DOI:10.1111/j.1365-2125.2004.02182.x

MDR1 haplotypes derived from exons 21 and 26 do not affect the steady-state pharmacokinetics of tacrolimus in renal transplant patients

Ingrid Mai,[*] Elke S. Perloff,[*] Steffen Bauer,[*] Mark Goldammer,[*] Andreas Johne,[*] Guido Filler,[‡] Klemens Budde[†] & Ivar Roots[*]

[*]Institute of Clinical Pharmacology and [†]Department of Internal Medicine and Nephrology, Charité-University Medicine Berlin, Germany, [‡]Division of Pediatric Nephrology, Department of Pediatrics, Children's Hospital of Eastern Ontario, University of Ottawa, Canada

Correspondence
Dr Ingrid Mai, Institut für Klinische Pharmakologie, Charité-Universitätsmedizin Berlin, Schumannstr. 20/21, 10098 Berlin, Germany.
Tel: +49-30-450 525187
Fax: +49-30-450 525932
E-mail: ingrid.mai@charite.de

Keywords
P-glycoprotein, *MDR1*, haplotype, tacrolimus, pharmacokinetics, patients

Received
4 June 2003
Accepted
1 March 2004

Aim
This retrospective study investigated the influence of *MDR1* haplotypes derived from the polymorphisms 2677G > T (exon 21) and 3435C > T (exon 26) on the pharmacokinetics of the immunosuppressant drug tacrolimus in 73 renal transplant patients.

Methods
Based on both variants of SNPs 2677 and 3435, four different haplotypes and eight different genotypes were identified in the study sample. Tacrolimus trough concentrations (C_0) were compared between different SNP variants and genotypes, as well as between carriers and noncarriers of each haplotype. Additionally, CYP3A5 genotype (6956G > A) was determined.

Results
No significant differences were observed between groups. Differences in mean tacrolimus C_0 values between carriers and noncarriers of each haplotype ranged from –0.04 μg/litre (95% confidence interval: –0.53 to 0.60) to –23 μg/litre (–1.07 to 1.53). No association was found between CYP3A5*1/*3 genotype and tacrolimus Co concentractions.

Conclusion
MDR1 haplotypes derived from the SNPs 2677G > T (exon 21) and 3435C > T (exon 26) do not influence the pharmacokinetics of tacrolimus in renal transplant patients.

Introduction

P-glycoprotein (P-gp) is an ATP-dependent drug efflux pump that is constitutively expressed in several human tissues (for example the epithelia of the small intestine, the blood–brain barrier endothelia, liver, kidney, testes, lymphocytes) [1, 2] as well as in certain cancer cells [3]. More than 50 single nucleotide polymorphisms have been identified in the P-gp encoding *MDR1* gene, some of which have been associated with differences in protein expression and function. However, findings on the association between *MDR1* genotype and drug disposition have been inconsistent. As recently reviewed by Fromm [4], the homozygous 3435TT variant has been associated with increased, decreased, or unchanged plasma concentrations of the P-gp substrates digoxin and fexofenadine [5–9].

Tacrolimus is a macrolide immunosuppressant frequently used after kidney, liver, and heart transplantations. Tacrolimus undergoes extensive hepatic metabolism by CYP3A4, and is also a substrate for P-gp [10]. These processes are the basis of a variety of drug–drug interactions between tacrolimus and certain

antibiotics, antiretrovirals, azol-antifungals, as well as the herbal antidepressant Saint John's wort [11–15].

Previous studies found no significant effect of the *MDR1* 2677G > T and 3435C > T polymorphisms on tacrolimus pharmacokinetics [16, 17]. However, recent findings indicate that analysis of *MDR1* haplotypes may be superior to that of single nucleotide polymorphisms in revealing genotype–phenotype associations both in pharmacokinetic studies [18] and in the assessment of disease risk [19]. The present retrospective study therefore investigated the influence of *MDR1* haplotypes derived from SNPs 2677G > T and 3435C > T on the steady-state pharmacokinetics of tacrolimus in renal transplant patients.

Methods

Patients

Seventy-five Caucasian renal transplant patients (42 men and 33 women) aged 18–72 years (mean age: 42, SD: 16) were studied. Patients were treated at the Department of Internal Medicine and Nephrology, University Medical Center Charité, Berlin, between September 1999 and November 2002. Mean body weight was 71 kg (SD: 16 kg) and ranged from 44 to 117 kg. Inclusion criteria were: at least 6 months post transplant, stable tacrolimus dose, and stable allograft function (creatinine clearance >30 ml min^{-1}). The study was approved by the ethics committee of the University Medical Center Charité, Humboldt University of Berlin, and all patients gave their written informed consent.

Genotyping

Genomic DNA was extracted from venous blood using a standard phenol/chloroform procedure and screened for the SNPs 2677G > T (exon 21) and 3435C > T (exon 26) of the *MDR1* gene using PCR-RFLP analysis [20]. SNP positions refer to the *MDR1* cDNA sequence with the first base of the ATG start codon set to 1 [21]. CYP3A5 genotype was determined as described previously [22].

Haplotype analysis

Haplotype analysis included the SNPs 2677G > T and 3435C > T on the basis of the linkage disequilibrium observed between both positions and on their possible functional relevance [8, 18]. Seventy-five patients were included in the study and all were genotyped. Two patients (2 men) carrying the rare 2677A variant were excluded from the analysis. Two patients carrying the rare 2677A variant were excluded from the analysis. Each genotype was assigned a haplotype pair. For individuals homozygous at both variants or heterozygous at only one position, the haplotypes could be assigned unambiguously. For one of the nine genotypes (genotype 11), two haplotype pairs, 11/22 and 12/21, are possible. However, haplotype 11/22 is much more likely based on haplotype frequencies that have been calculated [23] previously for a random sample of 687 subjects [18]. With the assumption that each haplotype is inherited dominantly, comparisons were performed between carriers and noncarriers of each particular haplotype. *MDR1* haplotypes and genotypes are shown in Table 1.

Tacrolimus dosage and measurement

Patients received tacrolimus (Prograf™, Fujisawa Healthcare, Inc., Deerfield, IL, USA) twice daily at stable and individually adjusted doses (mean: 4.5 mg day^{-1}, SD: 1.3 mg day^{-1}) for immunosuppressant therapy after renal transplantation. Tacrolimus trough concentrations were determined by the Microparticle Enzyme Immunoassay (MEIA) using an IM_X analyser (Abbott Laboratories, Chicago, IL, USA). Assay precision ranged from 7 to 16% (at concentrations of 2.4–21.4 ng ml^{-1}), and accuracy from 88 to 115% (2–25 ng ml^{-1}). The lower limit of determination was 1.5 ng ml^{-1}.

Data analysis

Tacrolimus trough concentrations (C_0) were corrected for the individual daily dose. Data were analysed using ANOVA (SPSS 10.0, SPSS Inc., Chicago, IL, USA) and differences were considered statistically significant at $P < 0.05$. Genotype frequencies and 95% confidence intervals were calculated using Systat 8.0 (SPSS Inc., Chicago, IL, USA).

Results

Of the 73 patients, 25 and 15 were carriers of the wild-type variants 2677GG and 3435CC, respectively. For the polymorphism at position 2677 (exon 21), 32 patients were heterozygous and 16 carried the homozygous 2677TT variant. For the polymorphism at position 3435 (exon 26), 38 patients were heterozygous and 20 carried the homozygous 3435TT variant. The frequencies of SNPs 2677 and 3435 in this study were consistent with previous findings in a larger ($n = 461$) randomly sampled Caucasian population [20] (Table 2), and the cohort followed Hardy–Weinberg equilibrium for both SNPs.

Different allelic combinations of both variants of SNPs 2677 and 3435 can result in four possible haplotypes and nine possible genotypes (Table 1). All possi-

Table 1
Four haplotypes and nine genotypes of *MDR1* derived from SNP 2677G > T (exon 21) and SNP 3435C > T (exon 26)

	Genotype 00		Genotype 01		Genotype 02		Genotype 10		Genotype 11		Genotype 12		Genotype 20		Genotype 21		Genotype 22	
Pos 2677	g	g	g	g	g	g	g	T	g	T	g	T	T	T	T	T	T	T
Pos 3435	C	C	C	T	T	T	C	C	C	T	T	T	C	C	C	T	T	T
Haplotype	11	11	11	12	12	12	11	21	11	22	12	22	21	21	21	22	22	22
or																		
Pos 2677									g	T								
Pos 3435									T	C								
Haplotype									12	21								

Positions refer to the MDR1 cDNA sequence with the first base of the ATG start codon set to 1. The rare 2677A variant was not considered in genotype and haplotype analysis. Genotype coding: 0 homozygous identical to reference sequence (2677G, 3534C), 1 heterozygous, 2 homozygous different from reference sequence. The first digit refers to position 2677, the second digit to position 3435. Haplotype coding: 1 identical to reference sequence (2677G, 3534C), 2 different from reference sequence. The first digit refers to position 2677, the second digit to position 3435. For genotype 11, a second haplotype pair (12/21) is possible, however haplotype pair 11/22 is much more likely based on haplotype frequencies.

ble haplotypes and eight genotypes were detected in the study population. The most frequent haplotypes were 11 and 22, both occurring with a frequency of more than 30%. Haplotypes 12 and 21 occurred with frequencies of 11% and 2.1%, respectively, and were only found in combination with either haplotype 11 or 22. Accordingly, genotypes 11, 00 and 22 were the most frequent and occurred in 36%, 19% and 19% of the study sample, respectively (Table 2). Genotype and haplotype frequencies observed in this study are in agreement with previous findings in a large ($n = 687$) randomly sampled Caucasian population [18] (Table 2). The different genotype groups were comparable with regard to age, gender, time post transplant, tacrolimus dose and comedications (data not shown).

In 67 out of the 73 subjects, the *CYP3A5* genotype was also determined. Nine patients were found to be heterozygous for the 6986G > A variant (*CYP3A5*1/*3*), 58 patients were found to be homozygous (*CYP3A5*3/3*) and no homozygous genotypes (*CYP3A5*1/*1*) were detected.

Tacrolimus concentrations were compared between carriers of different SNP variants and genotypes derived from haplotype pairs (Table 1), as well as between carriers and noncarriers of each haplotype. No significant differences were observed between groups (Table 2).

Additionally, there was no difference in tacrolimus C_0 values for individuals with the *CYP3A5*1/*3* genotype (mean ± SD: 1.79 ± 1.02 µg/litre) compared to (*CYP3A5*3/*3*) (1.74 ± 1.51 µg/litre) (95% confidence interval of the mean difference –0, 73 to 0, 83).

Discussion

Data on the effects of individual *MDR1* polymorphisms on P-gp transporter activity and drug disposition has been inconsistent, particularly for the P-gp substrates digoxin and fexofenadine [4]. Johne et al. [18] suggested recently that the combination of certain SNP variants into haplotypes might more accurately predict P-gp activity. The authors were able to demonstrate significant differences in digoxin pharmacokinetics between carriers and noncarriers of haplotype 12 (2677G/3435T) [18]. Different studies have failed to detect a significant effect of the *MDR1* genotype at positions 2677 and 3435 on tacrolimus pharmacokinetics [16, 17], thus we tested whether *MDR1* haplotypes derived from SNPs 2677G > T and 3534C > T could explain the large interindividual differences in tacrolimus trough concentrations in allograft recipients. Four different haplotypes and eight different genotypes were found in the study population, and the observed frequencies of SNP variants, genotypes and haplotypes were in agreement with those reported for large random samples [18, 20] (Table 2). The study confirmed that the SNPs at positions 2677 and 3435 do not affect tacrolimus pharmacokinetics. In addition, no significant differences between tacrolimus trough concentrations were found between carriers of different *MDR1* genotypes and

Table 2
Frequencies of *MDR1* polymorphisms at positions 2677 and 3435, genotypes, and haplotypes together with corresponding tacrolimus trough concentrations

	Frequency			Tacrolimus	
	Observed (n = 73)*	Random sample	n	C_0 (μg/litre)†	
Exon 21		(n = 461)‡			
2677GG	0.342 (0.210–0.484)	0.309	25	1.70 (1.20)	
2677GT	0.438 (0.294–0.580)	0.492	32	1.98 (1.18)	
2677TT	0.219 (0.111–0.352)	0.161	16	1.50 (0.68)	
Exon 26					
3435CC	0.205 (0.101–0.336)	0.208	15	1.88 (1.46)	
3435CT	0.521 (0.370–0.658)	0.505	38	1.77 (0.80)	
3435TT	0.274 (0.154–0.412)	0.286	20	1.72 (1.33)	
Genotype		(n = 687)§			
00	0.192 (0.080–0.338)	0.179	14	1.89 (1.51)	
01	0.137 (0.045–0.273)	0.109	10	1.53 (0.63)	
02	0.014 (0.000–0.974)	0.023	1	0.90	
10	0.014 (0.000–0.974)	0.019	1	1.80	
11	0.356 (0.204–0.515)	0.386	26	1.84 (0.86)	
12	0.068 (0.010–0.183)	0.105	5	2.74 (2.30)	
21	0.027 (0.000–0.121)	0.012	2	2.10 (0.99)	
22	0.192 (0.080–0.338)	0.167	14	1.41 (0.63)	
Haplotype		(n = 687)§			
Carrier 11	0.349 (0.405–0.279)	0.433	51	1.79 (1.02)¶	–0.53/0.60**
Noncarrier 11			22	1.75 (1.29)	
Carrier 12	0.110 (0.059–0.172)	0.133	16	1.87 (1.42)	–0.51/0.74
Noncarrier 12			57	1.76 (1.00)	
Carrier 21	0.020 (0.002–0.062)	0.018	3	2.00 (0.72)	–1.07/1.53
Noncarrier 21			70	1.77 (1.12)	
Carrier 22	0.322 (0.251–0.381)	0.416	47	1.82 (1.07)	–0.43/0.65
Noncarrier 22			26	1.71 (1.18)	

Two heterozygous carriers of the rare 2677A variant were excluded from the analysis.
**95% confidence intervals are in parentheses. †Values have been corrected for dose by dividing by each patient's daily tacrolimus dose. ‡Randomly drawn Caucasian sample [20]. §Randomly drawn Caucasian sample [18].* ¶Values are means with standard deviations in parentheses. No significant differences were detected using *ANOVA*. ***95% confidence interval of the difference. No significant differences were detected using a two-sided t-test. Demographic data and tacrolimus dose for the four most frequent genotypes.*

haplotypes. Unlike digoxin or fexofenadine, which are largely eliminated unchanged, tacrolimus is not only a P-gp substrate, but also undergoes extensive intestinal and hepatic metabolism, mainly by CYP3A [10]. Thus P-gp mediated intestinal efflux is not the only mechanism determining the bioavailability of tacrolimus, which may explain the lack of association between *MDR1* genotype or haplotype and its pharmacokinetics. Thervet et al. found that the *CYP3A5* genotype can influence tacrolimus dose requirements [24]. However, our study population did not include any homozygous (*CYP3A5*1/*1*) individuals and the heterozygous genotype (*CYP3A5*1/*3*) did not affect tacrolimus trough concentrations.

Recent studies also indicate that the *MDR1* polymorphisms at positions 2677 and 3435 may be related to the incidence of side-effects (of tacrolimus, amitriptyline) or predict treatment outcome (for antiretroviral combination therapy), presumably as a result of altered P-gp activity in blood–brain barrier endothelial cells [25–27]. Although the present study showed no significant association between *MDR1* haplotype and tacrolimus pharmacokinetics, an effect on tissue distribution or toxicity of the drug cannot be excluded.

It should also be noted that this cohort size was relatively small and a power analysis indicated that, in the case of the rare haplotype 21, detecting a phenotype/genotype association was unlikely. However, for haplotypes 11, 12 and 22, sample sizes were sufficiently large to have detected a 50% decrease in tacrolimus trough concentrations with a power of >0.8 and a type I error of 0.05 (based on a SD of 1.1 μg/litre).

Our study has limitations because of its retrospective nature. Given that the genotype and haplotype frequencies observed in this study are in agreement with those reported in large random samples, the study sample appears to be representative of the population as a whole. Trough concentrations of tacrolimus assessed in this study were corrected for each dose; however, differences in age, weight, diet, co-medication or underlying disease could not be controlled.

In summary, this retrospective study in renal transplant patients found no significant effect of *MDR1* haplotypes derived from the single nucleotide polymorphisms 2677G > T and 3435C > T on tacrolimus trough concentrations.

The authors would like to thank Dr Karla Köpke for advice on haplotype analysis.

There was no outside financial support for this study; it was funded solely by the Charité-University Medicine Berlin.

References

1 Cordon-Cardo C, O'Brien JP, Casals D, Rittman-Grauer L, Biedler JL, Melamed MR, Bertino JR. Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood–brain barrier sites. Proc Natl Acad Sci USA 1989; 86: 695–8.

2 Sugawara I, Kataoka I, Morishita Y, Hamada H, Tsuruo T, Itoyama S, Mori S. Tissue distribution of P-glycoprotein encoded by a multidrug-resistant gene as revealed by a monoclonal antibody, MRK 16. Cancer Res 1988; 48: 1926–9.

3 Ling V, Kartner N, Sudo T, Siminovitch L, Riordan JR. Multidrug-resistance phenotype in Chinese hamster ovary cells. Cancer Treat Rep 1983; 67: 869–74.

4 Fromm MF. The influence of MDR1 polymorphisms on P-glycoprotein expression and function in humans. Adv Drug Deliv Rev 2002; 54: 1295–310.

5 Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmöller J, Johne A, Cascarbi I, Gerloff T, Roots I, Eichelbaum M, Bnnkmann U. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. Proc Natl Acad Sci USA 2000; 97: 3473–8.

6 Drescher S, Schaeffeler E, Hitzl M, Hofmann U, Schwab M, Brinkmann U, Eichelbaum M, Fromm MF. MDR1 gene polymorphisms and disposition of the P-glycoprotein substrate fexofenadine. Br J Clin Pharmacol 2002; 53: 526–34.

7 Gerloff T, Schäfer M, Johne A, Oselin K, Meisel C, Cascorbi I, Roots I. MDR1 genotypes do not discriminate between absorptive pharmacokinetic parameters of a single oral dose of 1 mg dixogin in healthy white volunteers. Br J Clin Pharmacol 2002; 54: 610–6.

8 Kim RB, Leake BF, Choo EF, Dresser GK, Kubba SV, Schwarz UI, Taylor A, Xie HG, McKinsey J, Zhou S, Lan LB, Schuetz EG, Wilkinson GR. Identification of functionally variant MDR1 alleles among European Americans and African Americans. Clin Pharmacol Ther 2001; 70: 189–99.

9 Kurata Y, Ieiri I, Kimura M, Morita T, Irie S, Urae A, Ohdo S, Ohtani H, Sawada Y, Higuchi S, Otsubo K. Role of human MDR1 gene polymorphism in bioavailability and interaction of digoxin, a substrate of P-glycoprotein. Clin Pharmacol Ther 2002; 72: 209–19.

10 Hebert MF. Contributions of hepatic and intestinal metabolism and P-glycoprotein to cyclosporine and tacrolimus oral drug delivery. Adv Drug Deliv Rev 1997; 27: 201–14.

11 Christians U, Jacobsen W, Benet LZ, Lampen A. Mechanisms of clinically relevant drug interactions associated with tacrolimus. Clin Pharmacokinet 2002; 41: 813–51.

12 Ibrahim RB, Abella EM, Chandrasekar PH. Tacrolimus–clarithromycin interaction in a patient receiving bone marrow transplantation. Ann Pharmacother 2002; 36: 1971–2.

13 Mai I, Störmer E, Bauer S, Krüger H, Budde K, Roots I. Impact of St John's wort treatment on the pharmacokinetics of tacrolimus and mycophenolic acid in renal transplant patients. Nephrol Dial Transplant 2003; 18: 819–22.

14 Toda F, Tanabe K, Ito S, Shinmura H, Tokumoto T, Ishida H, Toma H. Tacrolimus trough level adjustment after administration of fluconazole to kidney recipients. Transplant Proc 2002; 34: 1733–5.

15 Jain AK, Venkataramanan R, Shapiro R, Scantlebury VP, Potdar S, Bonham CA, Ragni M, Fung JJ. The interaction between antiretroviral agents and tacrolimus in liver and kidney transplant patients. Liver Transpl 2002; 8: 841–5.

16 Goto M, Masuda S, Saito H, Uemoto S, Kiuchi T, Tanaka K, Inui K. C3435T polymorphism in the MDR1 gene affects the enterocyte expression level of CYP3A4 rather than Pgp in recipients of living-donor liver transplantation. Pharmacogenetics 2002; 12: 451–7.

17 Macphee IA, Fredericks S, Tai T, Syrris P, Carter ND, Johnston A, Goldberg L, Holt DW. Tacrolimus pharmacogenetics: polymorphisms associated with expression of cytochrome p4503A5 and P-glycoprotein correlate with dose requirement. Transplantation 2002; 74: 1486–9.

18 Johne A, Köpke K, Gerloff T, Mai I, Rietbrock S, Meisel C, Hoffmeyer S, Kerb R, Fromm MF, Bnntmann U, Eitchelbaum M, Brackmöller J, Cascorli I, Roots I. Modulation of steady-state kinetics of digoxin by haplotypes of the P-glycoprotein MDR1 gene. Clin Pharmacol Ther 2002; 72: 584–94.

19 Atanasova S, von Ahsen N, Dimitrov T, Armstrong V, Oellerich M, Toncheva D. MDR1 haplotypes modify BEN disease risk: a study in Bulgarian patients with Balkan endemic nephropathy compared to healthy controls. Nephron Exp Nephrol 2004; 96: e7–13.

20 Cascorbi I, Gerloff T, Johne A, Meisel C, Hoffmeyer S, Schwab M, Schaeffeler E, Eltchelbaum M, Brinkmann U, Roots I. Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter MDR1 gene in white subjects. Clin Pharmacol Ther 2001; 69: 169–74.

21 Chen CJ, Clark D, Ueda K, Pastan I, Gottesman MM, Roninson IB. Genomic organization of the human multidrug resistance (MDR1) gene and origin of P-glycoproteins. J Biol Chem 1990; 265: 506–14.

22 Gashaw I, Kirchheiner J, Goldammer M, Bauer S, Seidemann J, Zoller K, Mrozikiewicz PM, Roots I, Brockmöller J. Cytochrome p450 3A4 messenger ribonucleic acid induction by rifampin in human peripheral blood mononuclear cells: correlation with alprazolam pharmacokinetics. Clin Pharmacol Ther 2003; 74: 448–57.

23 Terwillinger J, Ott J. Handbook of Human Genetic Linkage. Baltimore: Johns Hopkins University Press, 1994.

24 Thervet E, Anglicheau D, King B, Schlageter MH, Cassinat B, Beaune P, Legendre C, Daly AK. Impact of cytochrome p450 3A5 genetic polymorphism on tacrolimus doses and concentration-to-dose ratio in renal transplant recipients. Transplantation 2003; 76: 1233–5.

25 Roberts RL, Joyce PR, Mulder RT, Begg EJ, Kennedy MA. A common P-glycoprotein polymorphism is associated with nortriptyline-induced postural hypotension in patients treated for major depression. Pharmacogenomics J 2002; 2: 191–6.

26 Yamauchi A, Ieiri I, Kataoka Y, Tanabe M, Nishizaki T, Oishi R, Higuchi S, Otsubo K, Sugimachi K. Neurotoxicity induced by tacrolimus after liver transplantation: relation to genetic polymorphisms of the ABCB1 (MDR1) gene. Transplantation 2002; 74: 571–2.

27 Fellay J, Marzolini C, Meaden ER, Decosterd LA, Furer H, Back DJ, Buclin T, Chave JP, Opravil M, Pontaleo G, Ratelska D, Ruiz L, Schinkel AH, Vernazza P, Eap CB, Talenti A. Response to antiretroviral treatment in HIV-1-infected individuals with allelic variants of the multidrug resistance transporter 1: a pharmacogenetics study. Lancet 2002; 359: 30–6.