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Effect of Tripterine on the pharmacokinetics of cyclosporine A and its mechanism, in rats

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Abbreviations: BCRP, Breast cancer resistance protein; BSEP, bile salt export pump; CAR, consistutive androstane receptor; CsA, Cyclosporine A; CYP, Cytochrome P450 proteins; DMEs, drug metabolizing enzymes; DTs, drug transporters; FXR, farnesoid X receptor; MRP, multi-drug resistance protein; NRs, nuclear receptors; NTCP, sodium taurocholate cotransporting polypeptide; OATP, organic anion transporting polypeptides; P-gp, P-glycoprotein; PXR, pregnane X receptor; UGT, UDP-glucuronosyltransferases.

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Abstract

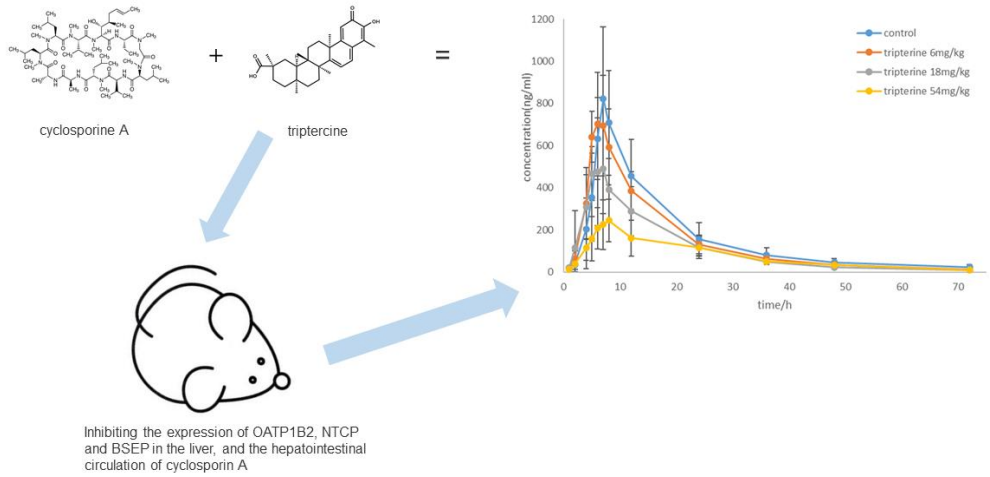
Tripterine is one of the main active components in tripterygium wilfordii polyglycosides tablets. Cyclosporine A (CsA) is an immunosuppressive agent commonly used in clinical organ transplantation. Combined application of Tripterygium wilfordii and cyclosporine A has been proved to enhance the immunosuppressive effect of cyclosporine and reduce its toxic effects. The present study investigated the effect of tripterine on the pharmacokinetics of CsA and its underlying mechanisms.LC-MS/MS was used to establish a detection method of the concentration of CsA in rat blood. Polymerase Chain Reaction (PCR) and Western Blot (WB) were used to determine the expression of tripterine on drug metabolizing enzymes (DMEs), drug transporters (DTs) and nuclear receptors (NRs). As the result, tripterine reduced the bioavailability of cyclosporin A. Compared with control group, the Cmax of CsA were reduced in all dosages of tripterine, and the AUC was significantly decreased in 18 $mg \cdot kg^{-1}$ and 54$mg \cdot kg^{-1}$ group. The results of PCR and WB showed that tripterine had inhibitory effects on CYP3A1, CYP3A2, UGT1A1, OATP1B2, P-GP, MRP2, BCRP, BSEP and NTCP. Therefore, we put forward that the inhibition effect of tripterine on NTCP, BESP and OATP1B2 in the liver could limit the uptake of cyclosporin A into the blood and the hepatointestinal circulation of cyclosporine A, resulting in the decrease of blood drug concentration of cyclosporin A.

Keywords Cyclosporine A; Tripterine; Drug metabolizing enzymes; Drug transporters; Nuclear receptors.

Significance

Tripterygium wilfordii and cyclosporine A have the prospect of joint use. We demonstrate that the inhibitory effect of tripterine on pharmacokinetics of cyclosporine A, which is significant for the combined use of these two drugs.

Visual Abstract



1. Introduction

Tripterygium wilfordii is a traditional Chinese medicine, widely used in the treatment of arthritis, dermatitis, lupus erythematosus and eczema (Lv et al., 2019; Liu et al., 2019; Wang et al., 2018; Lü et al.,2015). At present, many studies have found that tripterygium wilfordii extracts have potential application prospects in anti-tumor and immune regulation (Ramgolam et al.,2000; Jiang et al.,2013; Yang et al., 2019; Li et al., 2019). There are more than 100 chemical constituents of Tripterygium wilfordii have been identifiedc, which can be divided into three categories: alkaloids (Tripterygium wilfordii, Tripterygium wilfordii, Tripterygium wilfordii, etc.); diterpenes (triptolide, ethylamine, etc.); Triterpenes (tripterine et al.)(Liu et al., 2019). As a non-steroid immunosuppressive agent, the Tripterygium polycoride tablet were firstly developed and used in clinic in China since 1984 (Lipsky et al., 1997), which may exert its role by inhibiting the secretion of IL-2 and the expression of IL-2 receptors on T lymphocytes (Brinker et al., 2007), or by inducing apoptosis of lymphocytes and down-regulating T cell receptor signal (Nong et al., 2019).

Tripterine is an active substance extracted from tripterygium wilfordii. Under normal circumstances, tripterine is used as a main component of the Chinese patent medicine Tripterygium tablet, with the average content of 35 μg in each tablet (Wu et al., 2009).

A large number of studies have demonstrated its immunomodulatory and anti-tumor effects (Li et al., 2018; Chen et al., 2014; Xiong et al., 2018; Li et al., 2018; Chen et al., 2018; Zuo et al.,2019; Yuan et al., 2013). Tripterine had also been shown to regulate multiple drug metabolic enzymes and drug transporters. Jin et al. tested the effect of tripterine on five subtypes of cytopigment, including CYP1A2, CYP2C19, CYP2D6, CYP2E1 and CYP3A4, by cocktail method in human liver microsomes, and the results showed that tripterine inhibited the five subtypes to varying degrees (Jin et al.). Sun et al. found that tripterine had inhibitory effects on CYP1A2, CYP2C11, CYP2D6, CYP2E1 and CYP3A2, which were not only mixed inhibitors of CYP3A4 but also competitive inhibitors of CYP1A2 and CYP2C11 (Sun et al., 2014). The study of Zhang et al shown celastrol was a strong inhibitor of UGT1A6 and UGT2B7 (Zhang et al., 2012). In addition, Zhao et al. found that Tripterine can also up-regulate the expression of FXR in the liver (Zhao et al., 2019). Tripterine could affect the action of multiple drug-metabolizing enzymes and transporters, so it was likely to have drug-drug interactions with other drugs.

Cyclosporine A (CsA) is one of the most commonly used immunosuppressive agents in clinical practice and has a narrow therapeutic window. Many studies have proved that tripterygium wilfordii and cyclosporine A have the prospect of combined application. Han et al. reported that cyclosporin A combined with tripterygium glycosides can treat refractory primary immune thrombocytopenia in patients who have failed glucocorticoid therapy (Shin et al., 2019). Tripterygium wilfordii polyglycosides have synergistic effect with cyclosporine A in combating rejection after organ transplantation (Zhang et al., 2017; Li et al., 2019; Wang et al., 2018; Wang et al., 2016). This synergistic effect may be related to the regulatory effect of Tripterygium wilfordii on the immune system (Zhang et al., 2001). CsA is substrate of CYP3A, P-gp, UGT1A and MRP2 (Yu et al.,2016; Dupuis et al.,2012; Yigitaslan et al., 2016; Goldberg et al., 1988). Therefore, the simultaneous application of CSA with drugs, such as tripterine, that regulate these drug transporters (DTs) and drug metabolic enzymes (DMEs) may result in drug-drug interactions. However, to data, the effect of tripterine on cyclosporine A

has not been reported.

In present study, we investigated the effect of tripterine on the pharmacokinetics of CsA in rats. After 7 consecutive days of pretreatment of tripterine, CsA were orally administered, and the whole blood concentrations of CsA were determined periodically. The underlying mechanisms of drug interactions were explored by studying the effects of tripterine on certain DMEs and DTs and NRs involving with metabolism of CsA.

2. Material and methods

The method for this experiment was based on our previous developed experimental method (Huang et al., 2021).

2.1 Material

Triptolide, tripterine, triptolide ketone, ketoconazole, verapamil, rifampin, dexamethasone, cyclosporine A (CsA) and cyclosporine D (CsD) were purchased from Dalian Meilun Biotechnology Co., Ltd. (Dalian, China); Oleum extra virgin olive oil; sodium carboxymethyl cellulose (CMC-Na); saline.

2.2 Animals

Male Sprague-Dawley (SD) rats (weighing 180-220 g) were purchased from the Laboratory Animal Research Center of Tongji Medical College of Huazhong University of Science and Technology (Wuhan, China), and were given access to a commercial rat chow diet and tap water. The animals were housed, two per cage, and maintained at 22±2°C and 50-60% relative humidity, under a 12 h light-dark cycle. The experimeNRs were initiated after acclimation under these conditions for at least 1 week. The experimeNRs were performed in accordance with the “Guiding Principles in the Use of Animals in Toxicology” adopted by the Society of Toxicology (USA) in July 1989 and revised in March 1999.

2.3 Pharmacokinetic studies in rat

Experiment I Sixty rat were divided into 4 groups randomly (six rats a group): control group (pretreated with 0.5% CMC-Na); tripterine pretreated groups (6, 18 and 54 mg/kg, respectively). Tripterine were all prepared with the corresponding standard and 0.5%

CMC-Na as suspension. Shake well before gavage. Rats in the blank group were given 0.5% CMC-Na by gavage. All groups of rats were administered by oral gavage for 7 consecutive days. The rats were fasted for at least 12 hours (overnight) before the day of experiment and were given water freely. On the experimental day (day 7), 30min after the last dose of 0.5% CMC- Na or tripterine, CsA (10 mg/kg) was administered to the rats by oral gavage. Blood samples were collected after administration of CsA at 1，2，4，5，6，7，8，12，24，36，48，72 h. All the blood samples were stored at -80℃ until analysis.

Experiment II Rats were randomly divided into 4 groups consistent with Experiment I, but there were only 3 rats in each group. All of these rats were administered by oral gavage for 7 days. The rats were fasted for at least 12 hours (overnight) before the day of experiment and could drink water freely. After 2 hours of intragastric administration on the seven day, the rats were anesthetized and sacrificed. The liver, kidney and small intestine tissues were isolated, washed with saline and blotted dry, the samples were stored at -80℃ until use.

After the experiment, the rats were euthanized by overinjection of pentobarbital sodium.

2.4 Quantification of CsA

The method for the determination of CsA was based on our previous developed LC-MS/MS methods (Yang et al., 2018). The linear concentration range of CsA was 5-4000 ng/mL, and the lower limit of quantification was 5 ng/mL.

2.5 Pharmacokinetic analysis.

The blood concentration data were analyzed by the non-compartmental method using Drug and Statistics software (DAS, version 3.2.8, Shanghai BioGuider Medicinal Technology Co. Ltd., Shanghai, China). The peak blood concentration (C_{max}) and time to reach Cmax (T_{max}) of CsA were acquired directly from the concentration-time curve. The elimination rate constant (K_{el}) was calculated by log-linear regression of the phase-eliminated data. The area under the plasma concentration-time curve (AUC_{0-t}) from time zero to the time of last measured concentration (C_{last}) was calculated by the linear trapezoidal rule. The AUC zero to infinite ($AUC_{0-\infty}$) was obtained by the addition of

AUC_{0-t} and the extrapolated area determined by Clast/K_{el}. And the terminal half-life ($T_{1/2}$) was calculated by 0.693/K_{el}. The mean residence time (MRT) was calculated by AUMC/AUC, where AUMC represented the area under the first moment versus time curve. Apparent clearance (CL/F) was calculated by Dose/ $AUC_{0-\infty}$ and the apparent volume of distribution (V/F) was calculated by CL/K_{el}.

2.6 Measurement of mRNA expression.

Real-time PCR was used to quantify the mRNA expression of *BCRP, BSEP, CAR, CYP3A1, CYP3A2, FXR, MRP2, NTCP, OATPAB2, P-gp, PXR* and *UCT1A1* in the liver, the mRNA expression of *BCRP, CAR, CYP3A1, CYP3A2, FXR, MRP2, P-gp, PXR* and *UGT1A1* and the mRNA expression of *BCRP, MRP2* and *P-gp* in the kidney in the small intestine. Approximately 100 mg of tissue was taken and thoroughly ground in 1 mL of pre-chilled Trizol. RNA wasextracted with 250 μL of chloroform, precipitated with isopropanol, and washed with 75% ethanol. RNA was reverse transcribed to cDNA using the PrimeScript RT reagent kit with gDNA Eraser (RR047A; Takara Biotechnology Co., Ltd., Dalian, China). qRT-PCR assay was performed using SYBR ® Premix Ex Taq™ (Takara Biotechnology Co., Ltd.) and a StepOne Real-Time PCR System (Thermo Fisher Scientific, Inc.). The reaction procedure was first pre-denatured at 95 ℃ for 1 min, then 40 cycles of 15 seconds at 95 ℃, 20 seconds at 58 ℃, 45 seconds at 72 ℃, and finally the temperature rose from 60 ℃ to 95 ℃ at a rate of 1 ℃ per 20 seconds. The $2^{-\Delta\Delta CT}$ method was used to calculated the relative mRNA expression levels.

2.7 Measurement of protein expression.

The protein expression of *BCRP, BSEP, CAR, CYP3A1, CYP3A2, FXR, MRP2, NTCP, OATP1B2, P-gp, PXR* and *UCT1A1* in the liver, the protein expression of *BCRP, CAR, CYP3A1, CYP3A2, FXR, MRP2, P-gp, PXR* and *UGT1A1* in the small intestine and the protein expression of *BCRP, MRP2* and *P-gp* in the kidney of rats were analyzed by western blotting. After the total protein in the tissues was extracted, the protein samples were separated by 8% to 20% sodium dodecyl sulphate polyacrylamide gels (SDS-PAGE) and then transferred to polyvinylidene fluoride (PVDF) membranes

(Millipore, Esch-born, Germany). The PVDF membranes were added to the blocking solution for 1 h at room temperature and then incubated with the diluted primary antibody overnight at 4 °C. After the membranes were washed three times with TBST, the membranes were incubated with the diluted secondary antibody for 30 min at room temperature. The freshly prepared ECL mixed solution was added dropwise to the protein side of the membranes, and exposed in a dark room. The exposure conditions were adjusted according to different light intensities, and then development and fixing were performed. Finally, the film was scanned and archived, and the AlphaEaseFC software processing system was used to analyze the optical density of the target band.

2.8 Statistical analysis.

Experimental data are expressed as mean ± SD. Statistical analysis was performed using GraphPad Prism 5.0 software (GraphPad Software, Inc., La Jolla, CA, USA). The differences were considered statistically significant when P values were less than 0.05 by one-way ANOVA.

3 Result

3.1 Effect of tripterine on the pharmacokinetics of CsA

Figure 1 show the whole blood concentration profile and pharmacokinetics parameters of CsA after treatment of tripterine. The figure 2 showed tripterine had a dose-dependent inhibitory effect on the blood concentration of cyclosporin A. As table 1 shown, compared with the control group, AUC0-t, AUC0-∞, and Cmax of CsA reduced in all doses of tripterine administration groups. When the dose of tripterine was 54mg/kg, the AUC0-t, AUC0-∞, and Cmax of CsA decreased by 52.52%($P<0.05$), 37.53%($P<0.05$) and 66.74%($P<0.05$) respectively, and CLz/F of CsA increased by 105.44%($p<0.05$). Additionally, when the dosage of tripterine was 18mg/kg, the $AUC_{0-\infty}$ of CsA decreased by 33.34%($P<0.05$). There was no statistically significant difference in other data between the administration group and the control group.

3.2 Relative mRNA expression levels of DMEs, DTs and NRs in the liver, renal, and small intestine after the treatment of tripterine.

The mRNA expression of DMEs, DTs and NRs were determined by Real-time PCR. The results were shown in Figure 2.

3.2.1 mRNA expression levels of DMEs, DTs and NRs in the liver

As the figure 2 A shown, we measured the mRNA expression of CYP3A1, CYP3A2, UGT1A1, OATP1B2, P-gp, MRP2, BCRP, BSEP, NTCP, CAR, PXR and FXR in the liver. The mRNA expression of DMEs and DTs decreased, while the expression of NRs increased, compared to the control. The mRNA expression level of CYP3A1 in three tripterine pretreated groups (6, 18, 54mg·kg-1) was decreased by 24.92%, 46.62%, 57.54%, respectively. The decrease of the mRNA expression level of CYP3A2 in three tripterine pretreated groups was 29.26%, 39.42%, 79.25%. The mRNA expression level of UGT1A1 in three tripterine pretreated groups was decreased by 41.82%, 62.60%, 84.04%, separately. The mRNA expression level of OATP1B2 in three tripterine pretreated groups was decreased by 45.78%, 66.67%, 71.46%. The reductions of the mRNA expression level of P-gp in three tripterine pretreated groups was by 42.81%, 70.09%, 80.59%. The mRNA expression level of BCRP in three tripterine pretreated groups was decreased by 44.63%, 67.61%, 70.54% respectively. When it came to MRP2, the expression of mRNA reduced by 52.03%, 71.52%, 83.08% in three tripterine pretreated groups. BSEP mRNA expression decreased by 20.49%, 58.16% and 57.39% respectively in the three pretreatment groups. The mRNA expression level of NTCP in three tripterine pretreated groups was decreased by 38.46%, 46.04%, 66.12% respectively. The mRNA expression level of CAR in three tripterine pretreated groups was increased by 113.50%, 287.70%, 367.68% respectively. The mRNA expression level of PXR in three tripterine pretreated groups was raised by 74.04%, 221.33%, 235.20%. The increase of FXR mRNA expression in the three dose groups was respectively 145.40%, 272.55%, 275.28%.

3.2.2 mRNA expression levels of DMEs, DTs and NRs in the intestine

As the figure 2 B shown, we measured the mRNA expression of CYP3A1, CYP3A2, UGT1A1, P-gp, MRP2, BCRP, CAR, PXR and FXR in the intestine. The mRNA expression of genes in the small intestine was similar to that in the liver. The mRNA expression level of CYP3A1 in three tripterine pretreated groups (6, 18, 54mg·kg-1) was decreased by 45.07%, 64.55%, 71.89%, respectively. The decrease of the mRNA expression level of CYP3A2 in three tripterine pretreated groups was 40.48%, 60.33%, 67.96%. The mRNA expression level of UGT1A1 in three tripterine pretreated groups was decreased by 41.82%, 62.60%, 84.04%, separately. The reductions of the mRNA expression level of P-gp in three tripterine pretreated groups was by 42.81%, 70.09%, 80.59%. The mRNA expression level of Bcrp in three tripterine pretreated groups was decreased by 46.40%, 73.69%, 77.22% respectively. When it came to MRP2, the expression of mRNA reduced by 37.03%, 58.88%, 80.06% in three tripterine pretreated groups. The mRNA expression level of CAR in three tripterine pretreated groups was increased by 110.16%, 230.32%, 270.74% respectively. The mRNA expression level of PXR in three tripterine pretreated groups was raised by 72.16%, 120.29%, 220.24%. The increase of FXR mRNA expression in the three dose groups was respectively 71.02%, 163.56%, 184.31%.

3.2.3 mRNA expression levels of DTs in the kidney

As the figure 2 C shown, we measured the mRNA expression of P-gp, MRP2 and BCRP in the intestine. The mRNA expression level of P-gp in three tripterine pretreated groups was decreased by 38.60%, 58.70%, 79.73% respectively. The mRNA expression level of Bcrp in three tripterine pretreated groups was decreased by 37.52%, 59.82%, 76.82% respectively. The mRNA expression level of Mrp2 in three triptolide pretreated groups was decreased by 39.50%, 67.92%, 80.93% respectively.

3.3 Protein expression levels of DMEs, DTs and NRs in the liver, renal, and small intestine after the treatment of tripterine.

The protein expression of DMEs, DTs and NRs was determined by western blotting. The specific statistical analysis results were shown in Figure 3. The western blots of proteins in liver, renal and small intestine were presented in Figure 4.

3.3.1 Protein expression levels of DMEs, DTs and NRs in the liver

As the figure 3 A shown, we measured the Protein expression of CYP3A1, CYP3A2, UGT1A1, OATP1B2, P-gp, MRP2, BCRP, BSEP, NTCP, CAR, PXR and FXR in the liver. The Protein expression of DMEs and DTs decreased, while the expression of NRs increased, compared to the control. The Protein expression level of CYP3A1 in three tripterine pretreated groups (6, 18, 54mg·kg-1) was decreased by 49.25%, 91.47%, 86.88%, respectively. The decrease of the Protein expression level of CYP3A2 in three tripterine pretreated groups was 29.26%, 39.42%, 79.25%. The Protein expression level of UGT1A1 in three tripterine pretreated groups was decreased by 30.70%, 77.83%, 68.07%, separately. The Protein expression level of OATP1B2 in three tripterine pretreated groups was decreased by 41.84%, 74.97%, 69.80%. The reductions of the Protein expression level of P-gp in three tripterine pretreated groups was by 49.64%, 80.85%, 85.82%. The Protein expression level of BCRP in three tripterine pretreated groups was decreased by 40.00%, 52.18%, 72.50% respectively. When it came to MRP2, the expression of Protein reduced by 68.43%, 91.60, 92.62% in three tripterine pretreated groups. BSEP Protein expression decreased by 58.98%, 92.10%, 88.90% respectively in the three pretreatment groups. The Protein expression level of NTCP in three tripterine pretreated groups was decreased by 51.70%, 89.50%, 86.83% respectively. The Protein expression level of CAR in three tripterine pretreated groups was increased by 249.55%, 790.28%, 764.19% respectively. The Protein expression level of PXR in three tripterine pretreated groups was raised by 57.28%, 465.38%, 1231.11%. The increase of FXR Protein expression in the three dose groups was respectively 167.03%, 454.80%, 449.81%.

3.3.2 Protein expression levels of DMEs, DTs and NRs in the intestine

As the figure 3 B shown, we we measured the Protein expression of CYP3A1, CYP3A2, UGT1A1, P-gp, MRP2, BCRP, CAR, PXR and FXR in the intestine. The Protein expression of genes in the small intestine was similar to that in the liver. The Protein expression level of CYP3A1 in three tripterine pretreated groups (6, 18, 54mg·kg-1) was decreased by 62.90%, 90.53%, 90.34%, respectively. The decrease of the Protein expression level of CYP3A2 in three tripterine pretreated groups was 56.07%, 82.61%, 86.31%. The Protein expression level of UGT1A1 in three tripterine pretreated groups was decreased by 55.66%, 87.00%, 88.26%, separately. The reductions of the Protein expression level of P-gp in three tripterine pretreated groups was by 31.78%, 66.81%, 69.24%. The Protein expression level of Bcrp in three tripterine pretreated groups was decreased by 46.96%, 76.92%, 73.24% respectively. When it came to MRP2, the expression of Protein reduced by 60.64%, 81.78%, 81.36% in three tripterine pretreated groups. The Protein expression level of CAR in three tripterine pretreated groups was increased by 268.82%, 688.72%, 665.55% respectively. The Protein expression level of PXR in three tripterine pretreated groups was raised by 397.40%, 1055.17%, 1167.56%. The increase of FXR Protein expression in the three dose groups was respectively 94.91%, 158.49%, 215.79%.

3.3.3 Protein expression levels of DTs in the kidney

As the figure 3 C shown, we measured the Protein expression of P-gp, MRP2 and BCRP in the intestine. The Protein expression level of P-gp in three tripterine pretreated groups was decreased by 32.47%, 74.65%, 64.99% respectively. The Protein expression level of Bcrp in three tripterine pretreated groups was decreased by 47.61%, 69.27%, 70.29% respectively. The Protein expression level of Mrp2 in three triptolide pretreated groups was decreased by 52.41%, 76.12%, 77.17% respectively.

4 Discussion

Now more and more researchers are interested in traditional Chinese medicine. Tripterygium wilfordii as a kind of traditional Chinese medicine with a variety of pharmacological activities has attracted a lot of attention and widely used in

immunosuppression in clinic(Zhang et al., 2017; Li et al., 2019; Wang et al., 2018). CsA is one of the most commonly long-time used immunosuppressive agents in the clinic currently. Many studies have proved that combined application of tripterygium wilfordii and cyclosporine A after transplantation can enhance the immunosuppressive effect of cyclosporine A and reduce its toxic effects. In this study, we reported the inhibition effect of tripterine on the pharmacokinetics of d CsA in a dose-dependent manner in rats for the first time. The bioavailability of CsA was reduced in rats when pretreated with tripterine. The effects of tripterine on major drug transporters, drug metabolizing enzymes and nuclear receptors related to CsA in the small intestine, liver and kidney were systematically studied, and the mechanisms of tripterine on CsA pharmacokinetics were explained according to these results for the first time.

In our study, two important phase-one metabolic enzymes and the most widely distributed phase-two metabolic enzymes UGT1A1 were selected as the objects. CYP3A1 and CYP3A4 were mainly distributed in liver and small intestinal epithelial cells in rats, and metabolized most (70%) of CsA in the clinic setting. Hou et al. found that bioavailability of cyclosporin A decreased when P-GP and CYP3A4 were activated by glycyrrhizin (Huo et al., 2012). Therefore, theoretically, the bioavailability of cyclosporin A should enhance when CYAP3As are inhibited (Goldberg et al., 1998). However, our results showed the expression of CYP3A1 and CYP3A2 were decreased, and the blood concentration of CsA were also reduced after tripterine administered.

OATP1B2 was expressed in the hepatocyte sinusoidal membrane and the human homologs most closely related are OATP1B1 and 1B3 on human liver, involved in the transport of linear and circular peptides to the liver in the basal side of rat liver (Manikandan et al., 2018; Meier-Abt et al., 2004). OATP1B2 and NTCP were Influx transporters translocate specific molecules from blood into hepatic cytosol, mainly expressed in the basolateral membrane of hepatocytes (Pan et al., 2019). P-gp, MRP2, BCRP and BSEP were efflux transporters mediating the excretion of drugs and metabolites from hepatic cytosol to bile, distributed in the canalicular membrane of hepatocytes (Pan et al., 2019). Intestinal P-gp, MRP2 and BCRP, at the apical side of

the enterocytes, could pump drugs back into gut lumen and limit the oral absorption of CsA (Müller et al., 2017). In the kidney, P-GP, MRP2, and BCRP were distributed in the basolateral basolateral of proximal tubular cells and pumped drugs and their metabolites into the prourine (Ivanyuk et al., 2017). CsA has been demonstrated as a broad-spectrum modulator for multidrug resistance proteins such as P-gp, BCRP, MRP2, and OATP1B2 (Li et al., 2014; Xia et al., 2007; Akashi et al., 2006; Tang et al., 2008). Our results showed that tripterine was a strong inhibitor of OATP1B2, P-gp, BCRP, and MRP2. Contrary to our results, inhibition of P-gp and BCRP could increase the plasma concentration and decrease the clearance of P-gp substrates, such as CsA. We thought OATP1B2 was inhibited by tripterine could explain in part for the blood concentrations of CsA reduced after co-administration of tripterine. Once OATP1B2 was inhibited, the amount of CsA entering the liver from the hepatic portal vein decreassed, and CsA discharged from the mesenteric artery was stored in the hepatic portal vein and was mostly on the red blood cell membrane (Fahr, 1993). This resulted in a decrease in the amount of CsA that eventually enter the systemic circulation and an increased first-pass effect in the liver and intestine. The bioavailability of orally administrated cyclosporine A (CsA) is poor and is largely limited by the forst-pass effect.

Another explanation that may account for the lower bioavailability of oral CsA after co-administration of tripterine is tripterine inhibits both BESP and NTCP transporters, which played key roles in bile circulation, and inhibition of them by tripterine was likely to result in reduced bile secretion. CsA is a fat-soluble drug, and bile acids promote its absorption in the gastrointestinal (Wang et al., 2012; Stieger et al.,2011; Donkers et al., 2018) tract. Therefore, inhibition of BSEP and NTCP by tripterine could also lead to decreased bioavailability of CsA (Lindholm et al.,1991).

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Author Contributions

Participated in research design: S.j. Shi, Y.N. Liu, and R. Zhang.

Conducted experiments: X.X. Huang and Y. Wei.

Contributed new reagents or analytic tools: C.X. Yang, J.L. Zhou, T.Y. Yang, and P.P. Guo.

Performed data analysis: J.P. Zhou and R. Zhang.

Wrote or contributed to the writing of the manuscript: J.P. Zhou and R. Zhang.

Compliance with Ethical Standards

Competing interests

The authors declare that they have no competing interests.

Statement

All experimental protocols were approved by Ethics Committee of Huazhong University of Science and Technology. All methods were carried out in accordance with relevant guidelines and regulations. The study was carried out in compliance with the ARRIVE guidelines.

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Figures and Tables

Table 1 Pharmacokinetic parameters of CsA after intragastric administration of CsA (10 mg·kg^{-1}) in rats pre-treated with saline (control), tripterine (mean ± SD, n=6)

Parameters	control	Triperine 6mg/kg	Tripterine 18mg/kg	Tripterine 54mg/kg
AUC(0-t) (ug/L*h)	11534.533±3905.446	10312.868±1738.357	7907.875±2450.828	5476.198±1663.259 [a]
AUC(0-∞)(ug/L*h)	12014.629±3724.995	10473.354±1714.618	8008.644±2470.771 [a]	5705.554±1624.031 [a]
MRT(0-t) (h)	18.16±2.185	16.375±1.14	16.502±1.167	22.112±2.956
t1/2z(h)	18.995±8.132	12.4±2.116	11.77±2.945	15.86±7.006
Tmax(h)	7.167±0.408	6.167±1.169	6.167±1.472	7±1.265
Vz/F(L/kg)	28.537±24.462	17.799±5.386	23.422±10.932	47.161±38.51 [a]
CLz/F (L/h/kg)	0.919±0.347	0.98±0.185	1.362±0.446	1.888±0.593 [a]
Cmax (ug/L)	827±331.162	794.833±249.397	594.833±222.34	275±114.422a

[a] indicates P<0.05

*

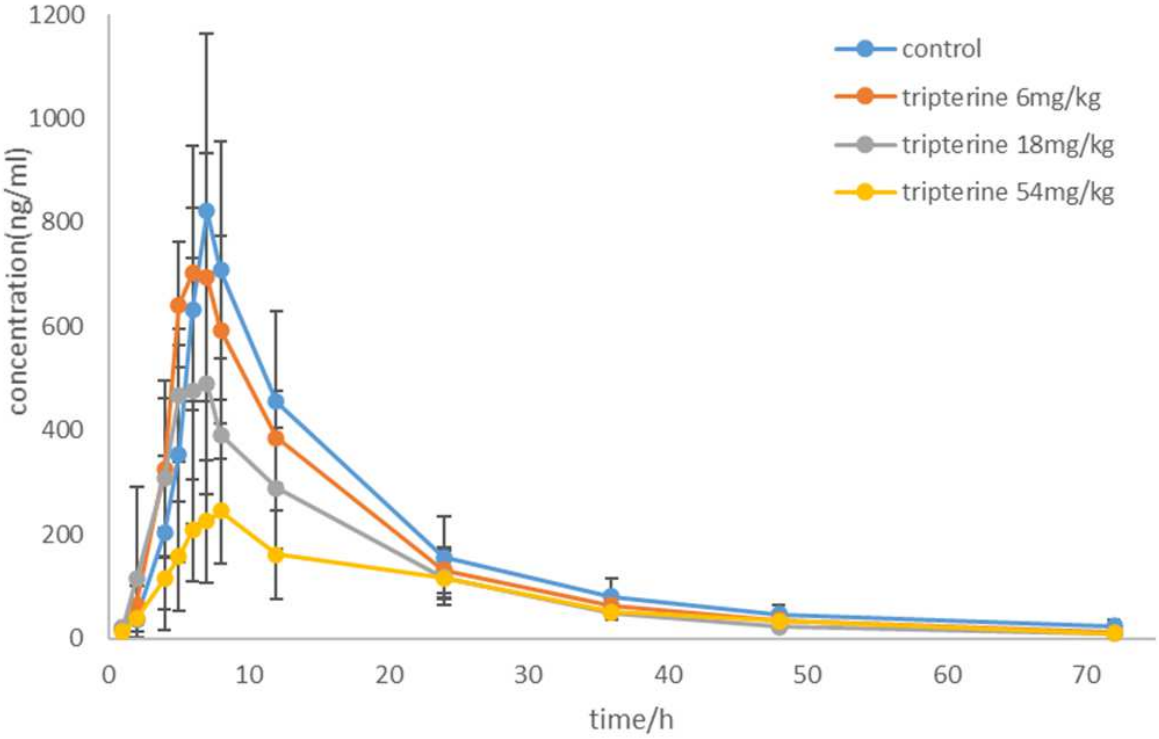
*** Figure 1 Mean blood concentration-time profiles of CSA.** The concentration of CSA was determined after its oral administration (10 mg·kg-1) pretreated with saline (control), tripterine (6, 18 and 54mg·kg-1) respectively to rats. Values are expressed as mean ± SD (n=6).

Figure 2 Effect of tripterine on the mRNA expression levels. A Effect of tripterine on the mRNA expression levels of CYP3A1, CYP3A2, UGT1A1, OATP1B2, P-gp, Bcrp, MRP2, Bsep,Ntcp, CAR, PXR, FXR in the liver. B Effect of tripterine on the mRNA expression levels of CYP3A1, CYP3A2, UGT1A1, P-gp, Bcrp, MRP2, CAR, PXR, FXR in the intestine. C Effect of tripterine on the mRNA expression levels of P-gp, Bcrp, MRP2 in the renal. Relative mRNA expression levels in rats in the control and different doses of tripterine groups were measured by Real-time PCR and calculated as comparative levels over control using 2-ΔΔCt method. Vertical bars represent mean ± SD (n=3).

Figure 3 Effect of tripterine on the protein expression levels. A Effect of tripterine on the protein expression levels of CYP3A1, CYP3A2, UGT1A1, OATP1B2, P-gp, Bcrp, MRP2, Bsep,Ntcp, CAR, PXR, FXR in the liver. B Effect of tripterine on the protein expression levels of CYP3A1, CYP3A2, UGT1A1, P-gp, Bcrp, MRP2, CAR, PXR, FXR in the intestine. C Effect of tripterine on the protein expression levels of P-gp, Bcrp, MRP2 in the renal. The protein expression levels in rats in the control and different doses of EGCG groups were assessed by western blotting. Vertical bars represent mean ± SD (n=3).

Figure 4 Western blots of DMEs, DTs and NRs in rats of each pretreatment group. Representative western blotting results in liver (A), in small intestine (B) and in kidney(C). CYP, cytochrome P450; UGT, uridine 5diphosphoglucuronosy-l transferase glucuronosyltransferase; P-gp, P-glycoprotein; Mrp2; multidrug resistance protein 2; CAR, constitutive androstane receptor; PXR, pregnane X receptor; FXR, farnesoid X

Figures



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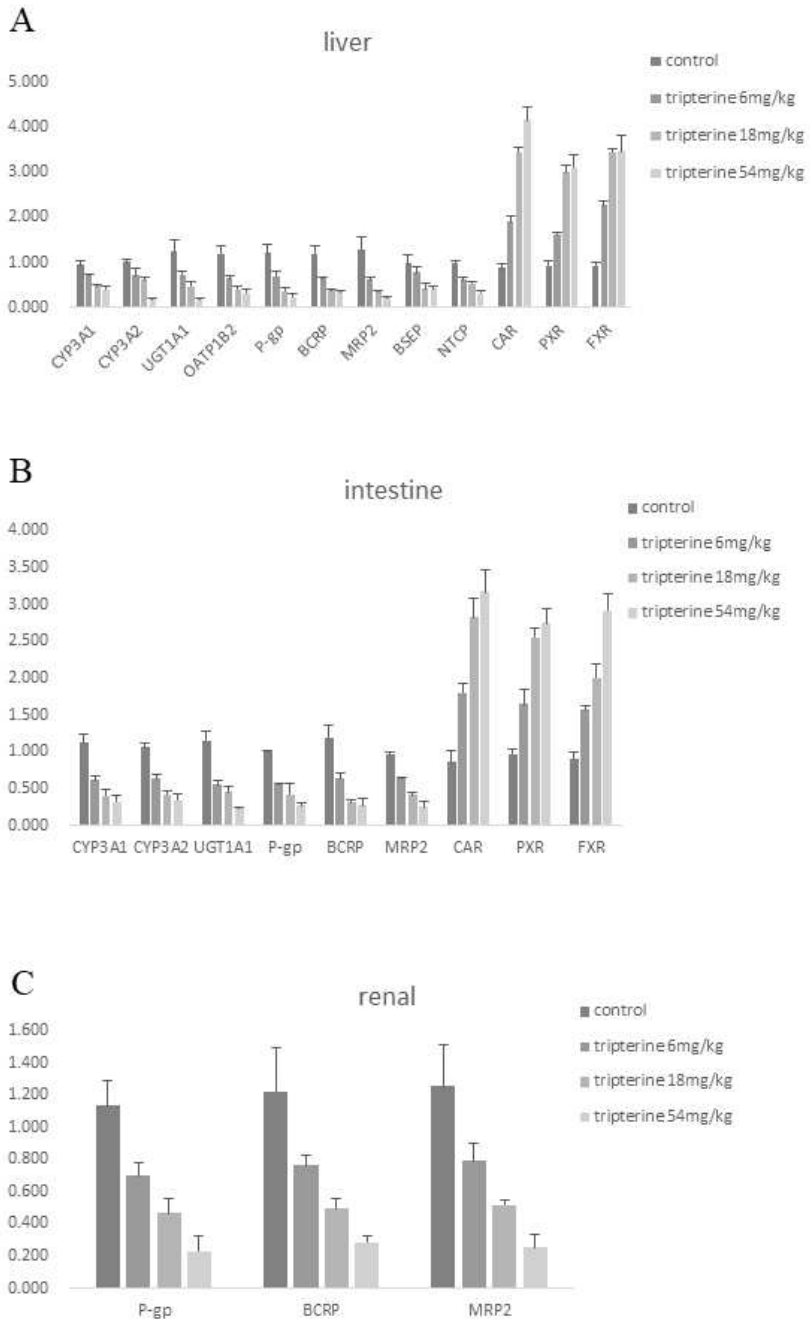

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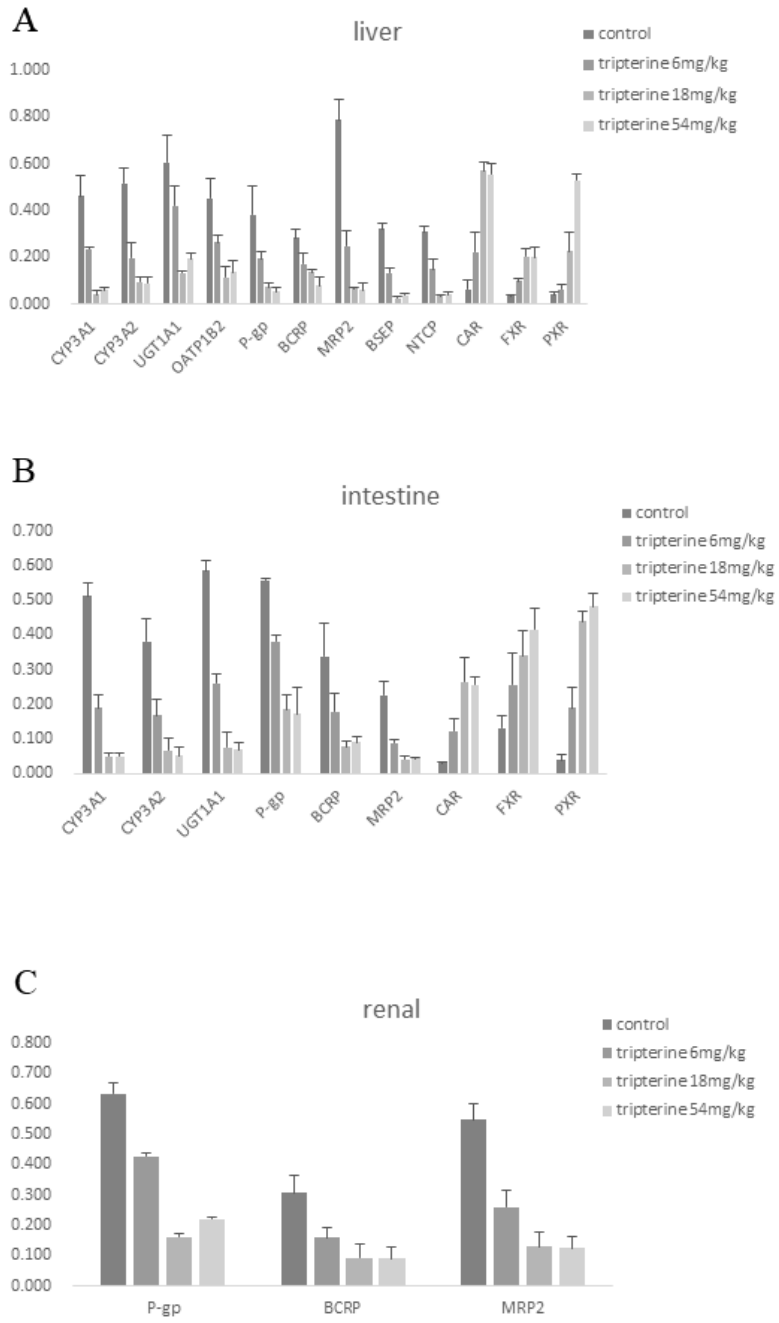

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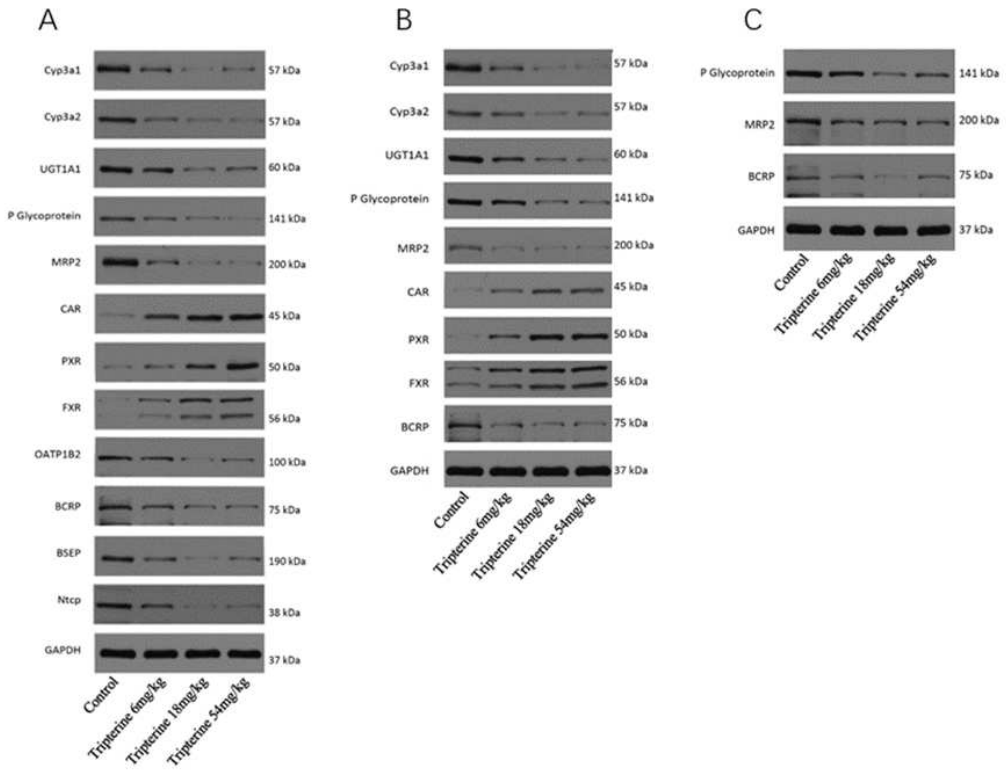

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