JPP Journal of Pharmacy and Pharmacology

JPP 2002, 54: 65–70

Received April 27, 2001
Accepted August 16, 2001
ISSN 0022-3573

Evaluation of increased bioavailability of tacrolimus in rats with experimental renal dysfunction

Hiromi Okabe, Ikuko Yano, Yukiya Hashimoto, Hideyuki Saito and Ken-ichi Inui

Abstract

The effects of renal failure on the hepatic and intestinal extraction of tacrolimus were evaluated to examine the mechanisms for the increased bioavailability of this drug in cisplatin-induced renal failure model rats. Tacrolimus extractions in the liver and intestine were evaluated by intravenous, intraportal and intraintestinal infusion. The intestinal metabolism and absorption rate were estimated by incubating the isolated intestine with drug solution and by an in situ loop method, respectively. Blood concentrations of tacrolimus following the intraintestinal infusion were significantly increased in rats with renal failure compared with those in normal rats. The blood concentration of tacrolimus during intraportal infusion in rats with renal failure showed non-linearity against dose, and was increased as compared with that in normal rats. The intestinal metabolism was not altered, but the absorption rate was significantly increased in the intestine from rats with renal dysfunction. These results suggest that the hepatic metabolism of tacrolimus is impaired in rats with renal failure, and that the accelerated absorption rate in the intestine in renal dysfunction is followed by partial saturation of hepatic extraction, which may be one of the mechanisms of increased bioavailability of tacrolimus.

Department of Pharmacy, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606-8507, Japan

Hiromi Okabe, Ikuko Yano, Hideyuki Saito, Ken-ichi Inui

Graduate School of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, Sugitani, Toyama 930-0194, Japan

Yukiya Hashimoto

Correspondence: Ken-ichi Inui, Department of Pharmacy, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606–8507, Japan. E-mail: inui@kuhp.kyoto-u.ac.jp

Funding: This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan, a Grant-in-Aid from Japan Health Sciences Foundation, and a Grant from the Yamanouchi Foundation for Research on Metabolic Disorders.

Introduction

Tacrolimus is a macrolide lactone with potent immunosuppressive properties that is used clinically for the prophylaxis of organ rejection after liver, heart, small bowel and kidney transplantation. Blood tacrolimus concentrations in patients have to be kept within a narrow therapeutic range (5–20 ng mL^{-1}) because of the side effects of tacrolimus, such as neurotoxicity and nephrotoxicity (Venkataramanan et al 1995). Tacrolimus is rapidly absorbed after oral administration and is extensively metabolized by cytochrome P450 (CYP)3A in the liver of humans and rats (Venkataramanan et al 1995; Iwasaki et al 1998). The bioavailability of tacrolimus is low in both humans and rats (Yasuhara et al 1995; Okabe et al 2000). Moreover, we studied the metabolism of tacrolimus in the intestine and showed that the small intestine, as well as the liver, contributes significantly to the first-pass effects of tacrolimus after oral administration in rats (Hashimoto et al 1998).

Although renal failure is commonly thought to affect only the renal elimination of drugs, it has a variety of influences on drug kinetics: it may reduce non-renal elimination, influence protein binding and alter the volume of distribution of some drugs (Gibson 1986). The bioavailability of some drugs is also affected by renal

failure. Propranolol, bufuralol and D-propoxyphene were reported to show increased bioavailability (Bianchetti et al 1976; Gibson et al 1977; Balant et al 1980). On the other hand, furosemide, pindolol and ciclosporin A were reported to show decreased bioavailability (Chau et al 1977; Tilstone & Fine 1978; Shibata et al 1999).

We previously reported that the bioavailability of tacrolimus is increased in rats with cisplatin-induced acute renal failure (ARF) (Okabe et al 2000). Our results showed that blood concentration in renal failure rats following intravenous infusion was only slightly higher (up to 1.3-fold) than that in normal rats. In contrast, the blood concentration in rats with renal failure after intraintestinal administration was significantly higher (about 2-fold) than that in normal rats. The bioavailability of tacrolimus was increased by about 35% in ARF rats as compared with normal rats.

The present study was designed to examine the mechanisms responsible for the increased bioavailability of tacrolimus in rats with cisplatin-induced acute renal failure. We evaluated the time course of blood tacrolimus concentration during intraintestinal, intraportal and intravenous infusion. In addition, we examined the effects of ARF on the metabolism of tacrolimus in the intestine and the absorption rate by an in situ loop method.

Materials and Methods

Chemicals

Tacrolimus was obtained from Fujisawa Pharmaceutical Co. Ltd (Osaka, Japan). Cisplatin (Randa injection, 0.5 mg mL^{-1}) was obtained from Nippon Kayaku Co. Ltd (Tokyo, Japan). All other chemicals were of the highest purity available.

Animals and induction of acute renal dysfunction

The animal experiments were performed in accordance with the Guidelines for Animal Experiments of Kyoto University. Male Wistar rats, 215–230 g, were used. Before the experiments the rats were housed in a temperature- and humidity-controlled room with free access to water and standard rat chow. ARF was induced by intraperitoneal administration of 5 mg kg^{-1} of cisplatin (Okabe et al 2000). Studies on rats with ARF were performed 72 h after injection of cisplatin. Non-treated normal rats served as controls. Each experiment was performed with 4–8 rats in each group.

Hepatic and intestinal extraction of tacrolimus following intraintestinal and intraportal infusion

Rats were anaesthetized with 50 mg kg^{-1} of sodium pentobarbital. Body temperature was maintained with appropriate heating lamps. The femoral artery was cannulated with a polyethylene tube (SP-31, Natsume Seisakusyo, Tokyo, Japan) for blood sampling. For intravenous infusion of tacrolimus, the jugular vein was cannulated with a polyethylene tube (PE-10, Becton Dickinson and Co., Parsippany, NJ). For intraportal infusion of tacrolimus, a catheter with a 24G needle was carefully fixed with cyanoacrylate glue into the portal vein. For intraintestinal infusion of tacrolimus, a catheter with a 24G needle was carefully fixed with cyanoacrylate glue into the middle part of the duodenum (Okabe et al 2000). To adjust the solution for intravenous (0.5 mg kg^{-1}), intraportal (0.5 and 1.0 mg kg^{-1}) and intraintestinal (1.5 mg kg^{-1}) infusion, tacrolimus injection solution (Prograf injection, 5 mg mL^{-1}) was diluted appropriately with saline. The solution was infused over a 30-min period (2.2 mL h^{-1}) by means of an automatic infusion pump (Natsume Seisakusyo, Tokyo, Japan). Blood samples for measurement of tacrolimus concentration were obtained 5, 15 and 30 min after initiation of infusion. The area under the time–concentration curve from 0 to 30 min (AUC_{0-30}) was calculated using the linear trapezoidal rule.

Intestinal metabolism of tacrolimus

Rats fasted overnight were anaesthetized with diethylether, and a 30-cm length of the small intestine was removed. Isotonic phosphate buffer (pH 6.5) containing disodium hydrogenphosphate (123 mM), sodium dihydrogenphosphate (163 mM), glucose (5 mM), and 0.3% bovine serum albumin was continuously bubbled with 95% O_2/5% CO_2. The intestinal tissues were incubated for 60 min at 37°C in 30 mL of buffer containing 822 ng mL^{-1} (1 μM) tacrolimus as acetonitrile solution (final, 3.3% acetonitrile). At the end of the incubation, the buffer solution was collected and the intestinal tissue was homogenized with 9 volumes of buffer for quantification of tacrolimus. The amount of metabolized tacrolimus was calculated by subtracting the amounts remaining in the incubation buffer and in the tissue

from the applied dose. In addition, tacrolimus is stable in mildly acidic media (Venkataramanan et al 1995), and we also confirmed that tacrolimus was stable in the phosphate buffer in the preliminary experiment.

Intestinal absorption of tacrolimus

Rats fasted overnight were anaesthetized with 50 mg kg^{-1} of sodium pentobarbital. The abdominal cavity was opened via a midline incision, and the upper site of the duodenum and 10 cm from the upper end were ligated twice with silk sutures. Tacrolimus injection solution was diluted with saline and was injected (4 mL kg^{-1}) into the middle part of the duodenum at a dose of 2 mg kg^{-1}. Ten minutes after injection, the contents in the lumen were collected with a 1 : 1 mixture of methanol and saline, and the intestinal tissue was homogenized with 9 volumes of saline for quantification of tacrolimus. The amount of absorbed plus metabolized tacrolimus was calculated by subtracting the amounts remaining in the luminal contents and in the tissue from the applied dose.

Assay of tacrolimus

Tacrolimus was assayed with an HPLC–EIA method (Okabe et al 2000). The detection limit of this assay system was 10 ng mL^{-1}, and the coefficient of variance was about 10%.

Statistical analysis

Values are expressed as mean±s.e.m. for n experiments, since the reliability of mean values is of interest. The statistical significance of the difference between mean values was calculated using a non-paired *t*-test provided that the variances were similar. If this was not the case, the Mann-Whitney U-test was applied. *P* values less than 0.05 (two-tailed) were considered to be significantly different.

Results and Discussion

Tacrolimus is a potent immunosuppressant that is mainly metabolized by CYP3A in the liver and intestine (Hashimoto et al 1998). We previously showed that the bioavailability of tacrolimus was increased by about 35% in rats with impaired renal function as compared with normal rats (Okabe et al 2000). In this study, we evaluated the mechanisms of increased bioavailability of tacrolimus in rats with cisplatin-induced renal dysfunction.

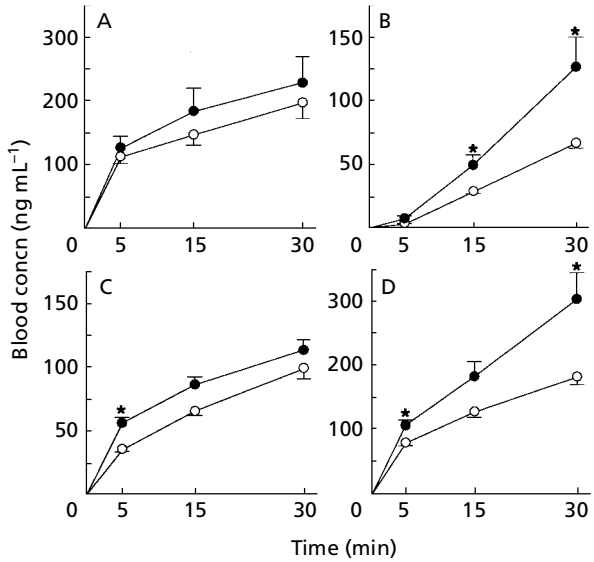

Figure 1 Blood tacrolimus concentration in rats during intravenous infusion at a dose of 0.5 mg kg^{-1} (A), intraintestinal infusion at a dose of 1.5 mg kg^{-1} (B) and intraportal infusion at a dose of 0.5 mg kg^{-1} (C) and 1.0 mg kg^{-1} (D). Each symbol and bar represents the mean±s.e.m. of results from four to eight rats: ○, normal rat; ●, ARF rats. *$P < 0.05$ compared with normal rats.

Table 1 AUC_{0-30} following intravenous, intraportal and intraintestinal infusion of tacrolimus to rats with or without renal failure.

	Dose (mg kg^{-1})	Normal (μg mL^{-1} min)	ARF (μg mL^{-1} min)
Intravenous	0.5	4.16±0.45	4.95±0.80
Intraportal	0.5	1.88±0.19	2.25±0.16
	1.0	3.58±0.20	5.38±0.64*
Intraintestinal	1.5	0.87±0.05	1.60±0.28*

Values are expressed as mean±s.e.m. of results from four to eight rats. *$P < 0.05$ compared with normal rats.

We examined the intestinal and hepatic extraction of tacrolimus by means of intravenous, intraportal and intraintestinal infusion. Figure 1 shows the whole blood concentrations during intravenous (0.5 mg kg^{-1}), intraintestinal (1.5 mg kg^{-1}) and intraportal (0.5 and 1.0 mg kg^{-1}) infusion of tacrolimus, and Table 1 shows AUC_{0-30} in normal and ARF rats. The AUC_{0-30} in ARF rats during the intravenous infusion was similar to that

in normal rats, whereas the AUC_{0-30} in rats with ARF during intraintestinal infusion was significantly higher than that in normal rats. The AUC_{0-30} in ARF rats during intraportal infusion at a lower dose (0.5 mg kg^{-1}) was similar to that of normal rats, while the value at a higher dose (1.0 mg kg^{-1}) was significantly increased. In addition, the AUC_{0-30} of tacrolimus during intraportal infusion showed non-linearity against the dose in rats with renal failure (Table 1). These results suggest that the hepatic metabolism is impaired in rats with renal failure, which may be one of the mechanisms for the increased bioavailability of tacrolimus in renal dysfunction.

The increased bioavailability of propranolol, which is subjected to extensive first-pass extraction in the liver, has been reported in rats with uranyl nitrate-induced or bilateral ureter-ligated experimental renal failure following oral administration (Terao & Shen 1983; Katayama et al 1984; Laganiere & Shen 1987). Despite the decreased presystemic clearance of propranolol in rats with uranyl nitrate-induced renal failure, no alterations were seen in the oxidative metabolic activity of rat liver (Hori et al 1985). In this case, decreased hepatic uptake (Hori et al 1985) and reduced hepatic metabolism due to the presence of an inhibitory factor in uremic blood (Terao & Shen 1985) were thought to be responsible for the decreased presystemic clearance of propranolol. On the other hand, the major metabolic enzyme of tacrolimus is CYP3A (Venkataramanan et al 1995), which is different from that of propranolol, CYP2D (Johnson et al 2000). In addition, the total liver CYP450 activity, which consists mainly of CYP2C11, 3A1 and 3A2, was reported to be decreased in 5/6 nephrectomized rats (Leblond et al 2000). Accordingly, the mechanisms for the impaired hepatic metabolism of tacrolimus in renal dysfunction may be different from that of propranolol. Additional studies are necessary to investigate the intrinsic hepatic clearance of tacrolimus in renal dysfunction and/or the effect of uremic substances on the metabolism of the drug.

We considered that the intestinal metabolism might also be responsible for the increased bioavailability in rats with renal failure, since the AUC_{0-30} during intraportal infusion of tacrolimus at a lower dose in ARF rats differed only slightly from that in normal rats (Table 1). We evaluated the intestinal metabolism of tacrolimus using the isolated tissue suspended in phosphate buffer. Table 2 shows the percentage of applied dose of tacrolimus remaining in the incubation buffer and in the tissue after 60 min of incubation. These values were not significantly different between ARF and normal rats. The amount of tacrolimus metabolized during the 60-min incubation period in the isolated intestine was not affected by ARF (Table 2). We therefore consider that the intestinal metabolism is not responsible for the increased bioavailability of tacrolimus in ARF rats.

Table 2 Amounts of tacrolimus remaining after incubation and metabolism in the intestine of normal and ARF rats.

	Normal	ARF
% remaining		
incubation buffer	69.6±1.6	73.2±2.0
tissue	6.4±1.7	5.6±1.9
% metabolized	24.0±2.5	21.1±3.2

Values are percentages of applied tacrolimus dose and are expressed as mean±s.e.m. of results from six rats.

Table 3 Absorption experiments in normal and ARF rats with the in situ loop method.

	Normal	ARF
% remaining		
fluid	53.9±2.1	36.0±4.2*
tissue	16.0±1.1	13.8±1.5
% absorbed and metabolized	30.2±2.1	50.2±4.1*

Values are percentages of applied tacrolimus dose and are expressed as mean±s.e.m. of results from five to six rats. *$P < 0.05$ compared with normal rats.

Another possible explanation for the decreased presystemic clearance is that the hepatic extraction of tacrolimus may be partially saturated as a result of the accelerated absorption rate in the intestine. In fact, we found a dose-dependent hepatic first-pass effect of tacrolimus following intraportal administration (Figures 1C and D). This finding was supported by the previous observation that the bioavailability of tacrolimus following intraintestinal administration was increased from 25.1% at a dose of 1.0 mg kg^{-1} to 32.2% at a dose of 3.0 mg kg^{-1} in normal rats; from 34.4% at a dose of 1.0 mg kg^{-1} to 42.8% at a dose of 3.0 mg kg^{-1} in rats with renal failure (Okabe et al 2000). In addition, Kimura et al (1984) reported that intestinal absorption rate of sulfanilic acid was increased in rats with $HgCl_2$-induced ARF, in rats with glycerol-induced ARF and in 5/6 nephrectomized rats as compared with control rats. We therefore evaluated the effect of ARF on the intestinal absorption rate of tacrolimus with an in situ loop method. Table 3 shows the intestinal absorption of

tacrolimus in rats with or without renal failure induced by cisplatin. Ten minutes after injection of tacrolimus (2 mg kg^{-1}) into the intestinal loop, the amount remaining in the loop of ARF rats was significantly decreased as compared with that of normal rats. Renal failure had no effect on the amount of tacrolimus remaining in the tissue. The sum of absorption and metabolism in the intestine was increased in ARF rats (Table 3). Since the intestinal metabolism was not altered by the renal failure (Table 2), the intestinal absorption rate in rats with renal failure was considered to be significantly increased as compared with that in normal rats. These findings suggest that the increased absorption rate in the intestine significantly contributes to the increased bioavailability of tacrolimus in ARF rats.

Kimura et al (1988) reported that the permeability of drugs with molecular weights lower than 1000 was increased in glycerol-induced renal failure. As the molecular weight of tacrolimus is 822, its absorption rate might be increased due to the increased non-specific permeability in the intestine in rats with renal failure. P-glycoprotein was reported to be associated with the absorption of tacrolimus in the intestine (Benet et al 1999; Masuda et al 2000). The function of P-glycoprotein was reported to be systemically suppressed in rats with glycerol-induced renal failure due to the accumulation of some endogenous substrates in plasma (Huang et al 2000). It is therefore possible that the absorption of tacrolimus via P-glycoprotein might be modulated in ARF rats. On the other hand, the intestinal absorption of ciclosporin A was reported to be decreased in rats with glycerol-induced renal failure (Shibata et al 1999). Although tacrolimus and ciclosporin A are both very lipophilic compounds and are substrates of CYP3A or P-glycoprotein, the effects of renal failure on their bioavailability seem to be different. The reasons for the difference remain to be clarified.

In conclusion, the increased bioavailability of tacrolimus is due not only to impaired hepatic metabolism, but also to partial saturation of hepatic extraction as a result of the accelerated absorption rate in the intestine of rats with renal dysfunction.

References

Balant, L., Francis, R. J., Tozer, T. N., Marmy, A., Tschopp, J. M., Fabre, J. (1980) Influences of renal failure on the hepatic clearance of bufuralol in man. *J. Pharmacokinet. Biopharm.* **8**: 421–438

Benet, L. Z., Izumi, T., Zhang, Y., Silverman, J. A., Wacher, V. J. (1999) Intestinal MDR transport proteins and P-450 enzymes as barriers to oral drug delivery. *J. Control. Release* **62**: 25–31

Bianchetti, G., Graziani, G., Brancaccio, D., Morganti, A., Leonetti, G., Manfrin, M., Sega, R., Gomeni, R., Ponticelli, C., Morselli, P. L. (1976) Pharmacokinetics and effects of propranolol in terminal uraemic patients and in patients undergoing regular dialysis treatment. *Clin. Pharmacokinet.* **1**: 373–384

Chau, N. P., Weiss, Y. A., Safar, M. E., Lavene, D. E., Georges, D. R., Milliez, P. L. (1977) Pindolol availability in hypertensive patients with normal and impaired renal function. *Clin. Pharmacol. Ther.* **33**: 505–510

Gibson, T. P. (1986) Influence of renal disease on pharmacokinetics. In: Evans, W. E., Schentag, L. J., Jusko, W. J. (eds) *Applied Pharmacokinetics*, 2nd edn. Applied Therapeutics, Washington, pp 83–115

Gibson, T. P., Giacomini, K. M., Briggs, W. A., Whitman, W., Levy, G. (1977) Pharmacokinetics of D-propoxyphene in anephric patient. *Clin. Pharmacol. Ther.* **21**: 103

Hashimoto, Y., Sasa, H., Shimomura, M., Inui, K. (1998) Effects of intestinal and hepatic metabolism on the bioavailability of tacrolimus in rats. *Pharm. Res.* **15**: 1609–1613

Hori, R., Okumura, K., Yasuhara, M., Katayama, H. (1985) Reduced hepatic uptake of propranolol in rats with acute renal failure. *Biochem. Pharmacol.* **34**: 2679–2683

Huang, Z., Murakami, T., Okochi, A., Yumoto, R., Nagai, J., Takano, M. (2000) Expression and function of P-glycoprotein in rats with glycerol-induced acute renal failure. *Eur. J. Pharmacol.* **406**: 453–460

Iwasaki, K., Shiraga, T., Hata, T. (1998) Metabolism of tacrolimus (FK506) and its metabolite by rat liver microsomes. *Xenobio. Metabol. Dispos.* **13**: 472–477

Johnson, J. A., Herring, V. L., Wolfe, M. S., Relling, M. V. (2000) CYP1A2 and CYP2D6 4-hydroxylate propranolol and both reactions exhibit racial differences. *J. Pharmacol. Exp. Ther.* **294**: 1099–1105

Katayama, H., Fujiwara, J., Yasuhara, M., Okumura, K., Hori, R. (1984) Increased availability of propranolol in rats with uranyl nitrate-induced acute renal failure. *J. Pharmacobiodyn.* **7**: 536–544

Kimura, T., Ikeda, K., Kobayashi, A., Nakayama, T. (1984) Effect of experimental acute renal failure on intestinal barriers to drug absorption. *Chem. Pharm. Bull.* **32**: 2471–2473

Kimura, T., Kobayashi, A., Kobayashi, M., Numata, K., Kawai, Y., Kurosaki, Y., Nakayama, T., Mori, M., Awai, M. (1988) Intestinal absorption of drugs in rats with glycerol-induced acute renal failure. *Chem. Pharm. Bull.* **36**: 1847–1856

Laganiere, S., Shen, D. D. (1987) Altered S-(−)-propranolol disposition in bilateral ureter-ligated rats. *Nephron* **46**: 305–311

Leblond, F. A., Giroux, L., Villeneuve, J.-P., Pichette, V. (2000) Decreased in vivo metabolism of drugs in chronic renal failure. *Drug Metab. Dispos.* **28**: 1317–1320

Masuda, S., Uemoto, S., Hashida, T., Inomata, Y., Tanaka, K., Inui, K. (2000) Effect of intestinal P-glycoprotein on daily tacrolimus trough level in a living-donor small bowel recipient. *Clin. Pharmacol. Ther.* **68**: 98–103

Okabe, H., Hashimoto, Y., Inui, K. (2000) Pharmacokinetics and bioavailability of tacrolimus in rats with experimental renal dysfunction. *J. Pharm. Pharmacol.* **52**: 1467–1472

Shibata, N., Ohmae, T., Hoshino, N., Minouchi, T., Yamaji, A. (1999) Influence of glycerol-induced acute renal failure on the pharmacokinetics of cyclosporin in rats. *J. Pharm. Pharmacol.* **51**: 397–404

Terao, N., Shen, D. D. (1983) Effect of experimental renal failure on the disposition kinetics of L-propranolol in rats. *J. Pharmacol. Exp. Ther.* **227**: 295–301

Terao, N., Shen, D. D. (1985) Reduced extraction of L-propranolol by perfused rat liver in the presence of uremic blood. *J. Pharmacol. Exp. Ther.* **233**: 277–284

Tilstone, W. J., Fine, A. (1978) Furosemide kinetics in renal failure. *Clin. Pharmacol. Ther.* **23**: 644–650

Venkataramanan, R., Swaminathan, A., Prasad, T., Jain, A., Zuckerman, A., Warty, V., McMichael, J., Lever, J., Burckart, G., Starzl, T. (1995) Clinical pharmacokinetics of tacrolimus. *Clin. Pharmacokinet.* **29**: 404–430

Yasuhara, M., Hashida, T., Toraguchi, M., Hashimoto, Y., Kimura, M., Inui, K., Hori, R., Inomata, Y., Tanaka, K., Yamaoka, Y. (1995) Pharmacokinetics and pharmacodynamics of FK506 in pediatric patients receiving living-related donor liver transplantations. *Transplant. Proc.* **27**: 1108–1110