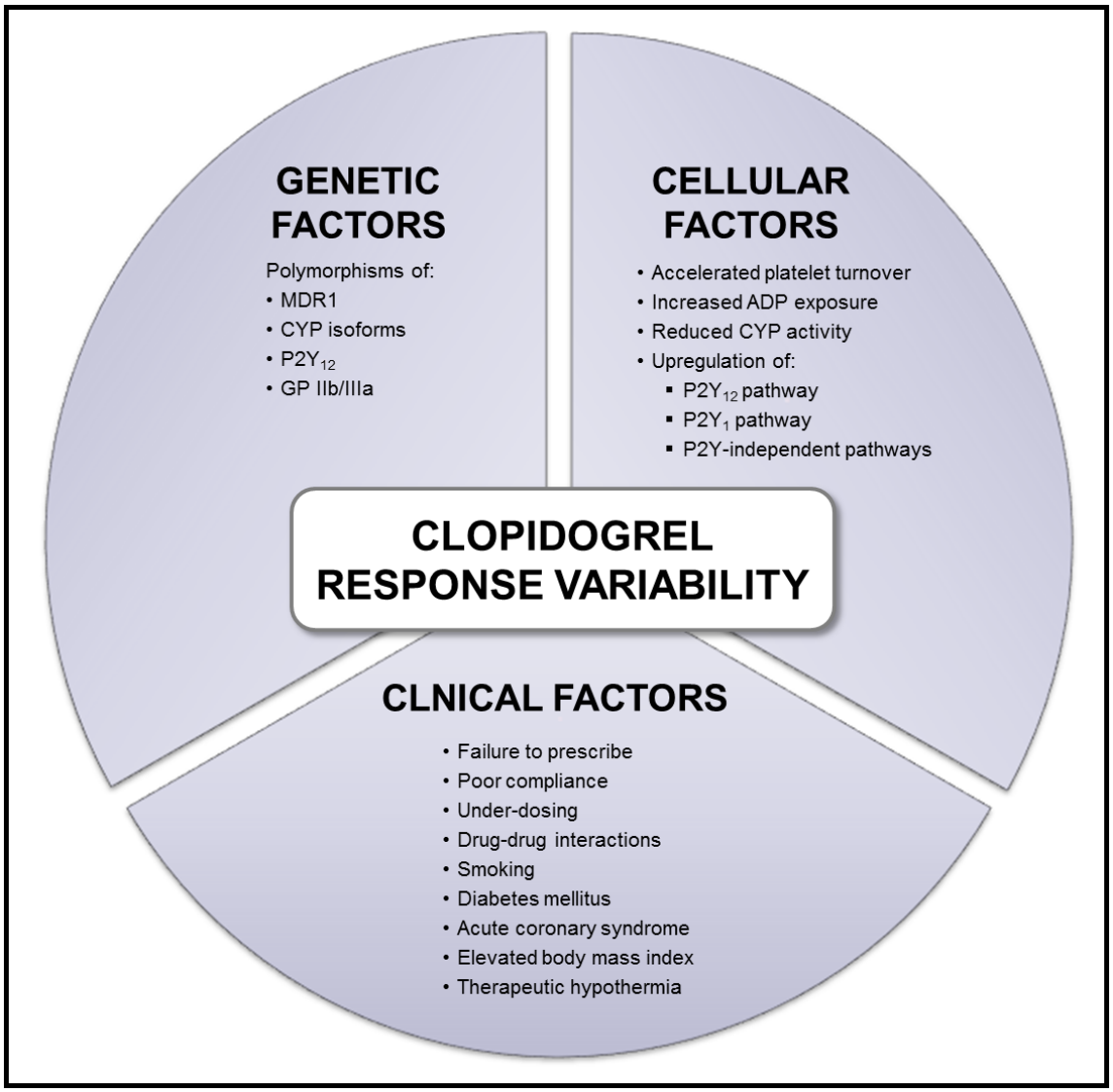
clopidogrel and omeprazole may overcome their interaction, since plasmatic concentrations of both drugs are nearly undetectable 6 to 8 hours after intake.

Smoking habit has also been associated with clopidogrel response variability. Although not properly a drug interaction, the underlying mechanism would be also related with the active metabolite generation by the CYP system. Cigarette smoking is a potent inducer of the CYP1A2 isoform and, therefore, it may increase clopidogrel biotransformation [76]. Some studies have reported that a heavy smoking habit may enhance clopidogrel-induced platelet inhibitory effects [77] and could improve clinical outcomes in clopidogrel-treated patients [78,79]. However, it is worth to remember that smoking is a major risk factor for atherothrombotic cardiovascular processes and smoking cessation is a class I recommendation for secondary prevention of ischemic events in patients with coronary artery disease. Whether smoking habit has an impact on clopidogrel efficacy is yet to be determined. Of note, functional studies suggesting this interaction did not assess cigarette smoking with objective measures, such as determining cotinine (a stable metabolite of nicotine).

Another clinical factor worth mentioning is therapeutic hypothermia, which could play a role in clopidogrel response variability and impact clinical outcomes of patients receiving this therapy. Mild therapeutic hypothermia (32 to 34ºC) is used in patients surviving a cardiac arrest (the most common cause is an ACS) who remain comatose with the objective of improving neurological prognosis and survival [80]. The results of recent investigations have suggested that hypothermia might increase platelet reactivity and reduce responsiveness to antiplatelet agents, particularly to clopidogrel [81,82]. The latter may have

clinical consequences since reports of case series have observed higher than expected rates of stent thrombosis in patients with therapeutic hypothermia after a primary PCI, despite receiving DAPT [83]. However, whether mild therapeutic hypothermia has an impact on platelet reactivity and increases the risk of ischemic events is nowadays matter of debate.

Figure 2. Mechanisms involved in clopidogrel response variability



ADP: adenosine diphosphate; CYP: cytochrome P450; GP: glycoprotein; MDR1: multidrug resistance transporter

Potent $P2Y_{12}$ receptor antagonists

The prognostic impact of suboptimal response to clopidogrel emphasizes the need for finding and using new antiplatelet strategies that achieve a more potent inhibition of the P2Y12 receptor with less variability in response (a more consistent effect), especially in high-risk ACS patients undergoing PCI. Three strategies have been suggested to overcome the problem of variability in response to clopidogrel: a) increasing clopidogrel dosing; b) adding a third antiplatelet agent to the combination of ASA and clopidogrel; and c) using newer and more potent $P2Y_{12}$ receptor antagonists.

Despite a modest pharmacodynamic improvement [84-86], neither increasing clopidogrel doses nor adding a third oral antiplatelet agent (e.g. cilostazol) have demonstrated an important benefit in clinical outcomes and these strategies have not been broadly implemented in daily practice [87,88]. Conversely, it has been proven advantageous the use of newer $P2Y_{12}$ antagonists such as prasugrel or ticagrelor, which have in common a faster onset of action, a more potent effect and less variability than clopidogrel. The superior efficacy of these agents on the ACS scenario, especially in the PCI setting, has been proven in large-scale clinical trials [89,90]. Consequently, these agents have been authorized for clinical use and are preferred over clopidogrel in current practice guidelines [7-9].

Prasugrel, like all thienopyridines, is an orally administered prodrug that needs hepatic biotransformation into its active metabolite to irreversibly block the $P2Y_{12}$ receptor. Prasugrel conversion into its active metabolite is more effective than that of clopidogrel. Since the active metabolites of both agents

are equipotent, the major production of active metabolite achieved by prasugrel provides greater platelet inhibition, in addition of having a faster onset of action and less interindividual variability in response than clopidogrel [91]. The benefit of prasugrel compared to clopidogrel was demonstrated in the TRITON-TIMI 38 (Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel- Thrombolysis In Myocardial Infarction 38) trial, which evaluated patients with moderate to high-risk ACS undergoing PCI [89]. In this trial, the use of prasugrel was associated with a 19% relative reduction of ischemic events (composite of cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke), which occurred at the cost of a small increase in TIMI (Thrombolysis in Myocardial Infarction) major bleeding not related to coronary artery bypass grafting. Certain subgroups such as patients with STEMI [92] and DM patients [93] benefit the most from prasugrel therapy without an increase in major bleeding risk. In contrast, no net benefit was observed in low-weight (<60 kg) and in elderly patients (≥75 years), while a net harm with prasugrel was observed in patients with prior history of stroke. The use of prasugrel is approved for treatment of ACS patients undergoing PCI and must be administered once coronary anatomy is known in NSTE-ACS subjects, whereas pretreatment is allowed in patients with STEMI.

Ticagrelor is a cyclopentyltriazolopyrimidine, the first developed agent of this new compound class, which directly and reversibly inhibits the $P2Y_{12}$ receptor. Ticagrelor has a faster onset of action and provides stronger platelet inhibition than clopidogrel, with less variability. It has a more rapid offset of action than clopidogrel, due to its reversible effects and a short plasmatic half-

life (twice daily dosing is required), although approximately 30-40% of ticagrelor effects are attributed to active metabolites generated in the liver. The efficacy and safety of ticagrelor compared to clopidogrel was evaluated in the PLATO (Platelet Inhibition and Patient Outcomes) trial, which included moderate- to high-risk ACS patients [90]. In this trial, ticagrelor therapy significantly reduced ischemic events (16% relative reduction) with no increase in protocol-defined major bleeding, although a small increase in TIMI major bleeding not related to coronary artery bypass grafting was observed (a similar 0,6% increase in absolute value than that observed in the pivotal trial of prasugrel when using the same definition). Of note, the greater efficacy of ticagrelor was consistent in patients undergoing an initial planned invasive strategy [96] and in those with an initial conservative approach with a non-invasive treatment strategy [97], with a particular benefit observed in patients with chronic kidney disease [98]. Ticagrelor is approved for clinical use in patients with ACS, including patients managed medically and those undergoing PCI.

The superior efficacy of prasugrel and ticagrelor over clopidogrel must be *sensu stricto* interpreted as applicable to populations with the same clinical characteristics as the study patients included in their respective clinical trials described above. However, it is noticeable that a particular benefit of these agents was observed in certain subgroups that are classically associated with higher platelet reactivity and worse response to clopidogrel, such as patients with STEMI, DM or even CKD. This may suggest that a more potent blockade of the $P2Y_{12}$ pathway could be able to overcome the platelet hyperreactivity

characteristic of these high-risk subgroups and achieve an adequate platelet inhibition that may, hence, contribute to improve clinical outcomes.

The antiplatelet agent that achieves the most potent $P2Y_{12}$ inhibition (well above 90%) is cangrelor, an intravenous ATP analog, which directly, without needing any biotransformation, and reversibly inhibits the receptor. Cangrelor has several interesting pharmacological properties, such as the following: a) rapid onset of action, reaching steady-state concentrations within few minutes; b) dose-dependent effects and, thus, predictable effects; and c) rapid offset of action, due to its extremely short half-life (3-6 minutes) caused by a rapid deactivation by plasmatic ectonucleotidases, returning to baseline platelet function within 30-60 minutes after stopping the infusion [99]. The CHAMPION (Cangrelor versus standard tHerapy to Achieve optimal Management of Platelet InhibitiON) program aimed to evaluate the efficacy and safety of cangrelor in patients undergoing PCI mostly presenting with an ACS. The first two trials that compared cangrelor (always administered before PCI was started) with clopidogrel, which was administered before the procedure in the CHAMPION-PCI study and immediately after PCI in the CHAMPION-PLATFORM study, were prematurely terminated for futility, failing to observe a significant difference between the two drugs in the primary endpoint (composite of death, myocardial infarction, or ischemia-driven revascularization at 48 hours) [100,101]. However, in a pooled analysis of the two studies using the universal definition of myocardial infarction instead of the original definition used in the trials, cangrelor was associated with a significant reduction of the rate of the primary endpoint [102]. In addition, the results of the CHAMPION-PHOENIX trial

showed a significant benefit of cangrelor compared to clopidogrel in terms of reducing ischemic events (composite of death by any cause, myocardial infarction, ischemia-driven revascularization, or stent thrombosis at 48 hours) in a population of patients undergoing PCI for stable angina or an ACS [103].Based on this evidence, cangrelor has been recently approved for clinical use both in the US and in Europe for treatment of patients undergoing PCI, with the particularity in Europe that it is indicated in patients who have not received an oral $P2Y_{12}$ inhibitor prior to the PCI procedure and in whom oral therapy with $P2Y_{12}$ inhibitors is not feasible or desirable [104]. Since it is the most potent developed $P2Y_{12}$ receptor antagonist, cangrelor is an appealing option to try overcoming the hyper-reactive platelet phenotype that characterizes certain high-risk subgroups, such as patients with DM.

Rationale

In spite of the development of newer and more potent agents (prasugrel and ticagrelor), it is important to remark that clopidogrel is still the most used $P2Y_{12}$ receptor antagonist in our real-life scenario in Spain [105]. Besides, prasugrel and ticagrelor are approved for clinical use in ACS subjects, but not in patients with stable coronary artery disease undergoing PCI, where clopidogrel remain the first antiplatelet treatment option, always in association with aspirin [7-9]. Therefore, the existence of a significant proportion of patients with ACS or undergoing PCI that are receiving clopidogrel therapy implies that a percentage of them may have a higher risk of suffering adverse ischemic events due to a suboptimal response to clopidogrel. This underscores the validity of the problem

and the relevance of deepen our knowledge of the mechanisms associated with the variability in response to clopidogrel and whether a potent blockade of the $P2Y_{12}$ receptor can overcome these mechanisms and achieve an optimal platelet inhibition.

As commented previously, a comprehensive understanding of clinical factors associated with augmented platelet reactivity and impaired clopidogrel-induced platelet inhibition is of critical relevance because it is possible to undergo some therapeutic actions at this level to minimize the deleterious impact of these factors. This is the reason why the efforts of this thesis have been mainly directed to strengthen our knowledge of several clinical mechanisms that may affect the antiplatelet efficacy of clopidogrel and, thus, impair the outcomes of patients with ACS or undergoing PCI receiving this agent. In particular, the mechanisms evaluated have been the following: a) drug interaction with omeprazole, a PPI metabolized primarily by CYP2C19 isoform, assessing its impact on clopidogrel-mediated platelet inhibition when both drugs are administered concomitantly or staggered (article I); b) drug interaction with pantoprazole, a PPI not metabolized primarily by CYP2C19 isoform, evaluating its impact on clopidogrel-mediated platelet inhibition when both drugs are administered concomitantly or staggered (article II); c) effect of cigarette smoking, objectively assessed by determining cotinine (a stable metabolite of nicotine), in clopidogrel efficacy in a cohort of DM patients (article III); d) impact of the presence of a STEMI on the initial efficacy of clopidogrel at the very moment of initiating a primary PCI procedure (article IV); and e) effect of mild hypothermia at therapeutic range on clopidogrel response (article V).

In addition, it has been evaluated whether the *in vitro* use of cangrelor, the most potent $P2Y_{12}$ receptor inhibitor, can achieve a similar platelet inhibition in patients with and without DM, thus, if a potent $P2Y_{12}$ receptor blockade may overcome the platelet dysfunction that characterizes patients with DM, a pathology in which several mechanisms contribute to platelet hyperreactivity and to a worse response to antiplatelet agents than non-DM subjects (article VI).

Finally, several review articles have been published as a result of the work related to this thesis and those considered the most relevant and interesting have been included in this manuscript. The main reasons for including these reviews were that they fit perfectly into the global theme of the thesis, their bibliometric impact and the crucial importance of the topics thoroughly revised in these papers, which are the following: a) $P2Y_{12}$ receptor antagonists, paying special attention to the mechanisms involved in variability in response to clopidogrel (article VII); b) platelet dysfunction and antiplatelet therapy in DM patients with an ACS (article VIII); and c) future perspectives of antiplatelet therapy, with special attention to novel agents recently available or still under development (article IX).

HYPOTHESIS

The main hypothesis of this thesis is that clopidogrel-induced pharmacodynamic antiplatelet effect is modified by the following mechanisms: a) impaired by the administration of omeprazole, mainly when both drugs are

administered concomitantly, whereas no pharmacologic interaction is produced with pantoprazole; b) increased with cigarette smoking in a dose-response manner; c) diminished by the occurrence of a STEMI; and d) reduced by *in vitro* generated mild hypothermia at therapeutic range.

A second hypothesis of this thesis is that *in vitro* administration of cangrelor achieves a great and similar degree of platelet inhibition in patients with and without DM, suggesting that a very potent $P2Y_{12}$ receptor blockade may overcome the effect of the various mechanisms that contribute to the hyper-reactive platelet phenotype which characterizes diabetic patients.

OBJECTIVES

The main and general objective of this thesis is to provide insights into the knowledge of several clinical factors that may be associated with platelet hyperreactivity and a suboptimal pharmacodynamic response to clopidogrel, in addition to assess whether the use of an agent that attains a very potent inhibition of the $P2Y_{12}$ pathway may overcome the effect of those clinical factors and achieve an optimal degree of platelet inhibition in patients at high risk of presenting a poor response to clopidogrel therapy.

The studies gathered in this thesis were performed in order to achieve the overall objective described above. However, they were conceived as independent investigations and the specific objectives of these studies were the following:

1. To evaluate the impact of the administration of omeprazole, a PPI metabolized primarily by CYP2C19, on clopidogrel-mediated platelet inhibition, assessing whether there is a differential effect when both drugs are administered concomitantly or staggered by 8 to 12 hours.
2. To examine whether the administration of pantoprazole, a PPI with low potential to inhibit CYP2C19, may impair clopidogrel-induced platelet inhibition, evaluating if there is a differential effect when both drugs are administered concomitantly or staggered by 8 to 12 hours.
3. To assess if there is a dose-response effect of cigarette smoking, as assessed by serum cotinine levels, on clopidogrel-mediated platelet inhibition in a cohort of patients with DM.
4. To determine the percentage of STEMI patients with HTPR at the very moment of initiating a primary PCI procedure after receiving a LD of clopidogrel at the moment of diagnosis and its association with the initial patency of the infarct-related artery.
5. To evaluate the *in vitro* effect of mild hypothermia at therapeutic range on the pharmacodynamic (PD) response to clopidogrel and aspirin in blood samples from STEMI patients undergoing primary PCI.
6. To analyze the *in vitro* pharmacodynamic efficacy of cangrelor in patients with and without DM, by comparing the platelet inhibition achieved in both groups, in addition to assess whether a potent blockade of the $P2Y_{12}$ receptor may modulate other platelet signaling pathways or platelet-derived thrombin generation processes.

RESULTS AND DISCUSSION

See **Section 4 "Publicaciones"** for a detailed description of methods, results and discussion of each and every study.

CONCLUSIONS

- Omeprazole impairs clopidogrel-induced antiplatelet effects in the maintenance phase of treatment irrespective of timing of drug administration (concomitantly or staggered by 8 to 12 hours).
- Pantoprazole therapy used at high doses is not associated with modulation of the antiplatelet efficacy of clopidogrel, irrespective of timing of drug administration.
- These two latter statements suggest that the pharmacodynamic interaction between clopidogrel and proton-pump inhibitors is not a class-specific effect but rather a drug-specific effect affecting PPIs metabolized primarily by CYP2C19.
- Cigarette smoking is associated with a dose-response effect on clopidogrel-induced antiplatelet effects and lower rates of high on-treatment platelet reactivity to clopidogrel in patients with diabetes mellitus.
- A high percentage of STEMI patients have inadequate levels of clopidogrel-induced and, to a lesser extent, aspirin-mediated platelet inhibition when starting a primary percutaneous coronary intervention.

Moreover, a poor response to clopidogrel might be associated with impaired initial patency of the infarct-related artery.

- Mild hypothermia at therapeutic range generated *in vitro* is associated with impaired clopidogrel-mediated platelet inhibition, with no effect on aspirin responsiveness.
- Cangrelor *in vitro* administration provides potent and dose-dependent blockade of the platelet $P2Y_{12}$ receptor, with no differential effect in patients with and without diabetes mellitus. In addition, *in vitro* cangrelor exerts moderate inhibitory effects on non-purinergic platelet signaling pathways, without modulating platelet-derived thrombin generation processes.

9. REFERENCIAS BIBLIOGRÁFICAS

El que lee mucho y anda mucho, ve mucho y sabe mucho.

MIGUEL DE CERVANTES

1. Libby P. Inflammation in atherosclerosis. Nature. 2002;420:868-874.

2. Fuster V, Badimon L, Badimon JJ, Chesebro JH. The pathogenesis of coronary artery disease and the acute coronary syndromes (1). N Engl J Med. 1992;326:242-250.

3. Fuster V, Moreno PR, Fayad ZA, Corti R, Badimon JJ. Atherothrombosis and high-risk plaque: part I: evolving concepts. J Am Coll Cardiol. 2005;46:937-54.

4. Davì G, Patrono C. Platelet activation and atherothrombosis. N Engl J Med. 2007;357:2482-94.

5. Furie B, Furie BC. Mechanisms of thrombus formation. N Engl J Med. 2008;359:938-49.

6. Tello-Montoliu A, Jover E, Rivera J, Valdés M, Angiolillo DJ, Marín F. New perspectives in antiplatelet therapy. Curr Med Chem. 2012;19:406-27.

7. Windecker S, Kolh P, Alfonso F, Collet JP, Cremer J, Falk V, et al. 2014 ESC/EACTS Guidelines on myocardial revascularization: The Task Force on Myocardial Revascularization of the European Society of Cardiology (ESC) and the European Association for Cardio-Thoracic Surgery (EACTS)Developed with the special contribution of the European Association of Percutaneous Cardiovascular Interventions (EAPCI). Eur Heart J. 2014;35:2541-619.

8. Roffi M, Patrono C, Collet JP, Mueller C, Valgimigli M, Andreotti F, et al. 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: Task Force for the Management of Acute Coronary Syndromes in Patients Presenting without Persistent ST-Segment Elevation of the European Society of Cardiology (ESC). Eur Heart J. 2015 Aug 29. pii: ehv320. [Epub ahead of print]

9. Steg PG, James SK, Atar D, Badano LP, Blömstrom-Lundqvist C, Borger MA, et al. ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation. Eur Heart J. 2012;33:2569-619.

10. Patrono C, García Rodríguez LA, Landolfi R, Baigent C. Low-dose aspirin for the prevention of atherothrombosis. N Engl J Med. 2005;353:2373-83.

11. Théroux P, Ouimet H, McCans J, Latour JG, Joly P, Lévy G, et al. Aspirin, heparin, or both to treat acute unstable angina. N Engl J Med. 1988;319:1105-11.

12. ISIS-2 (Second International Study of Infarct Survival) Collaborative Group. Randomised trial of intravenous streptokinase, oral aspirin, both, or neither among 17,187 cases of suspected acute myocardial infarction: ISIS-2. Lancet. 1988;2:349-60.

13. Kahner BN, Shankar H, Murugappan S, Prasad GL, Kunapuli SP. Nucleotide receptor signaling in platelets. J Thromb Haemost. 2006; 4:2317-26.

14. Storey RF, Newby LJ, Heptinstall S. Effects of P2Y(1) and P2Y(12) receptor antagonists on platelet aggregation induced by different agonists in human whole blood. Platelets. 2001;12:443-7.

15. Turner NA, Moake JL, McIntire LV. Blockade of adenosine diphosphate receptors P2Y(12) and P2Y(1) is required to inhibit platelet aggregation in whole blood under flow. Blood. 2001;98:3340-5.

16. Shankar H, Garcia A, Prabhakar J, Kim S, Kunapuli SP. P2Y12 receptor-mediated potentiation of thrombin-induced thromboxane A2 generation in platelets occurs through regulation of Erk1/2 activation. J Thromb Haemost. 2006;4:638-47.

17. Colombo A, Hall P, Nakamura S, Almagor Y, Maiello L, Martini G, et al. Intracoronary stenting without anticoagulation accomplished with intravascular ultrasound guidance. Circulation. 1995;91:1676-88.

18. Schömig A, Neumann FJ, Kastrati A, Schühlen H, Blasini R, Hadamitzky M, et al. A randomized comparison of antiplatelet and anticoagulant therapy after the placement of coronary-artery stents. N Engl J Med. 1996;334:1084-9.

19. Leon MB, Baim DS, Popma JJ, Gordon PC, Cutlip DE, Ho KK, et al. A clinical trial comparing three antithrombotic-drug regimens after coronary-artery stenting. Stent Anticoagulation Restenosis Study Investigators. N Engl J Med. 1998;339:1665-71.

20. Bertrand ME, Legrand V, Boland J, Fleck E, Bonnier J, Emmanuelson H, et al. Randomized multicenter comparison of conventional anticoagulation versus antiplatelet therapy in unplanned and elective coronary stenting. The full anticoagulation versus aspirin and ticlopidine (fantastic) study. Circulation. 1998;98:1597-603.

21. Urban P, Macaya C, Rupprecht HJ, Kiemeneij F, Emanuelsson H, Fontanelli A, et al. Randomized evaluation of anticoagulation versus antiplatelet therapy after coronary stent implantation in high-risk patients: the multicenter aspirin and ticlopidine trial after intracoronary stenting (MATTIS). Circulation. 1998;98:2126-32.

22. Bertrand ME, Rupprecht HJ, Urban P, Gershlick AH; CLASSICS Investigators. Double-blind study of the safety of clopidogrel with and without a loading dose in combination with aspirin compared with ticlopidine in combination with aspirin after coronary stenting: the clopidogrel aspirin stent international cooperative study (CLASSICS). Circulation. 2000;102:624-9.

23. Kazui M, Nishiya Y, Ishizuka T, Hagihara K, Farid NA, Okazaki O, et al. Identification of the human cytochrome P450 enzymes involved in the two oxidative steps in the bioactivation of clopidogrel to its pharmacologically active metabolite. Drug Metab Dispos. 2010;38:92-9.

24. von Beckerath N, Taubert D, Pogatsa-Murray G, Schömig E, Kastrati A, Schömig A. Absorption, metabolization, and antiplatelet effects of 300-, 600-, and 900-mg loading doses of clopidogrel: results of the ISAR-CHOICE (Intracoronary Stenting and

Antithrombotic Regimen: Choose Between 3 High Oral Doses for Immediate Clopidogrel Effect) Trial. Circulation. 2005;112:2946-50.

25. Angiolillo DJ, Fernández-Ortiz A, Bernardo E, Ramírez C, Sabaté M, Bañuelos C, et al. High clopidogrel loading dose during coronary stenting: effects on drug response and interindividual variability. Eur Heart J. 2004;25:1903-10.

26. Cuisset T, Frere C, Quilici J, Morange PE, Nait-Saidi L, Carvajal J, et al. Benefit of a 600-mg loading dose of clopidogrel on platelet reactivity and clinical outcomes in patients with non-ST-segment elevation acute coronary syndrome undergoing coronary stenting. J Am Coll Cardiol. 2006;48:1339-45.

27. Cadroy Y, Bossavy JP, Thalamas C, Sagnard L, Sakariassen K, Boneu B. Early potent antithrombotic effect with combined aspirin and a loading dose of clopidogrel on experimental arterial thrombogenesis in humans. Circulation. 2000;101:2823-8.

28. Yusuf S, Zhao F, Mehta SR, Chrolavicius S, Tognoni G, Fox KK; Clopidogrel in Unstable Angina to Prevent Recurrent Events Trial Investigators. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. N Engl J Med. 2001;345:494-502.

29. Mehta SR, Yusuf S, Peters RJ, Bertrand ME, Lewis BS, Natarajan MK, et al.; Clopidogrel in Unstable angina to prevent Recurrent Events trial (CURE) Investigators. Effects of pretreatment with clopidogrel and aspirin followed by long-term therapy in patients undergoing percutaneous coronary intervention: the PCI-CURE study. Lancet. 2001;358:527-33.

30. Sabatine MS, Cannon CP, Gibson CM, López-Sendón JL, Montalescot G, Theroux P, et al.; CLARITY-TIMI 28 Investigators. Addition of clopidogrel to aspirin and fibrinolytic therapy for myocardial infarction with ST-segment elevation. N Engl J Med. 2005;352:1179-89.

31. Chen ZM, Jiang LX, Chen YP, Xie JX, Pan HC, Peto R, et al.; COMMIT (ClOpidogrel and Metoprolol in Myocardial Infarction Trial) collaborative groupAddition of clopidogrel to aspirin in 45,852 patients with acute myocardial infarction: randomised placebo-controlled trial. Lancet. 2005;366:1607-21.

32. Steinhubl SR, Berger PB, Mann JT 3rd, Fry ET, DeLago A, Wilmer C, et al.; CREDO Investigators. Clopidogrel for the Reduction of Events During Observation. Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: a randomized controlled trial. JAMA. 2002;288:2411-20.

33. Ferreiro JL, Angiolillo DJ. Clopidogrel response variability: current status and future directions. Thromb Haemost. 2009;102:7-14.

34. Simon T, Verstuyft C, Mary-Krause M, Quteineh L, Drouet E, Méneveau N, et al.; French Registry of Acute ST-Elevation and Non-ST-Elevation Myocardial Infarction (FAST-MI) Investigators. Genetic determinants of response to clopidogrel and cardiovascular events. N Engl J Med. 2009;360:363-75.

35. Taubert D, von Beckerath N, Grimberg G, Lazar A, Jung N, Goeser T, et al. Impact of P-glycoprotein on clopidogrel absorption. Clin Pharmacol Ther. 2006;80:486-501.

36. Shuldiner AR, O'Connell JR, Bliden KP, Gandhi A, Ryan K, Horenstein RB, et al. Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. JAMA. 2009;302:849-57.

37. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Ramírez C, Cavallari U, Trabetti E, et al. Contribution of gene sequence variations of the hepatic cytochrome P450 3A4 enzyme to variability in individual responsiveness to clopidogrel. Arterioscler Thromb Vasc Biol. 2006;26:1895-900.

38. Suh JW, Koo BK, Zhang SY, Park KW, Cho JY, Jang IJ, et al. Increased risk of atherothrombotic events associated with cytochrome P450 3A5 polymorphism in patients taking clopidogrel. CMAJ. 2006;174:1715-22.

39. Brandt JT, Close SL, Iturria SJ, Payne CD, Farid NA, Ernest CS 2nd, et al. Common polymorphisms of CYP2C19 and CYP2C9 affect the pharmacokinetic and pharmacodynamic response to clopidogrel but not prasugrel. J Thromb Haemost. 2007;5:2429-36.

40. Hulot JS, Bura A, Villard E, Azizi M, Remones V, Goyenvalle C, et al. Cytochrome P450 2C19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects. Blood. 2006;108:2244-7.

41. Trenk D, Hochholzer W, Fromm MF, Chialda LE, Pahl A, Valina CM, et al. Cytochrome P450 2C19 681G>A polymorphism and high on-clopidogrel platelet reactivity associated with adverse 1-year clinical outcome of elective percutaneous coronary intervention with drug-eluting or bare- metal stents. J Am Coll Cardiol. 2008;51:1925-34.

42. Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT, et al. Cytochrome p-450 polymorphisms and response to clopidogrel. N Engl J Med. 2009;360:354-62.

43. Collet JP, Hulot JS, Pena A, Villard E, Esteve JB, Silvain J, et al. Cytochrome P450 2C19 polymorphism in young patients treated with clopidogrel after myocardial infarction: a cohort study. Lancet. 2009;373:309-17.

44. Sibbing D, Stegherr J, Latz W, Koch W, Mehilli J, Dörrler K, et al. Cytochrome P450 2C19 loss-of-function polymorphism and stent thrombosis following percutaneous coronary intervention. Eur Heart J. 2009;30:916-22.

45. Sibbing D, Koch W, Gebhard D, Schuster T, Braun S, Stegherr J, et al. Cytochrome 2C19*17 allelic variant, platelet aggregation, bleeding events, and stent thrombosis in clopidogrel-treated patients with coronary stent placement. Circulation. 2010;121:512-8.

46. Marín F, González-Conejero R, Capranzano P, Bass TA, Roldán V, Angiolillo DJ. Pharmacogenetics in cardiovascular antithrombotic therapy. J Am Coll Cardiol. 2009;54:1041-57.

47. Cesari F, Marcucci R, Caporale R, Paniccia R, Romano E, Gensini GF, et al. Relationship between high platelet turnover and platelet function in high-risk patients with coronary artery disease on dual antiplatelet therapy. Thromb Haemost. 2008;99:930-5.

48. Guthikonda S, Alviar CL, Vaduganathan M, Arikan M, Tellez A, DeLao T, et al. Role of reticulated platelets and platelet size heterogeneity on platelet activity after dual antiplatelet therapy with aspirin and clopidogrel in patients with stable coronary artery disease. J Am Coll Cardiol. 2008;52:743-9.

49. Ueno M, Ferreiro JL, Tomasello SD, Capodanno D, Tello-Montoliu A, Kodali M, et al. Functional profile of the platelet P2Y12 receptor signalling pathway in patients with type 2 diabetes mellitus and coronary artery disease. Thromb Haemost. 2011;105:730-2.

50. Lau WC, Gurbel PA, Watkins PB, Neer CJ, Hopp AS, Carville DG, et al. Contribution of hepatic cytochrome P450 3A4 metabolic activity to the phenomenon of clopidogrel resistance. Circulation. 2004;109:166-71.

51. Spertus JA, Kettelkamp R, Vance C, Decker C, Jones PG, Rumsfeld JS, et al. Prevalence, predictors, and outcomes of premature discontinuation of thienopyridine therapy after drug-eluting stent placement: results from the PREMIER registry. Circulation. 2006;113:2803-9.

52. Angiolillo DJ, Fernández-Ortiz A, Bernardo E, Barrera Ramírez C, Sabaté M, Fernandez C, et al. Platelet aggregation according to body mass index in patients undergoing coronary stenting: should clopidogrel loading-dose be weight adjusted? J Invasive Cardiol. 2004;16:169-74.

53. Sibbing D, von Beckerath O, Schömig A, Kastrati A, von Beckerath N. Impact of body mass index on platelet aggregation after administration of a high loading dose of 600 mg of clopidogrel before percutaneous coronary intervention. Am J Cardiol. 2007;100:203-5.

54. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Ramírez C, Sabaté M, Jimenez-Quevedo P, et al. Platelet function profiles in patients with type 2 diabetes and coronary artery disease on combined aspirin and clopidogrel treatment. Diabetes. 2005;54:2430-5.

55. Geisler T, Anders N, Paterok M, Langer H, Stellos K, Lindemann S, et al. Platelet response to clopidogrel is attenuated in diabetic patients undergoing coronary stent implantation. Diabetes Care. 2007;30:372-4.

56. Serebruany V, Pokov I, Kuliczkowski W, Chesebro J, Badimon J. Baseline platelet activity and response after clopidogrel in 257 diabetics among 822 patients with coronary artery disease. Thromb Haemost. 2008;100:76-82.

57. Soffer D, Moussa I, Harjai KJ, Boura JA, Dixon SR, Grines CL, et al. Impact of angina class on inhibition of platelet aggregation following clopidogrel loading in patients undergoing coronary intervention: do we need more aggressive dosing regimens in unstable angina? Catheter Cardiovasc Interv. 2003;59:21-5.

58. Sibbing D, von Beckerath O, Schömig A, Kastrati A, von Beckerath N. Platelet function in clopidogrel-treated patients with acute coronary syndrome. Blood Coagul Fibrinolysis. 2007;18:335-9.

59. Ferreiro JL, Angiolillo DJ. Challenges and perspectives of antiplatelet therapy in patients with diabetes mellitus and coronary artery disease. Curr Pharm Des. 2012;18:5273-93.

60. Stratmann B, Tschoepe D. Pathobiology and cell interactions of platelets in diabetes. Diab Vasc Dis Res 2005;2:16-23.

61. Vinik AI, Erbas T, Park TS, Nolan R, Pittenger GL. Platelet dysfunction in type 2 diabetes. Diabetes Care 2001;24:1476-85.

62. Ferroni P, Basili S, Falco A, Davì G. Platelet activation in type 2 diabetes mellitus. J Thromb Haemost 2004;2:1282-91.

63. Ferreiro JL, Gómez-Hospital JA, Angiolillo DJ. Platelet abnormalities in diabetes mellitus. Diab Vasc Dis Res. 2010;7:251-9.

64. Geisler T, Anders N, Paterok M et al. Platelet response to clopidogrel is attenuated in diabetic patients undergoing coronary stent implantation. Diabetes Care 2007;30:372-4.

65. Ferreiro JL, Cequier ÁR, Angiolillo DJ. Antithrombotic therapy in patients with diabetes mellitus and coronary artery disease. Diab Vasc Dis Res. 2010;7:274-88.

66. Lau WC, Waskell LA, Watkins PB, Neer CJ, Horowitz K, Hopp AS, et al. Atorvastatin reduces the ability of clopidogrel to inhibit platelet aggregation: a new drug-drug interaction. Circulation. 2003;107:32-7.

67. Neubauer H, Günesdogan B, Hanefeld C, Spiecker M, Mügge A. Lipophilic statins interfere with the inhibitory effects of clopidogrel on platelet function--a flow cytometry study. Eur Heart J. 2003;24:1744-9.

68. Saw J, Steinhubl SR, Berger PB, Kereiakes DJ, Serebruany VL, Brennan D, et al.; Clopidogrel for the Reduction of Events During Observation Investigators. Lack of adverse clopidogrel-atorvastatin clinical interaction from secondary analysis of a randomized, placebo-controlled clopidogrel trial. Circulation. 2003;108:921-4.

69. Saw J, Brennan DM, Steinhubl SR, Bhatt DL, Mak KH, Fox K, et al.; CHARISMA Investigators. Lack of evidence of a clopidogrel-statin interaction in the CHARISMA trial. J Am Coll Cardiol. 2007;50:291-5.

70. Lim MJ, Spencer FA, Gore JM, Dabbous OH, Agnelli G, Kline-Rogers EM, et al.; GRACE Investigators. Impact of combined pharmacologic treatment with clopidogrel and a statin on outcomes of patients with non-ST-segment elevation acute coronary syndromes: perspectives from a large multinational registry. Eur Heart J. 2005;26:1063-9.

71. Siller-Matula JM, Lang I, Christ G, Jilma B. Calcium-channel blockers reduce the antiplatelet effect of clopidogrel. J Am Coll Cardiol. 2008;52:1557-63.

72. Gremmel T, Steiner S, Seidinger D, Koppensteiner R, Panzer S, Kopp CW. Calcium-channel blockers decrease clopidogrel-mediated platelet inhibition. Heart. 2010;96:186-9.

73. Ho PM, Maddox TM, Wang L, Fihn SD, Jesse RL, Peterson ED, et al. Risk of adverse outcomes associated with concomitant use of clopidogrel and proton pump inhibitors following acute coronary syndrome. JAMA. 2009;301:937-44.

74. Juurlink DN, Gomes T, Ko DT, Szmitko PE, Austin PC, Tu JV, et al. A population-based study of the drug interaction between proton pump inhibitors and clopidogrel. CMAJ. 2009; 180:713-8.

75. Gilard M, Arnaud B, Cornily JC, Le Gal G, Lacut K, Le Calvez G, et al. Influence of omeprazole on the antiplatelet action of clopidogrel associated with aspirin: the randomized, double-blind OCLA (Omeprazole CLopidogrel Aspirin) study. J Am Coll Cardiol. 2008;51:256-60.

76. Zevin S, Benowitz NL. Drug interactions with tobacco smoking. An update. Clin Pharmacokinet. 1999;36:425-38.

77. Bliden KP, Dichiara J, Lawal L, Singla A, Antonino MJ, Baker BA, et al. The association of cigarette smoking with enhanced platelet inhibition by clopidogrel. J Am Coll Cardiol. 2008;52:531-3.

78. Desai NR, Mega JL, Jiang S, Cannon CP, Sabatine MS. Interaction between cigarette smoking and clinical benefit of clopidogrel. J Am Coll Cardiol. 2009;53:1273-8.

79. Berger JS, Bhatt DL, Steinhubl SR, Shao M, Steg PG, Montalescot G, et al.; CHARISMA Investigators. Smoking, clopidogrel, and mortality in patients with established cardiovascular disease. Circulation. 2009;120:2337-44.

80. Peberdy MA, Callaway CW, Neumar RW, Geocadin RG, Zimmerman JL, Donnino M, et al. Part 9: post-cardiac arrest care: 2010 American Heart Association Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. Circulation. 2010;122(18 Suppl 3):S768-86.

81. Bjelland TW, Hjertner Ø, Klepstad P, Kaisen K, Dale O, Haugen BO. Antiplatelet effect of clopidogrel is reduced in patients treated with therapeutic hypothermia after cardiac arrest. Resuscitation. 2010;81:1627-31.

82. Xavier RG, White AE, Fox SC, Wilcox RG, Heptinstall S. Enhanced platelet aggregation and activation under conditions of hypothermia. Thromb Haemost. 2007;98:1266-75.

83. Penela D, Magaldi M, Fontanals J, Martin V, Regueiro A, Ortiz JT, et al. Hypothermia in acute coronary syndrome: brain salvage versus stent thrombosis? J Am Coll Cardiol. 2013;61:686-7.

84. Montalescot G, Sideris G, Meuleman C, Bal-dit-Sollier C, Lellouche N, Steg PG, et al.; ALBION Trial Investigators. A randomized comparison of high clopidogrel loading doses in patients with non-ST-segment elevation acute coronary syndromes: the ALBION (Assessment of the Best Loading Dose of Clopidogrel to Blunt Platelet Activation, Inflammation and Ongoing Necrosis) trial. J Am Coll Cardiol. 2006;48:931-8.

85. von Beckerath N, Kastrati A, Wieczorek A, Pogatsa-Murray G, Sibbing D, Graf I, et al. A double-blind, randomized study on platelet aggregation in patients treated with a daily dose of 150 or 75 mg of clopidogrel for 30 days. Eur Heart J. 2007;28:1814-9.

86. Angiolillo DJ, Capranzano P, Goto S, Aslam M, Desai B, Charlton RK, et al. A randomized study assessing the impact of cilostazol on platelet function profiles in patients with diabetes mellitus and coronary artery disease on dual antiplatelet therapy: results of the OPTIMUS-2 study. Eur Heart J. 2008;29:2202-11.

87. CURRENT-OASIS 7 Investigators, Mehta SR, Bassand JP, Chrolavicius S, Diaz R, Eikelboom JW, Fox KA, et al. Dose comparisons of clopidogrel and aspirin in acute coronary syndromes. N Engl J Med. 2010;363:930-42.

88. European Medicines Agency [Internet]. European Medicines Agency recommends restricting use of cilostazol-containing medicines [cited 2013 March 22]. Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2013/03/news_detail_001746.jsp&mid=WC0b01ac058004d5c1

89. Wiviott SD, Braunwald E, McCabe CH, Montalescot G, Ruzyllo W, Gottlieb S, et al.; TRITON-TIMI 38 Investigators. Prasugrel versus clopidogrel in patients with acute coronary syndromes. N Engl J Med. 2007;357:2001-15.

90. Wallentin L, Becker RC, Budaj A, Cannon CP, Emanuelsson H, Held C, et al. for the PLATO Investigators. Ticagrelor versus Clopidogrel in Patients with Acute Coronary Syndromes. N Engl J Med. 2009; 361:1-13

91. Wiviott SD, Trenk D, Frelinger AL, O'Donoghue M, Neumann FJ, Michelson AD, et al.; PRINCIPLE-TIMI 44 Investigators. Prasugrel compared with high loading- and maintenance-dose clopidogrel in patients with planned percutaneous coronary intervention: the Prasugrel in Comparison to Clopidogrel for Inhibition of Platelet Activation and Aggregation-Thrombolysis in Myocardial Infarction 44 trial. Circulation 2007;116:2923-32.

92. Montalescot G, Wiviott SD, Braunwald E, Murphy SA, Gibson CM, McCabe CH, et al.; TRITON-TIMI 38 investigators. Prasugrel compared with clopidogrel in patients undergoing percutaneous coronary intervention for ST-elevation myocardial infarction (TRITON-TIMI 38): double-blind, randomised controlled trial. Lancet. 2009;373:723-31.

93. Wiviott SD, Braunwald E, Angiolillo DJ, Meisel S, Dalby AJ, Verheugt FW, et al.; TRITON-TIMI 38 Investigators. Greater clinical benefit of more intensive oral antiplatelet therapy with prasugrel in patients with diabetes mellitus in the trial to assess improvement in therapeutic outcomes by optimizing platelet inhibition with prasugrel-Thrombolysis in Myocardial Infarction 38. Circulation. 2008;118:1626-36.

94. Gurbel PA, Bliden KP, Butler K, Tantry US, Gesheff T, Wei C, et al. Randomized double-blind assessment of the ONSET and OFFSET of the antiplatelet effects of ticagrelor versus clopidogrel in patients with stable coronary artery disease: the ONSET/OFFSET study. Circulation. 2009;120:2577-85.

95. Storey RF, Husted S, Harrington RA, Heptinstall S, Wilcox RG, Peters G, et al. Inhibition of platelet aggregation by AZD6140, a reversible oral P2Y12 receptor antagonist, compared with clopidogrel in patients with acute coronary syndromes. J Am Coll Cardiol. 2007;50:1852-6.

96. Cannon CP, Harrington RA, James S, Ardissino D, Becker RC, Emanuelsson H, et al.; PLATelet inhibition and patient Outcomes Investigators.Comparison of ticagrelor with clopidogrel in patients with a planned invasive strategy for acute coronary syndromes (PLATO): a randomised double-blind study. Lancet. 2010;375:283-93.

97. James SK, Roe MT, Cannon CP, Cornel JH, Horrow J, Husted S, et al.; PLATO Study Group. Ticagrelor versus clopidogrel in patients with acute coronary syndromes intended for non-invasive management: substudy from prospective randomised PLATelet inhibition and patient Outcomes (PLATO) trial. BMJ. 2011;342:d3527

98. James S, Budaj A, Aylward P, Buck KK, Cannon CP, Cornel JH, Harrington RA, Horrow J, Katus H, Keltai M, Lewis BS, Parikh K, Storey RF, Szummer K, Wojdyla D, Wallentin L. Ticagrelor versus clopidogrel in acute coronary syndromes in relation to renal function: results from the Platelet Inhibition and Patient Outcomes (PLATO) trial. Circulation. 2010;122:1056-67.

99. Ferreiro JL, Ueno M, Angiolillo DJ. Cangrelor: a review on its mechanism of action and clinical development. Expert Rev Cardiovasc Ther. 2009;7:1195-201.

100. Harrington RA, Stone GW, McNulty S, White HD, Lincoff AM, Gibson CM, et al. Platelet Inhibition with Cangrelor in Patients Undergoing PCI. N Engl J Med. 2009;361:2318-29

101. Bhatt DL, Lincoff AM, Gibson CM, Stone GW, McNulty S, Montalescot G, et al.; CHAMPION PLATFORM Investigators. Intravenous Platelet Blockade with Cangrelor during PCI. N Engl J Med. 2009;361:2330-41.

102. White HD, Chew DP, Dauerman HL, Mahaffey KW, Gibson CM, Stone GW, et al. Reduced immediate ischemic events with cangrelor in PCI: A pooled analysis of the CHAMPION trials using the universal definition of myocardial infarction. Am Heart J. 2012;163:182-90.e4.

103. Bhatt DL, Stone GW, Mahaffey KW, Gibson CM, Steg PG, Hamm CW, et al.; CHAMPION PHOENIX Investigators. Effect of platelet inhibition with cangrelor during PCI on ischemic events. N Engl J Med. 2013;368:1303-13.

104. European Medicines Agency [Internet]. European public assessment report (EPAR) for Kengrexal [cited 2015 June 12]. Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/003773/human_med_001852.jsp&mid=WC0b01ac058001d124.

105. Bueno H, Sinnaeve P, Annemans L, Danchin N, Licour M, Medina J, et al.; on behalf of the EPICOR investigators. Opportunities for improvement in anti-thrombotic

therapy and other strategies for the management of acute coronary syndromes: Insights from EPICOR, an international study of current practice patterns. Eur Heart J Acute Cardiovasc Care. 2015 Jan 5. pii: 2048872614565912. [Epub ahead of print]

106. Gilard M, Arnaud B, Le Gal G, Abgrall JF, Boschat J. Influence of omeprazol on the antiplatelet action of clopidogrel associated to aspirin. J Thromb Haemost. 2006;4:2508-9.

107. Angiolillo DJ, Gibson CM, Cheng S, Ollier C, Nicolas O, Bergougnan L, et al. Differential effects of omeprazole and pantoprazole on the pharmacodynamics and pharmacokinetics of clopidogrel in healthy subjects: randomized, placebo-controlled, crossover comparison studies. Clin Pharmacol Ther. 2011;89:65-74.

108. Bates ER, Lau WC, Angiolillo DJ. Clopidogrel-drug interactions. J Am Coll Cardiol. 2011;57:1251-63.

109. Kreutz RP, Stanek EJ, Aubert R, Yao J, Breall JA, Desta Z, et al. Impact of proton pump inhibitors on the effectiveness of clopidogrel after coronary stent placement: the clopidogrel Medco outcomes study. Pharmacotherapy. 2010;30:787-96.

110. Stockl KM, Le L, Zakharyan A, Harada AS, Solow BK, Addiego JE, et al. Risk of rehospitalization for patients using clopidogrel with a proton pump inhibitor. Arch Intern Med. 2010;170:704-10.

111. Gaglia MA Jr, Torguson R, Hanna N, Gonzalez MA, Collins SD, Syed AI, et al. Relation of proton pump inhibitor use after percutaneous coronary intervention with drug-eluting stents to outcomes. Am J Cardiol. 2010;105:833-8.

112. O'Donoghue ML, Braunwald E, Antman EM, Murphy SA, Bates ER, Rozenman Y, et al. Pharmacodynamic effect and clinical efficacy of clopidogrel and prasugrel with or without a proton-pump inhibitor: an analysis of two randomised trials. Lancet. 2009;374:989-97.

113. Rassen JA, Choudhry NK, Avorn J, Schneeweiss S. Cardiovascular outcomes and mortality in patients using clopidogrel with proton pump inhibitors after percutaneous coronary intervention or acute coronary syndrome. Circulation. 2009;120:2322-9.

114. Ray WA, Murray KT, Griffin MR, Chung CP, Smalley WE, Hall K, et al. Outcomes with concurrent use of clopidogrel and proton-pump inhibitors: a cohort study. Ann Intern Med. 2010;152:337-45.

115. Bhatt DL, Cryer BL, Contant CF, Cohen M, Lanas A, Schnitzer TJ, et al.; COGENT Investigators. Clopidogrel with or without omeprazole in coronary artery disease. N Engl J Med. 2010;363:1909-17.

116. Hulot JS, Collet JP, Silvain J, Pena A, Bellemain-Appaix A, Barthélémy O, et al. Cardiovascular risk in clopidogrel-treated patients according to cytochrome P450 2C19*2 loss-of-function allele or proton pump inhibitor coadministration: a systematic meta-analysis. J Am Coll Cardiol. 2010;56:134-43.

117. Gremmel T, Steiner S, Seidinger D, Koppensteiner R, Panzer S, Kopp CW. Smoking promotes clopidogrel-mediated platelet inhibition in patients receiving dual antiplatelet therapy. Thromb Res 2009;124:588-91.

118. Jeong YH, Cho JH, Kang MK, Koh JS, Kim IS, Park Y, et al. Smoking at least 10 cigarettes per day increases platelet inhibition by clopidogrel in patients with ST-segment-elevation myocardial infarction. Thromb Res. 2010;126:e334-8.

119. Gagne JJ, Bykov K, Choudhry NK, Toomey TJ, Connolly JG, Avorn J. Effect of smoking on comparative efficacy of antiplatelet agents: systematic review, meta-analysis, and indirect comparison. BMJ. 2013;347:f5307.

120. Erlinge D, Varenhorst C, Braun OO, James S, Winters KJ, Jakubowski JA, et al. Patients with poor responsiveness to thienopyridine treatment or with diabetes have lower levels of circulating active metabolite, but their platelets respond normally to active metabolite added ex vivo. J Am Coll Cardiol. 2008;52:1968-77.

121. Park KW, Park JJ, Jeon KH, Kang SH, Oh IY, Yang HM, et al. Enhanced clopidogrel responsiveness in smokers: smokers' paradox is dependent on cytochrome P450 CYP1A2 status. Arterioscler Thromb Vasc Biol. 2011;31:665-71.

122. Ferreiro JL, Bhatt DL, Ueno M, Bauer D, Angiolillo DJ. Impact of smoking on long-term outcomes in patients with atherosclerotic vascular disease treated with aspirin or clopidogrel: insights from the CAPRIE trial (Clopidogrel Versus Aspirin in Patients at Risk of Ischemic Events). J Am Coll Cardiol. 2014;63:769-77.

123. Matetzky S, Shenkman B, Guetta V, Shechter M, Beinart R, Goldenberg I, et al. Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. Circulation. 2004;109:3171-5.

124. Heestermans AA, van Werkum JW, Taubert D, Seesing TH, von Beckerath N, Hackeng CM, et al. Impaired bioavailability of clopidogrel in patients with a ST-segment elevation myocardial infarction. Thromb Res. 2008;122:776-81

125. Bellemain-Appaix A, O'Connor SA, Silvain J, Cucherat M, Beygui F, Barthélémy O, et al.; ACTION Group. Association of clopidogrel pretreatment with mortality, cardiovascular events, and major bleeding among patients undergoing percutaneous coronary intervention: a systematic review and meta-analysis. JAMA. 2012;308:2507-16.

126. Biscaglia S, Tebaldi M, Vranckx P, Campo G, Valgimigli M. Effects of pre-hospital clopidogrel administration on early and late residual platelet reactivity in ST-segment elevation myocardial infarction patients undergoing primary intervention. J Thromb Haemost. 2013;11:192-4.

127. Bonello L, Berbis J, Laine M, Armero S, Bessereau J, Jacquin L, et al. Biological efficacy of a 600 mg loading dose of clopidogrel in ST-elevation myocardial infarction. Thromb Haemost. 2012;108:101-6.

128. Vlaar PJ, Svilaas T, Damman K, de Smet BJ, Tijssen JG, Hillege HL, et al. Impact of pretreatment with clopidogrel on initial patency and outcome in patients treated with primary percutaneous coronary intervention for ST-segment elevation myocardial infarction: a systematic review. Circulation. 2008;118:1828-36.

129. Steg PG, James S, Harrington RA, Ardissino D, Becker RC, Cannon CP, et al.; PLATO Study Group. Ticagrelor versus clopidogrel in patients with ST-elevation acute coronary syndromes intended for reperfusion with primary percutaneous coronary intervention: A Platelet Inhibition and Patient Outcomes (PLATO) trial subgroup analysis. Circulation. 2010;122:2131-41.

130. Joffre J, Varenne O, Bougouin W, Rosencher J, Mira JP, Cariou A. Stent thrombosis: an increased adverse event after angioplasty following resuscitated cardiac arrest. Resuscitation. 2014;85:769-73.

131. Chisholm GE, Grejs A, Thim T, Christiansen EH, Kaltoft A, Lassen JF, et al. Safety of therapeutic hypothermia combined with primary percutaneous coronary intervention after out-of-hospital cardiac arrest. Eur Heart J Acute Cardiovasc Care. 2015;4:60-3.

132. Högberg C, Erlinge D, Braun OO. Mild hypothermia does not attenuate platelet aggregation and may even increase ADP-stimulated platelet aggregation after clopidogrel treatment. Thromb J. 2009;7:2.

133. Berlin E, Shapiro SG, Friedland M. Platelet membrane fluidity and aggregation of rabbit platelets. Atherosclerosis. 1984;51:223-39.

134. Straub A, Krajewski S, Hohmann JD, Westein E, Jia F, Bassler N, et al. Evidence of platelet activation at medically used hypothermia and mechanistic data indicating ADP as a key mediator and therapeutic target. Arterioscler Thromb Vasc Biol. 2011;31:1607-16.

135. James S, Angiolillo DJ, Cornel JH, Erlinge D, Husted S, Kontny F, et al.; PLATO Study Group. Ticagrelor vs. clopidogrel in patients with acute coronary syndromes and

diabetes: a substudy from the PLATelet inhibition and patient Outcomes (PLATO) trial. Eur Heart J. 2010;31:3006-16.

136. Gurbel PA, Bliden KP, Guyer K, Aggarwal N, Tantry US. Delayed thrombin-induced platelet-fibrin clot generation by clopidogrel: a new dose-related effect demonstrated by thrombelastography in patients undergoing coronary artery stenting. Thromb Res. 2007;119:563-70.

137. Angiolillo DJ, Capranzano P, Desai B, Shoemaker SB, Charlton R, Zenni MM, et al. Impact of P2Y(12) inhibitory effects induced by clopidogrel on platelet procoagulant activity in type 2 diabetes mellitus patients. Thromb Res. 2009;124:318-22.

138. Leon C, Ravanat C, Freund M, Cazenave JP, Gachet C. Differential involvement of the P2Y1 and P2Y12 receptors in platelet procoagulant activity. Arterioscler Thromb Vasc Biol. 2003;23:1941-7.

139. Srinivasan S, Mir F, Huang JS, Khasawneh FT, Lam SC, Le Breton GC. The P2Y12 antagonists, 2-methylthioadenosine 5'-monophosphate triethylammonium salt and cangrelor (ARC69931MX), can inhibit human platelet aggregation through a Gi-independent increase in cAMP levels. J Biol Chem. 2009;284:16108-17.

140. Angiolillo DJ, Capranzano P, Ferreiro JL, Ueno M, Capodanno D, Dharmashankar K, et al. Impact of adjunctive cilostazol therapy on platelet function profiles in patients with and without diabetes mellitus on aspirin and clopidogrel therapy. Thromb Haemost. 2011;106:253-62.

141. Kirkby NS, Leadbeater PD, Chan MV, Nylander S, Mitchell JA, Warner TD. Antiplatelet effects of aspirin vary with level of $P2Y_{12}$ receptor blockade supplied by either ticagrelor or prasugrel. J Thromb Haemost. 2011;9:2103-5.

142. Armstrong PC, Leadbeater PD, Chan MV, Kirkby NS, Jakubowski JA, Mitchell JA, et al. In the presence of strong P2Y12 receptor blockade, aspirin provides little additional inhibition of platelet aggregation. J Thromb Haemost. 2011;9:552-61.