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11. Clinical pharmacokinetics of mycophenolic acid and its metabolites in solid organ transplant recipients

Helena Colom[1], Nuria Lloberas[2], Ana Caldés[2], Franc Andreu[2], Joan Torras[2]
Federico Oppenheimer[3], Jaime Sanchez-Plumed[4], Miguel A. Gentil[5]
Dirk R. Kuypers[6], Mercé Brunet[3], Henrik Ekberg[7] and Josep M. Grinyó[2]
[1]Department of Pharmacy and Pharmaceutical Technology, School of Pharmacy, University of Barcelona Barcelona, Spain; [2]Nephrology Service and Laboratory of Experimental Nephrology, Hospital Universitari de Bellvitge, Barcelona, Spain. [3]Hospital Clinic i Provincial, Barcelona, Spain; [4]Hospital La Fe, Valencia, Spain [5]Hospital Virgen del Rocío, Sevilla, Spain; [6]Department of Nephrology and Transplantation University Hospital, Leuven, Belgium; [7]Department of Nephrology and Transplantation University Hospital Malmö, Sweden

Abstract. Mycophenolate mofetil (MMF), an ester prodrug of the immunosuppressant mycophenolic acid (MPA), is widely used for maintenance immunosuppressive therapy and prevention of renal allograft rejection in renal transplant recipients.

MPA inhibits inosine monophosphate dehydrogenase (IMPDH), an enzyme involved in the "de novo" synthesis of purine nucleotides, thus suppressing both T-cell and B-cell proliferation. MPA shows a complex pharmacokinetics with considerable inter- and intra- patient by between- and within patient variabilities associated to MPA exposure. Several factors may contribute to it. The pharmacokinetic modeling according to the population pharmacokinetic approach with the non-linear mixed effects

Correspondence/Reprint request: Dr. Helena Colom, Department of Pharmacy and Pharmaceutical Technology School of Pharmacy, University of Barcelona, Avgda. Joan XXIII s/num 08028 Barcelona, Spain
E-mail: helena.colom@ub.edu

models has shown to be a powerful tool to describe the relationships between MMF doses and the MPA exposures and also to identify potential predictive patients' demographic and clinical characteristics for dose tailoring during the post-transplant immunosuppresive treatment.

Introduction

Mycophenolic acid based therapies are widely used in combination with calcineurin inhibitors (cyclosporine, tacrolimus, sirolimus) as maintenance immunosuppression in renal transplantation. Mycophenolate mofetil (MMF, brand name cellCept®, Hoffmann-La Roche, Basel, Switzerland) is one of the therapies currently used. In this context, there has been considerable interest in immunosuppressive regimens which permit reduction or elimination of calcineurin inhibitor (CNI)-associated and other chronic toxicities while maintaining adequate immune-suppresion [1]. MPA exposure values show a high variability partly attributable to its complex pharmacokinetics [2,3]. Several factors as albumin concentrations, renal function, co-medication and genetic polymorphism, may contribute to it [2-6]. Recent literature supports the notion that therapeutic drug monitoring (TDM) of MPA improves monitoring of kidney transplant patients on high risk of acute rejection or on calcineurin inhibitor minimization protocols [7,8]. The current review discusses the pharmacokinetics of MPA and its conjugated metabolites as well as the population pharmacokinetic models developed to describe it. Results of an integrated model recently developed by our group including the MPA protein binding, the pharmacokinetics (PK) of both metabolites (7-O-MPA glucuronide (MPAG) and acyl-glucuronide (AcMPAG) and the influence of co-medication and of multidrug resistance associated protein 2 (MRP2) polymorphism on the PK of MPA and its metabolites are also presented.

1. Chemistry of mycophenolic acid and its metabolites

Mycophenolic acid (MPA) is an antibiotic substance derived from *Penicillium stoloniferum*. Mycophenolate mofetil (MMF) is the 2,4-morpholinoethyl esther of MPA [9]. It was developed as a prodrug because the low oral bioavailability of MPA. Molecular structures of mycophenolic acid and its conjugated metabolites, i.e. the phenolic MPA 7-O-glucuronide (MPAG) and the acyl-glucuronide (AcMPAG) are summarized in Fig. 1.

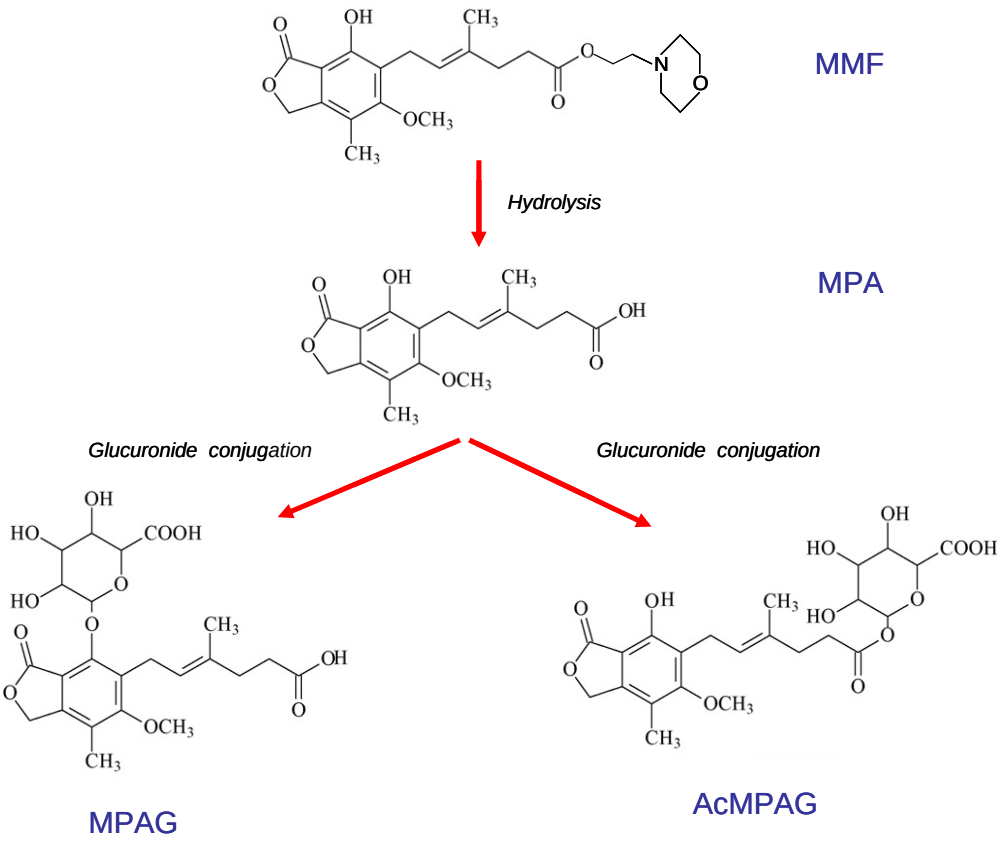

Figure 1. Molecular structures of mycophenolate mofetil (MMF), mycophenolic acid (MPA) and its metabolites, i.e. phenolic MPA 7-O-glucuronide (MPAG) and acyl-glucuronide (AcMPAG).

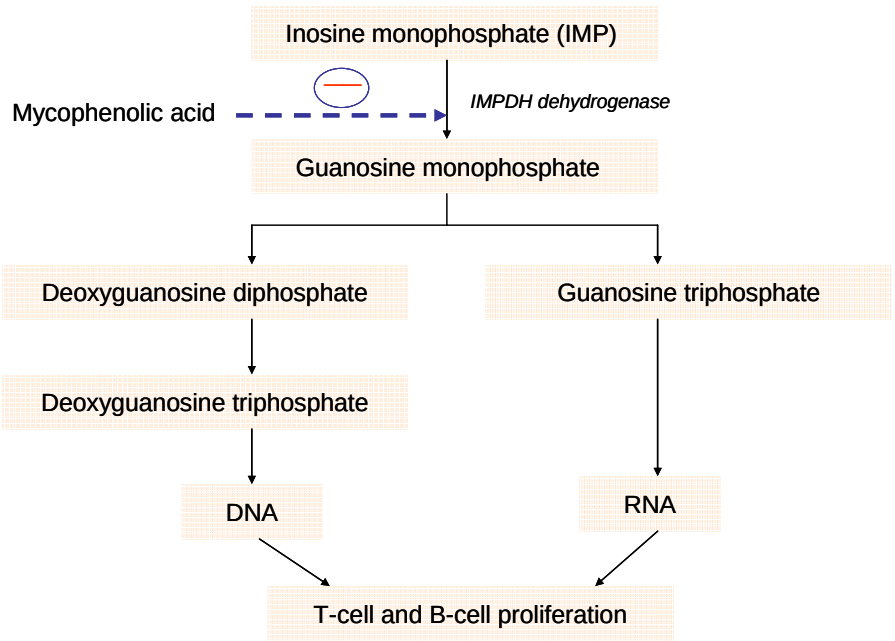

Figure 2. Purine biosynthetic pathways and mycophenolic acid activity.

2. Pharmacodynamics of mycophenolic acid

MPA blocks the “de novo” biosynthesis of purine nucleotides by inhibition of the enzyme inosine monophosphate dehydrogenase [10-12]. Mycophenolic acid is important because of its selective effects on the immune system. As displayed in Fig. 2, it prevents the proliferation of T-cells, lymphocytes, and the formation of antibodies from B-cells. It also may inhibit recruitment of leukocytes to inflammatory sites.

3. Pharmacokinetics of mycophenolic acid

The pharmacokinetic processes that take place when MMF is given orally are summarized in Fig. 3. After its oral administration, MMF is rapidly and essentially, completely absorbed, and then completely hydrolised to MPA, by estearases in the gut wall, blood, liver and tissue [2]. Oral bioavailability of MPA, after MMF administration ranges from 80.7% to 94% [3]. MPA is mainly metabolized by glucuronidation by several uridine diphosphate glucuronosyltransferases (UGTs) in the liver, gastrointestinal tract and kidneys into its inactive MPA 7-O-glucuronide (MPAG) and the pharmacologically active acyl-glucuronide (AcMPAG) [13]. Renal clearance of unchanged MPA is negligible [3] while MPAG and AcMPAG are mainly excreted into urine via active tubular secretion mediated by MRP2 (multidrug resistance-associated protein 2). In the liver MPA is taken up into hepatocytes, glucuronidated to MPAG which is then excreted into bile through MRP2 and then de-conjugated back to MPA by gut bacteria [14]. The formed MPA is then reabsorbed in the colon. Biliary secretion of MPAG, leading to this enterohepatic recirculation (EHC), contributes approximately 40% to the area under the plasma concentration-time curve (AUC) and is considered as the major cause of the secondary peak (observed from 6 to 12 h after oral administration) of MPA in plasma [15]. The EHC is also assumed to occur for the conjugated metabolite AcMPAG [16]. Ciclosporin causes a decrease in the biliary secretion of MPAG and thereby decreases MPA plasma levels and its exposure [14,17,18]. This interaction has been associated with MRP2 inhibition of biliary secretion of MPAG and MPA by ciclosporin [19,20]. On the other hand, MPA is extensively bound to serum albumin [21] and a protein binding ranging from 97% to 99% has been reported in patients with normal renal and liver functions [2]. Although lower, MPAG also displays a high albumin protein binding (82%) in stable patients [3]. Therefore, competition between MPA and MPAG by albumin binding may exist. The interplay between all the processes above described leads to large between-patient and within–patient variabilities associated to

the exposure of MPA and its metabolites in renal transplant patients, as it has been previously reported [2,3].

4. Pharmacokinetic variability

Renal function, serum albumin concentrations, haemoglobin levels, delayed graft function and immunosuppressive co-medication have been described as factors contributing to the variability and time-dependent pharmacokinetics described for MPA [6,22-27]. Previous studies [28] demonstrated that decreased albumin plasma concentrations lead to increased free fractions both of MPA and MPAG. As consequence, relatively more free MPA (fMPA) is available for elimination resulting in decreased total MPA (tMPA) exposure. However, the fMPA exposure is unaffected. This often happens with drugs of low extraction rate [29] as it is the case of MPA [30] and it implies that, in these cases, tMPA exposure could drop below the therapeutic values, so that MMF dose should be increased in order to avoid acute rejection. The influence of renal function on the pharmacokinetics of

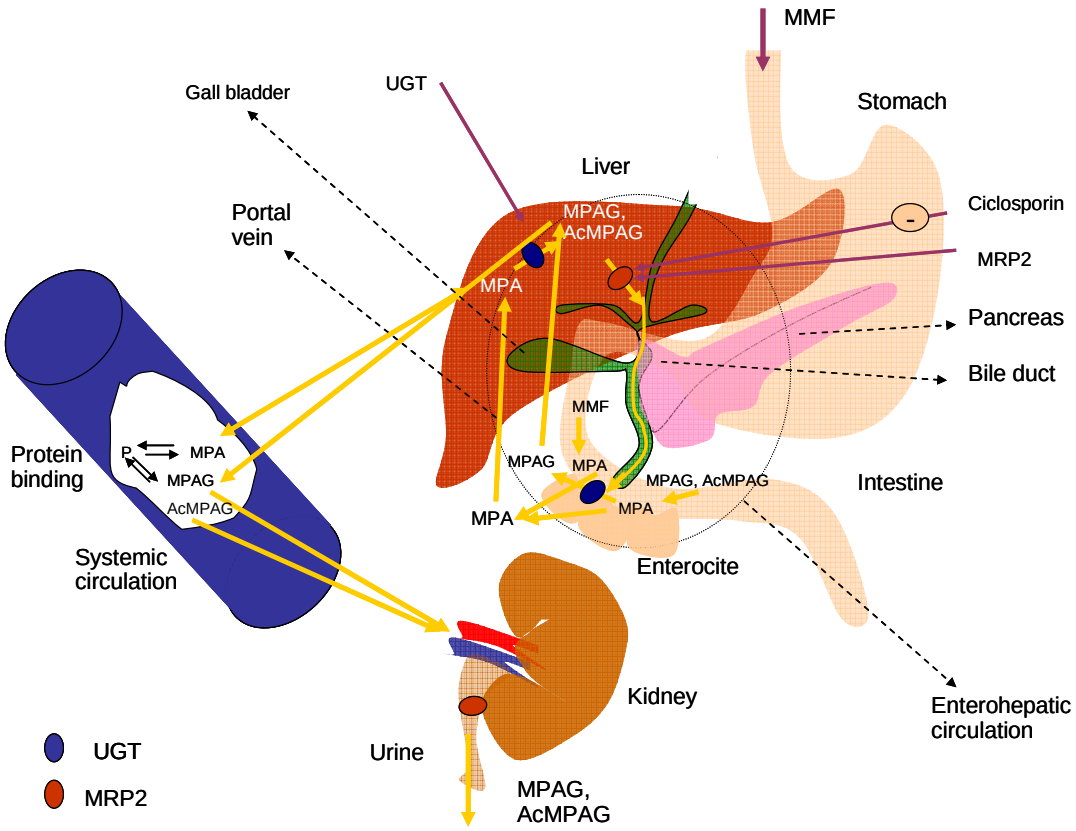

Figure 3. Pharmacokinetics of mycophenolic acid (MPA) and its metabolites, phenolic MPA 7-O-glucuronide (MPAG) and acyl-glucuronide (AcMPAG) after mycophenolate mofetil (MMF) oral administration.

MPA and its conjugates has also been widely reported [22,28]. As it should be expected, a very poor renal allograft function (i.e. CL_{CR} values around 10 mL/min), mainly occurring in the earliest phases after transplantation, have major effects on both MPAG and AcMPAG exposures than on exposures to tMPA or fMPA. According to De Winter et al. [28], depending on the calcineurin inhibitor (CNI) given to patients with impaired renal function, differential effects on tMPA and fMPA exposures can be observed. In the case of ciclosporin tMPA exposure decreases and fMPA exposure remains the same. In patients under tacrolimus an increased exposure to tMPA and a small increase in fMPA exposure are observed.

The justification of this is that under tacrolimus, accumulation of MPAG, as consequence of low renal function, results in increased transport of MPAG to the gallbladder leading to increased recirculation of MPAG to MPA. Because the extra-recirculation, MPAG does not accumulate to an extent where it can displace MPA from its protein binding sites. It results in increased tMPA and fMPA due to extra-recirculation and no change in unbound fraction of MPA. By contrast, in patients under ciclosporin the accumulated MPAG following renal impairment cannot be compensated by increased recirculation because MRP2 transport is inhibited by ciclosporin. As a result MPAG displaces MPA from its protein binding sites, leading to an increased fraction of fMPA. The increased fMPA exposure is immediately compensated for by an increase in MPA glucuronidation according to the theory of restrictively cleared drugs. Therefore, in this case the result is decreased tMPA exposure, unchanged fMPA exposure and an increased fMPA fraction. The genetic factors controlling the level of UGT-mediated MPA metabolism [31-34] and the MRP2-mediated conjugated metabolites transport [35] can also partly explain the observed variability in MPA exposure. The large variability and the MPA narrow therapeutic index lead to optimization of the immunosuppressive regimen in order to avoid the risk of acute rejection (AR), and to prevent adverse-effects associated with long-term immunosuppressive treatment.

5. Relationship between MPA exposure and clinical outcome

In vitro studies have suggested that the fMPA concentrations may more accurately reflect the degree of immunosuppressive action of the drug than does the concentrations of tMPA [21]. The fMPA concentrations have been shown to correlate with the risk of leukopenia and infection [24]. However, although a relationship between fMPA exposure and the risk for acute rejection should be expected, it has not been demonstrated yet. More information is needed about the relationship between fMPA exposure and the

risk for acute rejection and side effects to interpret the clinical effect of changes in protein binding of MPA. Alterations in both albumin concentrations and renal function have little effect on fMPA exposure and thereby little clinical relevance. However special attention must be paid on patients under tacrolimus with very poor renal function, as the increased fMPAG can cause elevated exposure to both tMPA and fMPA. Regarding to tMPA, a correlation between tMPA exposure and the risk of acute rejection has been reported [36]. The proper tMPA exposure (AUC) range to avoid acute rejection has been recommended to be 30-60 μg·h/mL when combined with ciclosporin [36-39]. Under this co-medication there is no further reduction in acute rejection at AUC values >60 (mg/L)·h [36]. Therefore, avoidance of higher exposure would seem prudent on the basis of these results. For patients under tacrolimus, the range of 30-60 μg·h/mL has also been suggested for MPA exposure [39]. Regarding to patients with low albumin concentrations or poor renal function, accompanied by low tMPA exposures, candidates to increased doses of MMF to avoid acute rejection, the dose increase will also increase the fMPA exposure. Therefore, the neutrofil count in these patients should be accurately monitored, as there may be a potential increased risk of leucopenia and infections. In that sense it should be noted that fMPA exposures > 0.14 (mg/L)·h, are expected to increase the risk of adverse events. This cut-off has been established in a pediatric study in the early post-transplant phase [24]. On the other hand, measurement of the pharmacologically active metabolite AcMPAG could also provide information about proper exposures to it in order to avoid the risk for adverse events. The population approach has shown to be a powerful tool for these purposes. The characteristics of this approach are reviewed below.

6. Population pharmacokinetic approach: Non linear mixed effects modeling

According to the FDA Guidance [40], population pharmacokinetics, is the study of the sources and correlates of variability in drug concentrations among individuals who are the target patient population receiving clinically relevant doses of a drug of interest. It allows to identify the determinants of pharmacokinetic variability, explaining between-patient differences in drug exposure and can provide clinically applicable models for dose tailoring. With this approach, the description of the pharmacokinetics of a given drug in the target population can be performed by determining a*)* what is the mean population pharmacokinetic behaviour, b*)* which factors influence the mean population PK behaviour and, c*)* what is the uncertainty degree associated to the mean population PK behaviour, i.e. the magnitude of between-patient variability and residual error can be quantified. Its advantages when compared

to the classical approach are numerous such as being able to analyze concentration-time data of parent compound and metabolites, data from several doses or plasmatic and urine data, simultaneously. Moreover, apart from analyzing dense data (intensive sampling in each individual) it allows to analyze unbalanced data and sparse data (few samples per individual) coming from observational studies, phase II/III/IV clinical studies or from special populations (neonates, children, elderly, transplantation, HIV) or from therapeutic drug monitoring (TDM).

Non linear mixed effects models implemented in several softwares are the most widely used for these purposes [41]. Once validated, the models developed with this approach will allow, a) description and a better knowledge of the pharmacokinetic profile of a given drug in the target population, b) simulations of new scenarios to predict exposures to different dosing regimens, to evaluate consequences of alternative sampling designs, or to evaluate the impact of changes in patients' characteristics, and c) calculation of the first dose and dose tailoring during the therapeutic drug monitoring, in order to achieve the target value of a given PK parameter (in general, AUC or area under the curve, peak or through concentrations) demonstrated to be the best marker of efficacy. Regarding to the last point, it should be noted that computer programs designed for dose optimisation for individual patients to be used as a part of routine clinical care, exist [42]. These programs require the implementation of the PK parameters estimated through the non linear mixed effects modeling.

In the mixed-effects modeling context [41], the collection of population characteristics is composed of population mean values (derived from fixed-effects parameters) and their variability within the population (generally the variance-covariance values derived from random-effects parameters). This approach allows to estimate directly the parameters of the population from the full set of individual concentration values. The individuality of each subject is maintained and accounted for, even when data are sparse. Fig. 4 summarizes the relationship between fixed and random effects in a population PK model. According to this and taking into account one of the PK parameters, i.e. clearance (CL), it is assumed that the deviations of individual plasmatic clearances of subject 1 (CL_1) or 2 (CL_2) from the mean population value ($\overline{CL}$) are given by η_1^{CL} and η_2^{CL}, respectively (left panel). The set of values of etas (η_i) of all individuals in the target population is assumed to follow a normal distribution of mean equal to zero and variance ω^2. Moreover, the set of deviations of the observed concentrations in each individual at a given sampling time j (open circles), from the individual predictions by the model (continuous lines or $f(CL_1)$, $f(CL_2)$, or f(CLi) for the i subject), given by $\varepsilon_{ij,}$ in general (i denoting the individual, i.e. i=1,2,..n; and

j the sampling time, i.e.,j=1,2,…t) are also assumed to follow a normal distribution of mean equal to zero and variance σ^2 (right panel). f($\overline{CL}$) represents the concentrations predicted for an individual of the target population showing the typical clearance value equal to the mean population value, $\overline{CL}$. In summary, the population parameters estimated by the non linear mixed approach are the following:

a) the fixed effects, i.e. the mean population pharmacokinetic parameters as $\overline{CL}$ or the corresponding regression parameters when there is a stististically significant relationship between the PK parameters and continuous (age, body weight, creatinine clearance, doses or exposure parameters corresponding to co-medication…) or discontinuous (gender…) covariates.
b) the variances of the η and ε distributions, that is ω^2 and σ^2, respectively.

During the estimation process the η and ε values for each individual will be calculated and thus the individual PK parameter values obtained to be used for dose tailoring during the TDM. However, before that, the validation or evaluation of the predictability of the model developed is required. Internal or external validation techniques can be applied for this purpose [40].

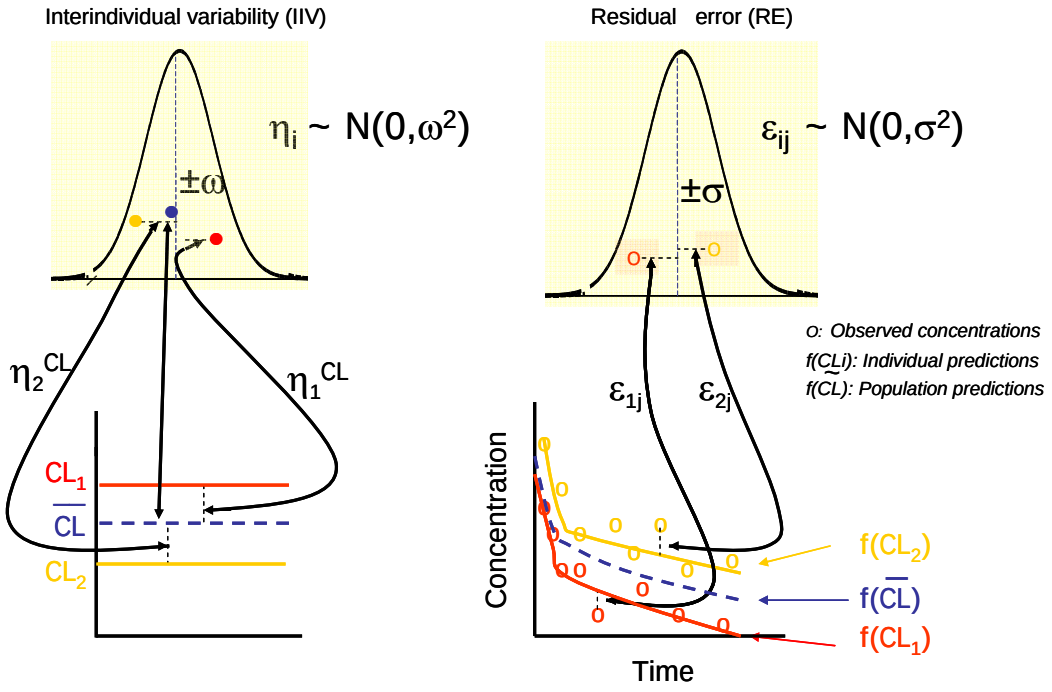

Figure 4. Relationship between fixed effects and random effects in the non linear mixed effects approach.

7. Population pharmacokinetic modeling of mycophenolic acid and its metabolites

Several MPA population pharmacokinetic models have been previously developed trying to identify the sources of variability of MPA pharmacokinetics after MMF oral administration [4,6,28,34,43-48]. Some of them have allowed to describe the second MPA plasma peak by including the EHC in the modeling process [34, 49], as the influence either of changes of protein binding [28,47], or of genetic polymorphism in UGT on MPA exposure [34]. Recently our group has addressed its work on the simultaneous modeling of total and free MPA as total MPAG and AcMPAG concentration vs time data proceeding from the PK sub-study of the Symphony study [50]. In the Symphony PK sub-study the effect of four different immunosuppressive therapies on the PK of MPA was evaluated. Briefly, patients randomized in four groups were given fixed doses of MMF (1 g twice daily) together with either standard doses of ciclosporin (group A), low doses of ciclosporin (group B) or low doses of the immunosuppressive macrolides that is to say tacrolimus (group C) or sirolimus (group D). PK sampling was performed on 5 occasions (on day 7 and at 1, 3, 6 and 12 months after transplantation) during the first year after transplantation and each time tMPA, fMPA, tMPAG and tAcMPAG exposures were measured. Results corresponding to the non-compartmental analysis of this study [50] indicated that tMPA and fMPA exposures were lower in patients receiving ciclosporin compared to those that were given macrolides. In contrast, exposures to the metabolites MPAG and acylMPAG were higher in patients treated with ciclosporin compared to the others (Fig. 5).

Moreover, in general, a trend to increased tMPA and fMPA exposures and decreased tMPAG and tAcMPAG exposures with post-transplant time was observed (Fig. 5). This was attributable to the decreasing and increasing of MPA and MPAG/AcMPAG clearances, respectively, with post-transplant time. On the other hand, Lloberas et al. [35], reported statistically lower tMPA and fMPA exposures in patients treated with macrolides being homozygous (TT) or heterozygous (CT) carriers of the MRP2 C24T single nucleotide polymorphism (SNP) vs non carriers (CC), after having investigated the influence of genetic polymorphism of MRP2-mediated transport on the MPA exposures in all groups of treatment. It should be noted that this effect could not be observed in the group of patients treated with ciclosporin. The time-dependent clearance found in this study was in agreement with results of previous studies [4,6,45]. As previously reported, ciclosporin dose tapering and improvement of the renal function along the post-transplant period can be among others, some of the causes of it. Since

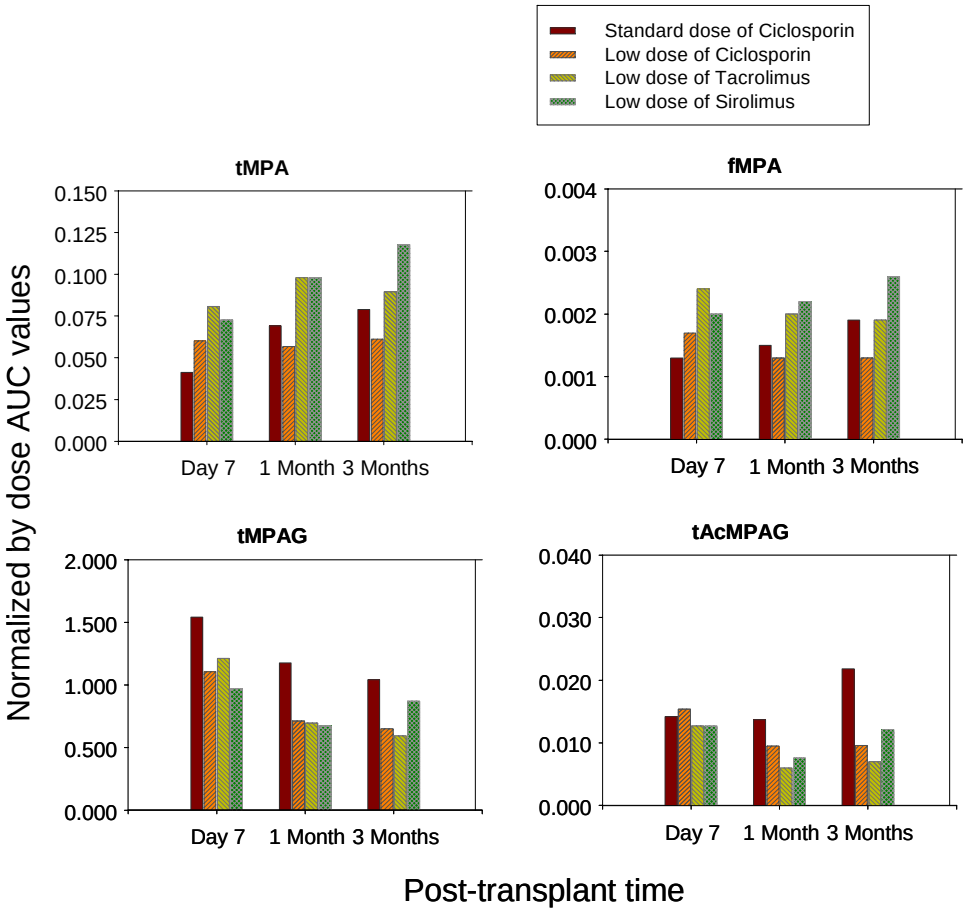

Figure 5. Normalized by dose exposure values given by AUCs (area under the the curve) observed for tMPA, fMPA, tMPAG and tAcMPAG, during the first three post-transplant months of the PK sub-study of the Symphony study [40].

MPA is a low extraction rate drug, the low albumin concentrations associated to a delayed graft function during the early post-transplant stages can also lead to higher MPA clearances values at the early stages vs the late. On the other hand, inhibition of MRP2 transport by ciclosporin, in patients treated with it, leads to decreased MPAG biliar excretion that in turns results in decreased EHC, increased MPAG and AcMPAG exposures and decreased MPA exposures when compared to the others. Regarding to the influence of C24T SNP, results of this study suggested a lower activity of transport of MPAG or AcMPAG through MRP2 in presence of C24T SNP that led to a decreased EHC, followed by lower MPA exposure and subsequently lower MPAG or AcMPAG exposures caused by its decreased formation, when compared to the non carrier (CC) genotype. This was only evidenced under macrolides when the effect of ciclosporin was not masking that of the SNP. Prompted by these results, a population pharmacokinetic model to allow the description of all these processes was developed (Fig. 6) (submitted for

publication). The pharmacokinetics of fMPA, tMPA, tMPAG and tAcMPAG were best described by an integrated model consisting on three linked compartment models; i.e. two two-compartment models for fMPA and tMPAG and a one-compartment model for tAcMPAG (Fig. 6). The model was parameterized in terms of volumes of distribution (V) and plasmatic (CL) and distributional (CL_D)clearances. An albumin compartment was also linked directly to the central compartment of fMPA to describe its binding to this plasmatic protein (K_B=binding rate constant). EHC could not be successfully modeled.

According to this model, after oral administration, MMF was transformed to and absorbed as MPA according to a time lagged first order kinetic process. Once in the systemic circulation MPA was simultaneously bound to albumin (bMPA) and the free fraction (fMPA) distributed and eliminated.

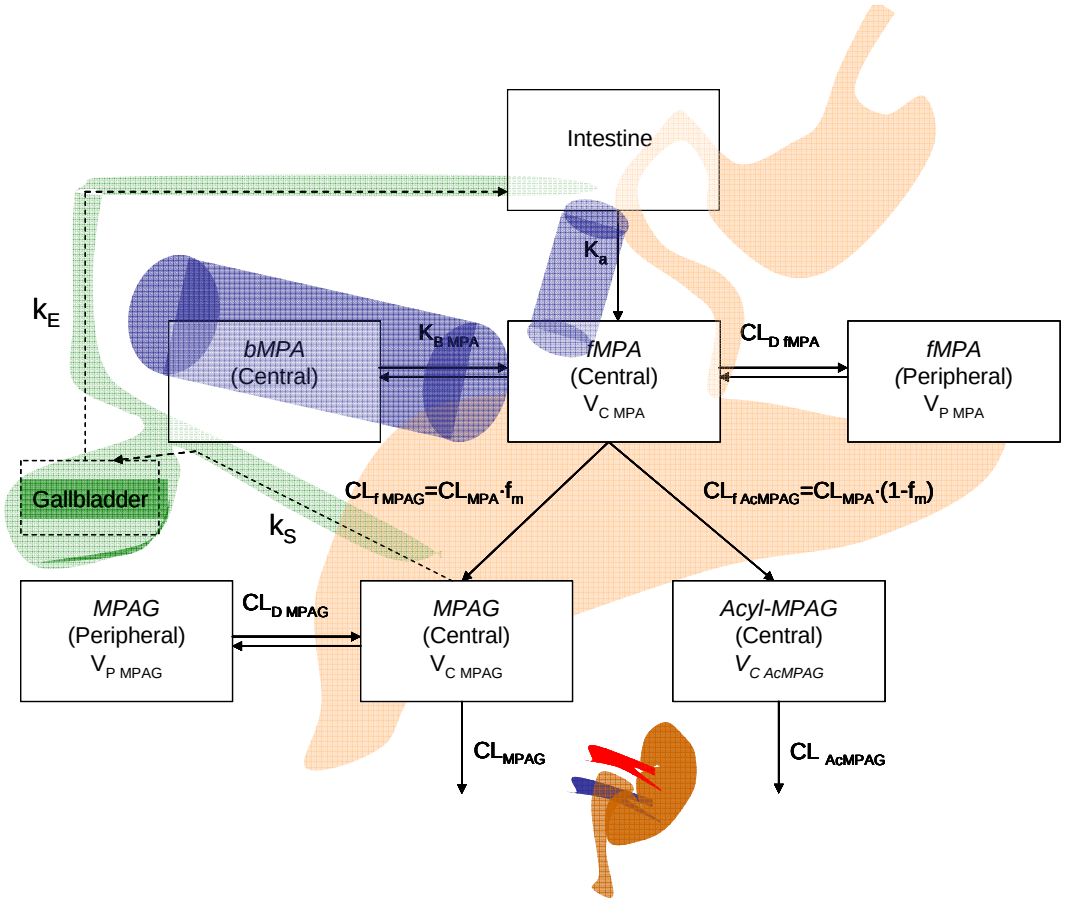

Figure 6. Schematic representation of the final pharmacokinetic model to simultaneously describe the protein binding of free mycophenolic acid (fMPA) and its conversion to its phenolic 7-O-glucuronide (MPAG) and acyl-glucuronide (AcMPAG) conjugates by first order processes, after oral administration of mycophenolate mofetil. Dashed lines correspond to the part that could not be successfully modeled. bMPA: MPA bound to albumin.

The fMPA protein binding was best described by a linear model ($bMPA=K_B \cdot fMPA$) as previously reported by Van Hest et al [46]. This might be due to the fact that the fMPA concentrations found in the current study (from 0.000019 to 0.005573 mmol/L) were far below the median plasma albumin concentrations of the studied population (42 g/L or 0.6087 mmol/L) and no saturation of the binding sites could be achieved. Obviously, taking into account at least one binding site to albumin, the protein binding would be hardly saturated with the current fMPA concentrations. It should be noted that fMPA and tMPA concentrations found in our study were around twice those observed by Van Hest et al [46] in which higher ciclosporin doses were given (around twice those of our study), and nevertheless the linear protein binding still described correctly our data. Effectively, unlike our results, Van Hest et al. [46], found statistically significant correlations between individual K_B values and albumin plasma levels and MPAG concentrations. This could be attributed to the fact that most of the patients included in the current study showed albumin plasma concentrations within the expected range of normal healthy adults (43 g/L, ranging from 35 to 53 g/L), even on day 7 of the study when delayed graft function should be expected to be more likely. Only 8 out of 56 patients and 2 out of 56 patients showed albumin plasma levels less than 35 and greater than 53 g/L, respectively; the albumin concentrations of the remaining patients were around the study population median level (42 g/L). Regarding to MPAG concentrations, these were lower in our study than those found by Van Hest el al. [46]. The stable renal function of patients and also the lower doses of ciclosporin in patients under this co-medication, might be the contributing factors to this fact. According to Bullingham et al. [3], MPA free fractions (fu) can increase as the MPAG concentrations increase to 475 mg/L (957 mmol/L). In our study peak MPAG concentrations were lower than 236 mg/L (479 mmol/L) in most patients, so that no displacement of bound MPA (bMPA) by MPAG should be expected.

Regarding to the elimination process of fMPA it took place by first-order kinetic processes as did the metabolites. According to the f_m value obtained (0.874), MPAG was the major metabolite, while only a 0.126 ($1-f_m$) of fMPA present in the blood stream was transformed to AcMPAG. These values were in agreement with that reported by Shipkova et al [16], The total clearance value of fMPA was the contribution of the clearance of formation MPAG ($f_m \cdot CL_{MPA}$) and the clearance of formation AcMPAG ($(1-f_m) \cdot CL_{MPA}$). Although EHC modeling could not be included, it does not seem to have a relevant impact from a clinical point of view, since the covariates that could affect the recycling rate constant (K_S) could be incorporated in both the MPA and its metabolites clearances. In effect, in agreement with results of the non-compartmental analysis, an statistically significant relationship was found

between CL_{MPA} and ciclosporin through concentrations in patients under ciclosporin, in such a way that CL_{MPA} increased with it and was higher in C24 SNP homozygous (TT) or heterozygous (CT) carriers vs non carriers (CC), in patients under macrolides. The time-dependent clearance of MPA in the target population was also confirmed by the model that suggested that CL_{MPA} on day 7 was higher than at the remaining monitoring days; however, gradual tapering of ciclosporin through concentrations along the post-transplant period was not sufficient to describe these changes over time nor other covariates whose significance could not be demonstrated as low graft function or acidosis or uremia associated to it and consequently low levels of albumin.

Regarding the metabolites, AcMPAG showed around a ten times faster elimination than MPAG, probably due to the highest hydrophilicity of the former. Plasmatic clearances of both metabolites were influenced by renal function through the estimated CL_{CR} according to the Cockcroft-Gault formula, as it should be expected, due to its elimination major pathway by urinary excretion [3, 16, 22]. Explicitly, both CL_{MPAG} and CL_{AcMPAG} were estimated to increase with renal function. Moreover, CL_{MPAG} decreased in a significant statistically way with increasing ciclosporin trough concentrations in patients under ciclosporin and similarly to $CL_{MPA,}$ it was higher in C24 SNP homozygous (TT) or heterozygous (CT) carriers vs non carriers (CC), in patients under macrolides. Regarding the influence of C24T SNP in CL_{AcMPAG}, it was statistically significant, but only in patients under macrolides. By contrast, no statistically significant effect of ciclosporin through concentrations on CL_{AcMPAG} could be evidenced.

Simulations performed once the model had been evaluated (Fig. 7), showed that after multiple fixed doses of MMF (1 g), at one month of the post-transplant period, both fMPA and tMPA exposures decreased significantly with increasing ciclosporin through concentrations from 100 to 300 ng/mL. In patients co-treated with macrolides (sirolimus or tacrolimus), fMPA and tMPA were significantly lower in homozygous and heterozygous variants of the C24T SNP vs wild-type. Among all patients, the highest exposure would be observed in wild-type or no variant alleles co-treated with sirolimus or tacrolimus while the lowest exposure would be found in patients co-treated with standard doses of ciclosporin. Regarding MPAG, exposures increased significantly with increasing ciclosporin through concentrations (from 100 to 300 ng/mL) and in patients co-treated with macrolides, they were significantly lower in homozygous and heterozygous variants of the C24T SNP vs wild-type. In the case of A_CMPAG, significantly lower exposures were observed in homozygous and heterozygous variants of the C24T SNP vs wild-type in patients co-treated with macrolides and also vs the

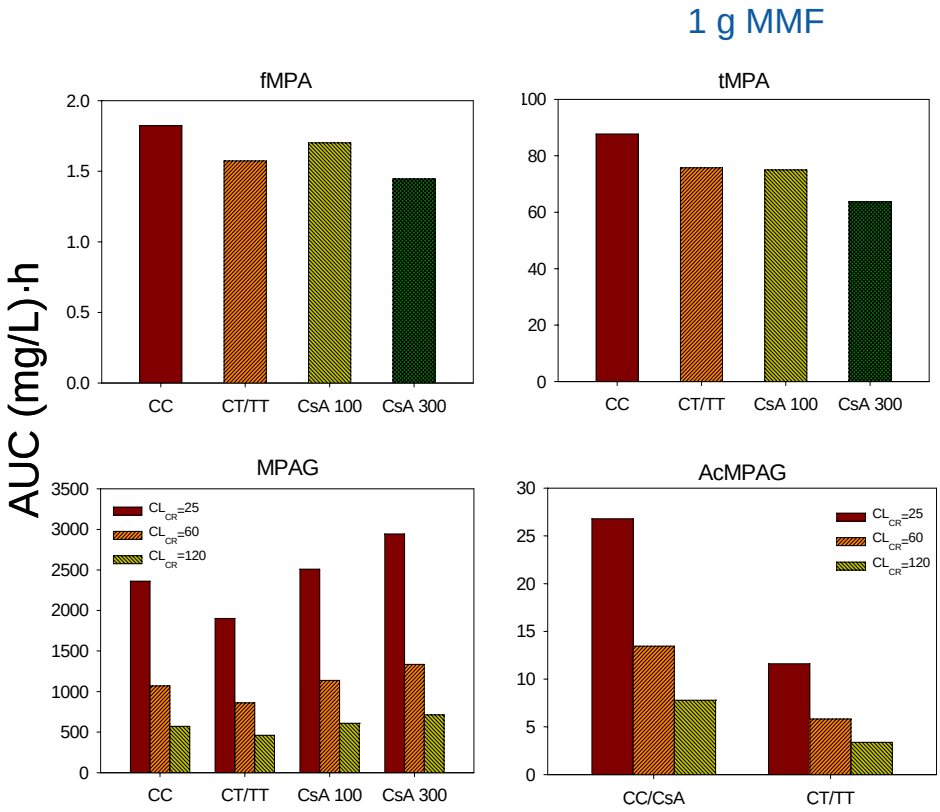

Figure 7. Median values of simulated exposures of fMPA, tMPA and MPAG and AcMPAG, after 1 g MMF given orally to patients under ciclosporin with trough concentrations of either 100 or 300 ng/mL and to patients under macrolides whether non carriers or CT/TT carriers of the C24T SNP of MRP2. The impact of renal function given by CL_{CR} estimated according to cockroft-Gault is also shown for MPAG and AcMPAG.

remaining patients. On the other hand, as expected, the major effects on both tMPAG and tAcMPAG exposures were due to changes in renal function given by CL_{CR} (estimated according to Crokroft-Gault). In effect, independently of the co-medication group, exposures significantly decreased with increasing CLCR values, the effect was higher for MPAG (around 55% from 25 mL/min to 60 mL/min and around 47% from 60 mL/min to 120 mL/min) than for AcMPAG (around 26% from 25 mL/min to 60 mL/min and around 42% from 60 mL/min to 120 mL/min). Then, renal function is the most influential covariate in both cases (CL_{MPAG} and CL_{AcMPAG}) followed by ciclosporin through concentrations and C24T SNP in the case of MPAG. The effect of C24T SNP is more relevant in CL_{AcMPAG} than in CL_{MAPG}. Unfortunately, the developed model did not allow the evaluation of the impact of changes in renal function on tMPA or fMPA exposures. Regarding changes in MPAG exposure with renal function observed in the current work, they are comparable to those found by De Winter et al. [28]. In summary we

built a population PK model to adequately describe plasma data of tMPA, fMPA and its currently known metabolites as a function of co-medication, C24T SNP of Mrp2 and renal function. According to it, patients under macrolides and non carriers of C24T SNP would require lower doses of MMF (around 40% less) than those under standard doses of ciclosporin with the same renal function. Since the model development does not allow evaluation of the impact of changes in albumin plasma levels and renal function on tMPA and fMPA it should be applied to patients with median albumin plasma levels of 42 g/L and median CLCR values of 60 mL/min , as the median of the population of the current study.

8. Conclusion

In the present chapter we summarize the relevance of modeling by using the population approach in order to describe the pharmacokinetics of mycophenolic acid and its metabolites. It allows, not only increasing our knowledge to better understand the clinical PK of this drug, but also it may prove useful in predicting the PK of MPA and all the characterized metabolites after various administration regimens of MMF.

Moreover, based on the protein binding model developed, precise predictions of fMPA concentrations can be made. This can be useful in at least two situations: a) for historical data were only tMPA concentrations are available, fMPA concentrations can be predicted and used in developing PK/PD relationships, and b) as the tMPA assay is considerably simpler than the fMPA assay, measuring only tMPA concentrations may be an alternative to measuring fMPA.

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References

1. Ekberg, H., Bernasconi, C., Tedesco-Silva, H., Vitko, S., Hugo, C. Demirbas, A., Reyes Acebedo, R., Grinyó, J., Frei, U., Vanrenterghem, Y., Daloze, P., Halloran, P. F. 2009. *Am. J. Transpl.*, 9, 1876.
2. Staatz, C. E., Tett, S. E. 2007. *Clin. Pharmacokinet.*, 46, 14.

3. Bullingham, R. E. S., Nicholls, A. J., Kamm, B. R.1998. *Clin. Pharmacokinet.*, 34, 429.
4. Shum, B., Duffull, P. J., Tett, S. E. 2003. *Br. J. Clin. Pharmacol.*, 56, 188.
5. Staatz, C. E., Duffull, S. B. 2005. *Eur. J. Clin. Pharmacol*., 61, 507.
6. Van Hest, R. M., Mathot, A. A., Pescovitz, M. D., Gordon, R., Mamelock, R. D., Van Gelder, T. 2006. *J. Am. Soc. Nephrol.*, 17, 871.
7. Tett, S. E., Saint-Marcoux, F., Staatz, C. E., Brunet, M., Vinks, A. A., Miura, M., Marquet, P., Kuypers, D. R., Van Gelder, T., Cattaneo, D. 2011. *Transplant. Rev.*, 25, 47.
8. Le Meur, Y., Borrows, R., Pescovitz, M. D., Budde, K., Grinyo, J., Bloom, R., Gaston, R., Walker, R. G., Kuypers, D., Van Gelder, T., Kiberd, B. 2011. *Transplant. Rev.*, 25, 58.
9. Srinivas, T. R., Kaplan, B., Meier-Kriesche, H. –U. 2003. *Expert. Opin. Pharmacother*.,4, 2325.
10. Allison, A. C., Almquist, S. J., Muller, C. D., Eugui, E. M. 1991. *Transplant. Proc.*, 23, 10.
11. Grailer, A., Nichols, J., Hullett, D., Sollinger, H. W., Burlingham, W. J. 1991. *Transplant. Proc.*, 23, 314.
12. Eugui, E. M., Mirkovich, A., Allison, A. C. 1991. *Transplant. Proc.*, 23, 15.
13. Picard. N., Ratanasavanh, D., Premaud, A., LeMeur, Y., Marquet, P. 2005. *Drug. Metab. Dispos*.,33, 139.
14. Westley, I. S., Brogan, L. R., Morris, R. G., Evans, A. M., Sallustio, B. C. 2006. *Drug. Metab. Dispos*., 34, 261.
15. Bullingham, R. E. S., Nicholls, A. J., Hale, M. 1996. *Transplant. Proc*.,28, 925.
16. Shipkova, M., Armstrong, V. W., Webber, L., Niedmann, P. D., Wieland, E., Haley, J., Tönshoff, B., Oellerich, M. 2002. *Ther. Drug Monit.*, 24, 390.
17. Van Gelder, T., Klupp, J., Barten, M. J., Christians, U., Morris, R. E. 2001. *J. Heart Lung Transplant*., 20,160.
18. Van Gelder, T., Klupp, J., Barten, M. J., Christians, U., Morris, R. E. 2001. *Ther. Drug Monit.*, 23, 119.
19. Kobayashi, M., Saitoh, H., Kobayashi, M., Tadano, K., Takahashi, Y., Hirano, T. 2004. *J. Pharmacol. Exp. Ther*., 309, 1029.
20. Hessenlink, D. A., Van Hest, R. M., Mathot, R. A., Bonthuis, F., Weimer, W., de Bruin, R. W., Van Gelder, T. 2005. *Am. J. Transplant*., 5, 987.
21. Nowak, I., Shaw, L. M. 1995. *Clin. Chem*., 41, 1011.
22. Naessens, M., De Loor, H., Vanrenterghem, Y., Kuypers, D. R. J. 2007. *Transplantation*, 84, 362.
23. Weber, L. T., Shipkova, M., Lamersdorf, T., Niedmann, P. D., Wiese, M., Mandelbaum, A., Zimmerhackl, L. B., Schütz, E., Mehls, O., Oellerich, M., Armstrong, V. W., Tonshoff, B. 1998. *J. Am. Soc. Nephrol.* 9, 1511.
24. Weber, L. T., Shipkova, M., Armstrong, V. W., Wagner, N., Schütz, E., Mehls, O., Zimmerhackl, L. B., Oellerich, M., Tonshoff, B. 2002. *J. Am. Soc. Nephrol.*, 13, 759.
25. Zucker, K., Rosen, A., Tsaroucha, A., De Faria, L., Roth, D., Ciancio, G., Esquenazi, V., Burke, G., Tzakis, A., Miller, J. 1997. *Transpl. Immunol.*, 5, 225.

26. Cattaneo, D., Perico, N., Gaspari, F., Gotti, E., Remuzzi, G. 2002. *Kidney Int*., 62, 1060.
27. Pou, L., Brunet, M., Cantarell, C., Vidal, E., Oppenheimer, F., Monforte, V., Vilardell, J., Roman, A., Martorell, J., Capdevila, L. 2001.*Ther Drug Monit*., 23, 35.
28. De Winter, B. C. M., Van Gelder, T., Sombogaard, F., Shaw, L. M., Van Hest, R. M., Mathot, R. A. A. 2009. *J. Pharmacokin. Pharmacodyn*., 36, 541.
29. Benet, L. Z., Hoener, B. A. 2002. *Clin. Pharmacol. Ther*., 71,115.
30. Bowlagaha, K., Miners, J. O. 2001. *Br. J. Clin. Pharmacol*., 52, 605.
31. Hesselink, D. A., Van Gelder, T. 2005. *Clin. Pharmacol. Ther*., 78, 317.
32. Kuypers, D. R. J., Naesens, M., Vermeire, S., Vanrenterghem, Y. 2005. *Clin. Pharmacol. Ther.*, 78, 351.
33. Levesque, E., Delage, R., Benoit-Biancamano, M. O., Caron, P., Bernard, O., Couture, F., Guillemette, C. 2007. *Clin. Pharmacol. Ther.*, 81, 393.
34. Jiao, Z., Ding, J. J., Shen, J., Liang, H. Q., Zhong, L. J., Wang, Y., Zhong, M. K., Lu, W. Y. 2008. *Br. J. Clin. Pharmacol*., 65, 893.
35. Lloberas, N., Torras, J., Cruzado, J. M., Andreu, F., Oppenheimer, F., Sánchez-Plumed, J., Gentil, M. A., Brunet, M., Eckberg H., Grinyó, J. M. 2011. *Nephrol. Dial. Transplant*., 26, 3784.
36. Hale, M. D., Nicholls, A. J., Bullingham, R. E., Hene, R., Hoitsma, A., Squifflet, J. P., Weimar, W., Vanrenterghem, Y., Van de Woude, F. J., Verpooten, G. A. 1998. *Clin. Pharmacol. Ther*., 64, 672.
37. Van Gelder, T., Hilbrands, L. B., Vanrenterghem, Y., Weimar, W., De Fitjer, J. W., Squifflet, J. P., Hene, R. J., Verpooten, G. A., Navarro, M. T., Hale, M. D., Nicholls, A. J. 1999. *Transplantation*, 68, 261.
38. Shaw, L. M., Holt, D. W., Oellerich, M., Meiser, B., Van Gelder, T. 2001. *Ther. Drug Monit.*, 23, 305.
39. Shaw, L. M., Figurski, M., Milone, M. C., Trofe, J., Bloom, R. D. 2007. *Clin. Am. Soc. Nephrol*., 2, 1062.
40. Guidance for Industry. Population Pharmacokinetics. U.S. Department of Health and Human Services.Food and Drug Administration.Center for Drug Evaluation and Research (CDER). Center for Biologics Evaluation and Research (CBER).February 1999 CP 1.
41. Beal, S. L., Sheiner, L. B. 1982. *CRC Critical Rev. Biomed. Eng.*, 8, 195.
42. Duffull, S., Kirkpatrick, C., Van den Berg, L. The University of Queenland. TCIWorks. Target concentration intervention software.
43. Van Hest, R. M., Van Gelder, T., Vulto, A., Mathot, A. A. 2005. *Clin. Pharmacokinet*., 44, 1083.
44. Payen, S., Zhang, D., Maisin, A., Popon, M., Bensman, A., Bouissou, F., Loirat, C., Gomeni, R., Bressolle, F., Jacqz-Aigrain, E. 2005. *Ther. Drug Monit*., 27, 378.
45. Van Hest, R. M., Van Gelder, T., Bouw, R., Goggin, T., Gordon, R., Mamelok, R. D., Mathot, R. A. 2007. *Br. J. Clin. Pharmacol.*, 63,741.
46. De Winter, B. C. M., Van Gelder, T., Glander, P., Cattaneo, D., Tedesco-Silva, H., Neuman, I., Hilbrands, L., Van Hest, R. M., Pescovitz, M. D., Budde, K., Mathot, R. A. A. 2009. *Clin. Pharmacokinet*., 47, 827.

47. Van Hest, R. M., Van Gelder, T., Vulto, A. G., Shaw, L. M., Mathot, R. A. 2009. *Clin. Pharmacokin*. 48, 464.
48. Guillet, B. A., Simon, N. S., Purgus, R., Botta, C., Morange, S., Berland, Y., Pisano, P. S. 2010. *Ther. Drug Monit.*, 32,427.
49. Sherwin, C. M. T., Fukuda, T., Brunner, H. I., Goebel, J., Vinks, A. A. 2011. *Clin. Pharmacokin*., 50, 1.
50. Grinyó, J. M., Ekberg, H., Mamelok, R. D., Oppenheimer, F., Sánchez-Plumed, J., Gentil, M. A., Hernandez, D., Kuypers, D. R., Brunet, M. 2009. *Nephrol. Dial. Transplant.*, 24, 2269.