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Effects of bile duct stricture on the pharmacokinetics of the immunosuppressant tacrolimus in rats

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Abstract

Background: To investigate the effects of bile duct stricture on the pharmacokinetics of the immunosuppressant tacrolimus, the pharmacokinetics of tacrolimus in rats with bile duct ligation were evaluated and the effects of the amount of intestinal bile on intestinal tacrolimus absorption were determined.

Methods: *In vivo* pharmacokinetic studies and *in vitro* metabolic studies in rats with bile duct ligation were performed. In addition, an *in situ* absorption study was performed.

Results: After an intravenous bolus injection of tacrolimus, the area under the blood concentration-time curve in rats with bile duct ligation was approximately 1.9-fold greater than that in control rats. The total clearance and steady-state volume of distribution decreased in the rats with bile duct ligation by 44.1% and 40.4%, respectively. Moreover, the production ratios of demethyl-tacrolimus and hydroxy-tacrolimus in the hepatic microsomes of rats with bile duct ligation were 59–62% and 21–43%, respectively, lower than the corresponding ratios in control rats. The area under the blood concentration-time curve after intraloop administration of tacrolimus with double-diluted bile or with saline was significantly lesser than that with undiluted bile. Significant positive linear correlations were observed between the amount of intestinal bile and the area under the blood concentration-time curve of tacrolimus ($r=0.999$, $p<0.05$).

Conclusions: The decrease in the hepatic intrinsic clearance of tacrolimus in rats with bile duct ligation suggests that the bile duct stricture might contribute to the clinically observed inter- and intra-patient pharmacokinetic variability. The amount of bile in the intestine is an important factor that should be considered during tacrolimus treatment. When the route of administration of tacrolimus is changed from injection to an oral one or during long-term oral administration of tacrolimus in patients with bile duct stricture, the decrease in absorption should be taken into account, and the dose should be adjusted accordingly. Taken together, the data indicate that personalized therapy is required for patients with bile duct stricture, and it is necessary to carefully evaluate therapeutic drug monitoring data for tacrolimus.

Keywords: Pharmacokinetics, bile ducts, tacrolimus, therapeutic drug monitoring

Introduction

Tacrolimus (TAC), an immunosuppressive agent (molecular weight of the non-hydrate form, 806), inhibits the signal transduction pathway involved in the activation of T-lymphocytes [1,2]. TAC is used for preventing or treating graft rejection after organ transplantation [3], and is superior to cyclosporine (CyA) in improving survival (patient and graft) and preventing acute rejection in living donor liver transplantation patients [4]. TAC is the drug of choice for living donor liver transplant recipients in our university hospital, Kyoto Prefectural University of Medicine. The pharmacokinetics (PK) of TAC are characterized by a marked and unexplained variability, particularly in the early period after transplantation [5,6]. TAC is highly lipophilic and insoluble in water, and is a substrate for the drug efflux pump, P-glycoprotein (Pgp), and the metabolizing enzyme, cytochrome P450 (CYP) 3A. These physicochemical properties

of TAC contribute to the large variation in oral absorption and extensive metabolic clearance from the body. In addition, TAC has a narrow therapeutic window and thus therapeutic drug monitoring (TDM) is essential for maintaining efficacy [7,8] and minimizing the toxicity of TAC [9,10].

Liver transplantation is a widely accepted treatment for patients with end-stage hepatic disease. Despite improvements in organ preservation technology, surgical technique, and immunosuppressive strategies, postoperative biliary stricture after liver transplantation is still a significant cause of morbidity and mortality [11]. Bile duct anastomotic stricture is a common problem after orthotopic liver transplantation, with an incidence of 15–20% [12]. Accumulation of bile acids in liver tissue because of biliary obstruction leads to production of free radicals, activation of hepatic stellate cells, and liver fibrosis [13–15]. In addition, cholestasis caused by bile duct ligation

(BDL) induces intestinal bile deficiency and accumulation of biliary compounds in the body [16]. Liver dysfunction or BDL decreases the availability of bile salts for solubilization of lipid-soluble drugs, and thus the intestinal absorption of TAC might decrease in patients with bile duct stricture. Therefore, we hypothesized that bile duct stricture after liver transplantation may influence the control of blood TAC concentration during TDM as well as the PK of TAC, especially through hepatic metabolism and intestinal absorption. Thus, the aim of our study was to elucidate the effects of BDL and the effects of different concentrations of bile in the intestine on the PK of TAC in rats with BDL.

Methods

Materials

TAC and CyA, which was used as an internal standard in liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis as described below, were purchased from Sigma-Aldrich Co. (Steinheim, Germany). Propylene glycol was obtained from Wako Pure Chemical Industries (Osaka, Japan). All other reagents were of analytical grade and used without further purification.

Animal preparation

All experimental protocols involving animals were performed in accordance with the Kyoto Pharmaceutical University Guidelines for Animal Experimentation and approved by the University's Animal Ethics Committee (animal protocol number: 030005). We purchased 10-week-old male Wistar rats (weighing 300 ± 10 g) from Nippon SLC Co., Ltd. (SLC, Hamamatsu, Japan). All rats were housed in a temperature-controlled facility with a 12-h light/12-h dark cycle for at least 5 days before use. The animals were fed standard rodent chow. Free access to food and water was allowed before the experiments. In this study, 36 rats were used and divided into 9 groups ($n = 4$ per group). BDL rats were prepared using a previously reported method with some modifications [17]. The animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (32 mg/kg body weight), and BDL was performed through flank incisions; subsequently, the muscle layers and the skin were sutured to close the wound. Sham surgeries were performed in control rats. After surgery, both control and BDL rats had free access to normal rat chow and tap water and were maintained as described above for 3 days. The BDL rats were used in the experiments on the fourth day after surgery. Hepatic function was determined by measuring the levels of total bilirubin (T-bil), glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), and alkaline phosphatase (ALP) in both the control and BDL rats at a commercial laboratory, Kyoto BIKEN (Kyoto, Japan) just before the experiment.

PK study of TAC in BDL rats

The PK of TAC was determined in BDL rats. Control and BDL

rats were fasted overnight but had free access to water. The rats were anesthetized using sodium pentobarbital (32 mg/kg body weight, intraperitoneal injection) and were placed in the supine position on a heating pad to maintain body temperature during all experiments. The dose of TAC in the PK studies was based on the clinical dose (0.1–0.3 mg/kg body weight) and blood concentration profiles observed in the clinical situation (1–50 ng/mL). The solutions of TAC for administration were prepared by dissolving TAC into propylene glycol (0.1 mg/mL). The TAC solution was administered into the right jugular vein of the control ($n = 4$) and BDL rats ($n = 4$) at a dose of 0.1 mg/kg body weight (1 mL/kg of TAC solution). Blood samples (200 μ L) were drawn from the external left jugular vein into heparinized microcentrifuge tubes at 0.25, 0.5, 1, 2, 4, and 6 h after drug administration and were immediately frozen at -80°C until analysis.

In vitro metabolic studies using hepatic microsomal fractions from rats

Metabolism of TAC was measured in a nicotinamide adenine dinucleotide phosphate (NADPH)-generating system according to a previously reported method [18]. Rat livers were rapidly excised and perfused with ice-cold potassium chloride (KCl) solution (1.15% w/v), and hepatic microsomes were prepared by differential ultracentrifugation [19]. Briefly, the rat liver was homogenized in 3 volumes of 1.15% w/v KCl in a glass-Teflon homogenizer maintained on ice. The homogenates were centrifuged at 4°C at $9,000 \times g$ for 15 min, and the supernatants were centrifuged again at $105,000 \times g$ for 1 h. Final microsomal pellets were resuspended in 0.1 M phosphate buffer at a concentration of 1.0 mg protein/mL. The concentration of protein in the microsomal preparations was determined using the method described by Lowry et al., bovine serum albumin was used as a standard [20]. We prepared and added 50 μ L of 10 mM NADPH in 0.1 M phosphate buffer (pH 7.4), 50 μ L of 100 mM glucose 6-phosphate (G6P) in 0.1 M phosphate buffer, 4 μ L of 500 units of G6P dehydrogenase in 0.1 M phosphate buffer, 50 μ L of 100 mM MgCl_2 in 0.1 M phosphate buffer, 736 μ L of 0.1 M phosphate buffer, and 10 μ L of TAC solution to a clean conical glass tube to produce final concentrations of 2.5, 5.0, 7.5, and 10 μM . After 5 min of preincubation in a water bath maintained at 37°C , the metabolic reaction was initiated by adding 100 μ L of rat liver microsomal suspension (final concentration, 0.8 mg protein/mL). After incubation for 10 min, the reaction was stopped by adding 200 μ L of ice-cold 2% ZnSO_4 in 50% methanol solution. The mixture was vigorously vortexed and centrifuged at $12,000 \times g$ for 10 min to precipitate the proteins. The resulting supernatant was used for the analysis of TAC metabolites, demethyl-TAC and hydroxy-TAC, and immediately frozen at -80°C until analysis.

In situ TAC absorption study

To investigate the effect of the amount of intestinal bile on the intestinal absorption of TAC, we performed an *in situ*

absorption study. Bile was collected from rats and pooled immediately before the experiment. Rats ($n = 12$) were divided into 3 groups ($n = 4$ per group): A, B, and C. In all groups, a 20-cm-long segment from the proximal end of the duodenum was surgically looped by placement of inlet and outlet silicon tubes (internal diameter [i.d.], 4.2 mm). After the contents of the looped intestine were washed 3 times with 25 mM glucose in phosphate-buffered saline, the solution remaining in the lumen was expelled with air at a rate of 1 mL/min. A polyethylene cannula (i.d., 0.47 mm) was introduced into the portal vein for obtaining blood samples. Then, 0.3 mg/

kg body weight of TAC (1 mL/kg of TAC solution for the *in situ* absorption study) and 1.28 mL/kg of undiluted bile (group A), 1.28 mL/kg of bile diluted with saline to double the volume (group B), or saline (group C) were injected into the looped intestine of each rat. Blood samples (200 μ L) were collected from the portal vein into heparinized microcentrifuge tubes 0.083, 0.25, 0.5, 0.75, 1, 1.5, and 2 h after drug administration and immediately frozen at -80°C until analysis.

Assay procedures using LC-MS/MS

Analysis of TAC levels in the blood and the level of demethyl-

Table 1. Body weights and hepatic function in control rats and rats with bile duct ligation.

	Control rats			BDL rats		
Body weight (g)	288.8	±	3.4	280.5	±	2.7
T-bil (mg/dL)	0.20	±	0.00	4.88	±	1.22**
GPT (IU/L)	107.3	±	18.5	900.5	±	178.6**
GOT (IU/L)	228.5	±	16.3	1641.3	±	511.2**
ALP (IU/L)	353.5	±	49.8	1367.8	±	243.8**

T-bil, total bilirubin; GPT, glutamic pyruvic transaminase; GOT, glutamic oxaloacetic transaminase; ALP, alkaline phosphatase; BDL, bile duct ligation

Each value represents the mean ± standard error (S.E.) of 4 rats.

** $p < 0.01$ statistically different from control in unpaired Student's t -tests

TAC and hydroxy-TAC in the hepatic microsomes was performed using LC-MS/MS [21]. The LC-MS/MS system (Applied Biosystems, Foster City, California) consisted of an LC-10AD micropump (Shimadzu Corporation, Kyoto, Japan) and an AS8020 automatic sample injector (Toso, Tokyo, Japan). MS was performed in the positive-ion electrospray ionization (ESI) mode. CyA was used as the internal standard. LC-MS/MS analyses were performed using Quicksorb ODS (2.1 mm × 150 mm, 5 μm; Chemco Scientific Co. Ltd., Osaka, Japan) maintained at 60°C for all separations. The mobile phase consisted of 10 mM ammonium acetate/acetonitrile (10:90 v/v) at a flow rate of 0.2 mL/min. The ion spray voltage and temperature were set at 5000 V and 300°C, respectively. The flow rates of the nebulizer gas, curtain gas, and collision gas were set at 15, 12, and 2.0 L/min, respectively. Multiple reaction monitoring analysis was performed with transition m/z 821.5→769.7 for TAC and m/z 1202.8→425.2 for CyA. We measured the levels of 2 metabolites of TAC, demethyl-TAC and hydroxy-TAC, in hepatic microsomes fortified with NADPH with transition m/z 807.5→755.7 for demethyl-TAC and m/z 837.5→785.7 for hydroxy-TAC. We confirmed the presence of 2 metabolites in hepatic microsomes after incubation for 10 min with NADPH, whereas these 2 metabolites were not observed in the hepatic microsomes after incubation without NADPH for 10 min (Figure 1). Standard samples were prepared by adding aliquots of TAC stock solutions to a drug-free matrix. The solid-phase extraction cartridge (Oasis® HLB; Waters Corp., Massachusetts, USA) was activated using 1 mL of acetonitrile followed by 1 mL of ion-exchanged water to prepare the solvent for use. Standard and unknown samples (100 μL) were added to 10 μL of the internal standard (CyA, 200 μg/mL in methanol) and 100 μL of 2% ZnSO₄ in 50% methanol solution, and the tube was vigorously vortexed for 15 s. After centrifugation for 5 min at 12,000 × g , the supernatant was applied to the solid-phase extraction cartridges and subsequently washed with 2 mL of ion-exchanged water. A 1.5mL clean tube was positioned below the solid-phase extraction cartridges, and

the compounds were eluted with 1 mL of acetonitrile. The eluate was dried under nitrogen at 37°C. The residue was redissolved with 100 μL of the mobile phase, and 50 μL was injected into the LC-MS/MS system for analysis.

PK analysis

Standard PK parameters were calculated from the blood concentration-time curve of TAC by using non-compartmental PK analysis using WinNonlin® Version 6.3 (Pharsight Co., Mountain View, CA). The terminal slope (λ_z) was determined using linear regression of at least 3 data points from the terminal portion of the blood concentration-time curve. The area under the blood concentration-time curve, extending from 0 to the last point measured (AUC_{0-last}), was calculated using the linear trapezoidal rule up to the last blood concentration measured ($C_{b(last)}$). The area under the blood concentration-time curve, extending from 0 to infinity ($AUC_{0-\infty}$), was extrapolated to infinity using a correction term, namely, $C_{b(last)}/\lambda_z$. The elimination half-life ($t_{1/2}$) was calculated using the equation $t_{1/2} = \ln 2/\lambda_z$. The area under the first-moment curve from 0 to infinity ($AUMC$) was calculated using the linear trapezoidal rule and the addition of the concentration term after the last measured point ($t_{(last)}$) to infinity, namely, $t_{(last)}C_{b(last)}/\lambda_z + C_{b(last)}/\lambda_z^2$. The mean residence time (MRT) was calculated using $AUMC/AUC_{0-\infty}$. Total blood clearance (CL_{tot}) was calculated using $D/AUC_{0-\infty}$, in which D represents the TAC dose administered. The steady-state volume of distribution (Vd_{ss}) was calculated by multiplying CL_{tot} with MRT .

Statistical analysis

All values are expressed as mean ± standard error (S.E.). The 2 groups were compared using Student's unpaired t -test. Correlations between the amount of bile in the intestinal loop and AUC_{0-2} of TAC after intraloop administration were assessed statistically using Pearson's correlation coefficient test. The differences between the means were considered statistically significant when $p < 0.05$.

Results

Biochemical parameters in BDL rats

The biochemical parameters of the control and BDL rats are shown in Table 1. No significant differences were observed in body weights between control and BDL rats. The level of T-bil in BDL rats was approximately 22-fold higher than that in control rats. Furthermore, the levels of GPT, GOT, and ALP were significantly higher in BDL rats than in control rats.

PK analysis of TAC in BDL rats

The mean blood TAC concentration-time curves after administration of an intravenous (i.v.) bolus injection of TAC to BDL rats are shown in Figure 2; the PK parameters of

Table 2. Pharmacokinetic parameters after intravenous administration of tacrolimus (0.1 mg/kg body weight) to control rats and rats with bile duct ligation.

Pharmacokinetic parameters	Control rats		BDL rats	
$t_{1/2}$ (h)	3.91	$\pm$ 0.12	4.31	$\pm$ 1.03
CL_{tot} (L/h/kg)	0.67	$\pm$ 0.01	0.38	$\pm$ 0.06*
Vd_{ss} (L/kg)	3.32	$\pm$ 0.13	1.98	$\pm$ 0.31**
$AUC_{0-\infty}$ (ng·h/mL)	150.0	$\pm$ 2.8	280.2	$\pm$ 32.9*

Each value represents the mean $\pm$ standard error (S.E.) of 4 rats.

** $p < 0.01$, * $p < 0.05$ significantly different from control in unpaired Student's t -tests BDL, bile duct ligation; $t_{1/2}$, elimination half-life; CL_{tot} , total blood clearance; Vd_{ss} , steady-state volume of distribution; $AUC_{0-\infty}$, area under the blood concentration-time curve, extending from 0 to infinity

TAC observed for each group are shown **Table 2**. After i.v. bolus injections of TAC, the $AUC_{0-\infty}$ in BDL rats was approximately 1.9-fold greater than that in control rats. CL_{tot} and Vd_{ss} were significantly lower in BDL rats than in control rats by 44.1% and 40.4%, respectively.

Metabolism of TAC in hepatic microsomes of BDL rats

The ratios of production of demethyl-TAC and hydroxy-TAC in the hepatic microsomes from BDL rats and control rats are shown in **Figure 3a** and **3b**, respectively. The production ratios of demethyl-TAC and hydroxy-TAC in hepatic microsomes

from BDL rats were 59–62% and 21–43%, respectively, lower than the corresponding ratios in control rats.

Effects of bile on the intestinal absorption of TAC

The mean whole blood concentration-time curves of TAC after intraloop administration of TAC with undiluted bile, double-diluted bile, or saline are shown in **Figure 4a**. The AUC_{0-2} of TAC after intraloop administration of TAC with double-diluted bile or with saline was significantly lower than that with undiluted bile. Significant positive linear correlations were observed between the amount of bile in the intestinal loop and the

Figure 4. Whole blood concentration-time curves of tacrolimus (TAC) after intraloop administration of TAC with undiluted bile, double-diluted bile, or saline (a), and the correlation between the amount of bile in the intestinal loop and area under the concentration-time curve (AUC_{0-2}) of TAC after intraloop administration (b), (■) Group A, 0.3 mg/kg body weight of TAC (1 mL/kg of TAC solution for *in situ* absorption study) and 1.28 mL/kg of undiluted bile; (▲) Group B, 0.3 mg/kg body weight of TAC (1 mL/kg of TAC solution for *in situ* absorption study) and 1.28 mL/kg of double-diluted bile with saline; (○) Group C, 0.3 mg/kg body weight of TAC (1 mL/kg of TAC solution for *in situ* absorption study) and saline. Results are presented as the mean $\pm$ standard error (S.E.) of 3–4 rats.

AUC_{0-2} of TAC ($r = 0.999$, $p < 0.05$; Figure 4b).

Discussion

BDL is an effective and a widely used method for the induction of liver cirrhosis in rats [22–24]. BDL stimulates the proliferation of biliary epithelial cells and oval cells, which are hepatocyte progenitors, and thus result in the proliferation of biliary ductules with accompanying portal inflammation and fibrosis [25]. Thus, the BDL rat model is a well-established, well-received, and widely used model of experimental bile duct stricture. For example, Espinosa et al., performed PK studies of ketorolac, a nonsteroidal anti-inflammatory agent, using BDL rats [26]. In this study, although no significant differences were observed in the body weight, the levels of GPT, GOT, ALP, and T-bil in BDL rats were significantly different from the corresponding levels in the control rats (Table 1). Our results are consistent with those reported in previous studies. Ji et al., and Fursule et al., reported that while BDL increased the levels of GPT, GOT, and ALP and the level of T-bil in the serum of BDL rats, no significant difference was observed in the body weights between the control and BDL rats throughout the experimental period [27,28]. These observations suggested the usefulness of BDL for inducing bile duct stricture in rats to investigate the effects of BDL on the PK of TAC.

Our results indicated that compared to control rats, the BDL rats showed alteration in the PK of TAC. Administration of an i.v. bolus injection of TAC to BDL rats increased the $AUC_{0-\infty}$ and decreased the CL_{tot} (Table 2). We measured the production ratio of demethyl- and hydroxy-TAC in the hepatic microsomes of BDL rats, because the main metabolite of TAC in the blood and urine of patients with a liver graft is demethyl-TAC, whereas the main metabolite of TAC in the bile is hydroxy-TAC [29]. Analysis of the enzyme kinetics of TAC in hepatic microsomes showed that the production ratios of both demethyl- and hydroxy-TAC were significantly lower in the BDL rats than in the controls, which indicates that the intrinsic clearance (CL_{int}) of TAC in the liver reduced in the BDL rats (Figure 3). Bastien et al., reported that BDL resulted in biliary cirrhosis with a reduction in the liver protein content and the activity of CYP3A, a primary metabolic enzyme of TAC [30]. Sasa et al., estimated the hepatic extraction ratio (E_h) of TAC using a physiologically based PK model [31]. The authors concluded that TAC could be considered as an intermediate hepatic extraction drug, and that CL_{tot} and E_h of TAC depend on both the hepatic blood flow and CL_{int} in the liver. Moreover, previously studies showed that the hepatic blood flow and hepatic arterial plasma flow increased in BDL rats [31,32]. On the basis of these findings, we can conclude that the decrease in the CL_{tot} in BDL rats occurs through a decrease in the CL_{int} induced by the decrease in hepatic CYP activity, which results in an increase in the $AUC_{0-\infty}$ and blood TAC concentration. Some studies have shown that the AUC values are important determiners for the clinical outcome and side effects of TAC. For example, the AUC values of TAC were significantly lower

in patients with acute rejection than in patients who had no acute rejection [33,34]. These observations suggest that the decrease in the CL_{int} of TAC during BDL would contribute to the inter- and intra-patient pharmacokinetic variability. Therefore, TDM should be performed, and the results of TDM should be carefully monitored in patients with bile duct stricture.

As shown in Table 2, Vd_{ss} after an i.v. bolus injection of TAC in BDL rats was approximately half of that in control rats. In addition, the blood concentration of TAC at 15 min after an i.v. bolus injection in BDL rats (70.1 ± 4.8 ng/mL) during the distribution phase was approximately 1.6-times higher than that in control rats (44.9 ± 3.7 ng/mL) (Figure 2). These findings clearly indicate that distribution of TAC from the systemic circulation to peripheral or central tissues is limited. Hyogo et al., examined the expression of hepatic adenosine triphosphate-binding cassette (ABC) transport proteins and their mRNA levels in canalicular membrane vesicles isolated from rat liver after BDL and observed a clear increase in the mRNA expression levels of multiple drug resistance-Pgp (MDR-Pgp) and MDR1b [35]. In addition, oxidative stress was observed in the blood, liver, intestine, kidney, and brain of BDL rats [36,37]. The findings of our previous studies indicate that oxidative stress-induced impairments may affect the affinity of lipophilic drugs (binding or penetrating) for erythrocyte membranes and thus increase the drug distribution to erythrocytes in a rat model of ferric-nitritolriacetate-induced oxidative stress [38-40]. Thus, although the liver is a highly perfused organ, the increase in Pgp-mediated excretion of TAC and increase in TAC distribution to erythrocytes in BDL rats may limit TAC distribution to the liver, peripheral, or central tissues and thus decrease the Vd_{ss} and increase the blood TAC concentration. Although blood TAC concentrations are high after bile duct stricture, the pharmacological effects of the drug are not changed because TAC does not reach the intended targets in the tissues. Moreover, the limited distribution to the liver in BDL rats might contribute to reduced hepatic metabolism, which decreases the CL_{tot} as described above. However, because we did not determine TAC distribution in the blood compartment and in the liver, peripheral, or central tissues in BDL rats, further studies are required to completely elucidate the alterations in TAC distribution in BDL rats.

Bile acids, such as ursodeoxycholic acid and chenodeoxycholic acid, may lead to formation of micelles with lipophilic drugs such as TAC and consequently enhance their absorption from the gastrointestinal tract [41]. To investigate the influence of reduction in the amount of intestinal bile on the intestinal absorption of TAC in bile duct stricture, we performed an *in situ* absorption study. We examined the total intestinal permeability using *in situ* intraloop administration rather than oral or enteral administration, because the former method excludes the effects of gastric acid and gastrointestinal motility. Since TAC is well absorbed from the upper section of the small intestine [42], we performed the

in situ absorption study using the upper section of the small intestine. The bile dose administered into the intestine (1.28 mL/kg) was based on the previously reported value of bile flow in rats (3.2 mL/h/kg) [43] and the blood sampling time of 2 h. Upon intraloop administration of TAC with undiluted bile, double-diluted bile, or saline, the AUC_{0-2} of TAC showed a positive correlation with the amount of bile (Figure 4 and Table 2). Sauer et al., reported that after BDL, the intestinal absorption rate of a bile acid taurocholate reduced and that the intestinal absorption of bile acids appears to be regulated by their systemic concentration [44]. Bile is necessary for solubilizing CyA, and a lack of bile after BDL decreases the CyA absorption [45,46]. These results are consistent with our suggestion that the intestinal absorption of TAC depends on the amount of bile in the intestine, and the lack of bile after BDL may result in decreased TAC absorption. Clinically, biliary drainage in patients receiving living donor liver transplantation plays an important role in the prevention of biliary anastomotic complications and the treatment of biliary anastomotic stenosis. When biliary drainage is performed at Kyoto Prefectural University of Medicine, half of the bile excreted from the drain is returned into the intestinal tract while the remaining half is discarded, which may decrease the TAC absorption and thus the blood TAC concentrations. In a related study, Deters et al., reported that daily treatment with TAC for 2 weeks reduced the bile flow [47]. This decrease in bile flow may reduce TAC absorption. Our findings are similar to those reported previously in that the amount of bile in the intestine is an important factor in TAC treatment. When the TAC formulation is changed from an injectable formulation to an oral one or during long-term oral administration of TAC in patients with bile duct stricture, the decrease in TAC absorption should be considered and the dose of TAC should be appropriately adjusted.

The findings of our study suggest that personalized treatment is required for patients with bile duct stricture, and the TDM data should be carefully examined. However, our study has a limitation. The BDL rat model does not completely represent the complexity associated with the human disease nor does it replace the studies performed using human tissues. However, the model is a valuable tool for developing and evaluating a variety of novel immunosuppressants or emerging therapeutic strategies that are difficult to address in humans.

Conclusions

In conclusion, BDL decreased the hepatic CL_{int} of TAC, and this decrease could contribute to the clinically observed inter- and intra-patient pharmacokinetic variability. This suggests that personalized treatment and careful examination of the TDM data are required in patients with bile duct stricture. Increase in the Pgp-mediated excretion of TAC in the liver and increase in the distribution of TAC to erythrocytes in BDL rats might limit TAC distribution to the liver, peripheral, or central

tissues and thus decrease the Vd_{ss} and increase in the blood TAC concentration. Although the blood TAC concentrations are high after bile duct stricture, the pharmacological effects of the drug are unchanged because TAC does not reach the intended targets in the tissues. This limited distribution to the liver in BDL rats might contribute to a decrease in hepatic metabolism and thus a decrease in CL_{tot} . Our findings suggest that the amount of bile in the intestine is an important factor in TAC treatment. Therefore, when switching from an injectable formulation of TAC to an oral one or during long-term oral administration of TAC in patients with bile duct stricture, the decrease in absorption and hepatic drug metabolism should be taken into account, and the dose of TAC should be accordingly adjusted.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Authors' contributions	SK	KF	YM	TK	HU	NY	NS	KT
Research concept and design	√	√	√	√	√	√	√	√
Collection and/or assembly of data	--	--	√	√	√	√	√	--
Data analysis and interpretation	√	√	√	√	√	√	√	√
Writing the article	√	√	√	--	--	--	√	--
Critical revision of the article	√	√	--	--	--	--	√	--
Final approval of article	√	√	√	√	√	√	√	√
Statistical analysis	√	√	√	--	--	--	√	--
Other (please specify)...	--	--	--	--	--	--	--	--

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References

- Kino T, Hatanaka H, Hashimoto M, Nishiyama M, Goto T, Okuhara M, Kohsaka M, Aoki H and Imanaka H. **FK-506, a novel immunosuppressant isolated from a Streptomyces. I. Fermentation, isolation, and physico-chemical and biological characteristics.** *J Antibiot (Tokyo)*. 1987; **40**:1249-55. | [Article](#) | [PubMed](#)
- Kino T, Hatanaka H, Miyata S, Inamura N, Nishiyama M, Yajima T, Goto T, Okuhara M, Kohsaka M, Aoki H and et al. **FK-506, a novel immunosuppressant isolated from a Streptomyces. II. Immunosuppressive effect of FK-506 in vitro.** *J Antibiot (Tokyo)*. 1987; **40**:1256-65. | [Article](#) | [PubMed](#)
- Plosker GL and Foster RH. **Tacrolimus: a further update of its pharmacology and therapeutic use in the management of organ transplantation.** *Drugs*. 2000; **59**:323-89. | [Article](#) | [PubMed](#)
- Haddad EM, McAlister VC, Renouf E, Malthaner R, Kjaer MS and Gluud LL. **Cyclosporin versus tacrolimus for liver transplanted patients.** *Cochrane Database Syst Rev*. 2006; **4**:CD005161. | [Article](#) | [PubMed](#)
- Staatz CE and Tett SE. **Clinical pharmacokinetics and pharmacodynamics of tacrolimus in solid organ transplantation.** *Clin Pharmacokinet*. 2004; **43**:623-53. | [Article](#) | [PubMed](#)
- Naesens M, Kuypers DR and Sarwal M. **Calcineurin inhibitor nephrotoxicity.** *Clin J Am Soc Nephrol*. 2009; **4**:481-508. | [Article](#) | [PubMed](#)
- Kershner RP and Fitzsimmons WE. **Relationship of FK506 whole blood concentrations and efficacy and toxicity after liver and kidney transplantation.** *Transplantation*. 1996; **62**:920-6. | [Article](#) | [PubMed](#)
- McMaster P, Mirza DF, Ismail T, Vennarecci G, Patapis P and Mayer AD. **Therapeutic drug monitoring of tacrolimus in clinical transplantation.** *Ther Drug Monit*. 1995; **17**:602-5. | [Article](#) | [PubMed](#)
- Boswell GW, Bekersky I, Fay J, Wingard J, Antin J, Weisdorf D, Maher R, Fitzsimmons W and Nash R. **Tacrolimus pharmacokinetics in BMT patients.** *Bone Marrow Transplant*. 1998; **21**:23-8. | [Article](#) | [PubMed](#)
- Jusko WJ, Piekoszewski W, Klintmalm GB, Shaefer MS, Hebert MF, Piergies AA, Lee CC, Schechter P and Mekki QA. **Pharmacokinetics of tacrolimus in liver transplant patients.** *Clin Pharmacol Ther*. 1995; **57**:281-90. | [Article](#) | [PubMed](#)
- Stratta RJ, Wood RP, Langnas AN, Hollins RR, Bruder KJ, Donovan JP, Burnett DA, Lieberman RP, Lund GB, Pillen TJ and et al. **Diagnosis and treatment of biliary tract complications after orthotopic liver transplantation.** *Surgery*. 1989; **106**:675-83. | [PubMed](#)
- Ostroff JW. **Post-transplant biliary problems.** *Gastrointest Endosc Clin N Am*. 2001; **11**:163-83. | [Article](#) | [PubMed](#)
- Sokol RJ, Devereaux M, Khandwala R and O'Brien K. **Evidence for involvement of oxygen free radicals in bile acid toxicity to isolated rat hepatocytes.** *Hepatology*. 1993; **17**:869-81. | [Article](#) | [PubMed](#)
- Poli G. **Pathogenesis of liver fibrosis: role of oxidative stress.** *Mol Aspects Med*. 2000; **21**:49-98. | [Article](#) | [PubMed](#)
- Tahan G, Akin H, Aydogan F, Ramadan SS, Yapicier O, Tarcin O, Uzun H, Tahan V and Zengin K. **Melatonin ameliorates liver fibrosis induced by bile-duct ligation in rats.** *Can J Surg*. 2010; **53**:313-8. | [Pdf](#) | [PubMed](#) | [Abstract](#) | [PubMed Full Text](#)
- Minich DM, Havinga R, Stellaard F, Vonk RJ, Kuipers F and Verkade HJ. **Intestinal absorption and postabsorptive metabolism of linoleic acid in rats with short-term bile duct ligation.** *Am J Physiol Gastrointest Liver Physiol*. 2000; **279**:G1242-8. | [Article](#) | [PubMed](#)
- Sanchez-Patan F, Anchuelo R, Corcuera MT, Casado I, Gomez-Aguado F, Aller MA, Cruz A, Alonso MJ and Arias J. **Biliary fibrosis in microsurgical extrahepatic cholestasis in the rat.** *Microsurgery*. 2008; **28**:361-6. | [Article](#) | [PubMed](#)
- Sugioka N, Sato K, Haraya K, Maeda Y, Yasuda K, Fukushima K, Ito Y and Takada K. **Effect of streptozotocin-induced diabetes mellitus on the pharmacokinetics of nelfinavir in rats.** *Biopharm Drug Dispos*. 2008; **29**:469-79. | [Article](#) | [PubMed](#)
- Hayes KA, Brennan B, Chenery R and Houston JB. **In vivo disposition of caffeine predicted from hepatic microsomal and hepatocyte data.** *Drug Metab Dispos*. 1995; **23**:349-53. | [Article](#) | [PubMed](#)
- Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. **Protein measurement with the Folin phenol reagent.** *J Biol Chem*. 1951; **193**:265-75. | [Article](#) | [PubMed](#)
- Koster RA, Dijkers EC and Uges DR. **Robust, high-throughput LC-MS/MS method for therapeutic drug monitoring of cyclosporine, tacrolimus, everolimus, and sirolimus in whole blood.** *Ther Drug Monit*. 2009; **31**:116-25. | [Article](#) | [PubMed](#)
- Maya-Mendoza A, Hernandez-Munoz R, Gariglio P and Aranda-Anzaldo A. **Gene positional changes relative to the nuclear substructure during carbon tetrachloride-induced hepatic fibrosis in rats.** *J Cell Biochem*. 2004; **93**:1084-98. | [Article](#) | [PubMed](#)
- Mikami K, Otaka M, Goto T, Miura K, Ohshima S, Yoneyama K, Lin JG, Watanabe D, Segawa D, Kataoka E, Odashima M and Watanabe S. **Induction of a 72-kDa heat shock protein and protection against lipopolysaccharide-induced liver injury in cirrhotic rats.** *J Gastroenterol Hepatol*. 2004; **19**:884-90. | [Article](#) | [PubMed](#)
- Antoine M, Tag CG, Wirz W, Borkham-Kamphorst E, Sawitz I, Gressner AM and Kiefer P. **Upregulation of pleiotrophin expression in rat hepatic stellate cells by PDGF and hypoxia: implications for its role in**

- experimental biliary liver fibrogenesis. *Biochem Biophys Res Commun*. 2005; **337**:1153-64. | [Article](#) | [PubMed](#)
25. Aziz TA, Aziz MA, Fouad HH, Rashed LA, Salama H, Abd-Alla S, Wehab MA and Ahmed T. Interferon-alpha gene therapy prevents aflatoxin and carbon tetrachloride promoted hepatic carcinogenesis in rats. *Int J Mol Med*. 2005; **15**:21-6. | [Article](#) | [PubMed](#)
26. Rivera-Espinosa L, Muriel P, Ordaz Gallo M, Perez-Urizar J, Palma-Aguirre A and Castaneda-Hernandez G. Ketorolac pharmacokinetics in experimental cirrhosis by bile duct ligation in the rat. *Ann Hepatol*. 2003; **2**:175-81. | [Article](#) | [PubMed](#)
27. Ji H, Jiang JY, Xu Z, Kroeger EA, Lee SS, Liu H, Shen H, Zhang M, Minuk GY, Choy PC and Gong Y. Change in lipid profile and impairment of endothelium-dependent relaxation of blood vessels in rats after bile duct ligation. *Life Sci*. 2003; **73**:1253-63. | [Article](#) | [PubMed](#)
28. Fursule RA and Patil SD. Hepatoprotective and antioxidant activity of *Phaseolus trilobus*, Ait on bile duct ligation induced liver fibrosis in rats. *J Ethnopharmacol*. 2010; **129**:416-9. | [Article](#) | [PubMed](#)
29. Christians U, Braun F, Kosian N, Schmidt M, Schiebel HM, Ernst L, Kruse C, Winkler M, Holze I, Linck A and et al. High performance liquid chromatography/mass spectrometry of FK 506 and its metabolites in blood, bile, and urine of liver grafted patients. *Transplant Proc*. 1991; **23**:2741-4. | [PubMed](#)
30. Bastien MC, Leblond F, Pichette V and Villeneuve JP. Differential alteration of cytochrome P450 isoenzymes in two experimental models of cirrhosis. *Can J Physiol Pharmacol*. 2000; **78**:912-9. | [Article](#) | [PubMed](#)
31. Sasa H, Hashimoto Y, Shimizu T and Inui K. Hepatic extraction of tacrolimus in rats with experimental liver diseases. *Biol Pharm Bull*. 1998; **21**:610-4. | [Article](#) | [PubMed](#)
32. Houdijk AP, Teerlink T, Bloemers FW, Wesdorp RI and van Leeuwen PA. Gut endotoxin restriction prevents catabolic changes in glutamine metabolism after surgery in the bile duct-ligated rat. *Ann Surg*. 1997; **225**:391-400. | [Article](#) | [PubMed Abstract](#) | [PubMed Full Text](#)
33. Undre NA. Pharmacokinetics of tacrolimus-based combination therapies. *Nephrol Dial Transplant*. 2003; **18 Suppl 1**:i12-5. | [Article](#) | [PubMed](#)
34. Undre NA, van Hooff J, Christiaans M, Vanrenterghem Y, Donck J, Heeman U, Kohnle M, Zanker B, Land W, Morales JM, Andres A, Schafer A and Stevenson P. Low systemic exposure to tacrolimus correlates with acute rejection. *Transplant Proc*. 1999; **31**:296-8. | [Article](#) | [PubMed](#)
35. Hyogo H, Tazuma S, Nishioka T, Ochi H, Yamaguchi A, Numata Y, Kanno K, Sakamoto M, Asamoto Y, Tsuboi K, Nakai K, Yasumiba S, Sunami Y and Kajiyama G. Phospholipid alterations in hepatocyte membranes and transporter protein changes in cholestatic rat model. *Dig Dis Sci*. 2001; **46**:2089-97. | [Article](#) | [PubMed](#)
36. Huang YT, Hsu YC, Chen CJ, Liu CT and Wei YH. Oxidative-stress-related changes in the livers of bile-duct-ligated rats. *J Biomed Sci*. 2003; **10**:170-8. | [Article](#) | [PubMed](#)
37. Grintzalis K, Papapostolou I, Assimakopoulos SF, Mavarakis A, Faropoulos K, Karageorgos N, Georgiou C, Chroni E and Konstantinou D. Time-related alterations of superoxide radical levels in diverse organs of bile duct-ligated rats. *Free Radic Res*. 2009; **43**:803-8. | [Article](#) | [PubMed](#)
38. Kobuchi S, Fukushima K, Aoyama H, Matsuda T, Ito Y, Sugioka N and Takada K. Effect of oxidative stress on the pharmacokinetics of clomipramine in rats treated with ferric-nitrilotriacetate. *Drug Metab Lett*. 2011; **5**:243-52. | [Article](#) | [PubMed](#)
39. Kobuchi S, Fukushima K, Aoyama H, Ito Y, Sugioka N and Takada K. Effects of oxidative stress on the pharmacokinetics and hepatic metabolism of atazanavir in rats. *Free Radic Res*. 2013; **47**:291-300. | [Article](#) | [PubMed](#)
40. Kobuchi S, Fukushima K, Ito Y, Sugioka N and Takada K. Pharmacokinetics and distribution of fluvoxamine to the brain in rats under oxidative stress. *Free Radic Res*. 2012; **46**:831-41. | [Article](#) | [PubMed](#)
41. Sasaki M, Maeda A, Sakamoto K and Fujimura A. Effect of bile acids on absorption of nitrendipine in healthy subjects. *Br J Clin Pharmacol*. 2001; **52**:699-701. | [Article](#) | [PubMed Abstract](#) | [PubMed Full Text](#)
42. Shimomura M, Masuda S, Saito H, Sakamoto S, Uemoto S, Tanaka K, Inui K. Roles of the jejunum and ileum in the first-pass effect as absorptive barriers for orally administered tacrolimus. *J Surg Res*. 2002; **103**(2):215-222. | [Article](#) | [PubMed](#)
43. Igarashi T, Yano I, Saito H and Inui K. Decreased cyclosporin A concentrations in the absorption phase using microemulsion preconcentrate formulation in rats with cisplatin-induced acute renal failure. *Biol Pharm Bull*. 2003; **26**:1591-5. | [Article](#) | [PubMed](#)
44. Sauer P, Stiehl A, Fitscher BA, Riedel HD, Benz C, Kloters-Plachky P, Stengelin S, Stremmel W and Kramer W. Downregulation of ileal bile acid absorption in bile-duct-ligated rats. *J Hepatol*. 2000; **33**:2-8. | [Article](#) | [PubMed](#)
45. Kobayashi M, Takaya S and Koie H. Effect of a stable analogue of prostacyclin on cyclosporine A-induced nephrotoxicity: morphological qualitative and quantitative studies. *Transplant Proc*. 1988; **20**:183-6. | [PubMed Abstract](#) | [PubMed Full Text](#)
46. Furukawa H, Imvitarza O, Venkataramanan R, Suzuki M, Zhu Y, Warty VS, Fung J, Todo S and Starzl TE. The effect of bile duct ligation and bile diversion on FK506 pharmacokinetics in dogs. *Transplantation*. 1992; **53**:722-5. | [Article](#) | [PubMed Abstract](#) | [PubMed Full Text](#)
47. Deters M, Klabunde T, Kirchner G, Resch K and Kaefer V. Sirolimus/cyclosporine/tacrolimus interactions on bile flow and biliary excretion of immunosuppressants in a subchronic bile fistula rat model. *Br J Pharmacol*. 2002; **136**:604-12. | [Article](#) | [PubMed Abstract](#) | [PubMed Full Text](#)

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