Use of a semi-physiological pharmacokinetic model to investigate the influence of itraconazole on tacrolimus absorption, distribution and metabolism in mice

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Abstract
1. The aim of this study was to investigate the influence of itraconazole (ITCZ) on tacrolimus absorption, distribution and metabolism by developing a semi-physiological pharmacokinetic model of tacrolimus in mice.
2. Mice were randomly divided into four groups, namely control group (CG, taking 3 mg kg⁻¹ tacrolimus only), low-dose group (LDG, taking tacrolimus with 12.5 mg kg⁻¹ ITCZ), medium-dose group (MDG, taking tacrolimus with 25 mg kg⁻¹ ITCZ) and high-dose group (HDG, taking tacrolimus with 50 mg kg⁻¹ ITCZ).
3. Liver clearance (CLli) decreased significantly (**p < 0.01) in LDG (35.3%), MDG (45.2%) and HDG (58.7%) mice compared to CG mice. With respect to gut clearance (CLgu), significant (**p < 0.01) decrease was also revealed in LDG (35.9%), MDG (50.2%) and HDG (64.6%) mice. A significant (**p < 0.01) higher tacrolimus brain-to-blood partition coefficient (Kt,br) was found in MDG (25.3%) and HDG (55.9%) mice than in CG mice. Moreover, a significant (*p < 0.05) increase (16.3%) was found in the absorption rate constant (Ka) in HDG mice compared to CG mice. There was a significant (**p < 0.01) association between ITCZ dose and the change in CLgu (DCLgu, r = 0.790), the change in CLli (DCLli, r = 0.787) and the change in Kt,br (DKt,br, r = 0.727), while the association between ITCZ dose and the change in Ka (DKa) was not significant (p > 0.05).
4. These findings could be useful in predicting the efficacy and toxicity of tacrolimus, and drug–drug interaction of ITCZ and tacrolimus in human.

Introduction
Fungal infection is one of the most fatal complications in organ transplantation (Brooks et al., 1985; Dummer et al., 1986; Kramer et al., 1991, 1993). In the setting of intense immune suppression immediately after transplantation, fungi, particularly Aspergillus and Candida, tend to colonise over the new anastomosis that is not yet well vascularised (Dummer et al., 1986; Kramer et al., 1993). The antifungal agent itraconazole (ITCZ) is routinely used as prophylaxis against Aspergillus and Candida infection and often administered to solid organ transplant and haematopoietic stem cell transplantation recipients receiving immunosuppressant (Kramer et al., 2011; Leather et al., 2006; Nara et al., 2013; Togashi et al., 2015). The mechanism of action of ITCZ is the same as the other azole antifungals: it inhibits the fungal-mediated synthesis of ergosterol by inhibiting cytochrome P450 3A (CYP3A) in fungi (Grant & Clissold, 1989; Heykants et al., 1989; De Beule & Van Gestel, 2001). In addition to inhibiting CYP3A, ITCZ is also metabolised extensively by CYP3A (Kunze et al., 2006; Peng et al., 2012). The metabolites of ITCZ, keto-ITCZ, hydroxy-ITCZ, and N-desalkyl-ITCZ, also contribute to CYP3A inhibition (Templeton et al., 2010). The persistent inhibition of CYP3A after ITCZ dosing is related to the effects of inhibitory metabolites with long half-life (Templeton et al., 2010). ITCZ and its metabolites have also been shown to be drug transporter P-glycoprotein (P-gp) inhibitors (Isoherranen et al., 2004; Shon et al., 2005; Templeton et al., 2010). Therefore, therapy with other medications metabolised by this route must be monitored for signs of toxicity.

Tacrolimus is a macrolide immunosuppressive agent used for the prevention of allograft rejection in organ transplantation, such as the lung, heart, liver, kidney, etc. (Fung, 2004;
Gupta et al., 2002; Hooks, 1994; Peters et al., 1993; Scott et al., 2003; Venkataramanan et al., 1995; Woo & James, 2005). Tacrolimus is metabolised by cytochrome P450 (CYP) 3A in human, and is also a substrate of P-gp (Hooks, 1994; Scott et al., 2003; Venkataramanan et al., 1995). Generally, inhibition of CYP3A-mediated metabolism of tacrolimus is regarded as the clinically most important drug interaction mechanisms. Drugs that inhibit this enzyme system, such as azole antifungals, bромокриптин, симетидин, calcium channel blockers, HIV-protease inhibitors and macrolide antibacterials may produce increased blood concentrations of tacrolimus (Capone et al., 1999; Christians et al., 1996; Hebert & Lam, 1999). Consequently, clinicians should pay particular attention to tacrolimus blood concentrations and tacrolimus-induced side effects when co-administered with these drugs. This is very important because overexposure of tacrolimus is connected with significant toxicity (neurotoxicity, nephrotoxicity, hematotoxicity, cardiac toxicity, posttransplant diabetes mellitus, increased risk of infections and malignancies) (Filler et al., 1997; Masuda & Inui, 2006; Iwasaki, 2007).

Physiologically based pharmacokinetic (PBPK) modelling, treating the body as anatomical compartments connected by blood flow, uses physiological and chemical-specific parameters, as well as mathematical equations to quantitatively describe the in vivo disposition of xenobiotics (Barrett et al., 2012; Lu et al., 2016). Compared with traditional compartmental modelling and non-compartmental analysis, which usually only focus on analysing concentration-time data in blood, PBPK modelling is a more mechanistic approach for studying xenobiotic absorption, distribution, metabolism and elimination (Nesterov, 2007). PBPK modelling is also capable of extrapolating across dose levels, formulations, routes of administration and species (Barrett et al., 2012; Lu et al., 2016; Rostami-Hodjegan, 2012). Therefore, one application of PBPK models is predicting xenobiotic exposure in humans based on that in experimental animals. In addition, this type of model may allow for the evaluation of the effects of different factors including age, disease, gender, genetics, drug–drug interaction, etc., on xenobiotic disposition (Edginton et al., 2008; Lu et al., 2016; Zhao et al., 2011). Combined with pharmacodynamic data, PBPK modelling also aids the understanding of therapeutic benefits and adverse effects of drugs, leading to optimised dosage regimens (Khalil & Läer, 2011). Because of these advantageous features, the interest in applying PBPK models in pharmaceutical industries and research academies has been rapidly growing in recent years (Rostami-Hodjegan, 2012).

Although antifungal agents have been shown to inhibit tacrolimus metabolism (Furlan et al., 1995), the reported studies concerning drug–drug interactions between ITCZ and tacrolimus in transplant recipients are mostly from case reports or small series (Billaud et al., 1998; Furlan et al., 1998; Outeda Macías et al., 2000). To date, no study systematically evaluated the influence of ITCZ on tacrolimus absorption, distribution and in vivo metabolism, all of which are important in predicting the efficacy and toxicity of tacrolimus. Therefore, we investigated the influence of ITCZ on tacrolimus absorption, distribution, hepatic and intestinal clearance by developing a semi-physiological pharmacokinetic (PK) model of tacrolimus in mice, in order that the findings in the present study could be useful in predicting the efficacy and toxicity of tacrolimus, and drug–drug interaction of itraconazole and tacrolimus in human.

Materials and methods

Chemicals, reagents and animals

Tacrolimus (purity > 98%) and ascomycin (purity > 98%) reference standards were purchased from the National Institutes for Food and Drug Control (Beijing, China). Tacrolimus raw materials (purity > 98%) used for preparing the solutions for gavage were provided by Struchem Co., Ltd. (Jiangsu, China), while ITCZ raw materials (purity > 98%) used for preparing the solutions for gavage were provided by Hubei Jusheng Technology Co., Ltd. (Tianmen, China). Methanol of HPLC grade was purchased from Fisher Scientific (Fair Lawn, NJ). Distilled water was prepared from demineralised water throughout the study. Other chemicals were of analytical grade.

Male Kunming strain mice (22 ± 2 g) were kindly provided by the Experimental Animal Centre of Shenyang Pharmaceutical University (Shenyang, China) and fed with unlimited access to food and water in an air-conditioned animal centre at a temperature of 22 ± 2°C and a relative humidity of 50 ± 10%, with a natural light–dark cycle for a week and then fasted with only access to water for 12 h prior to the experiment. The animal study was carried out in accordance with the Guideline for Animal Experimentation of Shenyang Pharmaceutical University, and the protocol was approved by the Animal Ethics Committee of the institution.

Preparation of tacrolimus and ITCZ solutions

The preparation procedure of tacrolimus solution used for gavage administration was as follows: the drug was supplied as 25 mg of tacrolimus in 1 mL of polysorbate 80. Before administration, the drug was diluted in distilled water maintaining a concentration of drug of about 0.165 mg mL⁻¹.

The preparation procedure of ITCZ solutions used for gavage administration was as follows: The drug was supplied as 100 mg of ITCZ in 2 mL of polysorbate 80. Before administration, the drug was diluted in distilled water maintaining a concentration of drug of about 0.685, 1.37 and 2.74 mg mL⁻¹, respectively.

Blood and tissue sample collection

A total of 144 mice were randomly divided into four groups, namely control group (CG), low-dose group (LDG), medium-dose group (MDG) and high-dose group (HDG). Animal numbers in each group was calculated based on the power analysis with a power of larger than 80% (effect size results see Table S1 and S2). Dose selection was based on the clinical dosages for both tacrolimus (0.3 mg kg⁻¹ day⁻¹) and ITCZ (100, 200 and 400 mg day⁻¹), and scaled to mice using the body surface area (BSA) method.

An oral dose of 12.5 (LDG), 25 (MDG) and 50 mg kg⁻¹ (HDG) ITCZ were first given to the mice and tacrolimus solution were given orally at a dose of 3 mg kg⁻¹ an hour later. Blood samples of about 0.6 mL were collected from each animal by cardiac puncture and placed into heparinised
centrifuge tubes, at 0.083, 0.5, 2, 8, 12 and 24 h after dosing. These samples were stored at $-80^\circ$C until analysis.

The tissue distribution study was also carried out on 144 mice, with the dosing regimen same as that mentioned in the blood sample collection procedure. After administration, the mice were sacrificed at 0.083, 0.5, 2, 8, 12 and 24 h, and tissues (brain, fat, gut, heart, kidneys, liver, lungs, muscle and spleen) were collected at the same time. The tissue samples were rinsed in ice-cold normal saline, blotted dry with filter paper, and then stored at $-80^\circ$C until analysis.

**Tacrolimus assay**

The quantitative analytical method of tacrolimus in mouse blood/tissues is established based on the liquid chromatography-mass spectrometry (LC-MS, Waters Co., Milford, MA) method. Briefly, chromatographic separation was performed using a Hypersil BDS C18 (50 mm $\times$ 2.1 mm, 3.0 $\mu$m particle size) (Elite Analytical Instruments Co., Ltd., Dalian, China) column kept at 25$^\circ$C with a constant flow rate of 0.2 mL min$^{-1}$. The mobile phase consisted of methanol-2 mmol L$^{-1}$ ammonium acetate (95:5, v/v). Tandem mass spectrometry detection was performed in the positive ion, multiple reaction monitoring (MRM) mode following the transition $m/z$ 821.7 $\rightarrow$ 768.9 for tacrolimus, and the transition $m/z$ 809.8 $\rightarrow$ 757.0 for internal standard (IS, ascomycin). The IS stock solution was prepared at a concentration of 809.8 m/z.

Samples was reconstituted with 100 mL mobile phase, and a volume of 3 mL ethyl acetate, vortex-mixed for 5 min and centrifuged at 4000 rpm for 10 min. The upper extract was then evaporated to dryness using a Hypersil BDS C18 (50 mm $\times$ 8 mm, 5.0 $\mu$m particle size) column kept at 35$^\circ$C for 10 min. The upper extract was then evaporated to dryness under nitrogen stream. The residue of blood/tissue samples was reconstituted with 100 mL mobile phase, and a 10 mL aliquot was injected onto the LC-MS system.

The retention times for tacrolimus and IS were approximately 1.8 and 1.4 min, respectively. No interference from any metabolites and endogenous substances was observed. The method was linear over the concentration range of 0.5–200 ng mL$^{-1}$ for mouse blood (2.5–5000 ng g$^{-1}$ for mouse tissues) (Table S3), with the lower limit of qualification (LLOQ) of 0.5 ng mL$^{-1}$ for mouse blood and 2.5 ng g$^{-1}$ for mouse tissues, respectively. The method showed good intra-assay precision and accuracy with relative standard deviation (% RSD) values from 5.5 to 10.3% and mean relative error (% MRE) from −2.5 to 3.2%, as well as good inter-assay precision and accuracy with RSD from 4.1 to 6.7%. The recoveries for all the bio-samples were over 66.7% (66.7–74.3%). There was no significant matrix effect for all the bio-samples were found to be within the range ±15% (Table S4 and S5).

**Development of the semi-physiological PK model**

The whole physiological model contains 12 single-organ models (Figure 1), which were selected and optimised based on a significantly drop of residual errors and biological plausibility of the PK parameters. All the single-organ compartments were connected by blood flow. As tacrolimus is extensively eliminated by metabolism in liver and gut (Iwasaki, 2007; Jeong & Chiu, 2006; Marchetti et al., 2007), both hepatic ($K_{l,l}$) and intestinal ($K_{l,u}$) elimination pathways, which were assumed to be involved in the clearance of tacrolimus, were included in the model structure. The following differential equations were used to describe the model structure for tacrolimus disposition:

**Lung (lu):**

$$V_{lu} \times \frac{dC_{lu}}{dt} = Q_{co} \times \left( C_{ve} - \frac{C_{lu}}{K_{l,lu}} \right) \quad (1)$$

**Artery (ar):**

$$V_{ar} \times \frac{dC_{ar}}{dt} = Q_{co} \times \left( \frac{C_{lu}}{K_{l,lu}} - C_{ar} \right) \quad (2)$$

**Brain (br):**

$$V_{br,v} \times \frac{dC_{br,v}}{dt} = Q_{br} \times \left( C_{ar} - C_{br,v} \right) - K_{p,br} \times \left( C_{br,v} - \frac{C_{br,e}}{K_{t,br}} \right) \quad (3)$$

$$V_{br,e} \times \frac{dC_{br,e}}{dt} = K_{p,br} \times \left( C_{br,v} - \frac{C_{br,e}}{K_{t,br}} \right) \quad (4)$$

**Heart (he), spleen (sp), kidney (ki), muscle (mu) and remainder (re):**

$$V_{tissue} \times \frac{dC_{tissue}}{dt} = Q_{tissue} \times \left( C_{ar} - C_{tissue} \right) \quad (5)$$

\[ \text{where} \]

- $Q_{co}$: blood flow rate to tissues; $Q_{tissue}$: tissue clearance.

- $K_{l,l}$ and $K_{l,u}$: hepatic and intestinal elimination rates.

- $C_{ve}$: central venous concentration.

- $V_{lu}$ and $V_{ar}$: volume of distribution in the lung and artery, respectively.

- $V_{br,v}$ and $V_{br,e}$: volumes of distribution in the brain in the vascular and extravascular spaces, respectively.

- $K_{p,br}$: brain perfusion rate.

- $K_{t,br}$: tissue to brain exchange rate.

- $C_{br,v}$ and $C_{br,e}$: concentrations in the brain vascular and extravascular spaces, respectively.

- $C_{tissue}$: tissue concentration.
Dosing compartment:
\[
\frac{dX}{dt} = -K_a \times X
\]  
(6)

Gut (gu):
\[
V_{gu} \times \frac{dC_{gu}}{dt} = K_d \times X + Q_{ar} \times \left( C_{ar} - \frac{C_{gu}}{K_{t,gu}} \right) - CL_{gu} \times C_{gu}
\]  
(7)

Liver (li):
\[
V_{li} \times \frac{dC_{li}}{dt} = \left( Q_{li} - Q_{sp} - Q_{gu} \right) \times C_{ar}
+ Q_{sp} \times \frac{C_{sp}}{K_{t,sp}} + Q_{gu} \times \frac{C_{gu}}{K_{t,gu}}
- Q_{li} \times \frac{C_{li}}{K_{t,li}} - CL_{li} \times C_{li}
\]  
(8)

Fat (fa):
\[
V_{fa} \times \frac{dC_{fa,c}}{dt} = Q_{fa} \times \left( C_{ar} - \frac{C_{fa,c}}{K_{t,fa}} \right) - K_{in,fa} \times C_{fa,c}
\times V_{fa} + K_{out,fa} \times X_{fa,p}
\]  
(9)

\[
\frac{dX_{fa,p}}{dt} = K_{in,fa} \times C_{fa,c} \times V_{fa} - K_{out,fa} \times X_{fa,p}
\]  
(10)

Vein (ve):
\[
V_{ve} \times \frac{dC_{ve}}{dt} = Q_{br} \times \frac{C_{br}}{K_{t,br}} + Q_{he} \times \frac{C_{he}}{K_{t,he}} + Q_{li} \times \frac{C_{li}}{K_{t,li}}
+ Q_{ki} \times \frac{C_{ki}}{K_{t,ki}} + Q_{mu} \times \frac{C_{mu}}{K_{t,mu}}
+ Q_{fa} \times \frac{C_{fa}}{K_{t,fa}} + Q_{re} \times \frac{C_{re}}{K_{t,re}} - Q_{co} \times \frac{C_{li}}{K_{t,li}}
\]  
(11)

where \( C_{ar} \) represents the tacrolimus concentration in artery blood; \( C_{tissue} \) represents the drug concentration in tissues; \( C_{ve} \) represents the tacrolimus concentration in venous blood; 
\( CL_{tissue} \) represents tissue clearance; \( K_a \) is the absorption rate constant; \( K_{in,fa} \) is the first-order association coefficient for fat; \( K_{out,fa} \) is the first-order dissociation coefficient for fat; \( K_{p,fa} \) is the permeability-limited distribution coefficient for brain; \( K_{tissue} \) is the tissue-to-blood partition coefficient; \( Q_{co} \) represents cardiac output; \( Q_{tissue} \) represents tissue blood flow; \( V_{ar} \) represents the volume of the artery blood pool, \( V_{tissue} \) represents tissue volume and \( V_{ve} \) represents the volume of the venous blood pool. The initial conditions for Equations (1)–(5) and (7)–(11) were all set to 0, and dosing compartment was regarded as the dosing site.

Physiological parameters, such as tissue volumes, blood flow rates to different organs and fractions of vascular space in tissues were fixed to literature values reported in Table 1 (Brown et al., 1997; Davies & Morris, 1993). All tissues that were not sampled were lumped into a remainder compartment. A density of 1 g cm \(^{-3}\) was assumed for all tissues.

### Data analysis

The semi-physiological PK model was constructed using MATLAB R2012b (The MathWorks, Natick, MA). All parameters were estimated using maximum likelihood, and the variance model was defined as:
\[
C_{obs}(t_i) = C_{pred}(\theta, t_i) + \epsilon_{i, obs}
\]  
(12)

where \( C_{obs}(t_i) \) is the observed concentration of the \( i \)th data point, \( C_{pred}(\theta, t_i) \) is the \( i \)th model-fitted concentrations and \( \epsilon_{i, obs} \) represents a normally distributed random variable with a mean of 0.

The areas under the concentration–time curve (AUCs) of tacrolimus were calculated by non-compartmental analysis of blood and tissue concentration versus time data using WinNonlin version 6.0 (Pharsight Corporation, Mountain View, CA). All statistical analyses were conducted using Matlab R2012b (MathWorks Corporation, Natick, MA).

Normality test of the PK parameters were performed using the Lilliefors test. The comparison of PK parameters between CG and the other three groups was possessed using independent samples \( t \)-test (data normally distributed) or the Mann–Whitney \( U \)-test (data non-normally distributed).

### Table 1. Physiological parameters for mouse.

| Tissue  | Tissue volume, %, of body weight | Blood flow, %, of cardiac output | Fraction of the vascular space, %, of tissue volume |
|---------|---------------------------------|---------------------------------|---------------------------------------------------|
| Lungs   | 0.73                            | 100                             | 0.50                                              |
| Brain   | 1.65                            | 3.30                            | 0.03                                              |
| Heart   | 0.50                            | 6.60                            | 0.17                                              |
| Spleen  | 0.35                            | 1.12                            | 0.17                                              |
| Liver   | 5.49                            | 16.1b                           | 0.31                                              |
| Gut     | 4.22                            | 12.9                            | 0.04                                              |
| Kidneys | 1.67                            | 9.10                            | 0.24                                              |
| Muscle  | 50.0                            | 11.4                            | 0.04                                              |
| Fat     | 7.00                            | 6.00                            | 0.04                                              |
| Rest of the body | 23.5c          | 47.5d                           | 0.04                                              |
| Blood   | 4.90                            | 100                             | –                                                 |

Except specified, all values are from Brown et al. (1997).

*from Davies & Morris (1993).

Hepatic venous blood flow.

*Calculated as body weight subtract sum of weight of lungs, brain, heart, spleen, liver, gut, kidneys, muscle and fat.

*Calculated as 100 subtract sum of blood flow of brain, heart, liver, kidneys, muscle and fat.
Figure 2. Observed and model-simulated concentration-time profiles in blood/different tissues of mouse following an oral dose (3 mg kg\(^{-1}\)) of tacrolimus. Circles and solid black lines: control group (CG); squares and solid grey lines: low-dose group (LDG); left triangles and dot grey lines: medium-dose group (MDG); right triangles and dot black lines: high-dose group (HDG).
Table 2. AUC_{0–24h} values in mouse blood/tissues after an oral dose of 3 mg kg⁻¹ tacrolimus with or without ITCZ. (Median ± SD, n = 6).

| Tissues   | CG       | LDG      | MDG      | HDG      |
|-----------|----------|----------|----------|----------|
| Brain     | 0.216 ± 0.0246 | 0.345 ± 0.0695** | 0.474 ± 0.0814** | 0.776 ± 0.410** |
| Fat       | 0.729 ± 0.173 | 1.26 ± 0.319** | 1.16 ± 0.226** | 2.61 ± 0.901** |
| Gut       | 3.00 ± 0.362  | 4.47 ± 0.396** | 4.75 ± 0.507** | 8.04 ± 1.78** |
| Heart     | 1.03 ± 0.419  | 1.55 ± 0.536*  | 1.72 ± 0.327*  | 1.67 ± 0.785*  |
| Kidney    | 0.805 ± 0.217  | 1.56 ± 0.733  | 1.40 ± 0.526*  | 2.06 ± 1.16*  |
| Liver     | 0.180 ± 0.159  | 0.461 ± 0.172  | 0.470 ± 0.205  | 0.709 ± 0.337* |
| Lung      | 1.90 ± 0.241  | 3.59 ± 1.09** | 3.41 ± 0.444** | 5.51 ± 0.952** |
| Muscle    | 1.12 ± 0.217  | 1.62 ± 0.410** | 1.60 ± 0.599** | 3.12 ± 0.588** |
| Spleen    | 0.615 ± 0.201  | 1.06 ± 0.296*  | 1.34 ± 0.298** | 2.70 ± 0.451** |
| Blood     | 6.91e-02 ± 9.63e-03 | 0.122 ± 0.0257** | 0.128 ± 0.0182** | 0.209 ± 0.0553** |

AUC_{0–24h}: area under the concentration–time curve from zero to the last measured time (24 h).

* p: the null hypothesis at the 5% significance level.
** p: the null hypothesis at the 1% significance level.

All statistical tests were two sided with *p < 0.05 as the probability required to declare a difference.

Results

The model-fitted concentrations versus time profiles were distributed randomly across the observations, indicating the physiological model adequately described the observed concentrations over the entire tacrolimus concentration range (Figure 2). After visual inspection of the concentration-time profiles, significant higher tacrolimus concentrations and AUCs were revealed in LDG, MDG and HDG mice compared to CG mice. For LDG mice, AUCs of tacrolimus in brain, fat, gut, heart, lung, muscle, spleen and blood increased by more than 44% (44.3–93.3%, *p < 0.05) than CG mice. MDG mice were also characterised by markedly higher AUCs (58.7–161%, **p < 0.05) in brain, fat, gut, heart, kidney, lung, spleen and blood than CG mice. A significant increase in AUCs (61.7–339%, *p < 0.05) in blood and all sampled tissues were also revealed in HDG mice compared to CG mice (Table 2). Changes in AUCs (ΔAUC, %) of tacrolimus in various tissues after ITCZ administration are shown in Figure 3. The correlation between ITCZ dose and ΔAUC, by using Pearson correlation analysis, was high (*p < 0.05) in lung, brain, spleen, gut, muscle, fat and blood, while no significant correlation was found between ITCZ dose and ΔAUC of tacrolimus in heart, liver and kidney. Changes in tacrolimus disposition in mice are also reflected in PK model parameters (Table 3). Liver clearance (CL_l) decreased significantly in LDG (35.3%, **p < 0.01), MDG (50.2%, **p < 0.01) and HDG (64.6%, **p < 0.01) mice compared to CG mice. With respect to gut clearance (CL_gu), significant decrease was also revealed in LDG (35.9%, **p < 0.01), MDG (50.2%, **p < 0.01) and HDG (64.6%, **p < 0.01) mice. There is no significant difference (p > 0.05) in tacrolimus brain-to-blood partition coefficient (K_{t,br}) between LDG and CG mice, while a significant higher K_{t,br} was found in MDG (25.3%, **p < 0.01) and HDG (55.9%, **p < 0.01) mice than CG mice. Moreover, a significant increase (16.3%, *p < 0.05) was found in the absorption rate constant (K_a) in HDG mice compared to CG mice. The association between ITCZ dose and the change of PK parameters (Δparameter, %) was shown in Figure 4.

There was a significant association (**p < 0.01) between ITCZ dose and ΔCL_gu (r = −0.790), ΔCL_l (r = −0.787) and ΔK_{t,br} (r = 0.727), while the association between ITCZ dose and ΔK_a was not significant (p > 0.05).

Discussion

Drugs that require metabolism by the same CYP enzymes or transport by the same influx/efflux transporters compete for metabolism by the CYPs or binding to the transporters. Therefore, in theory, any two drugs that are metabolised by identical CYP isoenzymes or transported by identical transporters have a potential for interaction. However, the clinical significance of this interaction will rely on the drug’s relative affinities for binding to these enzymes/transporters, dependence on CYP/transporter for elimination, concentrations achieved in the target tissue/organ after therapeutic doses, and therapeutic ratios (Slaughter & Edwards, 1995; Venkatakrishnan et al., 2000).

Tacrolimus is primarily metabolised by CYP3A isoenzymes, which are the most abundant isoforms of CYP, accounting for nearly 30% of the total CYP content in liver and as much as 70% in the gut wall (Hoensch et al., 1976; Iwasaki, 2007; Lin et al., 1999, 2002; Michalets, 1998; Obach et al., 2001; Shimada et al., 1994). Therefore, there is a potential for major drug interactions between itraconazole, a potent inhibitor of CYP3A, and tacrolimus. Our findings in the present study supported this speculation. The physiological PK analysis results showed that the liver and gut clearance of tacrolimus decrease significantly (**p < 0.01) in mice co-administered ITCZ, with the increase of tacrolimus AUCs in almost all tissues (**p < 0.05).

A strong gut clearance (CL_gu) was revealed based on the physiological PK analysis (Table 3), and there are two possible reasons that can explain this phenomenon. One of the explanations is that CYP3A was in a continuous saturation state at the beginning of drug absorption. In this stage, tacrolimus highly concentrated in the gut wall and cannot be metabolised timely. As CL_gu is the average of the gut clearance rate, the final estimate may be high due to the zero-order elimination at the beginning of drug absorption. The other explanation is the existence of P-gp in the gut wall. Tacrolimus is also a substrate of P-gp (Hooks, 1994;
Figure 3. Correlation between ITCZ doses with the changes of AUC (ΔAUC, %) in blood different tissues. Grey lines: median ΔAUCs versus ITCZ doses; Black lines: linear regression of ΔAUCs versus ITCZ doses.
Scott et al., 2003; Venkataramanan et al., 1995), which is a membrane efflux transporter that is normally expressed in mammalian tissues, such as the small intestine, brain capillary endothelial cells and renal proximal tubules (Thiebaut et al., 1987). The high concentration of tacrolimus in the gut wall at the beginning of drug absorption may also make P-gp continuously in its maximum transport capacity (zero-order elimination).

Although tacrolimus AUC rose significantly in almost every tissue as ITCZ dose increased, the magnitude of increase in AUCs (ΔAUC) was not proportional to ITCZ dose elevation. ITCZ dose adjusted ΔAUC (ΔAUC/Dose) was higher (*p<0.05) in lung, brain, liver, gut, kidney, muscle, fat and blood in HDG mice than MDG and LDG mice, which indicated that the increase of AUC accelerated when ITCZ dose reached 50 mg kg⁻¹. On the other hand, CLgu and CLli accelerated to decrease when ITCZ dose reached 50 mg kg⁻¹. Notably, Ka was also found to rise significantly (*p<0.05) in HDG mice, which indicated that high dose of ITCZ may accelerate tacrolimus absorption. Moreover, as high dose of

Table 3. Pharmacokinetic parameters of tacrolimus in mice after an oral dose of 3 mg kg⁻¹ with or without ITCZ (Median ± SD, n = 6).

| Parameters (Units) | CG       | LDG     | MDG     | CDG      |
|-------------------|----------|---------|---------|----------|
| Kt, lu            | 23.2 ± 4.21 | 21.3 ± 4.45 | 26.4 ± 5.57 | 25.8 ± 5.82 |
| Kp, br (L h⁻¹)    | 2.07e-03 ± 4.98e-04 | 2.30e-03 ± 2.74e-04 | 2.23e-03 ± 6.94e-04 | 1.64e-03 ± 6.94e-04 |
| Kt, br            | 2.81 ± 0.267 | 2.66 ± 0.396 | 3.52 ± 0.419** | 4.38 ± 0.300** |
| Kt, lu            | 11.0 ± 3.34 | 10.9 ± 4.28 | 9.41 ± 2.22 | 12.6 ± 1.34 |
| Kt, sp            | 9.27 ± 2.81 | 10.2 ± 4.10 | 8.85 ± 2.84 | 8.17 ± 2.98 |
| Kt, li            | 3.17 ± 0.941 | 2.95 ± 1.06 | 3.90 ± 0.749 | 3.17 ± 0.660 |
| CLi (L h⁻¹)       | 4.84e-02 ± 4.37e-03 | 2.93e-02 ± 3.03e-03** | 2.65e-02 ± 1.82e-03** | 1.84e-02 ± 2.09e-03** |
| Kt, lu            | 1.23 ± 0.136 | 1.18 ± 0.0979 | 1.17 ± 0.163 | 1.43 ± 0.0933 * |
| Kt, gu            | 30.4 ± 3.62 | 27.7 ± 3.54 | 30.6 ± 2.06 | 31.3 ± 6.16 |
| CLgu (L h⁻¹)      | 2.84e-02 ± 4.54e-03 | 1.86e-02 ± 1.80e-03** | 1.71e-02 ± 2.22e-03** | 1.07e-02 ± 1.20e-03** |
| Kt, lu            | 8.36 ± 2.63 | 7.88 ± 2.12 | 7.84 ± 3.36 | 9.58 ± 2.66 |
| Kt, lu            | 15.3 ± 2.86 | 13.9 ± 4.53 | 15.3 ± 3.90 | 15.0 ± 2.79 |
| Kt, lu            | 11.3 ± 2.24 | 8.90 ± 2.68 | 10.8 ± 2.92 | 10.8 ± 1.91 |
| Kt, lu            | 1.83 ± 0.722 | 2.00 ± 0.339 | 1.29 ± 0.735 | 1.61 ± 0.458 |
| Kt, lu            | 0.850 ± 0.205 | 0.929 ± 0.189 | 0.774 ± 0.174 | 0.877 ± 0.112 |
| Kt, re            | 3.02e-03 ± 5.03e-04 | 2.91e-03 ± 4.12e-04 | 2.86e-03 ± 5.66e-04 | 3.33e-03 ± 7.23e-04 |

CLgu: intestinal clearance; CLli: hepatic clearance; Kt: absorption rate constant; Ka: first-order dissociation coefficient for fat; Kp, br: permeability-limited distribution coefficient for brain; Kt, lu: partition coefficient for brain; Kt, sp: partition coefficient for gut; Kt, br: partition coefficient for heart; Kt, ki: partition coefficient for kidney; Kt, li: partition coefficient for liver; Kt, mu: partition coefficient for muscle; Kt, re: partition coefficient for the remainder compartment; Kt, sp: partition coefficient for spleen. *p: the null hypothesis at the 5% significance level; **p: the null hypothesis at the 1% significance level.

Figure 4. Correlation between ITCZ doses with the changes of PK parameters (Δparameter, %). CLgu: gut clearance; CLli: liver clearance; Kt: absorption rate constant; Kt, br: brain-to-blood partition coefficient. Grey lines: median Δparameters versus ITCZ doses; Black lines: linear regression of Δparameters versus ITCZ doses.

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ITCZ may significantly inhibit the activity of CYP3A and P-gp in the gut wall, it may also raise the bioavailability of tacrolimus, leading to a higher amount of tacrolimus absorption, distribution and metabolism. The results in the current study indicated that itraconazole at a dose of 50 mg kg\(^{-1}\) in mice may raise the absorption rate, raise the brain-to-tissue partition coefficient, and reduce the hepatic and intestinal clearance of tacrolimus. These findings could be useful in predicting the efficacy and toxicity of tacrolimus, and drug–drug interaction of itraconazole and tacrolimus in human. The developed physiological pharmacokinetic model could be further refined by combining more chemical- and/or species-specific data for predicting tacrolimus disposition in human.

Conclusion

A semi-physiological pharmacokinetic model was applied to investigate the influence of itraconazole on tacrolimus absorption, distribution and metabolism. The results in the current study indicated that itraconazole at a dose of 50 mg kg\(^{-1}\) in mice may raise the absorption rate, raise the brain-to-tissue partition coefficient, and reduce the hepatic and intestinal clearance of tacrolimus. These findings could be useful in predicting the efficacy and toxicity of tacrolimus, and drug–drug interaction of itraconazole and tacrolimus in human.

Declaration of interest

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Anderton MJ, Manson MM, Verschoyle R, et al. (2004). Physiological modelling of formulated and crystalline 3,3\(^{\prime}\)-diindolylmethane pharmacokinetics mainly focussed on several PK parameters, such as CL\(_{lu}\), CL\(_{gu}\), K\(_{tr}\) and K\(_{a}\). However, this did not cause any over- or underestimation of the observed concentrations. In fact, the model-fitted concentration-time profiles fit the observations very well. The reason of this phenomenon is that, although the single-organ models were integrated into a uniform physiological model, they were connected in a complex way (parallel, serial, circular and attenuate). In other words, a single PK parameter can influence the drug concentration in every single-organ model in a complex exponential manner. This is beneficial to improve the description ability of the PK model, especially when the observations are sparse, which is not the case when using the non-compartmental analysis. This means the physiological PK model is suitable for investigating the mechanisms of drug interaction. In addition, compared with the PK parameters in the classical compartmental models, the physiological PK parameters are characterised by more physiological significance and relatively stable in different species. These parameters can be further used for interspecies scaling, mainly for predicting the disposition procedure of a drug in human (Bradshaw-Pierce et al., 2007; Hu et al., 2014; Kagan et al., 2011; Lu et al., 2016).

However, we did not developed an advanced compartmental absorption and transit (ACAT) model (Sinha et al., 2012) for mouse or human in the present study because of the lack of P-gp content information in tissues. An alternative extrapolation (from mouse to human) method is to use the species-specific parameters (such as tissue clearance and permeability-limited distribution coefficient), which are scaled with body weight using empirical allometric exponents. This manner of extrapolation for species-specific parameters is routinely used in PBPK modelling (Anderton et al., 2004; Bradshaw-Pierce et al., 2007; Meno-Tetang et al., 2006), but may not always be accurate or valid because there are considerable differences in abundance and function of drug-metabolising enzymes, drug transporters and other molecules across species (Hu & Hayton, 2001; Sharma & McNeill, 2009). All these confounding factors can contribute to the discrepancies between the simulated and observed data. Collectively, the discrepancies between simulations and the observations imply that once available, more species-specific and/or chemical-specific parameters should be incorporated to achieve better model prediction. Therefore, the current semi-physiological PK model could be refined when more species-specific data and mechanisms regarding tacrolimus disposition become available.

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Supplementary material available online