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Pharmacokinetics and Bioavailability of Tacrolimus in Rats with Experimental Renal Dysfunction

HIROMI OKABE, YUKIYA HASHIMOTO AND KEN-ICHI INUI

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

Abstract

The effects of renal failure on the pharmacokinetics and bioavailability of tacrolimus were investigated in rats. Experimental renal dysfunction was induced by intraperitoneal injection of cisplatin ($5\,mg\,kg^{-1}$) into rats. The blood concentration of tacrolimus was measured after intravenous and intra-intestinal administration of the drug.

The blood concentration of tacrolimus after intravenous administration ($1\,mg\,kg^{-1}$) was slightly increased (up to 1·3 fold) by induction of renal dysfunction. In contrast, the peak tacrolimus concentration after intra-intestinal administration ($1\,mg\,kg^{-1}$ or $3\,mg\,kg^{-1}$) in rats with renal failure was about 2-fold higher than that in normal controls. The bioavailability was increased by about 35% in rats with impaired renal function as compared with normal controls. These results suggested that the bioavailability of tacrolimus, which is mainly metabolized in the liver and intestine after oral administration, is also influenced by renal function.

Tacrolimus is quite different from cyclosporin A in structure, but constitutes a cornerstone of maintenance immunosuppression together with cyclosporin A. The mechanism of action for both tacrolimus and cyclosporin A is binding to their intracellular receptors, immunophilins, creating composite surfaces that block the activity of specific targets, calcineurin. Inhibition of the action of calcineurin results in a complete block in the translocation of the cytosolic component of the nuclear factor of activated T cells, which is necessary for T-cell proliferation, such as IL-2 (Interleukin-2) (Ho et al 1996).

Tacrolimus is a very lipophilic compound, and is currently available as a solution for intravenous administration or as a solid dispersion formulation for oral use. Tacrolimus is rapidly absorbed after oral administration, and is extensively metabolized by CYP3A in the liver (Venkataramanan et al 1995). The oral bioavailability of tacrolimus is poor (around 25 %), and the drug shows large inter- and intra-individual pharmacokinetic variability (Venkataramanan et al 1995). We found that the clearance of tacrolimus after oral administration is related to the post-operation period in patients with living-related liver transplantation (Yasuhara et al 1995). Although there is a significant difference in clearance values between rats and human, we investigated the effects of liver function on the pharmacokinetics of tacrolimus, and our results showed that the bioavailability of tacrolimus increases in the case of liver dysfunction (Sasa et al 1998). Moreover, we studied the first-pass metabolism of tacrolimus in the intestine, and showed that the small intestine, as well as the liver, contributes significantly to first-pass metabolism following oral administration of tacrolimus (Hashimoto et al 1998). Accordingly, the variable metabolic activity in the liver or intestine (or both) seemed to be responsible for the large inter- and intra-individual pharmacokinetic variability of tacrolimus.

Although renal failure is commonly thought to have its sole effect on the renal elimination of drugs, it has a variety of influences on drug kinetics. Renal dysfunction may influence protein binding and alter the volume of distribution of some drugs (Gibson 1986). In addition, it is commonly thought that the metabolism of drugs that are less excreted in the urine is not affected by renal failure. However, there are several drugs, the bioavailability of which is changed with renal

Correspondence: K. Inui, Department of Pharmacy, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606-8507, Japan.

dysfunction. For example, Shibata et al (1999) showed that the bioavailability of cyclosporin A was decreased from 33·6% in controls to 11·8% in rats with glycerol-induced renal failure. Then, they reported that the reduction of the absorbed fraction of cyclosporin A contributed significantly to the decrease in bioavailability in rats with renal failure, and that renal failure decreased intestinal metabolism and increased hepatic metabolism.

The purpose of this study was to investigate the effects of renal function on the pharmacokinetics of tacrolimus. We chose cisplatin-induced acute renal failure (ARF) in rats, as a model system due to the simplicity of induction of renal dysfunction (Nakamura et al 1997). We measured the blood concentration of tacrolimus after intravenous and intra-intestinal administration to evaluate the pharmacokinetics and bioavailability of the drug in ARF rats.

Materials and Methods

Chemicals

Tacrolimus injection solution (Prograf, 5 mg mL^{-1}) was obtained from Fujisawa Pharmaceutical Co. Ltd (Osaka, Japan). Cisplatin (Randa injection, 0·5 mg mL^{-1}) was purchased from Nippon Kayaku Co. Ltd (Tokyo, Japan). Sodium pentobarbital was obtained from Abbott (Chicago, IL). All other chemicals were of the highest purity available.

Animals and induction of acute renal dysfunction

Male Wistar rats, 200–300 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 at approximately 09 00 h. Studies on rats with ARF were performed 72 h after injection of cisplatin. The animal experiments were performed in accordance with The Guidelines for Animal Experiments of Kyoto University.

Study protocols

Rats were anaesthetized with 50 mg kg^{-1} sodium pentobarbital. Supplemental doses of pentobarbital were administered as required. 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. The jugular vein was cannulated with a polyethelene tube (PE-10, Becton Dickinson & Co., Parsippany, NJ) for intravenous infusion of tacrolimus.

To adjust the solution for intravenous infusion to a dose of 1·0 mg kg^{-1}, tacrolimus injection solution was diluted with saline. The solution was infused over a 1-h period (2·2 mL h^{-1}) via the jugular vein by means of an automatic infusion pump (Natsume Seisakusyo, Tokyo, Japan) at approximately 09 00 h. Blood samples for measurement of tacrolimus concentration were obtained 15, 30, 60, 65, 75, 90, 120, 180 and 240 min after initiation of infusion.

For intra-intestinal administration of tacrolimus, the abdominal cavity of rats fasted overnight was opened via a midline incision, and the duodenum was exposed temporarily to administer the drug. To adjust the solution for intra-intestinal injection to a dose of 1·0 and 3·0 mg kg^{-1}, tacrolimus injection solution was diluted with saline. The solution was injected (2 mL kg^{-1} for a dose of 1·0 mg kg^{-1} and 3 mL kg^{-1} for a dose of 3·0 mg kg^{-1}) into the lumen of the duodenum. Blood samples were withdrawn 8, 15, 30, 45, 60, 75, 90, 120, 180 and 240 min after administration. In addition, the entire small intestine was removed by cutting across the upper end of the duodenum and the lower end of the ileum at the end of the 240-min experimental period. The small intestine was washed out with 5 mL of saline for quantification of tacrolimus remaining in the intestinal lumen. In addition, we confirmed that the mucosa of the intestine did not show sores or ulceration under these experimental conditions.

Assay of tacrolimus

Tacrolimus was assayed with a modified HPLC-EIA method (Sasa et al 1998). Briefly, blood samples (150 μL) were mixed with a precipitation reagent (300 μL) consisting of methanol (50%, v/v), ethylene glycol (30%, v/v), water (20%, v/v) and zinc sulphate (100 mM). Samples were stood for 30 min at −4°C, then centrifuged for 15 min at 14 000 rev min^{-1}. The supernatant (180 μL) was fractionated by HPLC in a system composed of a pump (LC-9A, Shimadzu Corporation, Kyoto, Japan), guard filter (Sumipax filter PG-ODS, Sumika Chemical Analysis Service, Ltd, Osaka, Japan), an analytical column (ChemcoPak, Chemcosorb 5-ODS-H, 4·6 × 150 mm, Chemco Scientific Co. Ltd, Osaka, Japan) and a spectrophotometric detector (SPD-2A, Shimadzu Corporation). The mobile phase consisted of methanol and water (80 : 20, v/v), the flow rate was 1 mL min^{-1}, and the column temperature was 60°C. The retention time of tacrolimus was measured by

injecting 1·8 μg of tacrolimus, which could be easily detected using the UV spectrophotometric detector. The HPLC fraction corresponding to unchanged tacrolimus was collected between 4·7 and 6·7 min, evaporated with an Automatic Environmental Speed Vac System (Savant Instruments Inc., Farmingdale, NY), and reconstituted with 1 mL of a solution consisting of saline (70%, v/v) and methanol (30%, v/v). This sample was quantified by an EIA method (IMx, Dainabot Co. Ltd, Tokyo, Japan). The detection limit of this assay system was 10 ng mL^{-1} and the coefficient of variance was about 10%.

Biochemical assays of plasma

The plasma concentrations of creatinine and urea nitrogen were measured using the Jaffé method and the urease indophenol method, respectively, with kits obtained from Wako Pure Chemical Industries Ltd (Osaka, Japan). The plasma concentrations of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvate transaminase (GPT) after each experiment were measured using the pyruvate oxidase *N*-ethyl-*N*-(2-hydroxy-3-sulfopropyl)-*m*-toluidine (POP.TOOS) method with a kit obtained from Wako Pure Chemical Industries Ltd (Osaka, Japan). Albumin and total protein after each experiment were measured with a kit obtained from Wako Pure Chemical Industries Ltd, using the bromocresol green (BCG) method and the Biuret method, respectively.

Pharmacokinetic analysis

The pharmacokinetic parameters of tacrolimus following intravenous infusion were estimated using the software package NONMEM (double precision NONMEM Version IV and PREDPP Version III). The two-compartment model was parameterized in terms of central volume of distribution (V_1), total clearance (CL), volume of distribution at steady state (Vdss) and intercompartmental clearance (Q), using the PREDPP subroutines ADVAN3 and TRANS3. The apparent clearance values (CL/F) expressed by the CL and bioavailability (F) after intra-intestinal injection were calculated from dose/area under the blood concentration–time curve (AUC). The AUC after intra-intestinal injection was calculated using the linear trapezoidal rule and extrapolated to infinity by adding the ratio of the last measurable tacrolimus concentration to the mean terminal disposition rate constant after intravenous administration at 1 mg kg^{-1} dose in each group. The mean F value following intra-intestinal injection was calculated from the CL and CL/F values.

Statistical analysis

Values are expressed as means ± s.e.m. Statistical significance was assessed by Student's *t*-test.

Results

Table 1 shows the biochemical parameters of rats with or without ARF induced by cisplatin. The plasma concentrations of creatinine and urea nitrogen in rats with ARF were 2- or 3-fold higher than those in normal rats. The indicators of hepatic function, GOT and GPT, in rats with ARF were not significantly different from those of normal controls. The concentrations of albumin and total protein in plasma were slightly higher in rats with renal dysfunction as compared with normal rats, but this was not significant.

The mean whole blood concentration–time profiles obtained after intravenous administration of tacrolimus in ARF rats were slightly higher (up to 1·3 fold) than those in normal rats (Figure 1), but this was not statistically significant. Table 2 lists the pharmacokinetic parameters calculated by NONMEM software using the data shown in Figure 1. CL and Q were decreased in the case of renal failure, whereas V_1 was increased in ARF rats.

Following the intra-intestinal injection of tacrolimus (1 and 3 mg kg^{-1}), the mean (± s.e.m.) whole blood concentrations in ARF rats were significantly higher than those in normal controls (Figure 2). The CL/F and F values of tacrolimus after intra-intestinal administration are listed in Table 3. The CL/F value in normal rats was dose-dependent: 167·3 ± 26·6 mL min^{-1} kg^{-1} at a dose of 1 mg kg^{-1} and 130·4 ± 16·7 mL min^{-1} kg^{-1} at a dose of 3 mg kg^{-1}. The CL/F value in ARF rats was also dose dependent, and F in rats with renal failure was

Table 1. Biochemical parameters of plasma obtained from normal and ARF rats.

	Normal	ARF
BUN (mg dL^{-1})	17·3 ± 1·2	51·9 ± 7·8*
Creatinine (mg dL^{-1})	0·75 ± 0·07	1·37 ± 0·19*
GOT (IU L^{-1})	66·7 ± 4·3	58·1 ± 3·5
GPT (IU L^{-1})	20·3 ± 3·2	27·7 ± 2·0
Albumin (g dL^{-1})	4·18 ± 0·04	4·35 ± 0·09
Total protein (g dL^{-1})	5·90 ± 0·12	6·28 ± 0·18

Values are means ± s.e.m. of results from 9–11 rats. *$P < 0·05$ compared with normal controls. BUN, Blood-urea nitrogen; GOT, glutamic oxaloacetic transaminase, GPT, glutamic pyruvate transaminase.

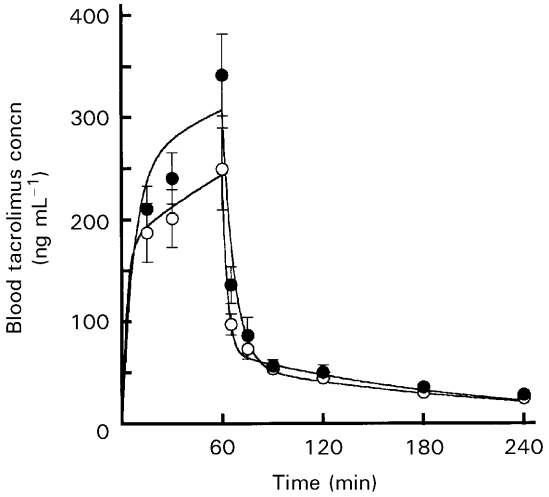

Figure 1. Blood tacrolimus concentration in rats following a 60-min intravenous infusion at a dose of 1·0 mg kg^{-1}. Each symbol with bar represents means ± s.e.m. of results from 5 rats: ○, normal rats; ●, ARF rats.

Table 2. Pharmacokinetic parameters of tacrolimus after intravenous administration to normal and ARF rats.

	Normal	ARF
CL (mL min^{-1} kg^{-1})	42·0 ± 1·3	34·8 ± 3·2*
V_1 (mL kg^{-1})	266 ± 2	453 ± 17*
Q (mL min^{-1} kg^{-1})	49·3 ± 4·8	34·3 ± 2·8*
Vdss (L kg^{-1})	3·82 ± 0·38	4·09 ± 0·15

Values are expressed as means ± s.e.m. of results from 5 rats. *$P < 0.05$ compared with normal controls.

Table 3. Bioavailability of tacrolimus after intra-intestinal administration to normal and ARF rats.

	Dose (mg kg^{-1})	Normal	ARF
CL/F (mL min^{-1} kg^{-1})	1·0	167 ± 27	101 ± 25
	3·0	130 ± 17	81·3 ± 16·7*
F (%)	1·0	25·1	34·4
	3·0	32·2	42·8

Values are expressed as means ± s.e.m. of results from 5 or 6 rats. *$P < 0.05$ compared with normal controls.

increased by about 35% as compared with normal controls (Table 3). The amount of tacrolimus remaining in the intestine at the end of the 240-min intra-intestinal injection experimental period was small (less than 0·1% of dose).

Discussion

Tacrolimus is a potent immunosuppressant that is mainly metabolized in the liver and intestine. To our knowledge, there have been no previous studies on the pharmacokinetics of tacrolimus in renal dysfunction. The pharmacokinetic studies described here clarified the relationship between the blood concentration changes of renal dysfunction induced by cisplatin, and tacrolimus. Our results showed that blood concentration in renal-failure rats following intravenous infusion was slightly higher (up to 1·3 fold) than that in normal rats. The blood concentration in rats with renal failure after intra-intestinal administration was significantly higher (about 2 fold) than that in normal controls.

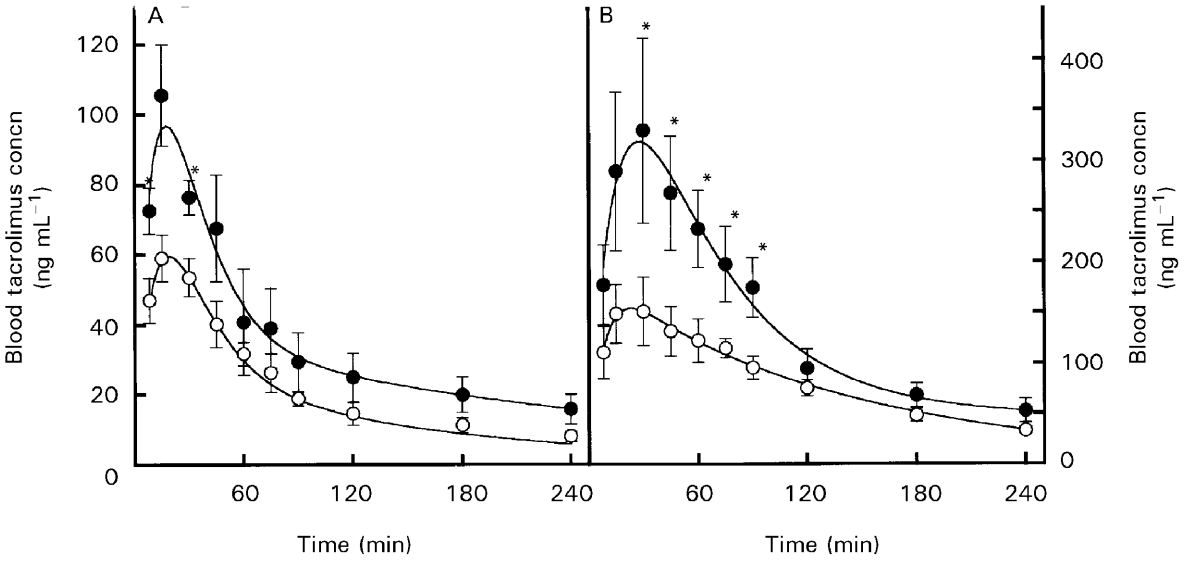

Figure 2. Blood tacrolimus concentration in rats after intra-intestinal administration at a dose of 1·0 mg kg^{-1} (A) or 3·0 mg kg^{-1} (B). Each symbol with bar represents means ± s.e.m. of results from 5 rats: ○, normal rats; ●, ARF rats. *$P < 0.05$ compared with normal controls.

The bioavailability of tacrolimus was increased by about 35% in ARF rats as compared with normal controls. These results were in contrast to those with cyclosporin A, where the bioavailability was decreased (Shibata et al 1999).

The absolute bioavailability of most drugs in patients with renal failure is unknown, but the first-pass metabolism of drugs such as propranolol (Lowenthal et al 1974; Bianchetti et al 1976), propoxyphene (Gibson et al 1977) and bufuralol (Balant et al 1980) has been reported to be reduced in such patients. The increased bioavailability of propranolol, which undergoes extensive first-pass extraction in the liver, has been investigated in rats with experimental renal failure after a single oral administration (Terao & Shen 1983; Katayama et al 1984; Laganiere & Shen 1987) or during repetitive dosing (Terao & Shen 1984). Katayama et al (1984) reported that the bioavailability of propranolol was increased from 12·0% in controls to 21·5% in rats with uranyl nitrate-induced ARF. Despite the decreased pre-systemic clearance of propranolol in rats with renal failure, there were no alterations in the intrinsic oxidative metabolic activity of the rat liver (Hori et al 1985). Decreased hepatic uptake or reduced hepatic metabolism due to the presence of an inhibitory factor in uraemic blood may be responsible for the decreased pre-systemic hepatic extraction of propranolol (Hori et al 1985; Terao & Shen 1985).

In this study, the bioavailability of tacrolimus was also shown to be increased in rats with renal failure (Figure 2 and Table 3). One possible explanation for the mechanism is the involvement of hepatic metabolism. In fact, we found decreased clearance of tacrolimus in ARF rats after intravenous administration (Table 2). It is reported that the hepatic blood flow rate does not change significantly in ARF rats (Katayama et al 1984). Therefore, the hepatic extraction ratio of tacrolimus may be decreased in ARF rats. It is necessary to investigate whether the mechanism of increased bioavailability of tacrolimus is the same as that of propranolol. That is, the major metabolic enzyme of propranolol is CYP2D6 (Yoshimoto et al 1995), which is different from that of tacrolimus, CYP3A4 (Vankataramanan et al 1995).

Another possible explanation for the mechanism of increased bioavailability of tacrolimus may lie with the change in the intestinal metabolic activity. Although tacrolimus is metabolized by CYP3A4, no information was available regarding the changes in intestinal metabolism in renal dysfunction. However, Shibata et al (1999) suggested the decreased intestinal metabolism of cyclosporin A with the intestinal everted sac method. Metabolism of cyclosporin A is mediated by the same enzyme as tacrolimus, CYP3A4 (Lo & Burckart 1999).

In addition, it is also possible that the hepatic extraction of tacrolimus may be partially saturated as a result of the accelerated absorption rate in the intestine in ARF rats. In fact, we found dose dependency of pharmacokinetics with intra-intestinal administration: F values were increased from 25·1% at a dose of $1\,mg\,kg^{-1}$ to 32·2% at a dose of $3\,mg\,kg^{-1}$ in normal rats (Table 3). Similarly, F values were increased from 34·4% at a dose of $1\,mg\,kg^{-1}$ to 42·8% at a dose of $3\,mg\,kg^{-1}$ in ARF rats (Table 3). On the other hand, Kimura et al (1988) reported that the permeability of drugs with molecular weights lower than 1000 is increased in renal failure.

In conclusion, tacrolimus and cyclosporin A showed different pharmacokinetic changes in renal dysfunction, despite their similar immunosuppressive mechanisms of action. This study provided useful information for determination of optimal dosage regimens of immunosuppressants in renal dysfunction.

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