JPP Journal of Pharmacy and Pharmacology

JPP 2004, 56: 1055–1059

Received March 22, 2004
Accepted May 4, 2004
DOI 10.1211/0022357043914
ISSN 0022-3573

Effect of lansoprazole and rabeprazole on tacrolimus pharmacokinetics in healthy volunteers with CYP2C19 mutations

Fumio Itagaki, Masato Homma, Kenji Yuzawa, Masuhiro Nishimura, Shinsaku Naito, Nobuhiko Ueda, Nobuhiro Ohkohchi and Yukinao Kohda

Abstract

The aim of this study was to investigate the effects of the proton pump inhibitors (PPIs), lansoprazole and rabeprazole, on tacrolimus pharmacokinetics in healthy volunteers with mutations in the cytochrome P450 (CYP) 2C19 gene (*CYP2C19*). An open-label crossover study was performed with 19 healthy subjects. Tacrolimus (2 mg) was administered orally with and without lansoprazole (30 mg per day for 4 days) or rabeprazole (10 mg per day for 4 days). Blood concentrations of tacrolimus were determined before and 1, 2, 4 and 8 h after dosing. Genotyping for *CYP2C19* was conducted by a polymerase chain reaction–restriction fragment length polymorphism method. Coadministration of lansoprazole significantly decreased the oral tacrolimus clearance, resulting in an increase in the area under the blood concentration–time curve (AUC_{0-8}) (control vs with lansoprazole: 29.7 ± 3.5 vs 44.1 ± 5.0 ng h mL^{-1}, $P < 0.05$). Large individual variation was observed in the effects of lansoprazole on tacrolimus AUC_{0-8} owing to *CYP2C19* genotype status. The percent change for tacrolimus AUC_{0-8} in subjects with and without *CYP2C19* mutant alleles was 81% and 29%, respectively. Coadministration of rabeprazole also increased the mean AUC_{0-8} of tacrolimus, but the difference was not statistically significant. These observations suggest that drug interaction between tacrolimus and lansoprazole occurs in subjects with higher lansoprazole blood concentrations corresponding to *CYP2C19* genetic status. In contrast, rabeprazole has minimal effect on tacrolimus pharmacokinetics regardless of *CYP2C19* genotype status.

Department of Pharmaceutical Sciences, Institute of Clinical Medicine, University of Tsukuba, Ten-nodai, Tsukuba, Ibaraki 305-8575, Japan

Fumio Itagaki, Masato Homma, Yukinao Kohda

Department of Surgery, Institute of Clinical Medicine, University of Tsukuba, Ten-nodai, Tsukuba, Ibaraki 305-8575, Japan

Kenji Yuzawa, Nobuhiro Ohkohchi

Division of Pharmacology, Drug Safety and Metabolism, Otsuka Pharmaceutical Factory, Inc., Muya-cho, Naruto, Tokushima 772-8601, Japan

Masuhiro Nishimura, Shinsaku Naito, Nobuhiko Ueda

Correspondence: M. Homma, Department of Pharmaceutical Sciences, Institute of Clinical Medicine, University of Tsukuba, Ten-nodai, Tsukuba, Ibaraki 305-8575, Japan. E-mail: masatoh@mail.hosp.tsukuba.ac.jp

Acknowledgements and funding: We thank the volunteers who kindly agreed to participate in this study. We thank Dr Katashi Fukao and Mr Takashi Tomita for their useful suggestions and discussions. This research was supported by a grant-in-aid for Scientific Research from the Japan Society for the Promotion of Science and a grant-in-aid from the University of Tsukuba Research Projects. This work was partly supported by Fujisawa Pharmaceutical Co, Ltd.

Introduction

Tacrolimus is a substrate for the drug metabolizing enzyme cytochrome P450 (CYP) 3A4 in the liver and intestine, and for the drug transport protein P-glycoprotein (P-gp) (Saeki et al 1993). Drug interaction is therefore assumed to occur when tacrolimus is coadministered with agents capable of modifying CYP3A4 and P-gp (Christians et al 2002). The proton pump inhibitors (PPIs) lansoprazole and rabeprazole, which are metabolized by CYP systems (2C19 and 3A4), are often given with tacrolimus for the prevention or treatment of upper gastrointestinal complications in renal transplant recipients (Logan et al 2002). We reported drug interaction between tacrolimus and PPIs in a renal transplant recipient who had *CYP2C19* gene mutation (Homma et al 2002; Itagaki et al 2002). The blood concentration of tacrolimus was dramatically increased by coadministration of lansoprazole but not rabeprazole. Because tacrolimus and lansoprazole share CYP3A4 for their intestinal and hepatic elimination, lansoprazole potentially interacts with tacrolimus in patients with *CYP2C19* gene mutation, which codes for another enzyme that metabolizes lansoprazole (Katsuki et al 1997; Lorf et al 2000; Furuta et al 2001). The increased blood concentration of lansoprazole in patients with *CYP2C19* mutant alleles inhibits tacrolimus metabolism by CYP3A4, resulting in elevation of blood tacrolimus concentrations. Unlike lansoprazole, rabeprazole did not seem to alter tacrolimus pharmacokinetics, because the blood concentration of rabeprazole was less affected by the *CYP2C19* genotype status (Ishizaki & Horai 1999; Adachi et al 2000; Ieiri et al 2001). Therefore, drug interaction of tacrolimus and PPIs may differ depending on the *CYP2C19* genotype.

In the present study, we examined the effects of the PPIs lansoprazole and rabeprazole on tacrolimus pharmacokinetics in healthy volunteers to clarify the drug interaction of tacrolimus and lansoprazole. The role of genetic variation in *CYP2C19* in this interaction was also investigated.

Materials and Methods

Subjects

Nineteen healthy volunteers (aged 22 to 47 years; 50–90 kg; 17 male and two female; 18 Japanese and one Chinese) were enrolled in the study. None of the subjects had taken any drugs for at least 2 weeks before or during the study. Each subject had physically normal conditions and no history of significant medical illness. Written informed consent was obtained from each subject. The study protocol was approved in advance by the ethics review board of Tsukuba University.

Study protocol

This study was conducted according to an open-label 3-period design. Each subject received a single oral dose of 2 mg tacrolimus as a capsule (Prograf; Fujisawa Pharmaceutical Co. Ltd, Osaka, Japan) to determine the baseline pharmacokinetic profile of tacrolimus. After a 1-week washout interval, each subject received 30 mg lansoprazole (Takepron; Takeda Chemical Industries, Ltd, Osaka, Japan) or 10 mg rabeprazole (Pariet; Eisai Co. Ltd, Tokyo, Japan) orally once daily for 3 days. On the fourth day, each subject received a single dose of 2 mg oral tacrolimus plus either 30 mg lansoprazole or 10 mg rabeprazole. In our previous case report, drug interaction of tacrolimus/lansoprazole was observed on the fourth day after starting lansoprazole coadministration. Therefore, we planned to test this drug interaction on the fourth day of PPI treatment. Venous blood samples for determining tacrolimus concentrations were obtained before and 1, 2, 4 and 8 h after administration of tacrolimus; plasma samples for determining lansoprazole concentrations were collected at the same intervals. No clinically undesirable signs or symptoms attributable to the study drugs were observed during the study period. Of the 19 volunteers, fifteen completed the 3-period and four participated in the tacrolimus alone and lansoprazole phase but not rabeprazole phase.

Genotyping for *CYP2C19*

CYP2C19 genotyping was conducted by a polymerase chain reaction–restriction fragment length polymorphism method with genomic DNA isolated from peripheral venous blood leukocytes by using the GenTLE kit (Takara Shuzo, Shiga, Japan). The wild-type allele, *CYP2C19*1*, and two mutant alleles, *CYP2C19*2* (G681A in exon 5) and *CYP2C19*3* (G636A in exon 4), were determined by the methods of de Morais et al (1994) and Edeki et al (1996). Subjects were classified into one of three genotype groups as follows: extensive metabolizer (*CYP2C19*1/*1)*, intermediate metabolizer (*CYP2C19* 1/*2, *1/*3*), and poor metabolizer (*CYP2C19*2/*2, *2/*3*).

Determination of tacrolimus concentration in whole blood

The tacrolimus concentration in whole blood was measured by microparticle enzyme immunoassays (Abbott Laboratories, Abbott Park, IL, USA). The detection limit for tacrolimus was 1.5 ng mL^{-1}. The intra- and inter-day assay precision determined at 5.0 ng mL^{-1} and 10.6 ng mL^{-1} were less than 6.1% and 3.4%, respectively.

Determination of lansoprazole concentration in plasma

The plasma concentration of lansoprazole was measured by high-performance liquid chromatography (HPLC) as described by Karol et al (1995) with minor modifications. In brief, 0.5 mL plasma containing 50 μL omeprazole (10 μg mL^{-1}) as an internal standard was diluted with 0.5 mL of 10 mM potassium phosphate buffer (pH 9.0). The mixture was extracted with 2.5 mL diethyl ether/methylene chloride (7:3, vol/vol). This extraction procedure was repeated twice. The combined organic layer was transferred to a clean glass test tube and evaporated to dryness with nitrogen gas flow at 40°C. The residue was reconstituted with 50 μL ethanol and 150 μL mobile phase, and a 50-μL aliquot was injected onto the HPLC system. The HPLC system consisted of an AS-8020 auto-sampler, DP-8020 pump, UV-8020 UV detector set at a wavelength of 285 nm, and TSK-GEL ODS-80Ts column (150 mm × 4.6 mm i.d.; Tosoh, Tokyo, Japan). The mobile phase solvent, consisting of acetonitrile, 50 mM potassium phosphate (pH 7.0) and triethylamine (36:64:1.5), was delivered at a flow rate of 1.0 mL min^{-1}. The detection limit of lansoprazole was 50 ng mL^{-1} plasma, and the coefficients of variation at 100 to 1500 ng mL^{-1} were less than 8%.

Pharmacokinetic analysis

Pharmacokinetic parameters (maximum blood concentration (C_{max}) and area under the blood concentration–time curve from 0 to 8 h (AUC_{0-8})) were calculated using the WinNonlin pharmacokinetic software package (Pharsight Corporation, Mountain View, CA). The AUC_{0-8} values for tacrolimus and lansoprazole were calculated with the trapezoidal rule. Apparent oral clearance (CL/F) for tacrolimus was calculated as dose/AUC_{0-8}.

Statistical analysis

Data are presented as mean ± s.e.m. and were analysed with the statistical program StatView for Windows, version 5.0 (SAS Institute Inc., Cary, NC, USA). Statistical analysis for tacrolimus pharmacokinetic parameters between tacrolimus alone, lansoprazole-treated, and rabeprazole-treated phase were performed using Dunnett's test. The statistical analysis between *CYP2C19* genotype groups was

performed using the Mann-Whitney *U*-test. Differences in lansoprazole AUC among the three different *CYP2C19* genotypes were determined by the one-way analysis of variance. *P* values less than 0.05 were considered to be statistically significant.

Results

Pharmacokinetic profiles in the subjects who were co-administered tacrolimus with and without the PPIs are shown in Figure 1. The mean pharmacokinetic parameters for tacrolimus are summarized in Table 1. In the lansoprazole phase, the mean AUC_{0-8} was significantly increased and CL/F was significantly decreased compared with the control phase. In the rabeprazole phase, the mean AUC_{0-8} was slightly increased and the CL/F was slightly decreased compared with the control phase, but the differences were not statistically significant.

The large inter-individual variation was observed in the effects of lansoprazole on tacrolimus AUC_{0-8} (Figure 2). To clarify the individual difference of this drug interaction, we examined the genotypes for *CYP2C19* and plasma concentrations of lansoprazole. Of the 19 volunteers, eight were extensive metabolizers (EM), seven were intermediate metabolizers (IM) and four were poor metabolizers (PM). The AUC_{0-8} values for plasma lansoprazole corresponded to *CYP2C19* genotype status ($P < 0.0001$); mean AUC_{0-8} values in EM, IM and PM were 1554 ± 266, 3594 ± 945 and 8414 ± 465 ng h mL^{-1}, respectively.

The effects of lansoprazole on tacrolimus AUC_{0-8} were compared between the subjects with and without *CYP2C19* mutant alleles (i.e. IM/PM vs EM). Coadministration of lansoprazole significantly increased tacrolimus AUC_{0-8} in both IM/PM and EM (Figure 2). The tacrolimus AUC_{0-8} values for the lansoprazole phase in IM/PM were significantly higher than those in EM (52.9 ± 6.8 vs 32.1 ± 5.2 ng h mL^{-1}, $P < 0.05$). The mean

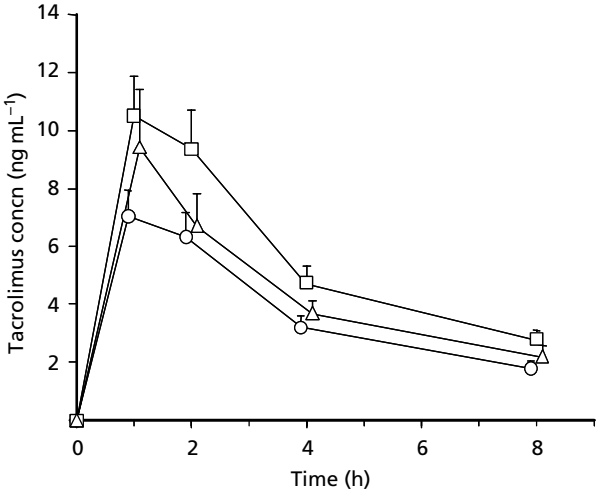

Figure 1 Pharmacokinetic profiles of tacrolimus coadministered with and without proton pump inhibitors in healthy volunteers: ○, tacrolimus alone (control, n = 19); □, tacrolimus plus 30 mg lansoprazole (n = 19); Δ, tacrolimus plus 10 mg rabeprazole (n = 15). Data are presented as mean ± s.e.m.

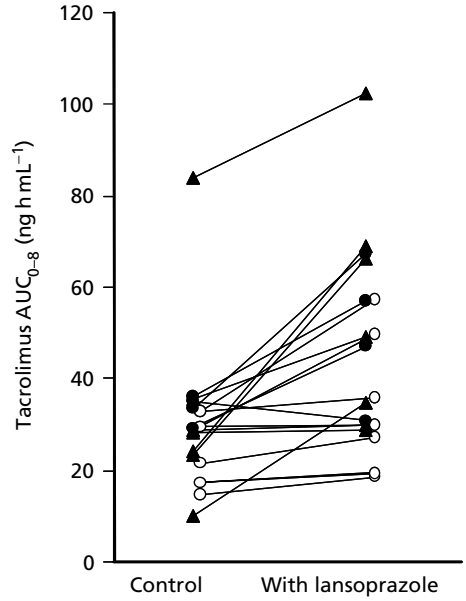

Figure 2 Effect of coadministration of lansoprazole on area under blood concentration–time curves (AUC_{0-8}) of tacrolimus: ○, extensive metabolizers; ▲, intermediate metabolizers; ●, poor metabolizers.

Table 1 Pharmacokinetic parameters of tacrolimus coadministered with and without proton pump inhibitors in healthy volunteers

Parameter	Control (n = 19)	With lansoprazole (n = 19)	With rabeprazole (n = 15)
AUC_{0-8} (ng h mL^{-1})	29.7 ± 3.5 (10.1–83.8)	44.1 ± 5.0* (18.5–101.8)	35.0 ± 4.7 (15.4–87.1)
CL/F (L h^{-1} kg^{-1})	1.23 ± 0.13 (0.48–2.96)	0.85 ± 0.09* (0.39–1.69)	1.03 ± 0.11 (0.44–1.88)
C_{max} (ng mL^{-1})	8.1 ± 0.9 (2.6–19.4)	11.3 ± 1.4 (3.3–27.7)	10.2 ± 1.9 (4.5–32.1)

AUC_{0-8}, area under the concentration–time curve; CL/F, clearance; C_{max}, maximum concentration of tacrolimus. Data represent mean ± s.e.m. (range). *$P < 0.05$ compared with control phase.

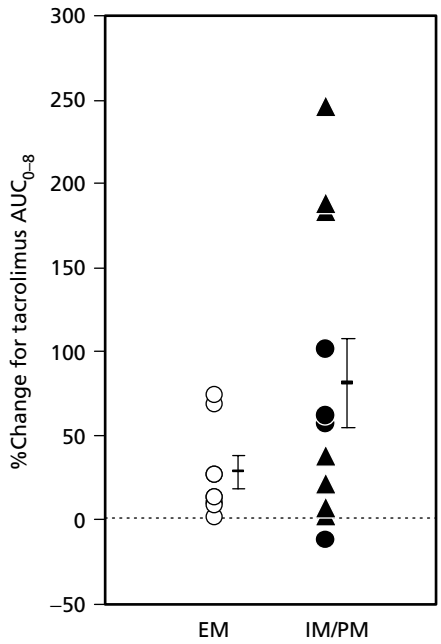

Figure 3 Percent change for area under blood concentration–time curves (AUC_{0-8}) of tacrolimus by coadministration of lansoprazole in different *CYP2C19* genotype status, extensive metabolizers (EM; ○), and intermediate metabolizers (▲) and poor metabolizers (●) (IM/PM). Bars indicate mean values with s.e.m.

percent changes for tacrolimus AUC_{0-8} were +81% (range −12% to +247%) in IM/PM and +29% (range +1% to +74%) in EM (Figure 3).

Discussion

Blood concentration-dependent adverse events, such as nephrotoxicity, neurotoxicity, glucose metabolism disturbances, gastrointestinal disturbances and infections, have been reported in tacrolimus treatment (Plosker & Foster 2000). The dose adjustment providing optimal blood tacrolimus concentrations is therefore required for safe multiple medication in renal transplantation.

The present study revealed that coadministration of lansoprazole significantly increased tacrolimus blood concentrations compared with tacrolimus alone. The degrees of interaction between tacrolimus and lansoprazole were varied depending on the genetic background.

In our previous case report, we hypothesized that the tacrolimus–lansoprazole drug interaction might be associated with the genotype of *CYP2C19* (Homma et al 2002; Itagaki et al 2002). We considered that the higher plasma concentrations of lansoprazole in subjects with *CYP2C19* mutant alleles inhibited tacrolimus metabolism by CYP3A4, resulting in elevation of blood tacrolimus concentrations. This hypothesis was prompted by the previous reports of Furuta et al (1999) and Kosuge et al (2001). Their data indicated that lansoprazole metabolism inclined to CYP3A4 in subjects with *CYP2C19* gene mutations and the risk of interaction between the drugs metabolized by CYP3A4 increased. The present results agree with the hypothesis as the marked elevation of blood tacrolimus when coadministered with lansoprazole was found in the subjects with *CYP2C19* mutated alleles (Figures 2 and 3).

The fact that some subjects without *CYP2C19* gene mutations experienced an increase in tacrolimus AUC implies the limitation of explaining this drug interaction by *CYP2C19* genotype status alone. Another factor, *MDR1*-encoded drug transporter P-gp, which affects tacrolimus absorption in the intestine, may be involved in this drug interaction. Hashida et al (2001) reported that the tacrolimus blood concentration was inversely related to *MDR1* expression in liver transplant recipients. Pauli-Magnus et al (2001) indicated that PPIs (omeprazole, pantoprazole and lansoprazole) were substrates and inhibitors of P-gp. Taken together, these reports suggest that lansoprazole enhances tacrolimus absorption by inhibiting P-gp-mediated intestinal efflux of tacrolimus. The inhibition might modify the drug interaction in both subjects with and without *CYP2C19* gene mutations.

Coadministration of rabeprazole, an alternative PPI, slightly increased tacrolimus AUC_{0-8}, but the effects were less than those of lansoprazole. Because rabeprazole uses a non-enzymatic pathway in addition to the CYP system for hepatic elimination, the effects of *CYP2C19* polymorphism on rabeprazole metabolism are minimal (Ishizaki & Horai 1999; Adachi et al 2000; Ieiri et al 2001). The lower blood concentrations of rabeprazole compared with lansoprazole (Ieiri et al 2001) may yield only slight effects on the CYP system, even in subjects with *CYP2C19* gene mutation.

Inter-ethnic variability in the allelic frequency of *CYP2C19* has been reported (Ishizaki & Horai 1999). Subjects with at least a one-gene mutation in *CYP2C19*, including IM and PM for PPIs, are found with higher frequency in Asians (58–72%) than in Caucasians (30–43%). Thus, Asians may have higher risk for the drug interaction of tacrolimus and lansoprazole, which was observed in the present study. Because the blood concentration of rabeprazole was less affected by the *CYP2C19* genotype status, rabeprazole can be used as a safe alternative PPI for patients with *CYP2C19* gene mutation.

Conclusion

Drug interaction between tacrolimus and lansoprazole is clinically significant in subjects with *CYP2C19* gene mutation. This observation suggests that drug interaction between tacrolimus and lansoprazole occurs in subjects with higher lansoprazole blood concentrations corresponding to *CYP2C19* genetic status. In contrast, rabeprazole has minimal effect on tacrolimus pharmacokinetics regardless of *CYP2C19* genotype status. To avoid this drug interaction, which occurs in subjects with decreased activity of CYP2C19, the *CYP2C19* genotyping test is useful for the optimal selection of PPIs in renal transplant recipients under tacrolimus treatment.

References

Adachi, K., Katsube, T., Kawamura, A., Takashima, T., Yuki, M, Amano K., Ishihara, S., Fukuda, R., Watanabe, M., Kinoshita, Y. (2000) CYP2C19 genotype status and intragastric pH during dosing with lansoprazole or rabeprazole. *Aliment. Pharmacol. Ther.* **14**: 1259–1266

Christians, U., Jacobsen, W., Benet, L. Z., Lampen, A. (2002) Mechanisms of clinically relevant drug interactions associated with tacrolimus. *Clin. Pharmacokinet.* **41**: 813–851

Edeki, T. I., Goldstein, J. A., de Morais, S. M. F., Hajiloo, L., Butler, M., Chapdelaine, P., Wilkinson, G. R. (1996) Genetic polymorphism of S-mephenytoin 4′-hydroxylation in African Americans. *Pharmacogenetics* **6**: 357–360

Furuta, T., Ohashi, K., Kobayashi, K., Iida, I., Yoshida, H., Shirai, N., Takashima, M., Kosuge, K., Hanai, H., Chiba, K., Ishizaki, T., Kaneko, E. (1999) Effects of clarithromycin on the metabolism of omeprazole in relation to CYP2C19 genotype status in humans. *Clin. Pharmacol. Ther.* **66**: 265–274

Furuta, T., Shirai, N., Xiao, F., Ohashi, K., Ishizaki, T. (2001) Effect of high-dose lansoprazole on intragastric pH in subjects who are homozygous extensive metabolizers of cytochrome P4502C19. *Clin. Pharmacol. Ther.* **70**: 484–492

Hashida, T., Masuda, S., Uemoto, S., Saito, H., Tanaka, K., Inui, K. (2001) Pharmacokinetic and prognostic significance of intestinal MDR1 expression in recipients of living-donor liver transplantation. *Clin. Pharmacol. Ther.* **69**: 308–316

Homma, M., Itagaki, F., Yuzawa, K., Fukao, K., Kohda, Y. (2002) Effects of lansoprazole and rabeprazole on tacrolimus blood concentration: case of a renal transplant recipient with CYP2C19 gene mutation. *Transplantation* **73**: 303–304

Ieiri, I., Kisimoto, Y., Okochi, H., Momiyama, K., Morita, T., Kitano, M., Morisawa, T., Fukushima, Y., Nakagawa, K., Hasegawa, J., Otsubo, K., Ishizaki, T. (2001) Comparison of the kinetic disposition of and serum gastrin change by lansoprazole versus rabeprazole during an 8-day dosing scheme in relation to CYP2C19 polymorphism. *Eur. J. Clin. Pharmacol.* **57**: 485–492

Ishizaki, T., Horai, Y. (1999) Cytochrome P450 and the metabolism of proton pump inhibitors: emphasis on rabeprazole. *Aliment. Pharmacol. Ther.* **13**: 27–36

Itagaki, F., Homma, M., Yuzawa, K., Fukao, K., Kohda, Y. (2002) Drug interaction of tacrolimus and proton pump inhibitors in renal transplant recipients with CYP2C19 gene mutation. *Transplant. Proc.* **34**: 2777–2778

Karol, M. D., Granneman, G. R., Alexander, K. (1995) Determination of lansoprazole and five metabolites in plasma by high-performance liquid chromatography. *J. Chromatogr. B* **668**: 182–186

Katsuki, H., Nakamura, C., Arimori, K., Fujiyama, S., Nakano, M. (1997) Genetic polymorphism of CYP2C19 and lansoprazole pharmacokinetics in Japanese subjects. *Eur. J. Clin. Pharmacol.* **52**: 391–396

Kosuge, K., Jun, Y., Watanabe, H., Kimura, M., Nishimoto, M., Ishizaki, T., Ohashi, K. (2001) Effects of CYP3A4 inhibition by diltiazem on pharmacokinetics and dynamics of diazepam in relation to CYP2C19 genotype status. *Drug. Metab. Dispos.* **29**: 1284–1289

Logan, A. J., Morris-Sriff, G. J., Bowrey, D. J., Jurewicz, W. A. (2002) Upper gastrointestinal complications after renal transplantation. *Clin. Transplant.* **16**: 163–167

Lorf, T., Ramadori, G., Ringe, B., Schwörer, H. (2000) The effect of pantoprazole on tacrolimus and cyclosporin. A blood concentration in transplant patients. *Eur. J. Clin. Pharmacol.* **56**: 439–440

de Morais, S. M. F., Wilkinson, G. R., Blaisdell, J., Nakamura, K., Meyer, U. A., Goldstein, J. A. (1994) The major genetic defect responsible for the polymorphism of S-mephenytoin metabolism in humans. *J. Biol. Chem.* **269**: 15 419–15 422

Pauli-Magnus, C., Rekersbrink, S., Klotz, U., Fromm, M. F. (2001) Interaction of omeprazole, lansoprazole and pantoprazole with P-glycoprotein. *Naunyn Schmiedebergs Arch. Pharmacol.* **364**: 551–557

Plosker, G. L., Foster, R. H. (2000) Tacrolimus: a further update of its pharmacology and therapeutic use in the management of organ transplantation. *Drugs* **59**: 323–389

Saeki, T., Ueda, K., Tanigawara, Y., Hori, R., Komano, T. (1993) Human P-glycoprotein transports cyclosporin A and FK506. *J. Biol. Chem.* **268**: 6077–6080