Effects of Traditional Chinese Medicine Wuzhi Capsule on Pharmacokinetics of Tacrolimus in Rats

Hua Wei, Xia Tao, Peng Di, Yingbo Yang, Jingxian Li, Xiaofeng Qian, Jin Feng, and Wansheng Chen

Department of Pharmacy, Changzheng Hospital, Second Military Medical University, Shanghai, People’s Republic of China

Received December 5, 2012; accepted April 24, 2013

ABSTRACT

Wuzhi capsule (WZC) is a preparation of an ethanol herbal extract of Schisandra sphenanthera (Nan-Wuweizi), with its main active ingredients that include schisandrin, schizandrol B, schisandrin A, schisanhenol, and deoxyxchizandrin. WZC and tacrolimus are often coadministered 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 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 [maximum plasma concentration (C_{max}) 18.87 ± 10.29 ng/ml; area under the plasma concentration-time curve from time zero to last sampling time (AUC_{0–t}) 40.98 ± 37.07 ng h/ml], a single intragastric administered dose of WZC increased the pharmacokinetic parameters of tacrolimus (C_{max} 59.42 ± 30.32 ng/ml; AUC_{0–t} 239.71 ± 28.86 ng h/ml) by 5-fold in rat plasma. After pretreatment with WZC for 12 days, there were still significant increases in AUC_{0–t} (from 40.98 ± 37.07 to 89.21 ± 26.39 ng h/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 tacrolimus C_{max} by WZC was dose-dependent. The effect of WZC on tacrolimus AUC_{0–t} also increased with dose, with a maximal effect observed at 450 mg/kg (825.34 ng h/ml). No further increases in tacrolimus AUC_{0–t} were observed at WZC dose above 450 mg/kg. It is suggested that, because of the effect of WZC on the pharmacokinetics of tacrolimus, the herb-drug interaction between WZC and tacrolimus should be taken into consideration in clinical practice.

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 Bowman, 2008). Tacrolimus has a narrow therapeutic index and, it oral pharmacokinetics, shows considerable variability among patients (Mancinelli et al., 2001; Christine and Susan, 2004). It is both a substrate for cytochrome P450 3A (CYP3A) and P-glycoprotein (P-gp) (Jeong and Chiou, 2006; Iwasaki, 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 absorption (Huang et al., 2007; Xin et al., 2007). It is therefore important to explore the effects of WZC on the pharmacokinetics of 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 neurasthenia because of its liver-protective, antioxidant, anti-inflammatory, 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 (Chen et al., 2002; Yu et al., 2006; Loo et al., 2007). WZC, with the main active ingredients including schisandrin, schizandrol B, schisantherin A, schisanhenol, and deoxyxchizandrin (Fig. 1), has liver-protective, anti-inflammatory, antioxidant, anti-tumor, and anti-HIV activities (Chen et al., 2002). It is popularly prescribed for patients with drug-induced hepatitis after renal or liver transplantation. Many investigators reported that WZC could enhance the plasma concentrations of tacrolimus and paclitaxel, probably because of its inhibitory effect on CYP3A and P-gp (Iwata et al., 2004; Qin et al., 2010a,b; Jin et al., 2011). In addition, WZC was also found to inhibit the blood concentration of other drugs or influence their absorption (Huang et al., 2007; Xin et al., 2007). It is therefore important to explore the effects of WZC on the pharmacokinetics of other drugs.

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

ABBREVIATIONS: AUC_{0–t}, area under the plasma concentration–time curve from time zero to infinity; AUC_{0–t,∞}, area under the plasma concentration–time curve from time zero to last sampling time; C_{max}, maximum plasma concentration; C_{tr}, target trough blood concentration; CMC-Na, sodium carboxymethyl cellulose; HPLC, high-performance liquid chromatography; LC-MS/MS, liquid chromatography–tandem mass spectrometry; LLOQ, the lower limit of quantification; P-gp, P-glycoprotein; t_{1/2}, apparent elimination half-life; T_{max}, time to reach the maximum concentration; TCM, traditional Chinese medicine; WZC, Wuzhi capsule.
Synergistic Effects of WZC on Pharmacokinetics of TAC

W.Z.C-tacrolimus interactions. First, several reports (Xin et al., 2007; Qin et al., 2010a; Xin et al., 2011) 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_{trough}) is important (Wallemacq et al., 2009). Therefore, it is necessary to determine whether the effect of WZC on tacrolimus pharmacokinetics is dose related. Third, several reports (Mu et al., 2006; Lai 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 to provide systematic insight into the effect of WZC on the pharmacokinetics of tacrolimus after oral administration of WZC as a single dose, 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 Pharmaceuticals, Northbrook, IL); internal standard ritonavir (National Institute for the Control of Pharmaceutical and Biologic Products of China, Beijing, China); WZC (including 0.14 mg/g schisandrin, 0.09 mg/g schizandrol B, 0.57 mg/g schisantherin A, 0.63 mg/g schisanhenol, and 5.69 mg/g deoxyschizandrin; Hezheng Pharmaceutical Company, Chengdu, China); the standards (purity >98%) containing schisandrin, schizandrol B, schisantherin A, and deoxyschizandrin (Shanghai R&D Center for Standardization of Traditional Chinese Medicine, Shanghai, China); high-performance liquid chromatography (HPLC)-grade acetonitrile and methanol (Merck, Darmstadt, Germany); and HPLC-grade formic acid (Tedia, Fairfield, OH). All other reagents were of analytical grade.

Animals

Male Sprague-Dawley 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°C with 55–60% relative humidity and a light cycle (12-hour light and 12-hour dark). They had free access to standard rodent chow and clean water ad libitum. The rats were fasted for 12 hours 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 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 was dissolved with 0.5% sodium carboxymethyl cellulose (CMC-Na).

Pharmacokinetic Experiments in Rats

Effect of Single-Dose WZC on the Pharmacokinetics of Tacrolimus

Five minutes after administration of tacrolimus (150 mg/kg) by gavage, tacrolimus (1.2 mg/kg) was administrated 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 at a volume of 10 ml/kg body weight.

Effect of Different Dose Levels of WZC on the Pharmacokinetics of Tacrolimus

Rats were equally randomized to seven dose groups of six rats per group. WZC at a dose of 0, 25, 100, 150, 450, 1000, and 1250 mg/kg was administrated by gavage to the animals. Five minutes later, all rats were given tacrolimus at a dose of 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 administrated by gavage. Five minutes later, one group was given 0.5% CMC-Na, and the other was given tacrolimus (1.2 mg/kg), both at a volume of 10 ml/kg body weight.

Collection and Treatment of Blood Samples. All rats were deprived of food for 12 hours before blood sample obtainment. Approximately 300 μl of...
ionization in the positive mode and using ion transitions compounds were detected by tandem mass spectrometry using electrospray using methanol/acetonitrile/0.1% formic acid – tacrolimus and m/z 721.1→296.1 for tacrolimus. The mixture was vortex-mixed for 30 seconds, centrifuged at 14,000g for 10 minutes, and stored at −20°C until use for analysis.

Liquid Chromatographic and Mass Spectrometric Conditions. WZC was determined using our previously developed liquid chromatography–tandem mass spectrometry (LC-MS/MS) methods in all samples. The blood samples were prepared as described in our previous article (Wei et al., 2010a).

Tacrolimus in the blood was collected into heparinized tubes via the jugular vein at 0, 2.5, 5.0, 10.0, 15.0, and 30.0 minutes and at 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0, 24.0, 36.0, and 48.0 hours. Plasma samples (100 μl) were pipetted into a microcentrifuge tube; 50 μl of ZnSO₄ solution and 300 μl of methanol/acetonitrile (50:50, v/v) were added. The mixture was vortex-mixed for 30 seconds, centrifuged at 14,000g for 10 minutes, and stored at −20°C until use for analysis.

Pharmacokinetic Calculation and Statistical Analysis. Pharmacokinetic parameters were calculated using a noncompartmental analysis by pharmacokinetic program (Data Access Service, DAS, version 2.0; Medical College of Wannan, Anhui, China). All results were expressed as mean ± S.D. The comparison of pharmacokinetic parameters was conducted using standard Student’s t test. Differences between groups were assumed to be 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 interday 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 single-dose WZC was low before 0.3 hour and then increased markedly, 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 coadministered with WZC (150 mg/kg, single dose) after 0.3 hour (Fig. 2). Table 1 shows the pharmacokinetic parameters after oral administration of tacrolimus with WZC. The area under the plasma concentration–time curve from time zero to last sampling time (AUC₀→∞) and area under the plasma concentration–time curve from time zero to infinity (AUC₀→∞) were increased by approximately 5-fold when tacrolimus was administered in combination with single-dose WZC and by approximately 2-fold during repetitive WZC dosing. The apparent elimination half-life (t₁/₂) of tacrolimus was longer when it was administered with single-dose WZC and prolonged when it was administered with multiple-dose WZC. The time to reach the maximum concentration (Tₘₚ) was delayed from 0.38 ± 0.21 to 1.54 ± 0.15 hours when tacrolimus was administered with single-dose WZC, and it was not different (0.38 ± 0.21 versus 0.32 ± 0.09 hours) when tacrolimus was administered with multiple-dose WZC for 12 consecutive days.

Effect of Different Doses of WZC on the Pharmacokinetics of Tacrolimus. The mean plasma concentration–time curve of tacrolimus coadministered with different doses of WZC is shown in Fig. 3. The mean plasma concentration of tacrolimus increased with the increase

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tacrolimus*</th>
<th>Tacrolimusb</th>
<th>Tacrolimusb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tₘₚ (h)</td>
<td>0.38 ± 0.21</td>
<td>1.54 ± 0.15**</td>
<td>0.32 ± 0.09**</td>
</tr>
<tr>
<td>Cₘₚ (ng/ml)</td>
<td>18.87 ± 10.29</td>
<td>59.42 ± 30.32**</td>
<td>43.16 ± 10.61**</td>
</tr>
<tr>
<td>t₁/₂ (h)</td>
<td>6.09 ± 1.68</td>
<td>8.31 ± 1.91</td>
<td>11.13 ± 2.55</td>
</tr>
<tr>
<td>AUC₀→∞ (ng h/ml)</td>
<td>40.98 ± 37.07</td>
<td>239.71 ± 28.86**</td>
<td>89.21 ± 26.39*</td>
</tr>
<tr>
<td>AUC₀→∞ (ng h/ml)</td>
<td>42.37 ± 36.70</td>
<td>254.37 ± 45.07**</td>
<td>89.66 ± 26.39*</td>
</tr>
</tbody>
</table>

*Tacrolimus at a dose 1.20 mg/kg was administered to rats (n = 6): control group.
**Tacrolimus (1.20 mg/kg) and WZC (150 mg/kg) were administered to rats at a single dose (n = 6).
*P < 0.05, compared with control. **P < 0.001, compared with control.
in WZC dose (ranging from 0 to 450 mg/kg). However, the mean plasma concentrations of tacrolimus coadministered with WZC (1250 mg/kg) was not the maximum, but the maximum mean plasma concentration of tacrolimus was found when coadministered with WZC at 450 mg/kg. In addition, \(AUC_{0\rightarrow t}\) and \(T_{\text{max}}\) of tacrolimus increased and prolonged gradually with the increase in the WZC dose, and the changes of \(C_{\text{max}}\) with WZC dose increase had the same tendency (Table 2). However, \(t_{1/2}\) of tacrolimus when coadministered with WZC at different doses (25, 100, 150, 450, 1000, and 1250 mg/kg) was longer than that in the control group (WZC, 0 mg/kg) (Table 2).

Moreover, the pharmacokinetic parameters of the main components in WZC were explored in the present study. Figure 4 showed the mean plasma concentration–time profiles after intragastric administration of 150 mg/kg WZC (equivalent to 21.51 \(\mu\)g/kg schisandrin, 14.62 \(\mu\)g/kg schizandrol B, 868.21 \(\mu\)g/kg schisandantherin A, 94.59 \(\mu\)g/kg schisanhenol, and 895.45 \(\mu\)g/kg deoxyschizandrin) (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, schizandrol B, schisantherin A, schisanhenol, and deoxyschizandrin before or after intragastric administration of WZC.

**Discussion**

It is reported that the metabolism of tacrolimus occurs in the small intestine and liver via CYP3A4 (Vincent et al., 1992; Lampen et al., 1995). In addition, the blood concentration of tacrolimus in rats was markedly increased after WZC administration because of the inhibitory effect of the WZC ingredients on the activity of P-gp and/or CYP3A4 (Qin et al., 2010a,b). These results are consistent with our findings that the concentration of tacrolimus was increased after single-dose administration of WZC. Likewise, the concentrations of tacrolimus was increased significantly after multiple-dose administration of WZC, albeit to a decreased extent compared with 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, probably by exerting a biphasic effect (short-term inhibition and long-term induction) on regulating CYP3A expression and activity, which was also observed with 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 hepatic CYP3A protein expression (Lai et al., 2009), suggesting that WZC exhibited a mechanism-based induction toward CYP3A. In combinatory considerations, we were interested in investigating the net effect of coadministration with WZC on exposure tacrolimus. Our results showed that tacrolimus blood concentration was increased when coadministered with single (or multiple) doses of WZC. In other words, WZC treatment exerts a much stronger inhibitory effect than inductive effect on P450 in rats.

However, there have been other studies reporting that Schisandra lignan could induce total P450s and increase metabolism of other drugs, such as warfarin, in rats when coadministered (Zhu et al., 2000; Mu et al., 2006). 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 *S. chinensis* and *S. sphenanthera* have the same name (i.e., Wuweizi).

Until 2000, we did not find that the fruits of these two plants are accepted by the Chinese Pharmacopoeia (Chinese Pharmacopoeia Commission, 2005) as two different crude drugs: Bei-Wuweizi (*S. chinensis*, Northern Magnoliavine Fruit) and Nan-Wuweizi (*S. sphenanthera*, Southern Magnoliavine Fruit). Phytochemical investigations have shown that the fruits of *S. chinensis* and *S. sphenanthera* are quite different in their chemical constituents and the content of bioactive components (Lu, Chen, 2009; Wei et al., 2010b). Most studies reporting the inductive effect of Wuweizi on P450 used Bei-Wuweizi as the research object, whereas those reporting the inhibitory effect of Wuweizi on P450 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 laboratory have shown that schisandrin, schizandrol B, and schisandrin B are the major constituents of *S. chinensis* fruits, and 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 most potent CYP3A inhibitor, and 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 et al. (2006) showed that schizandrol 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). 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 in maximum plasma concentration ($C_{\text{max}}$) depended on the WZC dosage, and AUC increased gradually with the increase in the WZC dose. $AUC_{0-\infty}$ increased to the maximum (825.34 ng h/ml) when WZC was administered at 450 mg/kg. Because of 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 advantage of WZC and use it as a tacrolimus-sparing agent to substantially reduce tacrolimus dose. The effect of WZC on the oral exposure of tacrolimus in healthy volunteers will be studied in the future.
Acknowledgments
The authors thank Daofeng Chen for the separation and purification of schisanchenol, Jinjong Peng and Zhanying Hong for useful discussions, and Shanghai R&D Center for Standardization of Traditional Chinese Medicines for their excellent assistance.

Authorship Contributions
Participated in research design: Wei, Chen.
Conducted experiments: Tao, Di, Yang, Qian.
Performed data analysis: Wei, Li, Feng.
Wrote or contributed to the writing of the manuscript: Wei, Chen.

References

Address correspondence to: Wansheng Chen, Department of Pharmacy, Changzheng Hospital, Second Military Medical University, Shanghai 200003, China. E-mail: chenws126@126.com.