

Influence of *ABCB1* Gene Polymorphisms and P-Glycoprotein Activity on Cyclosporine Pharmacokinetics in Peripheral Blood Mononuclear Cells in Healthy Volunteers

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Abstract: The calcineurin inhibitor cyclosporine is removed from lymphocytes by the drug efflux transporter P-glycoprotein (P-gp) encoded by the *ABCB1* gene for which several single nucleotide polymorphisms (SNPs) have been identified. Of a total of 87 healthy volunteers genotyped for *ABCB1* G2677T/A and C3435T SNPs, 10 GG-CC and 9 TT-TT individuals were selected and received a single oral dose of cyclosporine. Peripheral blood mononuclear cell (PBMC) *ABCB1* mRNA expression, P-gp activity in CD4⁺ and CD8⁺ cells and the 24h cyclosporine pharmacokinetics in PBMCs and whole blood were determined. No correlation was observed between cyclosporine PBMC and whole blood levels (AUC₀₋₂₄, Spearman, $r_s=0.09$, $p=0.71$). Intraindividual PBMC and whole blood levels followed parallel profiles that did not significantly differ with respect to t_{max} (Wilcoxon, $p=0.53$) and $t_{1/2}$ ($p=0.49$). Significant negative correlations between cyclosporine $t_{1/2}$ in PBMCs and P-gp activity in CD4⁺ ($r_s=-0.82$, $p=0.007$) and CD8⁺ ($r_s=-0.72$, $p=0.03$) were observed among TT-TT subjects. Similarly, a negative correlation was detected in the GG-CC group between P-gp activity in CD4⁺ and cyclosporine PBMC AUC₀₋₂₄ ($r_s=-0.69$, $p=0.03$), as well as PBMC to whole blood AUC₀₋₂₄ ratio ($r_s=-0.60$, $p=0.07$). Tested *ABCB1* genotypes had no influence on cyclosporine pharmacokinetic parameters in PBMCs and whole blood. The haplotypes investigated were neither significantly correlated with PBMC *ABCB1* mRNA expression nor with P-gp activity in CD4⁺ and CD8⁺. In conclusion, cyclosporine PBMC pharmacokinetics was influenced by P-gp activity and cyclosporine whole blood concentrations did not predict PBMC drug levels, suggesting that despite values in the therapeutic range, some subjects could have inadequate intracellular drug levels.

Key Words: Cyclosporine, PBMCs, *ABCB1*, P-Glycoprotein, pharmacokinetics, TDM, pharmacogenetics.

INTRODUCTION

Cyclosporine (INN, ciclosporin), a calcineurin inhibitor used after organ transplantation [1], has highly variable blood pharmacokinetics [2], notably due to its metabolism by the cytochromes P450 (CYP) 3A4/5 enzymes [3] and its transport by the P-glycoprotein (P-gp) [4]. Cyclosporine has a narrow therapeutic index and is subject to many drug-drug interactions [5]. Therefore, therapeutic drug monitoring is needed in order to predict clinical efficacy [6]. Due to high distribution into erythrocytes [7], cyclosporine quantification is currently performed in whole blood [8].

P-gp is a member of the adenosine triphosphate-binding cassette family and acts as an efflux pump that removes drugs from the intracellular compartment [9]. It is located in many tissues, including the small intestine, liver, kidneys, blood-brain barrier and placenta and plays an important role in drug absorption, distribution and elimination [10]. P-gp is also located in the membrane of lymphocytes [11] and therefore could regulate cyclosporine intracellular amounts.

P-gp is encoded by the human *ABCB1* gene, also called multidrug resistance gene (*MDR1*), for which 48 different single nucleotide polymorphisms (SNPs) have been identified. The most studied SNPs are the non-synonymous G2677T/A (exon 21) resulting in two amino acid changes (Ala893Ser/Thr) and the synonymous C3435T (exon 26). These SNPs are not strictly allelic, they exhibit strong linkage disequilibrium and account for the majority of the described haplotypes [12]. The impact of these polymorphisms on *ABCB1* mRNA levels, P-gp expression or activity in various tissues, as well as on the pharmacokinetics of different substrates revealed conflicting results which should be further investigated [13].

Kemnitz *et al.* have shown that the proportion of peripheral blood mononuclear cells (PBMCs) expressing P-gp correlated significantly with the incidence of acute rejection episodes in heart transplant patients under cyclosporine therapy [14]. More recently, a trend toward higher P-gp expression in PBMCs was observed in cyclosporine-treated liver transplant patients with acute rejection [15]. Furthermore, higher cyclosporine retention was observed in P-gp lacking tumoral lymphoid cell lines than in P-gp expressing cells [16]. Taken together, these observations suggest that P-gp might play a crucial role in the immunosuppressive effect of

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cyclosporine by removing this drug out of its main intracellular lymphocyte target.

In this work, the 24h cyclosporine pharmacokinetics within PBMCs and whole blood was investigated in healthy volunteers receiving a single oral dose. The impact of *ABCB1* G2677T/A-C3435T haplotypes and P-gp activity in CD4⁺ and CD8⁺ cells on cyclosporine pharmacokinetics in PBMCs and whole blood were evaluated. We also studied the influence of these haplotypes on PBMC *ABCB1* mRNA levels and P-gp activity in CD4⁺ and CD8⁺ cells. Our hypothesis was that individuals presenting the haplotype TT-TT would have lower mRNA levels and P-gp activity and higher PBMC cyclosporine levels than GG-CC.

METHODS

Ethics and Subjects

The study was conducted according to the revised Declaration of Helsinki. The protocol was approved by the Ethics Committee of the University Hospitals of Geneva and by the Swiss Agency for Therapeutic Products (Swissmedic, Bern). Written informed consent was obtained from all subjects.

Healthy adult male volunteers were included in the study. Before participation, all subjects underwent a complete history and clinical examination. The non-inclusion criteria were current drug treatment, known drug allergies, regular smokers and the presence of any current infectious disease, hypertension, asthma, liver, renal, neurological or psychiatric diseases. Before the second part of the study, the subjects were asked to refrain from alcohol, caffeine, chocolate and grapefruit juice for 48h.

Study Design

The study was set in two phases. The first part consisted of *ABCB1* genotyping (SNPs G2677T/A and C3435T). Individuals presenting the haplotypes 2677GG-3435CC (wild-type homozygote) or 2677TT-3435TT (mutant homozygote) were selected for participation to the second phase of the study.

In the second phase, the selected subjects were admitted to the University Hospitals of Geneva after an overnight fast. Each volunteer received a single 2 mg/kg oral dose of cyclosporine microemulsion (Sandimmun Neoral[®], Novartis, Switzerland) diluted in 2 dl of orange juice. Whole blood samples (11 ml) were collected through an intravenous catheter before cyclosporine intake and at 1, 2, 3, 4, 6, 8 and 24h after drug administration for the determination of the pharmacokinetics in PBMCs and whole blood. Additional blood samples (24 ml) were collected before drug intake for *ABCB1* mRNA quantification and P-gp activity determination. A glucose 5% perfusion was administered during the first 8h and blood pressure and heart rate were monitored regularly. A complete meal was served 4h after drug administration.

Genotyping of *ABCB1* G2677T/A and C3435T SNPs

Genomic DNA was extracted from whole blood (200 µl) using the QIAamp DNA blood mini kit (QIAGEN, Hombrechtikon, Switzerland). *ABCB1* (Genebank# AC005068) G2677T/A and C3435T polymorphisms were determined in

a single multiplex PCR with fluorescent probe melting temperature analysis on a LightCycler (Roche, Rotkreuz, Switzerland). For the G2677T/A variant, a sensor probe modified with a locked nucleic acid (LNA) was used to improve allelic discrimination [17]. The genotyping reaction mixture (20 µl) contained: 3 mM MgCl₂, 500 nM of each primer, 200 nM of each classical probe, 100 nM of the LNA-containing sensor probe, 60 ng of DNA and 2 µl of the LightCycler-FastStart DNA Master Hybridization probes kit (Roche). Primers and probes were obtained from TIB MOLBIOL (Berlin, Germany) and their sequences are indicated in Table 1. PCR was performed according to a previously described program [17]. The following mean melting temperatures were obtained: for G2677T/A, 48.2°C, 40.8°C and 52°C for the G, T and A, respectively; for C3435T, 65.7°C and 57.8°C for the C and T, respectively. Standard deviations were ≤ 0.3°C. The reliability of the method was confirmed by analyzing DNA samples of known G2677T/A and C3435T *ABCB1* genotype.

Table 1. Primers and Probes used for *ABCB1* Genotyping

SNP	Oligonucleotide Sequence
C3435T	Fw: 5'-tggcaagaataaacgac Rv: 5'-gactcgaagaagcatgta Anc: 5'-acaacagccgggtggtgca-FL Sens: 5'-LC705-ggaagagatcgtgagggcag-PH
G2677T/A	Fw: 5'-gcaggagttgtgaaatgaaatg Rv: 5'-cgctctttagttgactca Anc: 5'-LC640-ctttctattcttcagctgttcc-PH LNA_Sens: 5'-ttcccagTaccttet-FL

Fw, forward primer; rev, reverse primer; anc, anchor probe; sens, sensor probe; LNA-sens, sensor probe containing a locked nucleic acid (upper case letter); LC705, LightCycler Red 705 dye; LC640, LightCycler Red 640 dye; FL, fluorescein; PH, phosphate.

ABCB1 mRNA Quantification

PBMCs were isolated from freshly collected whole blood (8 ml) by density gradient centrifugation (2700 rpm, 30 min, room temperature) using BD Vacutainer[®] CPT[™] tubes (Becton Dickinson, Franklin Lakes, NJ) and washed twice with phosphate-buffered saline (PBS) solution. After extraction using the NucleoSpin[®] RNA II kit (Macherey-Nagel, Düren, Germany), total RNA (200 ng) was reverse-transcribed using the iScript cDNA synthesis kit (BioRad, Hercules, CA) in a 20 µl final reaction volume containing oligo(dT) and random hexamer primers. The relative abundance of *ABCB1* mRNA was assessed by Taqman quantitative PCR, and human large ribosomal protein (RPL0) was used to normalize data. Briefly, commercially available TaqMan[®] gene expression assays including pre-designed PCR primers and a FAM[™] dye-labeled TaqMan MGB probe (Applied Biosystems, Warrington, UK) were used: the Hs00184491m1 assay for *ABCB1* and the Hs99999902m1 assay for RPL0. Reactions were performed in Taqman Universal PCR Master Mix (Applied Biosystems) using an iCycler iQ detection system (BioRad).

P-gp Activity in CD4⁺ and CD8⁺ Cells

PBMCs were isolated from two 8 ml whole blood samples as describe above. After purification, cells were counted with a Sysmex® XE-2100 instrument (Sysmex Corporation, Kobe, Japan), suspended in Dulbecco's Modified Eagle Medium (DMEM, Invitrogen, Groningen, The Netherlands) containing 10% Fetal Bovine Serum (FBS, Biochrom AG, Berlin, Germany) and incubated for 30 min with 500 ng/ml of the P-gp fluorescent substrate rhodamine 123 (Rh123, Sigma-Aldrich, Saint Louis, MO) at 37°C and 5% CO₂. The cells were washed twice with PBS at 4°C and incubated (400'000 cells per aliquot) for 15, 30, 60 and 90 min in DMEM + FBS 10% at 37°C and 5% CO₂ to allow Rh123 efflux, in presence and absence of the potent P-gp inhibitor elacridar (GlaxoSmithKline, Stevenage, England) at 2 µM. After washing, cells were incubated for 15 min at 4°C with mouse IgG (Sigma-Aldrich). Subsequent staining were preformed incubating cells for 30 min at 4°C with monoclonal antibodies: allophycocyanin (APC)-labelled mIgG1 anti-CD4 (Immunotech Beckman Coulter, Marseille, France) or phycoerythrin (PE)-labelled mIgG1 anti-CD8 (DakoCytomation, Glostrup, Denmark) or appropriate isotypic controls: APC-conjugated or PE-conjugated mIgG1, κ (Becton Dickinson, San Jose, CA). The cells were washed twice with PBS at 4°C containing 2% FBS and 0.1% sodium azide (Sigma-Aldrich) and resuspended in the same buffer containing 10 µg/ml of 7-aminoactinomycine D (7-AAD, Sigma-Aldrich). Stained cells were analyzed for four colour fluorescence (Rh123, CD8-PE, CD4-APC and 7-AAD) with a FACSCalibur flow cytometer (Becton Dickinson). Using this method, the intracellular amount of Rh123 was specifically quantified in CD4⁺ and CD8⁺ cells. The amount of Rh123 expelled specifically by P-gp was calculated as the difference between median fluorescence of cells treated or not with elacridar for each efflux period and expressed as a percentage of median fluorescence in control cells with no efflux period. P-gp activity was expressed as the area under the efflux-time curve from 0 to 90 min using the trapezoidal method.

Cyclosporine Quantification in PBMCs and Whole Blood

PBMCs were isolated from freshly collected whole blood (8 ml) as describe above and whole blood samples were collected in 3 ml EDTA tubes. Samples were stored at -80°C until the day of analysis. Cyclosporine levels in PBMCs [18] and whole blood (Ansermot *et al.*, submitted) were determined using automated on-line solid phase extraction methods coupled with liquid chromatography, electrospray ionisation and mass spectrometry detection.

Cyclosporine Pharmacokinetic Analysis

Cyclosporine pharmacokinetics in PBMCs and whole blood was determined by a non-compartmental model using WinNonLin® version 4.1 (Pharsight Corporation, Mountain View, CA). The time to peak (t_{max}) and maximum peak concentration (C_{max}) were obtained from concentration-time profiles. The elimination half-life ($t_{1/2}$) was calculated as $\ln 2/\lambda$, where λ represents the slope of the linear terminal part of the concentration-time curve after semi-logarithmic transformation. Area under the concentration-time curve (AUC) over 24h (AUC₀₋₂₄) was calculated with linear trapezoidal

method. The ratio of the AUC in PBMCs versus whole blood was calculated.

Statistical Analysis

Statistical analyses were performed with non-parametric tests using SPSS version 11 (SPSS Inc, Chicago, IL). Differences between groups were assessed using Mann-Whitney *U* test for independent samples and Wilcoxon signed ranks test for matched data. Associations were assessed using the Spearman rank-order correlation coefficient r_s . A value of $p < 0.05$ was considered statistically significant (two-sided tests).

RESULTS

ABCBI Genotyping

Eighty-seven healthy men were selected for *ABCBI* SNPs G2677T/A and C3435T genotyping. The most frequent haplotypes were 2677GT-3435CT, 2677GG-3435CC and 2677TT-3435TT with 33 (37.9%), 20 (23.0%) and 13 (14.9%) individuals, respectively. Nineteen subjects (10 GG-CC and 9 TT-TT) were selected for investigating mRNA expression, P-gp activity and cyclosporine pharmacokinetics. No significant age or body weight difference was observed between the groups (age range 19-58 years; weight range 62-90 kg).

Relationship Between *ABCBI* Haplotypes, mRNA Expression and P-gp Activity

No significant difference in *ABCBI* mRNA level in PBMCs was observed in function of *ABCBI* G2677T/A-C3435T haplotypes. Median (range) mRNA amount normalized to RPL0 was 1.6 (0.9-2.2) in the GG-CC group and 1.4 (0.9-2.4) in the TT-TT group (Mann-Whitney *U*-test, $p=0.91$). Interindividual variability was important in both groups.

No significant difference in P-gp activity in CD4⁺ or CD8⁺ cells was observed in function of *ABCBI* G2677T/A-C3435T haplotypes. In CD4⁺, we observed a trend towards lower P-gp activity in the TT-TT group compared with the GG-CC group (median (range): 691% (465-955%) vs 780% (522-1030%), Mann-Whitney *U*-test, $p=0.13$). In CD8⁺, the activity was similar in TT-TT and GG-CC individuals (median (range): 4192% (3208-5364%) vs 3955% (2298-4877%), $p=0.36$). P-gp activity in CD4⁺ cells did not significantly correlate with the one in CD8⁺ cells ($r_s=0.21$, $p=0.38$).

PBMC *ABCBI* mRNA level did not correlate with P-gp activity in CD4⁺ or CD8⁺ cells ($r_s=-0.07$, $p=0.79$ for CD4⁺; $r_s=-0.12$, $p=0.64$ for CD8⁺).

Cyclosporine Pharmacokinetics in PBMCs and Whole Blood

Fig. 1 illustrates pharmacokinetic profiles in PBMCs and whole blood. Pharmacokinetic parameters are provided in Table 2. Whereas interindividual variability was moderate for cyclosporine pharmacokinetics in whole blood (2.1-fold for AUC₀₋₂₄), variability was large in PBMCs (10.8-fold for AUC₀₋₂₄). No correlation was found between AUC₀₋₂₄ in PBMC and whole blood ($r_s=0.09$, $p=0.71$). Interindividual variability of the intracellular distribution, assessed through the PBMC to whole blood AUC₀₋₂₄ ratio, was 14.3-fold. Pa-

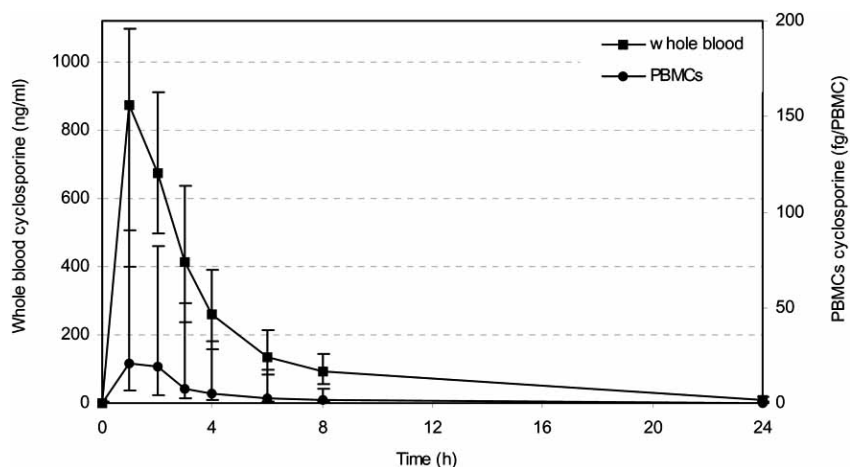


Fig. (1). Cyclosporine pharmacokinetic profiles in peripheral blood mononuclear cells (PBMCs) and whole blood in healthy volunteers after a single oral dose of 2 mg/kg (n=19). Curves represent median values and bars are minima and maxima.

rameters t_{max} and $t_{1/2}$ in PBMCs did not significantly differ from the estimates in whole blood (Wilcoxon signed ranks tests, $p=0.53$ and 0.49 respectively). No correlation was observed between age and cyclosporine pharmacokinetic parameters.

Influence of ABCB1 Haplotypes on Cyclosporine Pharmacokinetics

No significant difference was observed between 2677GG-3435CC and 2677TT-3435TT haplotypes for t_{max} , $t_{1/2}$, C_{max} and AUC_{0-24} , either in PBMCs or whole blood (Table 2). No significant difference was observed when considering PBMC to whole blood AUC_{0-24} ratio (Table 2, Mann-Whitney U-test, $p=0.49$).

Influence of P-gp Activity on Cyclosporine PBMC Pharmacokinetics

No correlation was observed between P-gp activity in $CD4^+$ or $CD8^+$ cells and cyclosporine pharmacokinetic pa-

rameters in PBMCs when considering all subjects together (Table 3). Among individuals with 2677TT-3435TT haplotype, subgroup analysis showed significant negative correlations between cyclosporine $t_{1/2}$ in PBMCs and P-gp activity in $CD4^+$ (Spearman $r_s=-0.82$, $p=0.007$) or $CD8^+$ ($r_s=-0.72$, $p=0.03$). Among subjects with 2677GG-3435CC haplotype, P-gp activity in $CD4^+$ correlated negatively with cyclosporine PBMC AUC_{0-24} ($r_s=-0.69$, $p=0.03$) and intracellular distribution (PBMC to whole blood AUC_{0-24} ratio, $r_s=-0.60$, $p=0.07$).

DISCUSSION

Relationship Between ABCB1 Haplotypes, mRNA Expression and P-gp Activity

Among the different identified ABCB1 SNPs, the exonic SNPs G2677T/A and C3435T are the most frequently studied and they have been shown to correlate with altered expression and activity of P-gp. In our study, we did not find any correlation between ABCB1 G2677T/A-C3435T haplo-

Table 2. Cyclosporine Pharmacokinetic Parameters in Whole Blood and PBMCs in Function of ABCB1 Haplotypes 2677GG-3435CC (n=10) and 2677TT-3435TT (n=9)

	Whole Blood			PBMCs		
	GG-CC	TT-TT	p	GG-CC	TT-TT	p
t_{max}	1.0 (1.0-2.0)	1.0 (1.0-2.0)	0.72	1.5 (1.0-4.0)	1.0 (1.0-3.0)	0.78
$t_{1/2}$	5.0 (4.2-6.2)	4.7 (3.9-6.4)	0.40	4.2 (3.3-7.7)	5.0 (2.9-13.8)	0.49
C_{max}	890 (619-1098)	750 (685-1081)	0.13	29.6 (13.4-82.6)	22.2 (7.3-90.0)	0.50
AUC_{0-24}	3564 (2262-4429)	3345 (2839-4825)	0.49	85.8 (41.3-303.4)	78.3 (28.0-172.8)	0.49
				GG-CC	TT-TT	p
PBMC AUC_{0-24} /whole blood AUC_{0-24}				0.0243 (0.0093-0.0829)	0.0203 (0.0058-0.0517)	0.49

Data are median (range). PBMC, peripheral blood mononuclear cell; t_{max} , time to peak (h); $t_{1/2}$ elimination half-life (h); C_{max} , maximum peak concentration (ng/ml blood or fg/PBMC); AUC_{0-24} , area under concentration-time curve from 0 to 24h (h*ng/ml blood or h*fg/PBMC); PBMC AUC_{0-24} /whole blood AUC_{0-24} [(h*fg/PBMC)/(h*ng/ml)]. No significant differences were observed between the groups.

Table 3. Spearman's Correlation Coefficients Between P-gp Activity in CD4⁺ and CD8⁺ Cells and PBMC Cyclosporine Pharmacokinetics

	P-gp Activity in CD4 ⁺			P-gp Activity in CD8 ⁺		
	whole sample	GG-CC	TT-TT	whole sample	GG-CC	TT-TT
t _{max}	-0.07	-0.14	0.02	-0.42	-0.36	-0.44
t _{1/2}	-0.22	0.58	-0.82**	-0.35	-0.03	-0.72*
C _{max}	-0.19	-0.49	-0.02	0.17	0.01	0.48
AUC ₀₋₂₄	-0.24	-0.69*	0.10	0.12	-0.16	0.53
PBMC AUC ₀₋₂₄ / whole blood AUC ₀₋₂₄	-0.22	-0.60*	0.10	0.10	-0.15	0.50

Whole sample n=19; GG-CC and TT-TT, *ABCB1* haplotypes G2677T/A-C3435T (n=10 and n=9); PBMC, peripheral blood mononuclear cell; t_{max}, time to peak (h); t_{1/2}, elimination half-life (h); C_{max}, maximum peak concentration (fg/PBMC); AUC₀₋₂₄, area under concentration-time curve from 0 to 24h (h·fg/PBMC); *p<0.05; **p<0.01.

types and *ABCB1* mRNA levels in PBMCs. Results are in accordance with those of Oselin *et al.* who did not observe any correlation between SNPs G2677T/A and C3435T and mRNA expression in CD4⁺, CD8⁺, CD19⁺ and CD56⁺ cells [19]. Other studies reported a trend to lower PBMC mRNA levels in volunteers with 3435TT genotype compared to 3435CC [20,21] and a significant decrease in *ABCB1* transcripts in PBMCs isolated from HIV-1-infected patients with 3435TT genotype [22]. However mRNA levels does not necessarily reflect protein surface expression.

No significant influence of *ABCB1* G2677T/A-C3435T haplotypes on P-gp activity in CD4⁺ or CD8⁺ cells was observed. However, TT-TT individuals tended to display lower P-gp activity in CD4⁺. Our results are in agreement with previous studies reporting a lack of correlation between SNPs G2677T/A or C3435T and P-gp activity in CD4⁺ or CD56⁺ cells [23,24]. Other studies have shown a significant decrease of P-gp activity in 3435TT CD56⁺ cells [20,21].

We have observed that PBMC *ABCB1* mRNA levels did not correlate with P-gp activity in CD4⁺ and CD8⁺ cells. This could be explained by the fact that the two methods investigated different cell populations and because no significant correlation was present between P-gp activity in CD4⁺ and CD8⁺ cells. In a previous study, no relationship was found between *ABCB1* mRNA levels and P-gp activity in PBMCs [25]. A correlation between P-gp cell-surface expression and P-gp activity in PBMCs was reported [26], but not confirmed [27].

Several other factors not evaluated in our study affect P-gp expression or activity, including genetic mutations, splicing, transcriptional regulation, stability of mRNA and post-translational modifications [28]. Furthermore, endogenous factors might also influence the transporter activity as reported for elevated cellular cholesterol levels that markedly increase P-gp activity in PBMCs [29].

Cyclosporine Pharmacokinetics in PBMCs and Whole Blood

The immunosuppressive action of cyclosporine is due to its binding to cyclophilin, a cytoplasmic receptor present in

T lymphocytes, resulting in an inhibition of the key enzyme calcineurin, leading to a decrease in cytokine production [30]. Calcineurin inhibition has been correlated with cyclosporine concentration in human PBMCs [31]. Thus, it could be relevant to measure drug amounts at the level of its target site, the intracellular compartment. To the best of our knowledge, the present investigation is the first description of simultaneous PBMC and whole blood single dose cyclosporine pharmacokinetics according to *ABCB1* genotype. Our results point to a large interindividual variability in PBMC cyclosporine pharmacokinetics and the absence of correlation between PBMC and whole blood levels, underlying the uncertainty of whole blood concentrations in predicting PBMC drug levels. These observations suggest that despite whole blood levels in the therapeutic range, some subjects could have inadequate amounts of drug in the target cells. Barbari *et al.* noticed lower cyclosporine PBMC trough or maximum levels in renal transplant patients with acute rejection compared to individuals without rejection, whereas no differences were observed in whole blood trough or maximum concentrations [32-35]. Our results also point to the fact that intraindividual PBMC and whole blood cyclosporine kinetic profiles were closely related. Our results are based on single dose pharmacokinetics and should be transposed with precaution to transplant patients receiving multiple doses. Single dose kinetics may not necessarily reflect the kinetics of the regimen at steady-state.

Influence of *ABCB1* Haplotypes on Cyclosporine Pharmacokinetics

No correlation was observed between *ABCB1* G2677T/A-C3435T haplotypes and the pharmacokinetic parameters in PBMCs and whole blood. These results indicate that the haplotypes evaluated in this study might not be useful to predict cyclosporine pharmacokinetics. The impact of *ABCB1* SNPs on whole blood cyclosporine pharmacokinetics differs among published studies. Significant higher whole blood concentration/dose ratios were observed for transplant patients with 3435TT genotype compared to 3435CC [36]. A non significant trend toward higher cyclosporine dose-normalized AUC₀₋₄ was observed in patients with 3435TT genotype compared to 3435CC [37]. When considering the

C1236T-G2677T/A-C3435T haplotypes, non significantly higher dose-adjusted AUC₀₋₄ and AUC₀₋₁₂ [38] or C_{max}, C_{min}, AUC₀₋₄ and AUC₀₋₁₂ [39] were reported in patients carriers of the variants alleles. Several other studies failed to observe an influence of ABCB1 SNPs on different cyclosporine pharmacokinetic parameters [40-43]. These contradicting observations could be partially explained by the fact that other factors, notably CYP3A4/5, are involved in the pharmacokinetics of this drug and thus it remains difficult to isolate the impact of P-gp alone.

Influence of P-gp Activity on Cyclosporine PBMC Pharmacokinetics

Subgroup analyses revealed significant negative correlations between cyclosporine PBMC t_{1/2} and P-gp activity in CD4⁺ and CD8⁺ cells, indicating that P-gp activity could have an influence on drug efflux. Furthermore, P-gp activity in CD4⁺ negatively correlated with cyclosporine PBMC AUC and with PBMC to whole blood AUC ratio, suggesting that individuals with high transporter activity could have lower intracellular drug levels. P-gp activity in CD4⁺ or CD8⁺ cells failed to correlate globally with pharmacokinetic parameters. This could be explained in part by the fact that CD4⁺ and CD8⁺ cells together represent only 49% ± 11% (mean ± SD) of the total PBMCs and a potential influence of P-gp could be masked by different activities in other PBMC subpopulations. No significant correlation was observed between P-gp activity in CD4⁺ and CD8⁺ cells, suggesting different efflux levels in each subpopulation. To assess more accurately the impact of P-gp activity on PBMC cyclosporine distribution, it would be of interest to quantify cyclosporine specifically in the same subpopulation where P-gp activity is evaluated. Another explanation is that in addition to P-gp, PBMCs express several proteins implicated in the transport of drugs. Batiuk *et al.* observed a higher recovery of calcineurin activity in cyclosporine-treated lymphocytes with high P-gp expression compared to low P-gp cells, suggesting an influence of P-gp in cyclosporine efflux. However, addition of a P-gp inhibitor did not prevent recovery, indicating the presence of other mechanisms affecting efflux [31]. For example, MRP1 and MRP2 transporters have been identified in PBMCs and it has been shown that cyclosporine was a substrate of these proteins [44]. Furthermore, cyclosporine is a potent inhibitor of P-gp and thus could reduce the potential effect of distinctive transporter activity [4]. Finally, cyclosporine distribution among different whole blood components depends on the presence of hydrophobic binding sites, including erythrocytes, lipoproteins and cyclophilin B [45,46]. The unbound drug concentration is also an important factor involved in the penetration of cyclosporine into lymphocytes [7].

In conclusion, the present study showed the absence of correlation between cyclosporine PBMC and whole blood levels, suggesting that whole blood concentration measured for therapeutic drug monitoring is not a good predictor of the target site concentration. The fact that cyclosporine PBMC and whole blood concentrations were closely related for each individual suggests that a single intracellular measurement might be an interesting complement to cyclosporine whole blood monitoring. Cyclosporine PBMC kinetics was influ-

enced by P-gp activity; significant negative correlations were observed between P-gp activity and cyclosporine t_{1/2} and levels in PBMCs. The clinical impact of these results needs however to be addressed in further controlled prospective clinical studies involving transplant patients taking multiple doses with cyclosporine concentrations at steady-state.

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ABBREVIATIONS

7-AAD	=	7-aminoactinomycine D
APC	=	Allophycocyanin
AUC	=	Area under the concentration-time curve
C _{max}	=	Maximum peak concentration
CYP	=	Cytochrome P450
DMEM	=	Dulbecco's modified eagle medium
FBS	=	Fetal bovine serum
LNA	=	Locked nucleic acid
MDR1	=	Multidrug resistance gene
PBMCs	=	Peripheral blood mononuclear cells
PBS	=	Phosphate-buffered saline
PE	=	Phycoerythrin
P-gp	=	P-glycoprotein
Rh123	=	Rhodamine 123
RPL0	=	Human large ribosomal protein
SNPs	=	Single nucleotide polymorphisms
t _{1/2}	=	Elimination half-life
t _{max}	=	Time to peak

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