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Manuscripts for DRUG METABOLISM AND DISPOSITION

Title: Effects of traditional Chinese medicine *Wuzhi* Capsule on Pharmacokinetics of Tacrolimus in Rats

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Running Title：Synergistic effects of WZC on Pharmacokinetics of TAC

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ABBREVIATIONS: WZC, *Wuzhi* capsule; Nan-Wuweizi, *Schisandra sphenanthera*; Bei-Wuweizi, *Schisandrae Chinensis*; LC-MS/MS, Liquid chromatography-tandem mass spectrometry; T_{max}, time to reach the maximum concentration; C_{max}, maximum plasma concentration; $t_{1/2}$,the apparent elimination half-life; $AUC_{0\rightarrow t}$,the area under the plasma concentration–time curve from time zero to last sampling time; $AUC_{0\rightarrow\infty}$,the area under the plasma concentration–time curve from time zero to infinity; CYP3A, cytochrome P450 3A; P-gp, P-glycoprotein; TCM, traditional Chinese medicine; C_{tro}, the target trough blood concentration; CMC-Na,sodium carboxymethyl cellulose; LLOQ, the lower limit of quantification; PXR, orphan nuclear receptor pregnane X receptor.

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Abstract

Wuzhi capsule (WZC) is a preparation of an ethanol herbal extract of *Schisandra sphenanthera*(Nan-Wuweizi), with its main active ingredients including schisandrin, schisandrol B, schisantherin A, schisanhenol and deoxyshisandrin. WZC and tacrolimus are often co-administered for the treatment of drug-induced hepatitis in organ transplant recipients in China. Recently, it was reported that WZC could significantly increase the blood concentration of tacrolimus. The purpose of this study was to investigate whether and how WZC affects the pharmacokinetics of tacrolimus in rats. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was used to determine the plasma concentration of tacrolimus. The results showed that WZC increased the mean plasma concentration of tacrolimus. Compared with administration of tacrolimus alone (C_{max}, 18.87±10.29 ng/mL; $AUC_{0\rightarrow t}$, 40.98±37.07), a single intragastric administered dose of WZC increased, the pharmacokinetic parameters of tacrolimus (C_{max}, 59.42±30.32 ng/mL; $AUC_{0\rightarrow t}$, 239.71±28.86) by five folds in rat plasma. After pretreatment with WZC for 12 days, there were still significant increases in $AUC_{0\rightarrow t}$ (from 40.98±37.07 to 89.21±26.39 h ng/mL; $p < 0.05$) and C_{max} (from 18.87±10.29 to 43.16±10.61 ng/mL; $p < 0.05$) of tacrolimus compared with oral of tacrolimus alone, suggesting that WZC increased the exposure of tacrolimus by one or more mechanisms. The increase in tacolimus C_{max} by WZC was dose dependent. The effect of WZC on Tacrolimus $AUC_{0\rightarrow t}$ also increased with dose with a maximal effect observed at 450 mg/kg (825.34 ng h/mL). No further increases in tacolimus $AUC_{0\rightarrow t}$ were observed at WZC dose above 450 mg/kg. It is suggested that due to the effect of WZC on the pharmacokinetics of tacrolimus, the herb-drug interaction between WZC and tacrolimus should be taken into considered in clinical practice.

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Introduction

Tacrolimus is a potent immunosuppressant agent that is used in the clinical treatment of solid organ transplantation (Mentzer et al., 1998; Staatz and Tett, 2004; Bowman and Brennan, 2008).Tacrolimus has a narrow therapeutic index and it oral pharmacokinetics shows considerable variability among patients (Mancinelli LM et al., 2001; Christine ES and Susan ET, 2004). It is both a substrate for cytochrome P450 3A (CYP3A) and P-glycoprotein (P-gp)(Jeong H and Chiou WL, 2006; Iwasaki K, 2007), which contribute to its variable oral pharmacokinetics. As a result, chemical medicines and herbs that inhibit or induce CYP3A or P-gp may increase or decrease the blood concentration of tacrolimus (Van GT, 2002). It is important to consider the drug interaction of tacrolimus and take measures to control additive effects caused by other drugs.

The ripe fruits of *Schisandra sphenanthera*, known as “Nan-Wuweizi”, have long been used as a traditional Chinese medicine (TCM) in China. Nan-Wuweizi is used for the treatment of hepatitis, hepatic/renal insufficiency, menstrual dysfunction and neuroasthenia owing to its liver-protective, anti-oxidant, anti-tumor, detoxificant, anti-HIV and platelet-activating factor antagonistic activities (Xiao et al., 2008). It has been made into various traditional Chinese medicinal preparations for clinical use. *Wuzhi* Capsule (WZC) is an ethanol extract from the ripe fruits of Nan-Wuweizi, and has been widely used to protect liver function in patients with chronic hepatitis and liver dysfunction patients (Loo et al., 2007; Yu et al., 2006; Chen et al.,2002). WZC, with the main active ingredients including schisandrin(1), schisandrolB(2), schisantherinA(3), schisanhenol(4) and deoxyshisandrin(5) (**Fig.1**), have liver-protective, anti-inflammatory, anti-oxidant, anti-tumor and anti-HIV activities (Chen et al., 2002). It is popularly prescribed for patients with drug-induced hepatitis following renal or liver transplantation. Many investigators reported that WZC could enhance the plasma concentrations of tacrolimus and paclitaxel, probably due to its inhibitory effect on CYP3A and P-gp (Jin at al., 2011; Qin et al., 2010a, 2010b; Iwata et al., 2004). In addition, WZC was also found to increase the blood concentration of other drugs or influence their absorption (Huang et al., 2007; Xin et al., 2007; Zhao et al., 2008). It is therefore important to explore the effects of WZC on the pharmacokinetics of other drugs.

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Although previous research has shed light on the effect of WZC on pharmacokinetics of tacrolimus, several critical issues remain unresolved in our understanding the clinical implications of WZC-tacrolimus interactions. First, several reports (Xin HW et al., 2007; Xin HW et al., 2011; Qin et al., 2010a) showed that WZC could markedly increase the blood concentrations of tacrolimus in patients and rats. However, it is unclear whether long-term consumption of WZC would also increase the blood concentration of tacrolimus. Second, tacrolimus has a narrow therapeutic index, and achieving and maintaining the target trough blood concentration (C_{tro}) are important (Wallemacq P et al., 2009). Therefore it is necessary to determine whether the effect of WZC on tacrolimus pharmacokinetics is dose-related. Third, several reports (Mu Y et al., 2009; Li L et al., 2009) showed that long-term oral pretreatment with WZC significantly induced both CYP3A and CYP2C expression. It is unclear whether the different chemical constituents in different species of genus Schisandra (Schisandraceae) lead to this discrepancy. Finally, Nan-wuweizi possesses many bioactive Schisandra lignans; however, which component in WZC primarily affects the pharmacokinetics of tacrolimus remains unknown.

The present study was attempted to answer these questions and provide a systematic insight in the effect of WZC on the pharmacokinetics of tacrolimus after oral administration of WZC at a single-dose level, multiple-dose and different-dose levels, contributing to the safer, more reasonable and more effective use of WZC as a clinical tacrolimus-sparing agent.

Materials and Methods

Chemicals and reagents. The chemicals and reagents used in this study included tacrolimus standard (Astellas, Ireland); internal standard (IS) ritonavir (National Institute for the Control of Pharmaceutical and Biological Products of China (Beijing, China); WZC (including 0.14 mg/g schisandrin, 0.09mg/g schisandrol B, 5.79 mg/g schisantherin A, 0.63 mg/g schisanhenol and 5.69 mg/g deoxyshisandrin) (batch no. 070601) (Hezheng Pharmaceutical Company, Chengdu, China); the standards (purity >98%) containing schisandrin, schisandrol B, schisantherin A and deoxyshisandrin (Shanghai R&D Center for Standardization of Traditional Chinese Medicines, Shanghai, China); and schisanhenol (purity 99%) isolated and purified from the ripe fruits of *Schisandra chinensis* by Prof.

Daofeng Chen (Department of Pharmacognosy, Fudan University School of Pharmacy, Shanghai, China); HPLC-grade acetonitrile and methanol (Merck, Darmstadt, Germany); and HPLC-grade formic acid (Tedia, Fairfield, USA). All other reagents were of analytical grade. Ultra-pure water was obtained from a Milli Q-plus system (Millipore, MA, USA).

Animals. Male Sprague-Dawley (SD) rats weighing 230-250 g were supplied by Shanghai Slac Laboratory Animal Co., Ltd. (Shanghai, China). The animals were kept in a room at 22-24℃ with 55-60% relative humidity and a light cycle (12 h light and 12 h dark). They had free access to standard rodent chow and clean water *ad libitum*. The rats were fasted for 12 h before the experiments. All procedures were done 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) and were approved by the Laboratory Animal Ethics Committee of the Second Military Medical University.

Preparation of the test substance. The powder obtained from WZC and tacrolimus capsules were dissolved with 0.5% sodium carboxymethyl cellulose (CMC-Na).

Pharmacokinetic experiments in rats

Effect of a single-dose WZC on the pharmacokinetics of tacrolimus. Five minutes after administration of WZC (150 mg/kg) by gavage, tacrolimus (1.2mg/kg) was administered in the same way in six rats, both at an administration volume of 10 ml/kg body weight.

Effect of repetitive-dose WZC on the pharmacokinetics of tacrolimus. WZC (150 mg/kg) was administered by gavage in six rats daily for 12 consecutive days. Five minutes after the last administration of WZC at day 12, tacrolimus (1.2 mg/kg) was administered with an administration volume of 10 ml/kg body weight.

Effect of different-dose levels WZC on the pharmacokinetics of tacrolimus. Rats were equally randomized to seven dose groups of 6 rats/group. WZC at a dose of 0, 25, 100, 150, 450, 1000, and 1250 mg/kg was administered by gavage to the animals. Five minutes later, all rats were given tacrolimus at a dose 1.2 mg/kg, with an administration volume of 10 ml/kg body weight.

Effect of tacrolimus on the pharmacokinetics of WZC. Twelve rats were equally

randomized into two groups. First, WZC (150 mg/kg) was administered by gavage. Five minutes after, one group was given 0.5% CMC-Na and the other was given tacrolimus (1.2 mg/kg), both with an administration volume of 10 ml/kg body weight.

Collection and treatment of blood samples. All rats were deprived of food for 12 h before blood sampling. About 300 μL blood was collected into heparinized tubes via the jugular vein at the time points of 0, 2.5, 5.0, 10.0, 15.0 and 30.0 min, and 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0, 24.0, 36.0 and 48.0h. Plasma sample (100 μL) was pipetted into a microcentrifuge tube, 50μL ZnSO4 solution and 300 μL methanol/acetonitrile (50:50, v/v) were added. The mixture was vortex-mixed for 30s and centrifuged at 1,4000×g for 10min and stored at -20℃ until use for analysis.

Liquid chromatographic and mass spectrometric conditions. WZC was determined using our previously developed LC–MS/MS methods in all samples. The blood samples were prepared as described in our previous paper (Wei et al., 2010a).

Tacrolimus in the samples was determined using LC-MS/MS methods. Briefly, the supernatants of samples were transferred to a 1.5 ml autosampler vial, and a 10μL aliquot was injected into the LC-MS/MS system and separated using methanol/acetonitrile/0.1% formic acid-water as the mobile phase. The compounds were detected by tandem mass spectrometry using electrospray ionization in the positive mode and following ion transitions m/z 826.5→616.3 for tacrolimus, m/z 721.1→296.1 for the ritonavir. The method was validated for selectivity, calibration curve, recovery, precision, the lower limit of quantification (LLOQ) and stability according to the FDA guideline for validation of bioanalytical methods (FDA, 2001).

Pharmacokinetic calculation and statistical analysis. Pharmacokinetic parameters were calculated using a non-compartmental analysis by pharmacokinetic program (Data Access Service，DAS, Ver. 2.0, Medical College of Wannan, China). All results were expressed as mean ± SD. The comparison of pharmacokinetic parameters was conducted using standard student's t-test. Differences between groups were assumed statistically significant for *P* values <0.05.

Results

Methodological validation of tacrolimus. No interfering peaks for tacrolimus were

seen in drug-free plasma in the analysis of selectivity. In addition, the calibration curve of tacrolimus in plasma of rats was established. The linearity was good when the concentration of tacrolimus was in the range of 0.20-100 ng/mL. The extraction recovery was 85.3% for tacrolimus in plasma. The intra- and inter-day precision of plasma tacrolimus was less than 14% for all quality control samples. LLOQ of the analytical method was 0.2 ng/mL for tacrolimus. Tacrolimus was stable under the conditions of stability examination.

Effect of a single-dose and multiple-dose WZC on the pharmacokinetics of tacrolimus. Pharmacokinetic interactions between WZC and tacrolimus in vivo were studied in rats. The mean plasma concentration of tacrolimus administered with a single-dose WZC was low before 0.3 h and then increased markedly as compared with that of tacrolimus without WZC (control group) (**Fig. 2**). However, after the administration of WZC (150 mg/kg) for 12 consecutive days, the mean plasma concentration of tacrolimus was above the level of tacrolimus without WZC and was lower than the concentration of tacrolimus co-administered with WZC (150 mg/kg, single dose) after 0.3h (**Fig. 2**). **Table 1** showed that the pharmacokinetic parameters after oral administration of tacrolimus with WZC. The $AUC_{0\rightarrow t}$ and $AUC_{0\rightarrow\infty}$ were increased by about 5 fold when tacrolimus was administered in combination with a single-dose WZC, and by about 2 fold during repetitive WZC dosing. The $t_{1/2}$ of tacrolimus was longer when it was administered with a single-dose WZC, and prolonged when it was administered with multiple-dose WZC. The T_{max} was delayed from 0.38±0.21 to 1.54±0.15h when tacrolimus was administered with a single-dose WZC, while it was not different (from 0.38±0.21 vs. 0.32±0.09) when tacrolimus was administered with multiple-dose WZC for 12 consecutive days.

Effect of different-dose WZC on the pharmacokinetics of tacrolimus. The mean plasma concentration-time curve of tacrolimus co-administered with different doses of WZC is shown in **Fig.3**. The mean plasma concentration of tacrolimus increased with the increase of WZC dose (ranging from 0 mg/kg to 450 mg/kg). However, the mean plasma concentrations of tacrolimus co-administered with WZC (1250 mg/kg) was not the maximum, but the maximum mean plasma concentration of tacrolimus was found when co-administered with WZC at 450 mg/kg. In addition, $AUC_{0\rightarrow t}$ and T_{max} of tacrolimus

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increased and prolonged gradually with the increase of the WZC dose, and the changes of $AUC_{0\rightarrow t}$ and T_{max} with WZC dose increase had the same tendency (**Table 2**). However, $t_{1/2}$ of tacrolimus when co-administered with WZC at different dosages ((25, 100, 150, 450, 1000, 1250 mg/kg) were longer than those of the control group (WZC 0 mg/kg) (**Table 2**).

Moreover, the pharmacokinetic parameters of the main components in WZC were also explored in the present study. **Fig.4** showed the mean plasma concentration-time profiles after intragastric administration of 150 mg/kg WZC (equivalent to 21.51μg/kg of schisandrin,14.62 μg/kg of schisandrol B, 868.21μg/kg of schisantherin A, 94.59 μg/kg of schisanhenol, and 895.45 μg/kg of deoxyshisandrin) (Wei et al., 2010a) without or with tacrolimus (1.2 mg/kg). The pharmacokinetic parameters obtained are indicated in **Table 3**. The results showed no significant differences in the pharmacokinetic parameters of schisandrin, schisandrol B, schisantherin A, schisanhenol and deoxyshisandrin before or after intragastric administration of WZC.

Discussion

It is reported that the metabolism of tacrolimus occurs in the small intestine and liver via cytochrome P450 (CYP3A4) (Lampen et al., 1995; Vincent et al., 1992). In addition, the blood concentration of tacrolimus in rats was markedly increased following WZC administration due to the inhibitory effect of the WZC ingredients on the activity of P-gp and/or CYP3A4 (Qin et al., 2010a, 2010b). These results are consistent with our findings that the concentration of tacrolimus was increased after a single-dose administration of WZC. Similarly, the concentrations of tacrolimus was increased significantly after multiple-dose administration of WZC, albeit to a decreased extent compared with a single-dose administration of WZC, suggesting that WZC may have a biphasic effect in regulating the pharmacokinetic parameters of tacrolimus. Previous reports (Mu et al., 2006) demonstrated that *Schisandra* lignan extract also induced both CYP3A and CYP2C expression through activating orphan nuclear receptor pregnane X receptor (PXR), probably by exerting a biphasic effect (short-term inhibition and long-term induction) on regulating CYP3A expression and activity, which was also observed by St. John's wort (Rengelshausen et al., 2005; Xie and Kim, 2005). More recently it was found that long-term administration of the *Schisandra* lignan extract induced both intestinal and

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hepatic CYP3A protein expression (Li et al., 2009), suggesting that WZC exhibited a mechanism-based induction toward CYP3A. In combinatorial considerations, we were interested in investigating the "net effect" of co-administered with WZC on exposure tacrolimus. Our results showed that tacrolimus blood concentration was increased when was co-administered with single (or multiple)-dose administration of WZC. In other words, WZC treatment exerts a much stronger inhibitory effect than inductive effect on CYP450 in rats.

However, there are also other studies reporting that *Schisandra* lignan could induce total CYP450s and increase metabolism of other drugs such as warfarin in rats when co-administered (Mu et al.,2006; Zhu et al.,2000). This mechanism does not seem to explain the interaction of WZC with tacrolimus, and therefore the significant increase in the blood concentration and bioavailability of tacrolimus after WZC administration may be attributable to some other mechanisms. A review of the literature reveals that the ripe fruits of *Schisandra chinensis* and *Schisandra sphenathera* have the same name, i.e. Wuweizi. Not until 2000 did we find that the fruits of these two plants are accepted by the Chinese Pharmacopoeia (ChP, 2005) as two different crude drug: "Bei-Wuweizi" (*Schisandrae Chinensis*, Northern Magnoliavine Fruit) and "Nan-Wuweizi" (*Schisandrae Sphenantherae*, Southern Magnoliavine Fruit). Phytochemical investigations have shown that the fruits of *Schisandrae Chinensis* and *Schisandrae Sphenantherae* are quite different in their chemical constituents and the content of bioactive components (Lu et al., 2006; Wei et al., 2010b). Most studies reporting the inductive effect of Wuweizi on CYP450 used Bei-Wuweizi as the research object, while those reporting the inhibitory effect of Wuweizi on CYP450 used Nan-Wuweizi as the research object, which could be the reason for the discrepancies of the findings. In our previous study, we also compared the chemical compositions of Bei-Wuweizi and Nan-Wuweizi. HPLC analysis showed that the bioactive lignans mainly exist in the ripe fruits of Bei-Wuweizi and Nan-Wuweizi. The results of extensive investigations in our lab have shown that schisandrin, schisandrol B and schisandrin B are the major constituents of *S. chinensis* fruits, while schisantherin A, deoxyschizandrin and (+)-anwulignan are the main constituents of *S. sphenanthera* fruits(Wei et al., 2010b). Among the lignan components tested, schisantherin A was the

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most potent CYP3A inhibitor, while deoxyschizandrin and schisandrin B showed a moderate and similar inhibitory effect. These results are largely consistent with the previous report, in which schisantherin A was identified as a very potent mechanism-based CYP3A inactivator (Iwata et al., 2004). However, the results of Mu's work (Mu et al., 2006) showed that schisandrol B, deoxyschizandrin and schisandrin B were more efficacious than schisandrin in inducing CYP3A4 mRNA expression. Therefore, it is necessary to regularly monitor the blood tacrolimus concentration when it is administered clinically together with the *Schisandra* lignan extract.

In addition, the intestinal CYP3A and P-gp contribute to a great extent to the first-pass metabolism of many CYP3A and P-gp substrates (Andersen et al., 2002; Paine et al., 2005; Hao et al., 2007;Kato, 2008). And the reduction of intestinal first-pass effect of tacrolimus by WZC through CYP3A and P-gp is extensive and contributes greatly to the increase in tacrolimus bioavailability (Venkataramanan et al., 1990; Qin et al., 2010b).Therefore it is useful to dissect the influence of WZC on regulating intestinal and hepatic CYP3A and P-gp for a better understanding of its differential effect in regulating pharmacokinetic profiles of tacrolimus. The related study will be addressed in our future study.

In summary, our results showed that single-dose and multiple-dose WZC could significantly increase blood tacrolimus concentration in rats. When WZC was administered at different doses, the change of C_{max} depended on the WZC dosage, and AUC increased gradually with the increase of the WZC dose. $AUC_{0\rightarrow t}$ increased to the maximum (825.34 ng h/mL) when WZC was given at 450mg/kg. Due to the effect of WZC on the pharmacokinetics of tacrolimus, the herb-drug interaction between WZC and tacrolimus should be considered in clinical practice. Our research hopes to take the advantage of WZC and use it as a tacrolimus-sparing agent to substantially reduce tacrolimus dosage. About dose-response of WZC on tacrolimus' oral-exposure in healthy volunteers will be studied in future.

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Authorship Contributions

Participated in research design: Hua Wei, Wansheng Chen.

Conducted experiments: Xia Tao, Peng Di, Yingbo Yang, Xiaofeng Qian.

Performed data analysis: Hua Wei, Jingxian Li, Jin Feng.

Wrote or contributed to the writing of the manuscript: Hua Wei, Wansheng Chen.

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Figures Legends:

Fig. 1 Chemical structures of schisandrin (A), schisandrol B (B), schisantherin A (C), schisanhenol (D), deoxyshisandrin (E)

Fig. 2 Mean plasma concentration-time profiles of tacrolimus. A: tacrolimus (1.20 mg/kg) was given to rats alone. B: tacrolimus (1.20 mg/kg) was administered with WZC at a dose of 150 mg/kg to rats simultaneously. C: tacrolimus (1.20 mg/kg) was administered to rats after 12-day administration of WZC (150 mg/kg).

Fig. 3 Mean plasma concentration-time profiles of schisandrin (A), schisandrol B (B), schisantherin A (C), schisanhenol (D) and deoxyshisandrin (E) before (after) co-administration of WZC (150 mg/kg) and tacrolimus (1.20 mg/kg) to rats.

Fig.4 Mean plasma concentration-time profiles of tacrolimus after the administration of WZC at the dose of 0 mg/kg (10 mL/kg 0.5%CMC-Na), 25 mg/kg, 100 mg/kg, 150 mg/kg, 450 mg/kg, 1000 mg/kg and 1250 mg/kg to rats.

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Table 1 Pharmacokinetic parameters of tacrolimus (1.20 mg/kg)

Parameter	Values		
	tacrolimus [m]	tacrolimus [n]	tacrolimus [k]
T_{max} (h) [a]	0.38±0.21	1.54±0.15**	0.32±0.09**
C_{max} (ng/mL) [b]	18.87±10.29	59.42±30.32**	43.16±10.61**
$t_{1/2}$ (h) [c]	6.09±1.68	8.31±1.91	11.13±2.55
$AUC_{0\rightarrow t}$ (ng h/mL) [d]	40.98±37.07	239.71±28.86**	89.21±26.39*
$AUC_{0\rightarrow\infty}$ (ng h/mL) [e]	42.37±36.70	254.37±45.07**	89.66±26.29*

[a] Time to reach the maximum concentration; [b] C_{max}: maximum plasma concentration; [c] The apparent elimination half-life; [d] The area under the plasma concentration–time curve from time zero to last sampling time; [e] The area under the plasma concentration–time curve from time zero to infinity.

[m] tacrolimus at a dose 1.20 mg/kg was administered to rats (n = 6):control group. [n] tacrolimus (1.20 mg/kg) and WZC (150 mg/kg) were administered to rats at a single dose (n = 6). [k] tacrolimus at a dose 1.20 mg/kg was given to rats after 12-day administration of WZC (150 mg/kg) (n = 6).

Compared with control: *P < 0.05; **P < 0.001.

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Table 2 The effect of dose level of co-administered WZC on the pharmacokinetics of tacrolimus (1.20 mg/kg) in rats (n = 6)

Parameter	Dose of WZC* (mg/kg)						
	0	25	100	150	450	1000	1250
T_{max} (h) [a]	0.18±0.03	0.28±0.14	0.40±0.30*	0.75±0.43**	1.86±1.18**	1.61±1.26**	2.28±0.93**
C_{max} (ng/mL) [b]	11.42±6.58	14.60±1.65	16.68±5.86	49.55±42.6**2	112.42±39.48**	67.69±42.23**	58.78±14.45**
$t_{1/2}$ (h) [c]	6.67±3.00	12.76±5.76*	13.98±7.12*	9.73±4.95	8.37±2.35	13.63±11.83**	10.78±6.42*
$AUC_{0\rightarrow t}$ (ng h/mL) [d]	20.14±10.51	55.02±19.32**	63.76±24.65**	198.69±97.96**	825.34±418.31**	435.95±226.76**	539.45±160.46**
$AUC_{0\rightarrow\infty}$ (ng h/mL) [e]	21.88±11.46	63.93±25.49**	71.19±20.63**	203.93±95.04**	835.91±416.79**	449.33±228.21**	551.40±156.73**

[a] Time to reach the maximum concentration;

[b] C_{max}: the maximum plasma concentration;

[c] The apparent elimination half-life;

[d] The area under the plasma concentration–time curve from time zero to last sampling time;

[e] The area under the plasma concentration–time curve from time zero to infinity;

* All dose groups were first given tacrolimus (1.20 mg/kg).

Compared with control: *P < 0.05; **P < 0.001.

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Table 3 Pharmacokinetic parameters of the main components in WZC after intragastric administration of WZC (150 mg/ kg) without or with tacrolimus (1.2 mg/kg) to rats (*n*=6)

Parameter		Values				
		schisandrin	schisandrol B	schisantherin A	schisanhenol	deoxyshisandrin
*T*max (h) [a]		0.50±0.02	0.25±0.01	0.71±0.06	0.46±0.10	0.50±0.01
	With TAC	0.67±0.26	0.25±0.01	0.71±0.33	0.46±0.10	0.46±0.19
*C*max (ng/mL) [b]		79.21±23.21	16.54±8.58	125.70±42.21	11.92±5.29	32.89±15.53
	With TAC	89.02±13.19	13.56±3.65	95.43±23.09*	10.55±2.50	41.01±10.54
$t_{1/2}$ (h) [c]		0.66±0.22	0.79±0.22	1.17±0.29	1.64±0.56	3.71±0.54
	With TAC	0.55±0.05	1.00±0.17	2.14±1.36	0.90±0.12	3.36±1.59
$AUC_{0\to t}$(ng h/mL) [d]		98.74±42.15	17.50±4.81	453.62±253.10	17.34±7.85	44.31±21.19
	With TAC	128.73±21.34*	18.94±3.90	359.64±148.45*	18.21±2.99	57.57±9.29
$AUC_{0\to\infty}$(ng h/mL) [e]		99.01±42.69	18.94±4.69	454.56±253.83	18.38±8.46	46.57±21.84
	With TAC	128.86±21.4	19.22±3.87	360.02±148.15*	18.48±3.11	59.41±8.56

[a] Time to reach the maximum concentration; [b] C_{max}: the maximum plasma concentration;

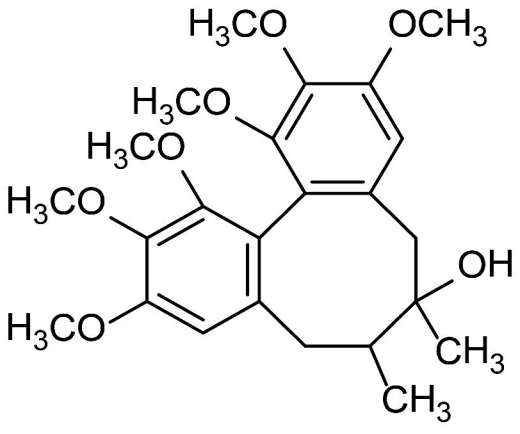
[c] The apparent elimination half-life;

[d] The area under the plasma concentration–time curve from time zero to last sampling time;

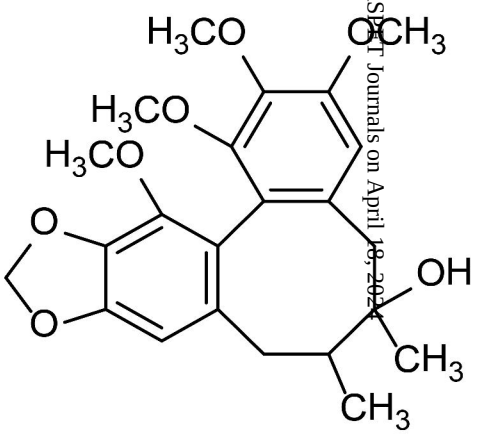
[e] The area under the plasma concentration–time curve from time zero to infinity;

Without TAC: control group; Compared with control: *P < 0.05; **P < 0.001.

Fig. 1



Schisandrin (A)



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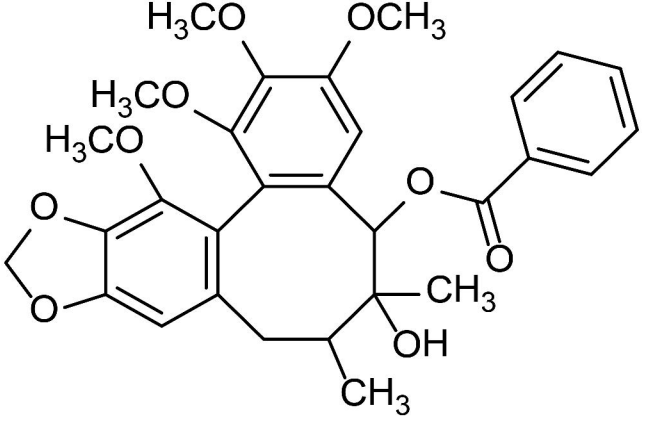
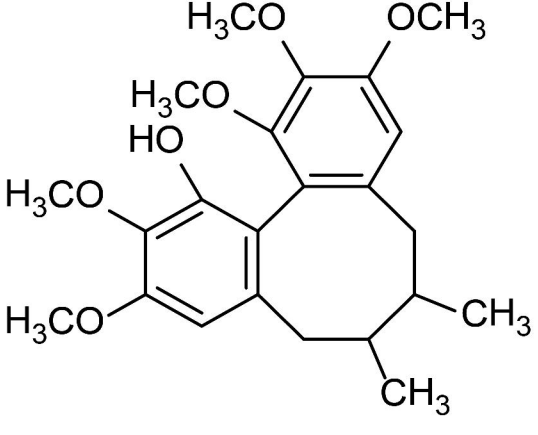
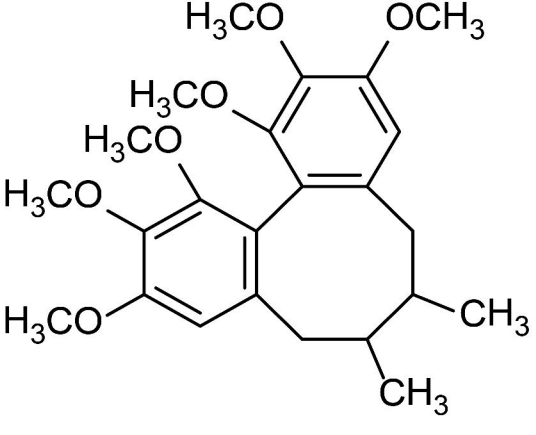


Schisantherin A (C)



Schisanhenol (D)



Deoxyshisandrin (E)

Fig.2

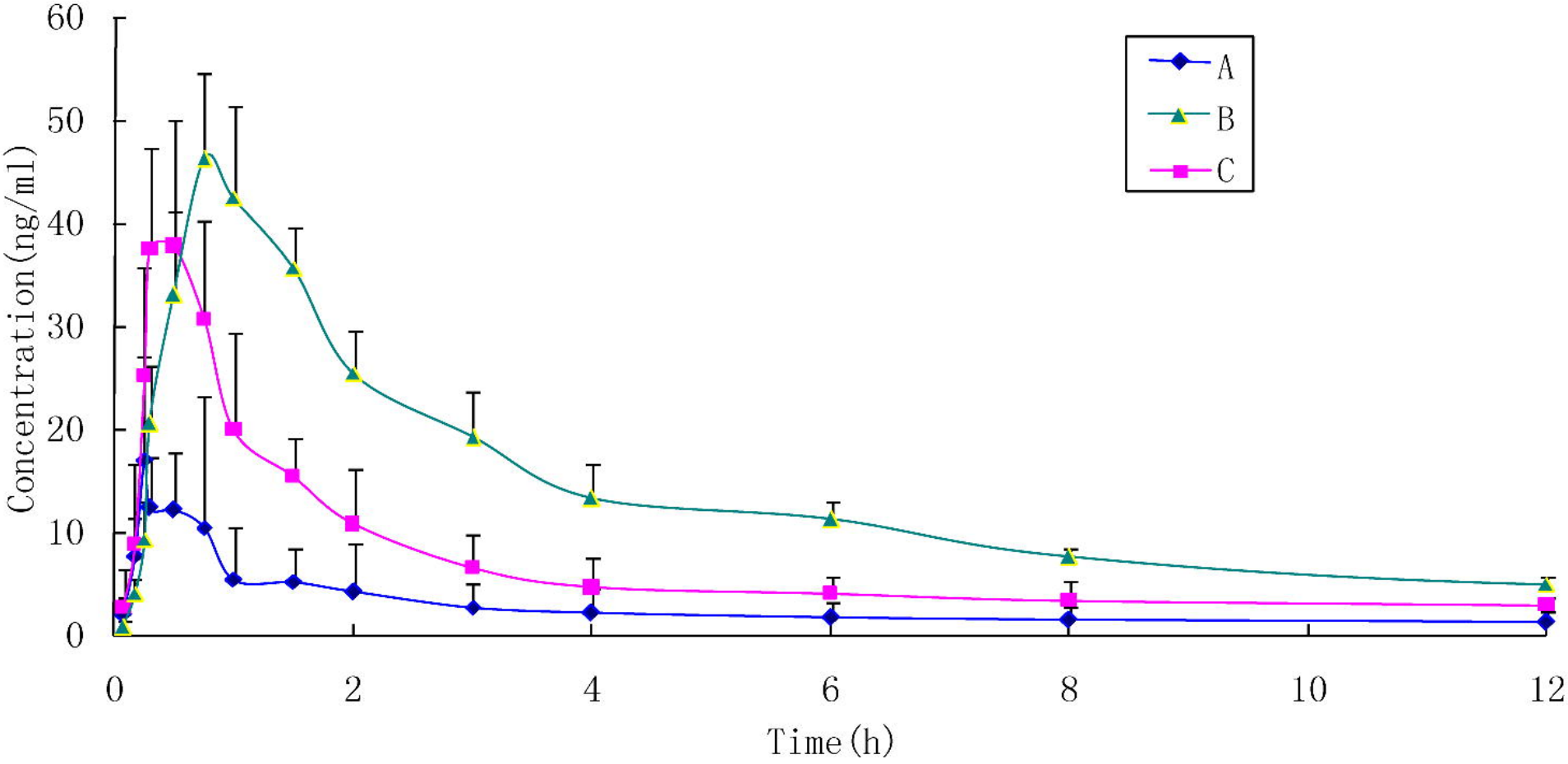


Fig.3

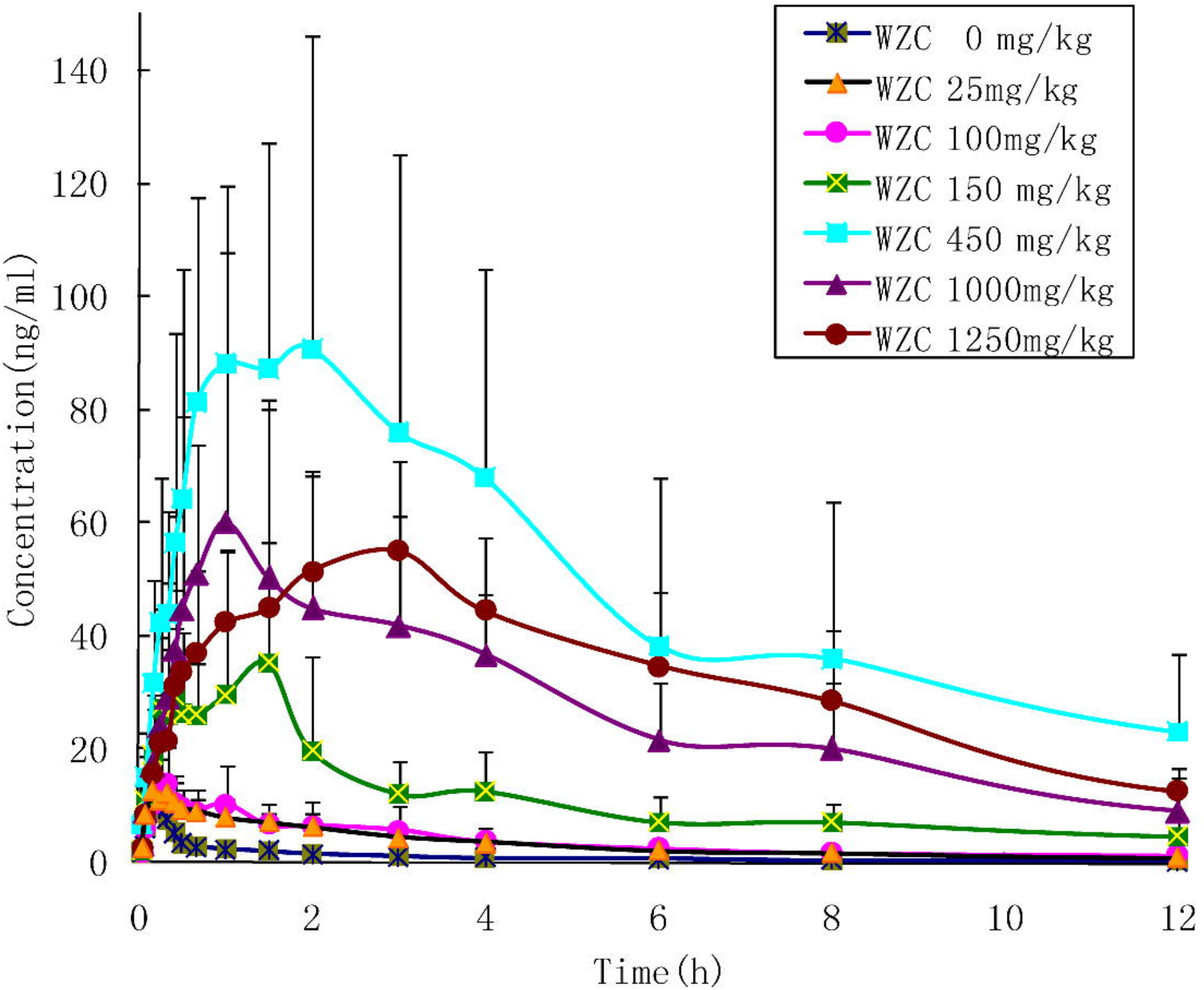

WZC 0 mg/kg
WZC 25mg/kg
WZC 100mg/kg
WZC 150 mg/kg
WZC 450 mg/kg
WZC 1000mg/kg
WZC 1250mg/kg
Concentration(ng/ml)
0
20
40
60
80
100
120
140
0
2
4
6
8
10
12
Time(h)

Fig.4