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Research Article

Metabolism and transporter based drug-drug interaction of tacrolimus with nine co-medicated injections

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Abstract

Backgroud: Tacrolimus, also named FK506, is a well-known potent immunosuppressant agent, which is usually co-medicated with multiple drugs in solid organ transplant. The objective of this study was to evaluate DDI between tacrolimus and nine injections co-administered in patients receiving liver organ transplant.

Methods: In this study, the cytochrome P450 enzymes (CYPs) inhibition assay were conducted by using pooled human liver microsomes, for supporting the evaluation of metabolism-mediated DDI. The transport substrate assay used to determine the transporter-mediated DDI, was conducted in the transfected HEK293 cell line expressing specific human transport proteins.

Results: All the tested drugs, especially Alprostadil, Methylprednisolone, and Pantoprazole showed apparent inhibition on CYP3A4/5 with the half-maximal inhibitory concantration (IC_{50}) from 0.5 μg/mL to 89.6 μg/mL, which also inhibited metabolism of tacrolimus in human liver microsomes, whose half time ($T_{1/2}$) was prolonged from 5.05 to 35.3 min. However, tacrolimus was not the substrates of solute carrier (SLC) transporters including OATP1B1, OATP1B3, OAT1, OAT3, and OCT2, which indicated that the risk of transport-mediated DDI between tacrolimus and these nine drugs was likely to be low.

Conclusion: These findings indicate that the metabolism-based DDI between tacrolimus and co-administered nine drugs seems to be the major cause of the high individual variability of tacrolimus exposure in patients with solid organ transplant and should be further studied.

More Information

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Introduction

Tacrolimus, which is available as an injection formulation, capsules, or an ointment, has been marked as an immunosuppressive agent for preventing or treating graft rejection in organ transplantation patients. The metabolism of tacrolimus is predominantly mediated by CYP3A, and the major metabolite identified in incubations with human liver microsomes is 13-demethyl tacrolimus, which has been reported to have the same activity as tacrolimus [1,2]. In human, less than 1% of the dose administered is excreted unchanged in urine. When administered by IV, fecal elimination accounted for 92.6%, urinary elimination accounted for 2.3% [2]. Before and after organ transplantation, patients are co-medicated with multiple drugs like antibiotics, antivirus, anti-thrombus drugs, to prevent rejection of the transplanted organ. Due to the narrow safety margin of tacrolimus, drug-drug interaction (DDI) studies are essential for the clinically optimal usage of this drug [3]. Moreover, it has been known that the human disease like liver or kidney injury, can elicit a wide range of effects on the expression and function of CYP450 and transport proteins [4,5]. As CYP3A4/5 is the main enzyme responsible for the metabolism of tacrolimus, strong CYP3A inducers like rifamapin and rifabutin used as antimycobaterials, may increase the risk of rejection due to decrease the plasma exposure of tacrolimus. On the contrary, the strong CYP3A inhibitors such as nelfinavir, voriconazole and nefazodone may improve the risk of serious

adverse reactions by increasing the plasma concentration of tacrolimus [2]. Moreover, many reports on clinical pharmacokinetics indicate that dose levels of tacrolimus need to be adjusted in transplant patients with CYP3A polymorphism [6]. It is also well estabolished that tacrolimus is the substrate of P-glycoprotein (P-gp) transporter, and recent studies have focused on the relationship between transporters polymorphisms and clinical pharmacokinetics of tacrolimus, which indicate that several genetic variants for both efflux transporters like P-gp, multidrug resistance protein 2 (MRP2), and influx transporters like organic anion transporting polypeptides (OATP1B1, OATP1B3), organic cation transporter (OAT3) and organic cation transporter (OCT1), have been associated with tacrolimus pharmacokinetics variability or the occurrence of toxicity [7,8]. However, there are relatively few studies devoted to determination of the above transporters, especially influx transporter-based DDI of tacrolimus. This study aims to evaluate the potential DDI between tacrolimus and nine usually co-medicated drugs including methylprednisolone, cefoperzone, pantoprazole, meropenem, alprostadil, omeprazole, ganciclovir, salvia miltrorrhiza, and codonopsis on both metabolism and transporter-mediated DDI by using human liver microsomes and HEK293 cell lines specifically expressing transporter proteins.

Materials and methods

Materials

Tacrolimus (Product No. 160411) was produced by Hua Dong Medicine Company (Hangzhou, China). Methylprednisolone (Product No. N68462) and Cefoperzone (Product No. R36425) were produced by Pfizer (USA). Pantoprazole (Product No. 313481) was produced by Takeda (Japan). Meropenem (Product No. 9160629101) was produced by Haibin Pharmaceutical Company (Shenzhen, China). Alprostadil (Product No. 3B096K) was produced by Tide Pharmaceutical Company (Beijing, China). Omeprazole (Product No. 20160818G9) was produced by Changzhou Siyao Pharmaceuticals Co., Ltd. (Changzhou, China). Ganciclovir (Product No. 160805-1) was produced by China Meheco Keyi Pharma Co., Ltd (Wuhan, China), Salvia miltrorrhiza injection (Product No. 1603283; Chiatai Qingchunbao, Hangzhou, China) and Codonopsis injection (Product No. 1603211; Lei Yun Shanghai, Changshu, China) were provided by Zhongshan Hospital, Fudan University. The probe substrates of CYP3A4/5 and co-factors like NADPH were purchased from Sigma-Aldrich (St Louis, MO, USA). Ketoconazole (Product No. K0045) was supplied by TCI. The probe substrates of transporters were provided by GenoMembrane (Yokohama, Japan).

Trypsin-EDTA (Product No. 25300-062), Penicillin-Streptomycin (Product No. 15140-122), Fetal bovine serum (Product No. 10099-141) were supplied by Gibco. BCA Protein Assay Kit (Product No. 23225) was supplied by Thermo. 24-well Lysine-coated plate (Product No. 354414) was supplied by CORNING.

Pooled human Liver Microsomes (Product No. VIN) used in this study were purchased from BIOIVT (Baltimore, USA). HEK293 cell lines stably expressing transporter proteins including OAT1 (HEK293-OAT1), OAT3 (HEK293-OAT3), OCT2 (HEK293-OCT2), OATP1B1 (HEK293-OATP1B1) and OATP1B3 (HEK293-OATP1B3), and MOCK transfected with empty vector (HEK293-MOCK) were supplied by GenoMembrane (Yokohama, Japan).

Cell cultures

HEK293 cells stably transfected with transporters listed above were maintained in DMEM medium supplemented with 10% (v/v) fetal bovine serum, penicillin-streptomycin solution (100 U/mL and 0.1 mg/mL) and 0.5 mg/mL of geneticin at 37 °C and 5% CO_2. For the process of thawing and seeding cells, firstly, the vitals were incubated in water bath until the vital was -90% thawed, where there should be a small, visibly frozen portion remaining in the vials. Then, the vital was decanted into a 15 mL conical tube containing pre-warmed culture medium, followed by centrifuged at 220×g for 5 min. The supernatant was removed and cell pellet was suspended with 12.5 mL fresh medium. 0.5 mL cell suspension was plated into each well of 24-well Lysine-coated plate and incubated at 37 °C and 5% CO_2. Daily changes of medium were performed, and the transport experiment can be performed when the confluence of the cells reached 80% ~ 90%. The details of the transporter experiment is described in 2.6.

Metabolic stability assay

The object of the experiment was to determine the disappearance of tacrolimus in the presence or absence of ketoconazole, the selective inhibitor of CYP3A. The reaction mixture contained 1.0 μM tacrolimus, 1 mg/mL liver microsomal protein, 0.1 M potassium phosphate buffer (pH7.4), and 1mM NADPH. The reaction was started by the addition of the NADPH and was allowed to incubate for a time course up to 60 or 120 min at 37°C water bath, which was stopped by adding ice-cold methanol containing internal standard, and the supernatants were collected for quantification of parent tacrolimus by the LC-MS/MS method shown in 2.7.

CYP3A4/5 inhibition assay

This study was conducted to evaluate the potential inhibition of the nine co-medicated drugs on CYP3A4/5 activity by using human liver microsomes. In brief, the incubations were conducted at 37°C in 200 μL incubation mixtures containing 0.5 mg/mL liver microsomes, 0.1 M potassium phosphate buffer (pH7.4), 1mM NADPH, testosterone (the probe substrate of CYP3A4/5) and nine

drugs with different dosing concentrations. The microsomes solution was pre-incubated with the dosing solution of different co-medicated drug or blank buffer used for negative control (NC), for 15 min. Then, testosterone and NADPH were added to start reaction and incubated for another incubation time. The reaction was stopped by adding ice-cold methanol containing internal standard followed by centrifugation. The supernatant was collected and used for determining the formation of the metabolite, 6-β hydroxylation of testosterone by a LC-MS/MS method.

Inhibition assay of nine drugs on tacrolimus metabolism

This study was aimed to detect whether nine drugs had potential inhibition on tacrolimus metabolism in human liver microsomal incubations. In brief, the process was same as CYP3A4/5 inhibition assay beside the probe substrate was replaced by tacrolimus.

Transporter substrate assay

This study was conducted to determine the uptake of tacrolimus by SLC transporter using HEK293 cell lines shown above. In general, the working solutions of. tacrolimus and probe substrates were prepared from the stock solution by dilution using DMSO, and the dosing solutions were prepared by further dilution using Transporter Buffer (TB) (pH7.4) containing 125 mM NaCl, 4.8 mM KCl, 5.6 mM D-(+)-glucose, 1.2 mM $CaCl_2 \cdot 2H_2O$, 1.2 mM KH_2PO_4, $MgSO_4 \cdot 7H_2O$ and 25 mM HEPES. After cell monolayers had been formed, the DMEM medium was removed from wells of the plate, and the cells were washed twice with 37 °C pre-warmed TB buffer. At second washing, the buffer was kept in the wells and incubated at 37 °C for 5 min, which followed by being replaced by 0.25 mL of pre-warmed TB containing tacrolimus or the probe substrates, then was incubated for an additional 10 min. The uptake assay was stopped by removing incubation solution followed by washing the cells with 0.5 mL of ice-cold TB for three times. 0.3 mL of distilled water was added to each well of the plates, and the cells were completely lysed by repeated freezing and thawing with liquid nitrogen for 3 times. Finally, 100 µL cell lysate and methanol containing internal standard were precipitated at ratio 1:4 and followed by centrifugation at 12000 rpm and 4 °C for 5 min. The supernatants were collected for measurement of tacrolimus or probe substrates by an LC-MS/MS method presented in 2.7. The part of cell lysate was also used to determine the cell protein concentration by BCA Protein assay kit. The probe substrate of OATP1B1 and OATP1B3 used in this assay was estradiol-17-beta-glucuronide, and the probe substrates of OAT1, OAT3 and OCT2 were p-aminohippuric acid, estrone sulfate and metformin, respectively.

LC-MS/MS methods

The LC-MS/MS system used in this study included a Agilent 1100 and LEAP CTC HTS PAL liquid chromatography. Mass spectrometric analysis was performed using API 4000 (triple-quadrupole) instrument from Applied Biosystems Inc. (USA) with an ESI interface. The data acquisition and control system were created using Analyst® 1.6.3 software from Applied Biosystems Inc. The chromatographic conditions used to analyze tacrolimus was as followings: the separation was achieved using a Dikma, Inspire C18, (50 × 2.1 mm, 5 µm) column, whose temperature was set at 40°C. The mobile phase used was 0.1% formic acid in water (v/v) containing 5mM ammonium acetate as eluent A and 0.1% formic acid in methanol (v/v) as eluent B; the flow rate was 450 µL/min. The gradient eluted program consisted of the following: analysis time 0 min, 20%B; analysis time 1.50 min, 98%B; analysis time 3.50 min, 98%B; analysis time 3.51 min, 20%B; analysis time 5.00 min, 20%B. The injection volume was 10 µL. The mass conditions was shown as followings: Scan type was ESI+, MRM; The CAD, curtain gas, Gas1, Gas2, Ionspray voltage and temperature were 6 psi, 20 psi, 50 psi, 50 psi, 5000 volts and 500 °C, respectively. The MS/MS parameters was as followings: Q1: 821.6; Q3: 768.6; Dwell time: 200 ms; DP, EP, CE and CXP (v) were 60, 10, 30 and 25, respectively. Osalmide was used as internal standard.

Data analysis

Metabolic stability assay: The parent remaining (% of 0 min), the half-time ($T_{1/2}$) and the *in vitro* hepatic intrinsic clearance (CL_{int}) were calculated by the following equation (1), (2) and (3), respectively.

(1) Parent remaining (% of 0 min) = amount of parent compound at Tx/ amount of parent compound at T0 × 100%, where Tx was each incubation time point and T0 was 0 min.

(2) The half-time ($T_{1/2}$) = 0.693/K, where K represented the depletion rate constant, from the slope of the natural algorithm of the percent of parent compound remaining at each time point, namely K=-2.303×Slope.

(3) *In vitro* intrinsic clearance (CL_{int}) = (0.693/$T_{1/2}$) × (1/(microsomal protein) × scaling factors), where the physiology parameter of microsomal protein per gram of liver and liver weight per kilogram of body weight were 48.8 mg/g and 25.7 g/kg, respectively, according to the reference [9].

CYP3A4/5 inhibition assay: The relative activity (% of NC) and IC_{50} were calculated using the formula (4) and (5), respectively.

(4) Relative activity (% of NC) = (the concentration of metabolites in co-medicated group/that in negative controls) × 100.

(5) Y = 100/ (1 + 10^(X-LogIC_{50})), where X was the test concentrations (Log µM), Y was the relative activity (% of NC), which was processed by Prism Software.

Transporter substrate assay: The uptake rate and the uptake ratio (UR) were calculated using the equation (6) and (7), respectively.

(6) The uptake rate (U) = $C_{lysate}/(P \times T)$, where C_{lysate} was the substrate content in cell lysate (pmol), P was the cell protein content (mg), and T was the time of incubation (min).

(7) The uptake ratio (UR)=mean of U/ mean of U_{MOCK}, where U was the uptake rate of the substrates in SLC transporter-expressing cells, and U_{MOCK} was the uptake rate in MOCK cells. According to USFDA DDI Guidance, it is considered that the test article is likely to be a substrate of transporter when the uptake ratio is equal to or more than 2.0-fold and significantly inhibited by specific inhibitor by more than 50% [10].

The calculation of R1 value: The equation (8) to calculate the R1 value for basic models of reversible inhibition is shown as followings:

(8) $R1 = 1+(I_{max,u}/K_{i,u})$, where $I_{max,u}$ is the maximal unbound plasma concentration of the interacting drug at steady state, and $K_{i,u}$ is the unbound inhibition constant predicted by $IC_{50}/2$ determined in CYP3A4/5 inhibition assay [10-13].

Results

Metabolism of tacrolimus in human liver microsomes

In human liver microsomes, after being incubated for 0, 15, 30 and 60 min, the parent remaining of 1 μM tacrolimus was 100%, 11.40%, 6.39% and 5.60%, respectively, of which the calculated $T_{1/2}$ and CL_{int} were 5.04 min and 215 mL/min/kg, respectively (Figure 1 and Table 1), suggesting that tacrolimus was extensively metabolized in human liver microsomes. However, the metabolism of tacrolimus was apparently inhibited by ketoconazole, the selective inhibitor of CYP3A4/5, of which the $T_{1/2}$ and CL_{int} were 450 min and < 20 mL/min/kg (Figure 1 and Table 2), indicating the major metabolic pathway of tacrolimus was CYP3A4/5. This results is consistent with the observation from the previous studies and reports [2,3].

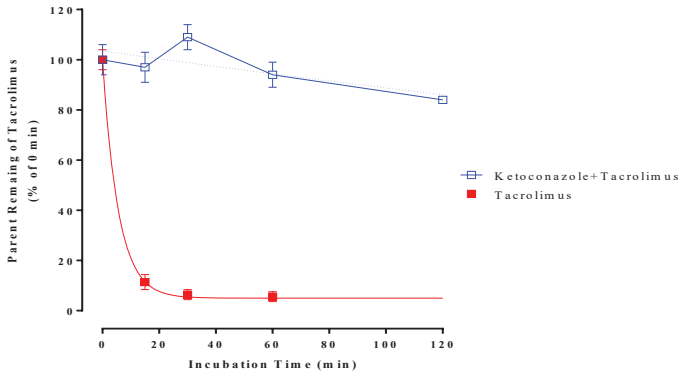

Figure 1: The metabolism of tacrolimus in pooled human liver microsomes with (open circle) or without (filled square) of ketoconazole, the specific inhibitor of CYP3A4/5. The parent amount of tacrolimus was determined by a LC-MS/MS method present in 2.7. Each value represents the mean ± S.D (*n* = 3).

Table 1: The metabolism of tacrolimus in pooled human liver microsomes with or without ketoconazole.

Incubation time (min)	Parent remaining of tacrolimus					
	Without ketoconazole			With ketoconazole		
	Mean	SD	% of 0 min	Mean	SD	% of 0 min
0	0.873	0.092	100	0.912	0.084	100
15	0.100	0.011	11.4	0.889	0.103	97.48
30	0.056	0.009	6.39	0.995	0.087	109.10
60	0.044	0.007	5.06	0.861	0.121	94.41
$T_{1/2}$ (min)	5.04			> 60		
CL_{int} (mL/min/kg)	215			NA		

Potential inhibition of nine co-medicated drugs on CYP3A4/5

In this study, the potential inhibition of nine co-mediated drugs on CYP3A4/5 activity was detected by using the method presented in 2.4. From the results shown in figure 2 and table 3, it was observed that meropenem, cefoperzone, alprostadil, genciclovir, methylprednisolone, pantoprazole, omeprazole, salvia miltrorrhiza and codonopsis showed potential CYP3A4/5 inhibition with the IC_{50} of 6660, 539, 0.5005, 4075, 89.6, 7.112, 36.732, 8.137 and 380.1 μg/mL, respectively.

Potential inhibition of nine co-medicated drugs on the metabolism of tacrolimus

The findings raise the possibility that these nine co-medicated drugs might affect the metabolism of tacrolimus

Table 2: Effects of co-medicated drugs on the metabolism of tacrolimus in human liver microsomes. A: Alprostadil; B: Methylprednisolone; C: Pantoprazole; D: Salvia miltrorrhiza

Drugs	$T_{1/2}$ (min)	CL_{int} (mL/min/kg)	Fold change of $T_{1/2}$
Tacrolimus	5.04	215	NA
Tacrolimus + Ketoconazole	450	< 20	89.29
Tacrolimus + Alprostadil injection (A)	35.3	44.2	7.00
Tacrolimus + Methylprednisolone injection (B)	16.5	63.7	3.27
Tacrolimus + Pantoprazole injection (C)	15.1	70.2	3.00
Tacrolimus + Salvia miltrorrhiza injection (D)	5.92	203	1.17
Tacrolimus + ABCD	>120	< 20	>23.81

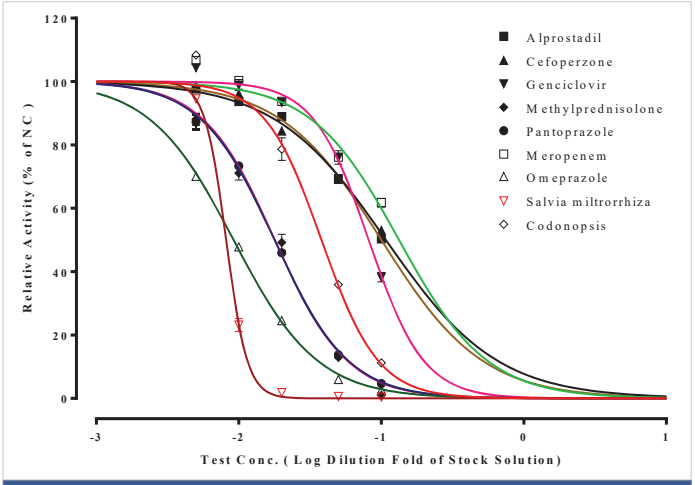

Figure 2: The inhibition of nine co-medicated drugs on CYP3A4/5 activity. The activity of CYP3A4/5 was determined by the formation rate of 6-β testosterone by using a LC-MS/MS method. Each value represents the mean ± S.D (*n* = 3).

through inhibiting the enzyme activity of CYP3A4/5. Therefore, we selected four co-medicated drug including alprostadil, methylprednisolone, pantoprazole and salvia miltrorrhiza, which showed apparent inhibition potential on CYP3A4/5 As the results shown in table 2 and figure 3, the $T_{1/2}$ of tacrolimus in alprostadil, methylprednisolone, pantoprazole and salvia miltrorrhiza groups were changed from 5.04 min to 35.3 min, 16.5 min, 15.1 min and 5.93 min, respectively, namely the fold change of $T_{1/2}$ in these test groups mentioned above were 7.00, 3.27, 3.00 and 1.17-fold of the tacrolimus alone. Another interesting finding is that not only the mixture of these four co-medicated drugs, but also the mixture of all nine co-mediated drugs, apparently inhibited the metabolism of tacrolimus, with the $T_{1/2}$ more over than 120 min (Table 2 and Figure 4).

Transporter substrate assay of tacrolimus

The SLC (solute carrier) uptake transporters, including organic anion transport polypeptides of OATP1B1 and OATP1B3, organic anion transporters of OAT1 and OAT3, organic cation transporter of OCT2, play important roles in absorption, distribution, excretion and metabolism of drugs within the body. OATP1B1 and OATP1B3 are expressed in the liver and are involved in the distribution of drugs from blood to hepatocytes. OAT1, OAT3 and OCT2 are expressed in the kidney and co-transport drugs excreted in the kidney [14]. Previous studies have revealed that tacrolimus is the substrate of P-glycoprotein (P-gp) transporter [15], which is also known as MDR1, however, to our knowledge, there have been no reports about the substrate determination of SLC transporter listed above. Thus, we conducted a study aiming to evaluate whether tacrolimus was a substrate of these SLC transporters. According to the data expressed in table 4, at the concentrations of 1 and 10 μM, the OAT1-mediated uptake ratios of tacrolimus were 1.22-fold and 0.93-fold, respectively; the OAT3-mediated uptake ratios were 1.34-fold and 1.05-fold, respectively; the OCT2-mediated uptake ratios were 1.71-fold and 1.06-fold, respectively; the OATP1B1-mediated uptake ratios were 0.704-fold and 1.49-fold, respectively; the OATP1B3-mediated uptake ratios were also less than 2.0, the cut off determined as a substrate of transporters according to USFDA DDI Guidance. However, the uptake ratio of the probe substrates were all more than

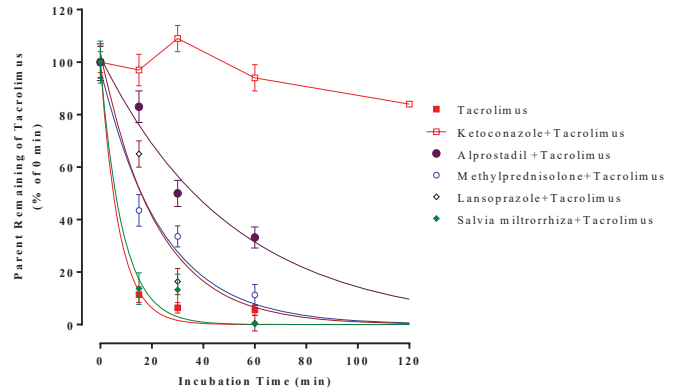

Figure 3: The inhibition of four co-medicated drugs on the metabolism of tacrolimus. These four drugs are alprostadil (filed circle), methylprednisolone (open circle), lansoprazole (open diamond) and salvia meltrorrhiza (filed diamond). The parent amount of tacrolimus was determined by a LC-MS/MS method present in 2.7. Each value represents the mean ± S.D (n = 3).

Table 3: Effects of co-medicated drug on the enzyme activity of CYP3A4/5. Dilution factor means the dilution fold of injection, e.g. 0.1332 was 13.32% of injection; C_{max} means the maximal unbound plasma concentration of the tested drugs. PPB means human plasma protein binding. C_{max} and PPB were obtained from FDA label and related reference, while the C_{max} and PPB data of herb medicines including salvia miltrorrhiza and codonopsis can not be confirmed, so of which the R1 values are not available (NA). R1 values were calculated according to the method shown in 2.8.4.

Drugs	IC_{50} (Dilution factor)	IC_{50}(μg/mL)	C_{max}(μg/mL)	PPB(%)	R1 value
Meropenem	0.1332	6660	46.9	2	1.013
Cefoperzone	0.1078	539	150	93	1.039
Alprostadil	0.1001	0.5005	0.0000168	98	1.000
Genciclovir	0.0815	4075	9	2	1.004
Methylprednisolone	0.01792	89.6	0.41	76.8	1.002
Pantoprazole	0.01778	7.112	5.52	98	1.031
Omeprazole	0.009183	36.732	1.458	95	1.004
Salvia miltrorrhiza	0.008137	8.137	NA	NA	NA
Codonopsis	0.03801	380.1	NA	NA	NA

Table 4: The uptake rate and uptake ratio of 1 and 10 μM of tacrolimus and probe substrates by SLC transporters including OAT1, OAT3, OCT2, OATP1B1 and OATP1B3. The methods used for calculating the data were present in 2.8.3. Each value represents the mean ± S.D (n = 3).

Transporters	Uptake Rate (pmol/mg/min)									
	OAT1		OAT3		OCT2		OATP1B1		OATP1B3	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
MOCK-probe substrates	1.30	0.13	2.09	1.35	455	187	2.73	0.95	2.77	0.311
SLC-probe substrates	143	20.80	101	5.70	5152	408	58.6	17.9	16.0	0.217
Uptake Ratio	111		48.6		11.13		21.5		5.78	
MOCK-Tacrolimus 1 μM	14.3	3.89	16.4	3.85	14.6	5.60	7.04	3.07	2.26	0.40
SLC-Tacrolimus 1 μM	17.4	2.83	21.9	6.90	17.1	3.79	4.95	1.66	1.78	0.27
Uptake Ratio	1.22		1.34		1.71		0.704		0.788	
MOCK-Tacrolimus 10 μM	131	36.50	143.5	21.92	180	20.20	0.225	0.102	BLQ	NA
SLC-Tacrolimus 10 μM	122	46.00	150.0	23.30	190	9.00	0.335	0.173	BLQ	NA
Uptake Ratio	0.931		1.05		1.06		1.49		NA	

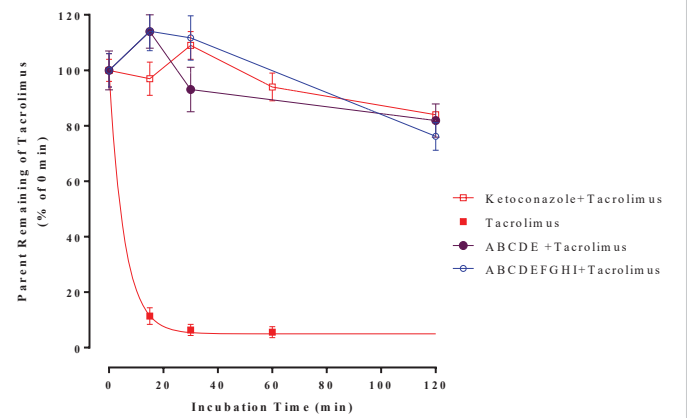

Figure 4: The inhibition of the mixture of co-medicated drugs on the metavolism of tacrolimus. There are two mixture groups tested, one is the group containing five co-medicated drugs, represented as ABCDE (filed circle), another is the group containing nine co-medicated drugs, represented as ABCDEFGHI (open circle), where A: Alprostadil, B: Methylprednisolone, C: Pantoprazole, D: Salvia miltrorrhiza, E: Cefoperzone, F: Omeprazole, G: Codonopsis, H: Ganciclovir, I: meropenem. The parent amount of tacrolimus was determined by a LC-MS/MS method present in 2.7. Each value represents the mean ± S.D (n = 3).

2.0, indicating that the test systems were qualified for this study. On the basis of this result, it can be concluded that tacrolimus is not be a substrate of these SLC transporters, hence there seems to be less risk in transporter-mediated DDI between tacrolimus and co-medicated nine drugs.

Discussion

It is well established that tacrolimus is characterized by high pharmacokinetic individual variability and a narrow therapeutic window [16,17], so the DDI studies are critical for its clinical use with other co-medicated drugs. In this study, we focused on the metabolism-mediated and transported mediated DDI between tacrolimus and nine drugs including antibiotics, antivirus drugs, anti-thrombus drugs, adrenal corticosteroids, cardiovascular drugs, and herb medicines for improving liver functions.

Firstly, we confirmed that CYP3A4/5 is the main enzyme responsible for the metabolism of tacrolimus by using human liver microsomes, which is in good consistent with the previous studies. Then, it was observed that the tested nine drugs had potential inhibition on the activity of CYP3A4/5, which raised the possibility of the inhibition on the metabolism of tacrolimus. Moreover, to further evaluate the clinical significance, we collected human pharmacokinetic information of these nine drugs from FDA label or related reference [18-23], and calculated the ratio of intrinsic clearance values of a probe substrate for CYP3A4/5 pathway in the absence and in the presence of the interacting nine drugs by using basic models. This ratio is referred to as R1 value, whose analysis method is present in 2.8.4. As the results shown in Table 3, only the R1 value of cefoperzone and pantoprazole were more than 1.02, implying the potential clinical significance. Additionally, we attempted to confirm this hypothesis and detected that the apparent inhibition on tacrolimus metabolism by both one drug alone and the mixtures of drugs, which indicates that the exposure of tacrolimus is likely to be increased by these co-medicated drugs, which may cause some serious adverse reactions like QT prolongation, myocardial hypertrophy and neurotoxicity, especially in slower metabolizers with polymorphism of CYP3A5.

Considering that the function and expression of SLC transporter may be affected in kidney and liver injury patients, and there is no further studied focused on the SLC transport-based DDI of tacrolimus, we hence determined whether these transporter played a role in tacrolimus transport. From the results we have obtained, one can conclude that tacrolimus is not a substrate of the SLC transporters including OATP1B1/OATP1B3 and OAT1/OAT3/OCT3, mainly expressing on liver and kidney, respectively. On the basis of these findings, it can be concluded that these co-medicated drugs may affect the exposure of tacrolimus though metabolism-based DDI rather than transport-based.

However, it should be mentioned that this work is limited in the effects of nine co-medicated drugs on tacrolimus, so future work will focus on the effect of tacrolimus on these nine drugs. Moreover, it is generally believed that tacrolimus is a substrate of P-gp, thus the inhibition or induction potential of these nine drugs should be further determined.

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