



Reversal of Rivaroxaban-Induced Alterations on Hemostasis by Different Coagulation Factor Concentrates

– In Vitro Studies With Steady and Circulating Human Blood –

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Background: Despite the good safety of rivaroxaban, there is limited information on strategies for urgent reversal of its antihemostatic effects.

Methods and Results: Alterations of hemostasis induced by rivaroxaban (230 ng/ml) were assessed by using several tests applied to steady and circulating human blood. Effects on thrombin generation (TG) and thromboelastometry (TEM) parameters were measured. Modifications in platelet adhesive, aggregating and procoagulant activities were evaluated in studies with circulating blood. The potential reversal of prothrombin complex concentrates (PCCs; 50 IU/kg), activated PCCs (aPCCs; 75 IU/kg), or recombinant factor VIIa (rFVIIa; 270 µg/kg) was evaluated. Impairment of TG parameters induced by rivaroxaban were corrected by the different concentrates (aPCC≥PCC>rFVIIa). Prolonged clotting times and reduced clot firmness caused by rivaroxaban on TEM tests were improved by different concentrates (rFVIIa≥aPCC>PCC). Rivaroxaban significantly reduced platelets and fibrin interactions with damaged vascular surfaces in perfusion studies. While alterations of platelet interactions were favourably counteracted by rFVIIa or aPCCs, reductions in fibrin formation were only partially restored by the different factor concentrates (rFVIIa>aPCC≥PCC).

Conclusions: Rivaroxaban-induced alterations on coagulation parameters measured through assays performed under static conditions were easily reversed by the different concentrates. Studies under flow conditions revealed that these concentrates normalized the action of rivaroxaban on platelets, and significantly improved fibrin formation; although in the later case, levels were not restored to the pre-treatment value. (*Circ J* 2015; **79**: 331–338)

Key Words: Activated prothrombin complex concentrates; Prothrombin complex concentrates; Recombinant factor VIIa; Rivaroxaban

Anticoagulation is essential for the prevention and treatment of different conditions, such as atrial fibrillation, deep vein thrombosis or pulmonary embolism. Rivaroxaban is a new oral anticoagulant with a selective inhibitory action on factor Xa.¹ Different clinical trials have investigated the efficacy and safety of rivaroxaban compared with standard therapy in different clinical settings, and it is currently indicated for the prevention of stroke and systemic embolism in patients with non-valvular atrial fibrillation, prevention of venous thromboembolism in adult patients under-

going elective hip or knee replacement surgery, treatment of deep vein thrombosis and pulmonary embolism and the prevention of recurrent deep vein thrombosis and pulmonary embolism in adults.^{2–10} Despite the good safety profile of rivaroxaban shown in clinical trials,^{9,11,12} no definite information is available about possible strategies for the reversal of its antihemostatic effects in patients presenting with medical or surgical emergencies.^{13–16} The summary of the product characteristics for rivaroxaban recommends that in cases in which bleeding cannot be controlled, the use of PCC, aPCC or

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rFVIIa should be considered,¹⁷ although no clinical evidence is available to support such a recommendation.

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Investigations of the subtle mechanisms involved in the antithrombotic action of rivaroxaban preventing formation of occlusive thrombi at the level of the damaged vasculature and reversal of its anticoagulant action are difficult to perform in patients that are receiving treatment while in clinical trials. The direct inhibitory action of rivaroxaban facilitates the development of experimental approaches *in vitro* to generate evidence on detailed mechanisms of action and possible reversal.¹⁸ In the present study, we evaluated the effects of the average Cmax concentration (230 ng/ml) achieved with a standard rivaroxaban dose of 20 mg/day, on platelet- and coagulation-mediated mechanisms of hemostasis. We applied a series of laboratory tests performed on steady blood or plasma samples, and performed additional studies with circulating human blood. After establishing the effects of rivaroxaban in the different biomarkers of coagulation, we explored the effects of different coagulation factor concentrates, including prothrombin complex concentrates (PCCs), activated PCCs (aPCCs) or recombinant factor VIIa (rFVIIa), to reverse the alterations of hemostasis previously induced by rivaroxaban.

Methods

Ethics Statement

Our investigations were performed in accordance with the Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes, and the principles outlined in the Declaration of Helsinki. The study was approved by the Hospital Clinic Ethical Committee of Clinical Investigation. The protocol to isolate rabbit aortas to be used as thrombogenic substrata was also approved by the Animal Ethical Committee of the University of Barcelona.

Study Material

The study group consisted of 8 healthy volunteers who agreed to donate blood samples after written informed consent was obtained. Individuals who had received acetylsalicylic acid, non-steroidal anti-inflammatory or antiplatelet drugs within 7 days before blood sampling were excluded. Blood samples were collected into tubes (BD Vacutainer, Franklin Lakes, NJ, USA) containing citrate (final concentration of 13 mmol/L). Rivaroxaban was kindly provided by Bayer HealthCare.

Experimental Design

Rivaroxaban was initially dissolved in ethanol, and subsequently diluted in saline. Aliquots of rivaroxaban dilutions were added to blood samples to achieve a Cmax-equivalent plasma concentration of rivaroxaban after a steady 20 mg/day dose (230 ng/ml).¹⁹

Whole blood samples spiked with rivaroxaban were used to evaluate modifications in: (1) viscoelastic parameters of clot formation in whole blood using thromboelastometry (ROTEM, TEM International GmbH, Munchen, Germany); (2) dynamics of thrombin generation (TG) in plasma using the fluorogenic assay, Technothrombin TGA (Technoclone GmbH, Wien, Austria); and (3) perfusion studies with whole blood circulated through damaged vascular segments at a shear rate of 600/s, equivalent to that found in medium-sized cerebral arteries.

Commercially available coagulation factor concentrates were

tested for determining their ability to reverse the antihemostatic actions of rivaroxaban at doses approved in the prescribing information for each concentrate: rFVIIa: Novoseven® 270 µg/kg (NovoNordisk, Bagsvaerd, Denmark); aPCC: Feiba® 75 U/kg (Baxter); and PCC: Beriplex® 50 IU/kg (CSL Behring GmbH, Marburg, Germany). Doses of these concentrates added to blood were calculated assuming a blood volume of 4,900 ml in an adult weighing 70 kg. Aliquots of the different concentrates were spiked in the blood samples to evaluate their potential corrective effect on the different laboratory tests.

TG Assay (TGA)

TG was evaluated in citrated platelet-poor plasma (PPP) samples. TG on citrated PPP was assessed with the fluorogenic assay, Technothrombin TGA™, by following the manufacturer's instructions (Technoclone GmbH, Austria).^{20,21} The activation of the coagulation cascade was triggered by 2 different commercial reagents, Technothrombin® RC Low (RCL) containing a low concentration micelles of negatively charged phospholipids and recombinant human tissue factor, and Technothrombin® RD (RD) containing negatively charged phospholipids.

Thromboelastometry Studies

The dynamic thrombelastography of whole blood coagulation, using the ROTEM Analyser (PentapharmGmbH, Munchen, Germany) was investigated.²² For simplicity, the study focused on the analysis of the exTEM test (Rotem Thromboelastometry, Biometra, Spain) in TEM studies with citrated blood, recalcified with 6 mmol/L CaCl₂. Three of them were assessed for the purpose of our studies: clotting time (CT), the time (s) elapsed from the measurement start until the amplitude of the forming clot reaches 2 mm; clot formation time (CFT), the time (s) from the start of clot formation until the tracing reaches 20 mm of amplitude; and maximum clot firmness (MCF), the maximum amplitude of the tracing reached (in mm).^{23,24} CT and CFT were indicators of the dynamics of clot formation. The MCF or clot amplitude gave information about clot strength and stability. ROTEM analyses were performed for a minimum of 45 min.

Perfusion Studies

Aliquots of blood were perfused through annular chambers exposing damaged vascular segments, as thrombogenic substrata. Aortas were extracted from young female New Zealand rabbits (2.8–3.0 kg) previously euthanized according to protocols approved by the Animal Ethical Committee of the University of Barcelona (number DAAM: 6632). Vessels were cleaned, everted, cut into segments and maintained in PBS.²⁵ Perfusion studies were performed at a shear rate of 600/s for 10 min. Before it entered the flow chamber, citrate-anticoagulated blood was mixed with 6 mmol/L CaCl₂. Perfused vessels were rinsed with PBS (0.15 mol/L), fixed with 2.5% glutaraldehyde (in 0.15 mol/L PBS) at 4°C for 24 h and processed histologically for further morphometric evaluation. Fibrin deposition and platelet interactions were evaluated as previously described.²¹

Statistical Analysis

Data were expressed as mean±standard error of the mean (SEM). The SPSS statistical package 17.0.0 (SPSS Inc, Chicago, IL, USA) was used for all analyses. Statistical analysis was performed with raw data using ANOVA. Comparative statistics were performed with respect to values in control studies

Table 1. Effects of Rivaroxaban in Thrombin Generation Kinetics in Plasma Samples Triggered With Different *Technothrombin®* Reagents

	Lag phase (min)	Thrombin peak (nmol/L)	Time peak (min)
A) Phospholipids micelles and tissue factor (<i>Technothrombin®</i> RCL)			
Control	19.5±1.6	159.0±26.0	34.6±2.8
RIV	47.0±3.3**	30.5±6.5**	80.2±3.1**
RIV+rFVIIa	17.1±1.3††	103.1±13.4††	41.6±4.7††
RIV+aPCC	14.7±1.3*††	138.0±20.5††	39.5±4.9††
RIV+PCC	34.3±9.6	73.9±24.5*	74.0±6.4*
B) Phospholipids micelles (<i>Technothrombin®</i> RD)			
Control	4.0±0.5	510.6±19.2	7.8±0.5
RIV	4.1±0.5	468.5±18.4*	11.3±0.9**
RIV+rFVIIa	3.7±0.3	445.9±15.7**	8.8±0.6*††
RIV+aPCC	5.2±0.5*,†	931.6±33.2*,††	11.3±1.7*
RIV+PCC	4.6±0.5	847.7±93.3*,††	13.3±2.1*

RIV, Rivaroxaban 230 ng/ml; recombinant factor VIIa (rFVIIa), Novoseven® 270 µg/kg; activated prothrombin complex concentrates (aPCC), Feiba® 75 U/kg; prothrombin complex concentrates (PCC), Beriplex® 50IU/kg. *P<0.05 or **P<0.01 vs. Control; †P<0.05 or ††P<0.01 vs. RIV (mean±SEM, n=8).

(samples not exposed to rivaroxaban) and blood samples exposed to rivaroxaban. Initial levels of statistical significance were established at P<0.05.

Results

Influence of Rivaroxaban on TG

Rivaroxaban at 230 ng/ml caused a marked inhibition in the parameters that define the kinetics of TG when the activation of coagulation was triggered by phospholipid micelles and rTF (RCL reagent). As summarized in **Table 1A** and **Figure 1A**, a significant delay in the average time to reach the maximum thrombin peak, with a concomitant reduction in the maximum peak of TG, was observed in rivaroxaban-treated samples (P<0.01 vs. control values, in both cases). Effects of rivaroxaban on TG were less evident when the activations were triggered by phospholipid micelles alone (RD reagent). As shown in **Table 1B** and **Figure 1B**, the maximum thrombin peak and the time to peak were still significantly altered (P<0.05 and P<0.01, respectively, vs. control values).

The addition of the different coagulation factor concentrates improved the alterations in TG induced by rivaroxaban, but with different intensity depending on the activating agent. Thus, when the activation of the coagulation cascade was triggered by phospholipids and tissue factor (RCL reagent), thrombin peak and time to peak were improved by concentrates with the following order of efficacy aPCC≥rFVIIa>PCC (**Figure 1A**). Both PCC and aPCC were more efficient at restoring thrombin peak altered after rivaroxaban when only phospholipid micelles (RD reagent) were used as activators (**Figure 1B**). Thrombin peaks achieved with aPCC and PCCs significantly exceeded (P<0.05) the baseline values observed in control blood samples not exposed to rivaroxaban. Recombinant factor VIIa did not affect the thrombin peak, but significantly shortened the time to reach the thrombin peak (P<0.01 vs. rivaroxaban) in this experimental setting. **Table 1** shows detailed statistics.

Modifications by Rivaroxaban in Viscoelastic Properties of Clots

Rivaroxaban (230 ng/ml) prolonged parameters related to clot formation (CT and CFT) and reduced MCF in TEM studies

using tissue factor as the activator (P<0.05 vs. control studies). Alterations in these parameters were improved by the different factor concentrates according to the following order of efficacy rFVIIa≥aPCC>PCC (**Table 2**). Both PCC and aPCC proved especially effective at shortening the prolongation of CT and CFT observed after treatment with rivaroxaban, reaching values below those observed in baseline blood samples.

Perfusion Studies

Rivaroxaban quantitatively reduced platelet and fibrin interactions with damaged vascular surfaces in perfusion studies. The inhibitory action of rivaroxaban on fibrin deposition was more intense and evident. Representative micrographs in **Figure 2** illustrate these modifications. Data from the morphometric analysis presented in **Figure 3A** revealed that the total surface covered by platelets was reduced in perfusion studies with blood samples exposed to rivaroxaban (9.1±1.5 vs. 16.4±1.7% in controls; P<0.01). Moreover, treatment with rivaroxaban caused a marked statistically significant reduction in the percentage of the vessel covered by fibrin (15.6±5.3 vs. 60.3±10.4% in controls; P<0.01), with a concomitant decrease in the average size of the fibrin masses (62.5±23.2 vs. 390.6±144.7 µm² in controls; P<0.05) deposited on the vessel surface (**Figure 3B**).

Alterations induced by rivaroxaban on platelets and fibrin components of the hemostasis in studies with flowing blood were partially counteracted by the different factor concentrates, showing a more pronounced correction of platelet interactions with rFVIIa and aPCCs (P<0.05 vs. rivaroxaban alone). Fibrin formation was also variably compensated for by the factor concentrates with efficacies following this order rFVIIa>aPCC≥PCC (**Figures 2,3A**). Despite the significant improvement in fibrin coverage observed with the different concentrates, a detailed analysis of the modifications in the size of the fibrin masses formed on the exposed subendothelium is required because values never returned to the original levels in non-treated blood (**Figure 3B**).

Discussion

In the present studies, rivaroxaban at 230 ng/ml inhibited TG, altered viscoelastic parameters during clot formation, and re-

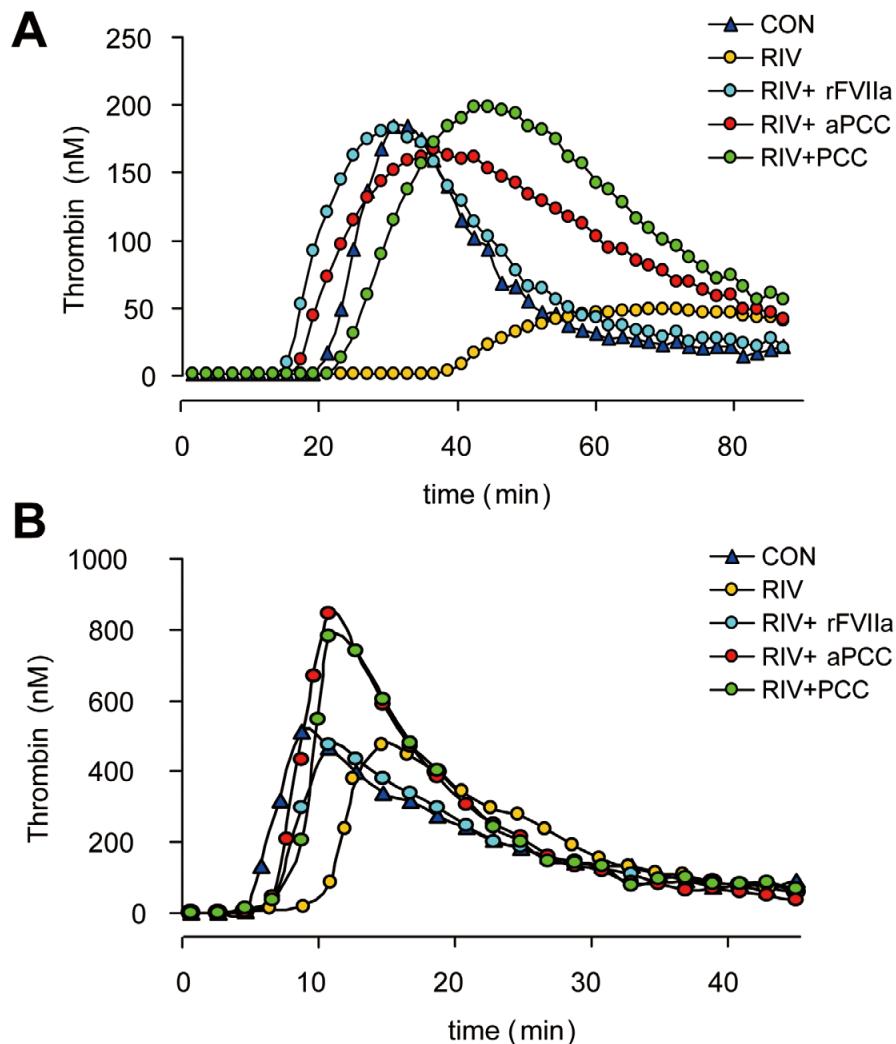


Figure 1. Thrombin generation kinetics in recalcified plasma activated with phospholipids and tissue factor or phospholipid micelles. Representative thrombograms showing the kinetics of thrombin generation (TG) in experiments performed triggering thrombin generation in recalcified plasma with (A) phospholipids and tissue factor (RCL reagent) or (B) phospholipid micelles (RD reagent). Rivaroxaban (230 ng/ml) delayed and reduced thrombin generation kinetics in plasma. Effects of rivaroxaban on thrombin generation were very evident when activation was initiated by tissue factor (A) and less marked when activation was initiated by phospholipids (B). All the coagulation factor concentrates tested improved the alterations in TG induced by rivaroxaban, with activated prothrombin complex concentrates (aPCCs) being more efficacious than recombinant factor VIIa (rFVIIa) or prothrombin complex concentrates (PCCs) at restoring TG altered by rivaroxaban when TG was initiated by tissue factor (A), and PCCs and aPCCs dramatically enhancing TG peaks above the levels observed in baseline control experiments when activation was initiated by phospholipids (B). See Table 1 for more detailed statistics. CON, Control; RIV, Rivaroxaban 230 ng/ml; rFVIIa, Novoseven® 270 µg/kg; aPCC, Feiba® 75 U/kg; PCC, Beriplex® 50IU/kg.

duced platelet and fibrin deposition on damaged vessels in studies with circulating blood. Strategies with the different coagulation factor concentrates variably compensated or even reversed the alterations in coagulation induced by rivaroxaban, although responses to these concentrates were not homogeneous in all tests. In fact, PCCs and aPCCs were more efficient in TG, whereas rFVIIa seemed more effective in ROTEM and perfusion studies. These data suggest that any of these concentrates might be useful to reverse or at least improve the alterations in coagulation induced by rivaroxaban.

Despite the demonstrated efficacy of rivaroxaban and its

good safety profile in multiple clinical trials, treatment with rivaroxaban is associated with an increased risk of bleeding. Although rivaroxaban has a short half-life (5–13 h), and a simple discontinuation of the treatment might be enough in the majority of cases to control mild to moderate bleeding, it is felt that immediate reversal of the anticoagulant effect of rivaroxaban might be necessary in case of emergencies.²⁶ Several studies performed in animals and also in humans have investigated whether different coagulation factor concentrates, particularly PCC, aPCC and rFVIIa, used to control bleeding in severe hemostatic disorders or reverse the effects of classic

Table 2. Effects of Rivaroxaban and Reversing Strategies in Thromboelastometry Parameters During Clot Formation in Recalcified Citrated Blood

EXTEM reagent	CT (s)	CFT (s)	MCF (mm)
Control	196.8±27.3	292.3±54.2	54.0±1.9
RIV	711.2±282.3*	885.8±271.9*	33.4±9.3*,†
RIV+rFVIIa	117.5±11.2*,†	148.5±20.2*,†	60.5±2.3*,†
RIV+aPCC	156.8±13.3	141.1±16.2*,†	61.7±1.1*,†
RIV+PCC	355.8±83.9	486.2±144.4	57.8±2.6

CT, clotting time; CFT, clot formation time; MCF, maximum clot firmness; RIV, Rivaroxaban 230 ng/ml; recombinant factor VIIa (rFVIIa), Novoseven® 270 µg/kg; activated prothrombin complex concentrates (aPCC), Feiba® 75 U/kg; prothrombin complex concentrates (PCC), Beriplex® 50 IU/kg. *P<0.05 or **P<0.01 vs. Control; †P<0.05 or ††P<0.01 vs. RIV (mean±SEM, n=8).

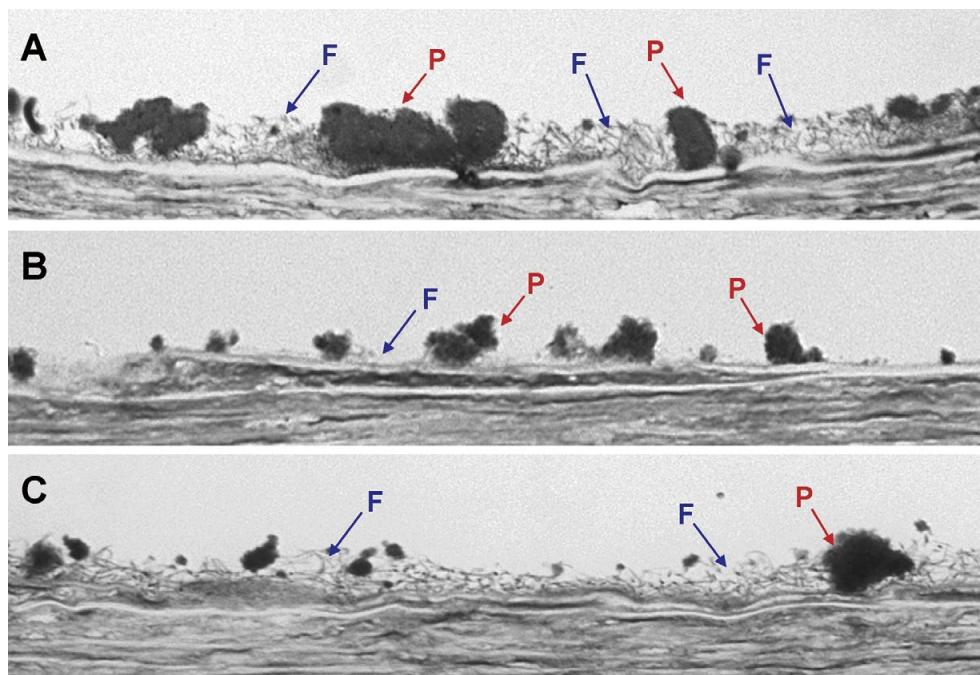


Figure 2. Effect of rivaroxaban on platelet and fibrin interactions with damaged vessels exposed to flowing blood. Light microscopy images showing platelet and fibrin interactions on cross-sections of the perfused vessels. Perfusion studies with recalcified blood were performed at a shear rate of 600/s with flowing blood for 10 min. Representative micrographs of (A) control studies and (B) blood incubated with rivaroxaban 230 ng/ml. (B) Rivaroxaban caused a reduction in fibrin formation and platelet interactions with the damaged vessels. The size of platelet aggregates was apparently decreased in samples exposed to rivaroxaban. (C) Recombinant factor VIIa (rFVIIa), activated and non-activated prothrombin complex concentrates (aPCCs and PCCs, respectively), and partially restored levels of fibrin deposited on the subendothelium previously reduced by rivaroxaban treatment. Both rFVIIa and aPCC were capable of improving levels of platelet interactions with the damaged vessel. P, platelet aggregates (in red); F, fibrin (in blue). Magnification for cross-sections micrographs was $\times 1,000$.

vitamin K antagonists, could be applied to counteract the antihemostatic effects of rivaroxaban. In a recent study performed in rats and baboons, the administration of PCC, aPCC or rFVIIa had the potential to reverse the anticoagulant and anti-hemostatic effects of rivaroxaban.^{27,28} In a randomized study in humans, 12 healthy volunteers received rivaroxaban 20 mg twice daily or dabigatran 150 mg twice daily for 2.5 days, followed by either a single bolus of 50 IU/kg PCC or a similar volume of saline.²⁹ After a washout period, volunteers received the other anticoagulant treatment. As expected from its direct anti-Xa action, rivaroxaban significantly prolonged the pro-

thrombin time and inhibited TG.^{27,30} Both alterations were normalized by PCCs. By contrast, dabigatran increased the activated partial thromboplastin time, ecarin CT, and thrombin time, but the administration of PCC did not have a significant impact on these coagulation tests. In another randomized cross-over ex vivo study performed in 10 healthy white male volunteers, subjects were randomized to receive rivaroxaban (20 mg) or dabigatran (150 mg) in a single oral administration. Reversal of anticoagulation was tested in *in vitro* analyzing TG parameters using PCC, rFVIIa or aPCC at various concentrations. Rivaroxaban altered quantitative and kinetic param-

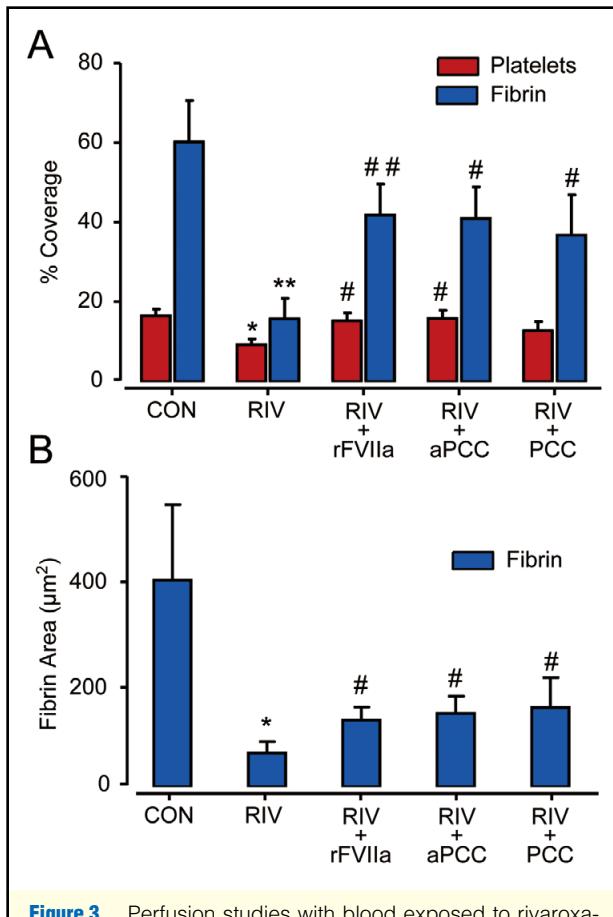


Figure 3. Perfusion studies with blood exposed to rivaroxaban and the different reversing strategies. **(A)** Diagram bars in represent morphometric values of platelets (in red) and fibrin (in blue) interactions with damaged vascular surfaces in perfusion studies with citrated whole blood. Blood was recalcified and perfused through an annular chamber containing damaged vascular segments at a shear rate of 600/s. **(B)** Diagram bars represent a subanalysis of the average size of fibrin masses deposited (Fibrin area in blue) expressed as μm^2 . Coagulation factor concentrates improved levels of fibrin formation and size of fibrin masses previously reduced by rivaroxaban, but values never returned to the baseline levels in non-treated samples. CON, Control; RIV, Rivaroxaban 230 ng/ml; recombinant factor VIIa (rFVIIa), Novoseven® 270 $\mu\text{g}/\text{kg}$; activated prothrombin complex concentrates (aPCC), Feiba® 75 U/kg; prothrombin complex concentrates (PCC), Beriplex® 50 IU/kg. * $P<0.05$ or ** $P<0.01$ vs. CON; # $P<0.05$ or ## $P<0.01$ vs. RIV (mean \pm SEM, n=8).

eters of the TG. Both PCCs and aPCC corrected the total amount of thrombin generated; however, rFVIIa only modified the kinetic parameters.²⁸ In another study, PCC increased the area under the curve of TG, but only rFVIIa and aPCC corrected the altered lag-time after rivaroxaban.³¹

The favourable results on rivaroxaban reversal in the previously mentioned studies have not been completely supported by additional investigations in animal models of hepatosplenic bleeding. In a rabbit model of bleeding and thrombosis, rivaroxaban increased blood loss, ear bleeding time, thrombelastographic CT, and decreased TG. Recombinant FVIIa and PCC partially improved thromboplastin time as well as thrombelastographic CT, but showed no efficacy at controlling blood loss after hepatoesplenic section.³² In another study, rabbits

were subjected to the anticoagulant action of apixaban. Treatment with this new anticoagulant, which acted on factor Xa, reduced TG and the viscoelastic parameters of forming clots. Recombinant FVIIa, PCC, and fibrinogen concentrates improved alterations of laboratory parameters but did not reverse apixaban-induced bleeding.³³ Results of the later studies illustrate the existence of discrepancies between normalization of coagulation biomarkers measured by assays on steady blood samples and the control of bleeding after new oral anti-coagulants.

In our present studies, alterations in biomarkers of coagulation after rivaroxaban, analyzed through assays performed under static conditions (TG and ROTEM) or subsequent modifications after exposure to reversal strategies, were basically compatible with those previously communicated.^{29,31} The information provided by our studies under flow condition offers additional insights on the antithrombotic action of rivaroxaban and the potential reversal of its antihemostatic action of coagulation factor concentrates. In our study, the effects of rivaroxaban on platelet- and coagulation-mediated mechanisms of hemostasis using an experimental approach with circulating human blood, revealed statistically significant reductions in platelet interactions and fibrin deposition on the subendothelium. The reduction of platelet interactions in flowing blood observed in our studies might explain the beneficial effects of rivaroxaban shown not only in the prevention and treatment of thromboembolic events but also in other situations such as acute coronary syndromes.³⁴

Remarkably, our studies with circulating blood demonstrated that rivaroxaban, at the concentration reached at standard Cmax, caused a dramatic reduction not only on the surface of the vessel covered by fibrin, but also in the average size of the fibrin masses deposited on the damaged vessel surface. The different strategies applied to reverse the antihemostatic action of rivaroxaban demonstrated to statistically improve the reduction in fibrin formation after this direct anticoagulant. Despite the statistical improvement observed with the different concentrates tested, none of these strategies were capable to restore levels of fibrin formation to their baseline value. Our results in studies under flow conditions might help explain the discrepancies between results of the TG test and correction of bleeding that might require restoration of platelet and fibrin components of hemostasis for full bleeding correction.

Prolongation of PT in plasma-based assays, although valuable as an estimate of coagulation status in patients receiving rivaroxaban,³⁰ only evaluates a small amount of thrombin formed during the initiation of coagulation.^{35,36} In contrast with PT, TG assays have proven useful in the evaluation of alterations of coagulation in severe congenital deficiencies,³⁷ although there is a reasonable concern on whether correction of alterations in the kinetics of TG in vitro assays could be predictive of a bleeding risk after new oral anticoagulants or its potential reversals.^{38,39} In our studies, effects of rivaroxaban were very evident when TG was activated through the extrinsic pathway (RCL reagent) and less evident when triggering the intrinsic pathway using the phospholipid-based activator (RD reagent). Interestingly, the effectiveness of reversal therapies at restoring TG differed strongly depending on the activator, with very mild responses to PCCs with the TF containing reagent, and explosive responses to PCCs and aPCCs within the phospholipid-based activator. In a recent study, Dinkelaar et al⁴⁰ investigated the applicability of PCCs to the reversal of rivaroxaban-induced alterations on TG. These authors concluded that responses to different TG tests were clearly dependent on the assay conditions, and the amount of

PCC required for normalization of TG depended on the concentration of TF and the presence of phospholipids. It is very likely that normalization or even overcompensation in TG observed after some reversal strategies do not necessarily correspond with a complete restoration of fibrin generation previously altered by new oral anticoagulants.^{21,32} The basic problem with coagulation assays such as PT or TG is that they disregard rheological conditions that regulate cellular and plasma interactions, with damaged vascular surfaces resulting in the initiation and consolidation of hemostasis. Studies in flow perfusion chambers allow the evaluation of platelet and fibrin components of hemostasis under shear stress conditions, offering additional information not provided by laboratory tests performed under low shear or steady conditions.⁴¹

A limitation of our experimental approach is that our studies were performed at a single concentration. Conversely, the dose chosen for our studies (230 ng/ml), corresponds to the average Cmax obtained in patients under standard treatment with rivaroxaban, and as such, should be effective and safe. The novelty of our present contribution is the use of models of hemostasis and thrombosis with circulating blood. In vitro flow devices based on the perfusion of whole blood over a damaged vascular surface at physiologic shear rates have been applied to evaluate bleeding situations, and their correction transfusion therapies have demonstrated throughout the years a good correspondence with the clinical situation.^{25,42–44} Under the perspective of this correspondence, the lack of normalization in fibrin formation after the different reversal strategies tested might indicate that the strategies with coagulation factor concentrates might compensate, although not completely neutralize, the antihemostatic action exerted by rivaroxaban. On the basis of our present study results with flowing blood, it would be uncertain to expect a full restoration of the alterations of hemostasis caused by overdoses of rivaroxaban. Target-specific antidotes for novel oral anticoagulants are in development.^{45,46} These antidotes might prove more efficient at correcting the coagulopathy observed after rivaroxaban in studies with circulating blood.

Notwithstanding the fact that the 3 procoagulant concentrates tested showed similar abilities at correcting platelet and fibrin formation in perfusion studies, there is concern regarding the potential thrombogenicity of rFVIIa and aPCCs used out of their approved indications.^{47,48} PCCs showed a significant potential to compensate the antihemostatic actions of rivaroxaban in perfusion studies. There is wide experience with the use 4-factor PCCs in the reversal of the anticoagulant effect of vitamin K antagonists, and rates of thrombotic complications seem to be acceptable.⁴⁹ Based on our studies and their accepted good safety profile, PCCs could be considered as the first-line reversal agent for rivaroxaban until other most specific therapies demonstrate efficacy and safety in the clinical setting. In the mean time, the efficacy and safety of PCCs in the reversal of the anticoagulant action of rivaroxaban should be confirmed by adequately powered clinical trials.

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