In Vivo to In Vitro Effects of Six Bioactive Lignans of Wuzhi Tablet (Schisandra Sphenanthera Extract) on the CYP3A/P-glycoprotein-Mediated Absorption and Metabolism of Tacrolimus

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Received July 24, 2013; accepted November 6, 2013

ABSTRACT

We recently reported that Wuzhi tablet (WZ; Schisandra sphenanthera extract) can inhibit P-glycoprotein (P-gp)-mediated efflux and CYP3A-mediated metabolism of tacrolimus (FK506) and thus increase the blood concentrations of FK506. Major active lignans of WZ include schisandrin A, schisandrin B, schisandrin C, schisandrol A, schisandrol B, and schisantiserin A. Whether and how these six lignans affect the pharmacokinetics of FK506 remains unclear. Therefore, this study aimed to investigate the effects of these lignans on the first-pass absorption and metabolism of FK506 and the involved mechanisms in vitro and in vivo. The results showed that whole-blood concentrations of FK506 were increased to different degrees following coadministration of the six lignans, respectively. Schisandrol B showed the strongest effect on the increase of the area under the concentration-time curve, the oral bioavailability, the gut processes affecting availability, and the hepatic availability of FK506. The reduction of intestinal first-pass effect contributed most to the increase in oral bioavailability of FK506 when coadministered with schisandrol B. In vitro transport experiment showed that schisandrin A, schisandrin B, and schisandrol B inhibited P-gp-mediated efflux of FK506. In vitro metabolism study showed that the inhibitory effect of these six lignans on FK506 metabolism was dose-dependent. In conclusion, the exposure of FK506 in rats was increased when coadministered with these lignans, and schisandrol B showed the strongest effect. Lignans of WZ inhibited P-gp-mediated efflux and CYP3A-mediated metabolism of FK506, and the reduction of intestinal first-pass affected by the lignans was the major cause of the increased FK506 oral bioavailability.

Introduction

Tacrolimus (FK506) (3S,4R,5S,8R,9E,12S,14S,15R,16S,18R,19R,26aS)-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-Hexadecahydro-5,19-dihydroxy-3-[(1E)-2-[(1R,3R,4R)-4-hydroxy-3-methoxycyclohexyl]-1-methylethenyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-8-(2-propenyl)-15,19-epoxy-3H-pyrido[2,1-e][1,4]oxazacloctricosine-1,7,20,21(4H,23H)-tetrone is a potent immunosuppressant which has been widely used to prevent acute allograft rejection and improve long-term graft survival after solid organ transplantation (Mentzer et al., 1998; Staatz and Tett, 2004). FK506 is metabolized by the CYP3A subfamily, and its absorption is further limited because it is a substrate of an efflux transporter, P-glycoprotein (P-gp) (Jeong and Chiou, 2006; Iwasaki, 2007). Therefore, inhibitors or inducers of CYP3A/P-gp can cause significant interactions with FK506, and thus increase or decrease the blood levels of FK506 (van Gelder, 2002). On the other hand, transplant patients must be treated with an immunosuppressant for their whole lives, and long-term treatment with FK506 brings patients huge financial burden. Thus, FK506-sparing agents that could reduce its dose while maintaining the desired therapeutic concentration are in high demand.

Recently, many studies, including our previously published works, have indicated that blood concentrations of FK506 were increased in the presence of Schisandra sphenanthera extract in transplant recipients and in rats (Xin et al., 2007; Jiang et al., 2010; Qin et al., 2010a,b, 2013; Xin et al., 2011; Wei et al., 2013), suggesting preparations of Schisandra sphenanthera extract and its active components might be a promising FK506-sparing agent. Wuzhi tablet (WZ) is a preparation of an ethanol extract of Wu Wei Zi (Schisandra sphenanthera) containing 7.5 mg of schisanterin A per tablet. WZ is a prescribed

ABBREVIATIONS: AP, apical; AUC, area under the concentration-time curve; AUC$_{0-24}$, area under the concentration-time curves from 0h to 24h; AUC$_{0-t}$, the area under the concentration-time curves from zero to infinity; BL, basolateral; ER$_{na}$, hepatic extraction ratio; F$_{h}$, hepatic availability; FK506, tacrolimus (3S,4R,5S,8R,9E,12S,14S,15R,16S,18R,19R,26aS)-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-Hexadecahydro-5,19-dihydroxy-3-[(1E)-2-[(1R,3R,4R)-4-hydroxy-3-methoxycyclohexyl]-1-methylethenyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-8-(2-propenyl)-15,19-epoxy-3H-pyrido[2,1-e][1,4]oxazacloctricosine-1,7,20,21(4H,23H)-tetrone; F$_{o}$, oral bioavailability; HBSS, Hanks’ balanced salt solution; LC-MS/MS, liquid chromatography–tandem mass spectrometry; P$_{app}$, the apparent permeability coefficient; P-gp, P-glycoprotein; TEER, transepithelial electric resistance; WZ, Wuzhi tablet.
drug (registration number in China: Z20025766) which is widely used to treat viral and drug-induced hepatitis in China. WZ is often co-administered with FK506 when drug-induced hepatitis or liver dysfunction occurs in transplant patients (Qin et al., 2010a,b, 2013; Xin et al., 2007, 2011). Our previous study in rats indicated that a concomitant administration of WZ significantly increased the FK506 blood concentration with only a slight change in FK506 tissue distribution, suggesting WZ was a promising FK506-sparing agent for transplant patients (Qin et al., 2010a,b). Our further study demonstrated that WZ could inhibit P-gp-mediated efflux and CYP3A-mediated metabolism of FK506, and the reduction of the intestinal first-pass effect by WZ was the major cause of the increased FK506 oral bioavailability (Qin et al., 2010b). The major active components of WZ are the bioactive lignans with an uncommon structure derived from dibenzocyclooctadiene such as schisandrin A, schisandrin B, schisandrol A, schisandrol B, and schisantherin A (Huyke et al., 2007). We further identified and determined the content of six major active constituents in WZ, including schisandrin A, schisandrin B, schisandrin C, schisandrol A, schisandrol B, and schisantherin A (their chemical structures are shown in Fig. 1) using a validated liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis (Qin et al., 2013). The results showed that the amounts (milligram per gram tablet) of lignans in WZ were as follows: schisantherin A, 12.97 ± 1.20 mg/g; schisandrin A, 12.57 ± 1.59 mg/g; schisandrin B, 0.027 ± 0.0031 mg/g; schisandrin C, 0.047 ± 0.0054 mg/g; schisandrol A, 0.077 ± 0.0086 mg/g; and Schisandrol B, 0.89 ± 0.10 mg/g, respectively. Schisantherin A, schisandrin A, and schisandrol B were the three highest bioactive lignans in WZ; other lignans were much lower than the aforementioned contents (Qin et al., 2013). Although the effect of WZ on P-gp-mediated efflux and CYP3A-mediated metabolism of FK506 was clearly elucidated in our previous studies, the effects of these six identified bioactive lignans from WZ on the absorption and metabolism of FK506 remain unknown; most importantly, the most potent lignan in WZ which can increase FK506 bioavailability is unknown. Finally, to our knowledge, there is no published study that could clearly clarify the interaction and the involved mechanisms between FK506 and these six bioactive lignans.

Therefore, the current study aimed to 1) investigate the effects of these six bioactive lignans on bioavailability of FK506 in rats, 2) examine the effect of these six lignans on the CYP3A-mediated FK506 metabolism and P-gp-mediated exsorption of FK506, and 3) differentiate the effect of these six lignans on the extent of intestinal FK506 first-pass effect from that of the liver metabolism.

Materials and Methods

Schisandrin A, schisandrin B, schisandrin C, schisandrol A, schisandrol B, and schisantherin A were all produced by Shanghai Winherb Medical Science and Technology Development Co. Ltd. (Shanghai, China; http://www.winherb.cn/). WZ (each tablet containing 7.5 mg of schisantherin A) was produced by Fang Lue Pharmaceutical Company (Guangxi, China). Tacrolimus (FK506) with a purity of 98% as determined by high-performance liquid chromatography with UV detection was synthesized and provided by Toronto Research Chemicals Inc. (Toronto, Canada). Ascomycin (FK520, as the internal standard) with a purity of 95% as determined by high-performance liquid chromatography with UV detection was synthesized and provided by BIOMOL Research Laboratories Inc. (Plymouth Meeting, PA). Tacrolimus capsules (Prograf capsules, each capsule contained 1 mg of tacrolimus) and tacrolimus injection (5 mg/1 ml) were produced by Astellas Ireland Co., Ltd. (Dublin, Ireland).

Fig. 1. Chemical structures of lignans in Wuzhi tablet.
All other reagents or materials were the same as described in our previous study (Qin et al., 2010b).

Animals. Male Sprague-Dawley rats (250–330 g) were supplied by the Laboratory Animal Service Center at Sun Yat-sen University (Guangzhou, China). The animals were kept in a room at 22–24°C with a light/dark cycle of 12/12 hours and 55%–60% relative humidity. They had free access to standard rodent chow and clean tap water. The rats were fasted for 12 hours before the experiments. All procedures were in accordance with the Regulations of Experimental Animal Administration issued by the Ministry of Science and Technology of the People’s Republic of China (http://www.most.gov.cn). The animal study protocols were approved by the Institutional Animal Care and Use Committee at Sun Yat-sen University.

Cell Culture. Caco-2 cells were obtained from the American Type Culture Collection (Manassas, VA). Cells were cultured and used according to procedures described in our previous papers (Zhang et al., 2006; Qin et al., 2010b).

Preparation of Rat Liver Microsomes. Hepatic tissues were collected from healthy male Sprague-Dawley rats (230–310 g) and stored at −80°C. Liver microsomes were prepared by differential centrifugation as previously described in our report (Bi et al., 2008; Qin et al., 2010b). Human liver microsomes were purchased from BD Biosciences (Franklin Lakes, NJ). All liver microsomes were stored at −80°C until use. The amount of protein in liver microsomes was measured using BCA Protein Assay Kit (Beyotime, Shanghai, China).

Effect of the Six Lignans on In Vivo Pharmacokinetics of Tacrolimus (FK506). On the day before the experiment, a light surgery on the right jugular vein of the rats was performed as described in our previously published paper (Bi et al., 2008; Qin et al., 2010b). Afterward, the rats were placed individually in cages to recover and were fasted for 12 hours before the pharmacokinetic study. The rats were randomly divided into several groups.

For oral dosing (each group contained 5 rats, n = 5), FK506 dissolved in pure water was given to rats by gavage at a dose of 1.89 mg/kg. Schisandrin A, schisandrin B, schisandrin C, schisandrol A, schisandrol B, and schisantherin A (0.024 mM/kg) dissolved in pure water were given, respectively, by gavage 1 minute before the administration of FK506. Rats receiving FK506 alone were given an equivalent volume of vehicle. Blood samples of about 220 μl were withdrawn via the right cannulated jugular vein before (0 hour) and at 5, 15, 30, and 45 minutes, and 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours postdosing. For i.v. dosing (each group contained 4 rats, n = 4), FK506 injection diluted in saline was injected via the right jugular vein at a dose of 0.2 mg/kg (2 ml/kg), followed by a 0.5 ml heparinized saline solution (50 U/ml) flush. Schisandrol B dissolved in pure water was also given by gavage at a dose of 0.024 mM/kg 1 minute before the administration of FK506. Rats receiving FK506 alone were given an equivalent volume of vehicle. Blood samples of about 220 μl were withdrawn via the right jugular vein before (0 hour) and at 2, 5, 10, 15, 30, 45, and 60 minutes, and 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours postdosing.

After blood sampling, the cannula was flushed with an equal volume of heparinized saline solution (50 U/ml) to prevent coagulation and to replace blood loss. Blood samples of 200 μl were immediately injected into a preheparinized tube and stored at −20°C until analysis.

Effect of the Six Lignans on Transport of Tacrolimus (FK506) in Caco-2 Cells. The transport of FK506 across the Caco-2 monolayer was investigated by the methods described in our previous reports (Zhang et al., 2006; Bi et al., 2008; Qin et al., 2010b) with small modifications. Briefly, Caco-2 cells were seeded at 1.0 × 10⁵ cells/cm² on polycarbonate membrane Transwell inserts (Corning Inc., Corning, NY) and cultured for 21 days. The integrity of the cell monolayer was confirmed by the transepithelial electric resistance (TEER) value. The cells with TEER values exceeding 250 Ω × cm² were used in the transport experiment. At the beginning of the transport study, they were washed three times with Hanks’ balanced salt solution (HBSS); after the third wash, the plates were incubated at 37°C for 30 minutes, then the TEER values were measured. Preincubation was carried out for 30 minutes in the presence or absence of the lignans (100 μM) in HBSS buffer at both the apical (AP) and the basolateral (BL) sides of the monolayers. The buffer was then replaced with fresh HBSS buffer on one side of the cell layer and FK506 solution (1 μM/ml) in HBSS buffer on the other side, and incubation was performed at 37°C. The bidirectional transport studies of FK506 were conducted by loading FK506 solution (1 μM/ml) to either the apical or basolateral side of the Caco-2 monolayers. Samples of 50 μl were taken from the receiver side at 30, 60, and 90 minutes, and an equal volume of HBSS buffer was replenished. All samples were stored at −20°C until analysis, according to our previously developed LC-MS/MS methods (Li et al., 2008; Qin et al., 2010b). Both AP to BL and BL to AP directions were tested in triplicate in parallel experiments. At the end of the transport experiment, the cell monolayers were replaced with fresh HBSS buffer, incubated for 30 minutes at 37°C, and TEER values were measured. The TEER values were not significantly different before and after the transport experiment.

Effect of the Six Lignans on Metabolism of Tacrolimus (FK506) by Rat and Human Liver Microsomes. The metabolism of FK506 in human and rat liver microsomes was investigated by the methods described previously (Qin et al., 2010b). Briefly, the incubation system, with a total volume of 200 μl, contained 100 mM potassium phosphate buffer (pH 7.4), rat/human liver microsomes (final concentration: 0.02 mg/ml), and FK506 (final concentration: 5 ng/ml) with or without schisandrin A, schisandrin B, schisandrin C, schisandrol A, schisandrol B, or schisantherin A (1, 10, or 100 μM). The following steps were the same as described previously (Qin et al., 2010b). All incubations were performed in triplicate. The metabolism ratio was calculated by comparing the initial drug concentration with the concentration after incubation.

Quantification of Tacrolimus (FK506) by LC-MS/MS. FK506 in all samples was determined using our previously reported LC-MS/MS methods (Li et al., 2008; Qin et al., 2010a,b, 2013). The biological samples (blood, liver microsomes, and HBSS samples) were prepared using a single-step liquid-liquid extraction procedure described in our previously published papers (Li et al., 2008; Qin et al., 2010a,b, 2013). After a single acetic ether-induced liquid-liquid extraction step with or without protein precipitation, FK506 and FK20 (3,5,4,5,8,9,10,11,12,13,14,15,16, 17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[(1E)-2-[1(R,3R,4R)-4-hydroxy-3-methoxycyclohexyl]-1-methylethenyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3H-pyridin-2(1H)-one] were subjected to LC/MS/MS analysis using positive electrospray ionization in multiple reaction monitoring mode. The linearity of the calibration curves over the concentrations of 0.5–300, 0.5–500, and 0.5–20 ng/ml for FK506 in blood, HBSS buffer, and microsomes buffer, respectively, were all good, with a correlation coefficient r² > 0.99. The lower limit of quantitation of the method was 0.5 ng/ml for FK506. The intra- and interbatch precision and accuracy were less than 15% for all quality control samples in blood, HBSS buffer, and microsomes buffer, respectively.

Data Analysis. Pharmacokinetic analysis was performed using a non-compartmental analysis by the Pharmacokinetics and Bioavailability Program Package (version 2.1; Institute of Clinical Pharmacology, School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou, China) (Bi et al., 2008; Qin et al., 2010b, 2013; Jin et al., 2011; Xue et al., 2013). The derived pharmacokinetic parameters of FK506, including the absolute bioavailability, were calculated by the methods described previously (Qin et al., 2010b). The apparent permeability coefficient (Papp) in cellular monolayers was calculated according to our previous report (Qin et al., 2010b).

The results were expressed as the mean ± S.D. Statistical significance was evaluated using Student’s t tests or the Wilcoxon two-sample test. Statistical analyses were performed using SAS 8.1 software (SAS Institute, Inc., Cary, NC). A P value <0.05 was considered statistically significant.

Results

Effect of the Six Lignans on the Pharmacokinetics of Tacrolimus (FK506). The different effects of the six lignans of Wuzhi tablet (schisandin A, schisandrin B, schisandrin C, schisandrol A, schisandrol B, or schisantherin A) on the pharmacokinetics of FK506 following oral and intravenous administration were studied to determine the one with the strongest effect to increase FK506 exposure. The mean FK506 blood concentration versus time curves obtained after oral or intravenous administration of FK506 with or without the six lignans, respectively, are shown in Figs. 2 and 3; the pharmacokinetic...
parameters of FK506 are presented in Tables 1 and 2. With the coadministration of a single oral dose of schisandrin A, schisandrin B, schisandrin C, schisandrol A, schisandrol B, or schisantherin A, the area under the concentration-time curve (AUC) of the oral dose of FK506 was increased 152.0%, 109.6%, 46.4%, 41.4%, 598.4%, respectively, compared with that of the FK506 alone group. Schisandrol B showed the strongest effect, with an increase of FK506 area under the concentration-time curves from 0h to 24h (AUC0-24h) by 6.8-fold; schisantherin A and schisandrin A increased the AUC0-24h of FK506 1.5- and 1.4-fold, respectively. In the presence of lignans, the blood clearance of oral doses (CL/F) of FK506 was decreased from 30.0 ± 11.9 l/h/kg to 11.9 ± 4.5 (schisandrin A), 19.1 ± 13.5 (schisandrin C), 14.9 ± 6.1 (schisandrin B), 21.7 ± 8.6 (schisandrin C), 22.8 ± 12.0 (schisandrol A), and 5.1 ± 3.4 l/h/kg (schisandrol B), respectively. In our previous report (Qin et al., 2010b), the mean residence time (MRT) of FK506 (5.7 hours) was significantly extended to 7.3 hours in the presence of Wuzhi tablet (the whole extract); however, these single lignans of the whole extract showed little effect on the MRT of FK506.

Furthermore, the effect of schisandrol B, the one with the strongest effect on FK506 exposure, on the pharmacokinetics of FK506 after intravenous administration was studied. When coadministered with schisandrol B, the AUC0-24h value of FK506 was only increased by 38.8% from 109.0 ± 13.5 to 151.3 ± 20.8 ng/ml·h for intravenous FK506 dosing, and the total intravenous blood clearance was decreased from 1.5 ± 0.2 to 1.2 ± 0.1 l/h/kg, indicating that whole-blood concentrations of FK506 were increased with a slower elimination, but the extent was much smaller than that of the oral administration route. The different effect of schisandrol B on oral and intravenous doses of FK506 suggested that oral concomitant administration of schisandrol B in rats showed a more potent effect on the increasing intestinal bioavailability of FK506 than that of liver bioavailability.

**Effect of Schisandrol B on the Oral Bioavailability and Gut/Liver Metabolic Extractions of Tacrolimus (FK506).** Among the bioactive lignans of Wuzhi tablet, schisandrol B showed the strongest effect on the increase of FK506 exposure. To investigate the effect of schisandrol B on the oral bioavailability and gut/liver metabolic extractions of FK506, FK506 was administered to the rats intravenously in the presence of schisandrol B, and the derived pharmacokinetic parameters were calculated and compared. The derived pharmacokinetic parameters of FK506 in the presence of schisandrol B are presented in Table 3. In the presence of schisandrol B, the calculated total drug intrinsic clearance (fu × CLint) was decreased by 40.5% from 2.84 ± 0.54 to 1.69 ± 0.33 l/h/kg (P < 0.0001), the Foral of FK506 was increased from 5.40% ± 2.74% to (27.45% ± 10.53%) (P < 0.0001), and the gut processes affecting availability of FK506 was increased from 0.10 ± 0.009 to 0.41 ± 0.03 (P < 0.0005), whereas the FH of FK506 was only increased from 0.54 ± 0.05 to 0.66 ± 0.04 (P < 0.005). In the presence of schisandrol B, the Foral of FK506 was markedly increased by 408.3% (P < 0.0005), and the gut processes affecting availability of FK506 was 310.0% greater than that of the FK506 alone group (P < 0.0005), suggesting that concomitant administration of schisandrol B could markedly increase gut bioavailability of FK506. However, the FH of FK506 was only 22.2% greater than that of the FK506 alone group, indicating the reduction of the intestinal first-pass effect was the major cause of increased oral bioavailability of FK506 when coadministered with schisandrol B.

**Effects of the Six Lignans on the Transport of Tacrolimus (FK506) in Caco-2 Cells.** The transport ratio of FK506 pretreated with lignans is shown in Table 4. When pretreated with 100 μM of schisandrin A, schisandrin B, and schisandrol B, the transport ratio of FK506 was 1.0, 1.5, and 1.3, respectively, which was lower than the ratio without pretreatment (1.9). Schisandrin C, schisandrol A, and schisantherin A showed poor effect on transportation of FK506 in Caco-2 cells. In the presence of 100 μM of schisandrin C, schisandrin B, or schisandrol B, the Papp of FK506 from AP to BL across Caco-2 cell monolayers (Papp AP-BL) was increased (from 4.5 ± 1.4 to 6.9 ± 1.6, 4.8 ± 0.4, and 6.1 ± 0.9 × 10⁻⁶ cm/s), whereas the Papp BL-AP was decreased (from 8.3 ± 1.6 to 6.6 ± 0.7, 7.1 ± 1.3, and 7.6 ± 2.3 × 10⁻⁶ cm/s). One hundred micromolars schisandrin A, schisandrin B, and schisandrol B elevated the AP-to-BL transport of FK506 and reduced the BL-to-AP transport of FK506.

**Effects of the Six Lignans on the Metabolism of Tacrolimus (FK506) in Rat and Human Liver Microsomes.** The effects of the six lignans on the metabolism of FK506 in rat and human liver microsomes were studied with the system described in our previous report (Qin et al., 2010b). The effects of different concentrations of lignans on the metabolism of FK506 are shown in Fig. 4. The inhibitory effect of the six lignans on the metabolism of FK506 was dose-dependent. Schisandrol B showed the most potent effect on metabolism of FK506 in liver microsomes, and schisantherin A came next. One hundred micromolars ketoconazole and schisandrol B almost completely inhibited FK506 metabolism in rat and human liver microsomes.
CYP3A in vivo and in vitro (Iwata et al., 2004; Qiangrong et al., 2005; the effect of xenobiotics on CYP3A or P-gp significant first-pass metabolism. Thus, it is important to well define venting saturation of efflux transporters and CYP3A and facilitating limitation of concentrations entering into the enterocytes, thereby pre-

...drugs. In clinical practice, the recommended oral dose of FK506 is 0.15–0.3 mg/kg twice a day for renal transplant patients. Thus, for a 70-kg subject, taking the volume of the gastrointestinal tract (3–5 l) into consideration (Egashira et al., 2004), the concentration of FK506 in the intestinal surface would be 1–3.5 μg/ml. In addition, a previous study (Pawarode et al., 2007) showed that 1 μM (about 0.8 μg/ml) of FK506 could enhance cellular drug uptake in cells overexpressing P-gp, multidrug resistance-associated protein 1 (MRP-1), or Breast cancer resistance-related protein (BCRP). Thus, to limit the inhibition effect of FK506 on P-gp activity and better mimic the clinical herb-drug interaction, the concentration of FK506 used in the transport experiment was set at 1 μg/ml (Qin et al., 2010b).

### Discussion

Many reports, including our previous studies, have indicated that blood concentrations of FK506 were increased in the presence of *Schisandra sphenanthera* extract in transplant recipients and in rats (Xin et al., 2007; Jiang et al., 2010; Qin et al., 2010a, b, 2013; Xin et al., 2011; Wei et al., 2013), but the effect of active lignans in *Schisandra sphenanthera* extract on pharmacokinetics of FK506 remains unclear. Our results indicated that the whole-blood concentrations of FK506 were increased when orally coadministered with schisandrin A, schisandrin B, schisandrin C, schisandrol A, schisandrol B, or schisandrol A, respectively. Schisandrol B showed the most remarkable effect on the increase of FK506 exposure and significantly reduced the intestinal first-pass effect of FK506. Further, in vitro evidence showed that schisandrol B significantly inhibited CYP3A-mediated FK506 metabolism and P-gp-mediated FK506 efflux.

FK506 was well defined as a substrate of CYP3A and P-gp (Jeong and Chiu, 2006; Iwasaki, 2007). CYP3A/P-gp-mediated first-pass absorption and metabolism played an important role in the bioavailability of FK506. Since FK506 has the characteristics of a class II compound according to the biopharmaceutics drug disposition classification system proposed by the Food and Drug Administration (Tamura et al., 2002; Custodio et al., 2008), its high permeability will allow ready access to the gut membrane, but the low solubility will limit the concentrations entering into the enterocytes, thereby preventing saturation of efflux transporters and CYP3A and facilitating significant first-pass metabolism. Thus, it is important to well define the effect of xenobiotics on CYP3A or P-gp-mediated first-pass absorption and metabolism of FK506. Previous reports had shown that Wu Wei Zi extracts and its components may affect P-gp and/or CYP3A in vivo and in vitro (Iwata et al., 2004; Qiangrong et al., 2005; Pan et al., 2006; Wan et al., 2006; Fong et al., 2007; Sun et al., 2007; Yoo et al., 2007). It was reported that, after oral intragastric administration with varied doses of schisandrin A to rats, the Cmax and AUC of Midazolam (MDZ) (a specific CYP3A substrate) was dose dependently increased, suggesting that schisandrin A was an inhibitor of CYP3A (Li et al., 2011). Another study showed that schisandrol A and schisandrol B inhibited CYP3A4 and P-gp activity without altering the cellular glutathione level in HepG2 and HepG2-D8 cells (Wan et al., 2006, 2010). In light of these observations and our current results, it may be reasonable that schisandrol B inhibited the activity of P-gp and CYP3A together in the intestine and liver, and then reduced the intestinal and hepatic first-pass effect of FK506. Furthermore, the reduction of the intestinal first-pass effect was the major cause of increased FK506 oral bioavailability when coadministered with schisandrol B.

To confirm whether and how the six lignans influenced P-gp-mediated FK506 efflux, the effects of the six lignans (schisandrin A, schisandrin B, schisandrin C, schisandrol A, schisandrol B, or schisandrol A) on the transport of FK506 in the Caco-2 cell monolayer model were investigated. In clinical practice, the recommended oral dose of FK506 is 0.15–0.3 mg/kg twice a day for renal transplant patients. Thus, for a 70-kg subject, taking the volume of the gastrointestinal tract (3–5 l) into consideration (Egashira et al., 2004), the concentration of FK506 in the intestinal surface would be 1–3.5 μg/ml. In addition, a previous study (Pawarode et al., 2007) showed that 1 μM (about 0.8 μg/ml) of FK506 could enhance cellular drug uptake in cells overexpressing P-gp, multidrug resistance-associated protein 1 (MRP-1), or Breast cancer resistance-related protein (BCRP). Thus, to limit the inhibition effect of FK506 on P-gp activity and better mimic the clinical herb-drug interaction, the concentration of FK506 used in the transport experiment was set at 1 μg/ml (Qin et al., 2010b).

### Table 1

Pharmacokinetic parameters of tacrolimus (FK506) after a single oral dose of tacrolimus (1.89 mg/kg) in rats with and without an oral dose of lignans (0.024 mM/kg). Data are the mean ± S.D. (n = 5).

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameters of FK506</th>
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<tbody>
<tr>
<td></td>
<td>Cmax (ng/ml)</td>
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<tr>
<td>Con.</td>
<td>15.1 ± 11.2</td>
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<tr>
<td>+ Schisandrin A</td>
<td>31.2 ± 13.9</td>
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<tr>
<td>+ Schisandrin B</td>
<td>39.8 ± 30.0</td>
</tr>
<tr>
<td>+ Schisandrin C</td>
<td>24.8 ± 14.3</td>
</tr>
<tr>
<td>+ Schisandrol A</td>
<td>11.4 ± 4.2</td>
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<tr>
<td>+ Schisandrol B</td>
<td>78.4 ± 16.1</td>
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**CL/F**, blood clearance of oral doses; **Con.**, Control group (FK506 alone); **MRT**, mean residence time; **t1/2**, terminal half-life; **Tmax**, time to peak blood concentration; **Vd/F**, apparent volume of distribution of oral doses.

### Table 2

Pharmacokinetic parameters of tacrolimus (FK506) after a single intravenous dose of tacrolimus (0.2 mg/kg) in rats with and without an oral dose of schisandrol B (0.024 mM/kg). Data are the mean ± S.D. (n = 4).

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameters of FK506</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cmax (ng/ml)</td>
</tr>
<tr>
<td>Con.</td>
<td>100.0 ± 13.5</td>
</tr>
<tr>
<td>+ Schisandrol B</td>
<td>132.0 ± 14.2</td>
</tr>
</tbody>
</table>

**CLint**, total intravenous blood clearance; **MRT**, mean residence time; **t1/2**, terminal half-life; **Vd**, volume of distribution.

### Table 3

Summary of derived pharmacokinetic parameters of tacrolimus (FK506) after a single oral dose (1.89 mg/kg) or intravenous dose (0.2 mg/kg) of tacrolimus in rats with and without an oral dose of schisandrol B (0.024 mM/kg).

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameters of FK506</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vd/F</td>
</tr>
<tr>
<td>Con.</td>
<td>2.84 ± 0.54</td>
</tr>
<tr>
<td>+ Schisandrol B</td>
<td>5.40 ± 2.74</td>
</tr>
</tbody>
</table>

**Fabs**, free drug concentrations affecting availability; **LClint**, Total drug intrinsic clearance.

* P < 0.05; **P < 0.005; ***P < 0.0005; significant difference as compared with the FK506 alone group.
The chosen concentration of lignans was based on the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and referred to the published paper (Qiangrong et al., 2005; Pan et al., 2006; Wan et al., 2006; Fong et al., 2007; Sun et al., 2007; Yoo et al., 2007). The results showed that, for pretreatment with 100 μM schisandrin A, schisandrin B, or schisandrol B, the transport ratio of FK506 was 1.0, 1.5, and 1.3, respectively, which was lower than the ratio without pretreatment (1.9), suggesting schisandrin A, schisandrin B, and schisandrol B could inhibit P-gp-mediated efflux of FK506.

To further confirm whether and how the six lignans influence CYP3A-mediated FK506 metabolism, the effects of the six lignans on the metabolism of FK506 in rat and human liver microsomes were investigated. The incubation time, protein concentration, and FK506 concentration were optimized by the method described previously (Qin et al., 2010b). In the liver microsome incubation study, the concentration of FK506 was set as 5 ng/ml for the following reasons. First, the therapeutic range of FK506 was 5–15 ng/ml (Venkataramanan et al., 1995). Second, high concentration of FK506 showed an inhibited effect on CYP3A. It was reported that FK506 competitively inhibited CYP3A in human liver microsomes, showing inhibition constants (Ki) of 0.61 μM (Amundsen et al., 2012). Third, the reported concentration of FK506 used in a liver-microsome–based metabolism study of FK506 was 3 ng/ml (Egashira et al., 2004). Thus, to mimic the clinical herb-drug interaction between WZ and FK506, 5 ng/ml of FK506 was chosen in the metabolism study.

The results from the in vitro metabolism study indicated that the inhibition of lignans on FK506 metabolism was dose-dependent. Schisandrol B showed the most potent effect on metabolism of FK506 in liver microsomes, and schisantherin A came next, which is in line with our in vivo pharmacokinetic result that schisandrol B was the most potent lignan to increase the AUC of FK506. Schisandrol B had an inhibition ability equivalent to that of ketoconazole, a potent inhibitor of CYP3A. Metabolism of FK506 was almost completely inhibited by 100 μM schisandrol B and ketoconazole (it was reported that 25 μM ketoconazole caused an 80% inhibition of FK506 metabolism) (Lampen et al., 1996), indicating a potent inhibition of FK506 metabolism by schisandrol B and ketoconazole. Previously published work had shown that Schisandra fruit extract, gomisin B (schisandrol B), gomisin C (schisantherin A), and gomisin G were potent inhibitors of CYP3A4, and the inhibitory effect of gomisin C (schisantherin A) was stronger than that of ketoconazole (Ki = 0.070 μM) (Iwata et al., 2004), which showed a small difference from our study. Iwata et al. (2004) used erythromycin and testosterone as substrates of CYP3A, whereas in our study, we evaluated the effect of lignans on the metabolism of FK506 in liver microsomes. Finally, in our study, 100 μM schisandrol B almost completely inhibited FK506 metabolism in rat and human liver microsomes, which indicated that schisandrol B potently inhibits the CYP3A-mediated metabolism of FK506.

All of the aforementioned results indicate that the dramatic increase in oral bioavailability of FK506 in the presence of lignans (especially schisandrol B) was mostly due to the inhibition of the intestinal first-pass effect. In the presence of schisandrol B, the absorption of FK506 was increased significantly, and the transport ratio was decreased, indicating the inhibition of P-gp activity. Whereas the blood clearance of oral doses (CL/F) of FK506 was decreased markedly, in addition, the FK506 metabolism was inhibited by lignans, suggesting a significant inhibition of CYP3A by lignans, especially by schisandrol B. Therefore, the inhibition of intestinal and hepatic P-gp and CYP3A by these lignans contributed together to the increase of FK506 bioavailability in rats. This study also suggested that these lignans (especially schisandrol B) could be potential FK506-sparing agents for future clinical application. However, whether the first-pass effect in the intestine is more related to CYP3A-mediated metabolism than to P-gp–mediated drug exsorption is still unknown; further studies are needed to elucidate the exact interaction of P-gp and CYP3A involved in the FK506 pharmacokinetic process. In addition, our present study only indirectly showed that these lignans inhibited P-gp–mediated efflux and CYP3A-mediated metabolism of FK506 to increase FK506 oral bioavailability. The direct evidence of the effect of lignans on CYP3A and P-gp activity and the involved mechanisms are still unknown, thus the effect of these lignans on the hepatic/intestinal CYP3A and P-gp activity and the underlying mechanisms need to be further elucidated.

### Table 4

<table>
<thead>
<tr>
<th>Groups</th>
<th>( P_{\text{app}} ) AP-BL ( \times 10^{-5}\text{cm/s} )</th>
<th>( P_{\text{app}} ) BL-AP ( \times 10^{-5}\text{cm/s} )</th>
<th>Transport Ratio (BL-AP/BL-AP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.5 ± 1.4</td>
<td>8.3 ± 1.6</td>
<td>1.9</td>
</tr>
<tr>
<td>Verapamil</td>
<td>4.2 ± 0.9</td>
<td>6.5 ± 0.2</td>
<td>1.5</td>
</tr>
<tr>
<td>Schisandrin A</td>
<td>6.9 ± 1.6</td>
<td>6.6 ± 0.7</td>
<td>1.0</td>
</tr>
<tr>
<td>Schisandrin B</td>
<td>4.8 ± 0.4</td>
<td>7.1 ± 1.3</td>
<td>1.5</td>
</tr>
<tr>
<td>Schisandrin C</td>
<td>4.2 ± 0.5</td>
<td>7.8 ± 0.3</td>
<td>1.9</td>
</tr>
<tr>
<td>Schisantherin A</td>
<td>3.8 ± 0.6</td>
<td>8.7 ± 0.06</td>
<td>2.3</td>
</tr>
<tr>
<td>Schisandrol A</td>
<td>6.1 ± 0.9</td>
<td>7.6 ± 2.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Schisantherin A</td>
<td>4.3 ± 0.8</td>
<td>8.7 ± 0.5</td>
<td>2.0</td>
</tr>
<tr>
<td>250 μM schisandrol B</td>
<td>5.6 ± 0.7</td>
<td>5.4 ± 0.3</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*Groups Papp AP-BL P app BL-AP Transport Ratio (BL-AP/BL-AP) = 3.*
Effect of Lignans on Absorption and Metabolism of Tacrolimus

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Authorship Contributions

Participated in research design: Bi, Huang, Qin, Chen.

Conducted experiments: Qin, Wang (third author), Xue, Wang, Li.

Contributed new reagents or analytic tools: Huang, Wang, X. D. Wang, C. X. Wang.

Performed data analysis: Qin, Bi, Zhong.

Wrote or contributed to the writing of the manuscript: Qin, Bi, Yang.

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