Wuzhi Tablet (*Schisandra sphenanthera* Extract) Is a Promising Tacrolimus-Sparing Agent for Renal Transplant Recipients Who Are CYP3A5 Expressers: a Two-Phase Prospective Study

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ABSTRACT

Tacrolimus is a potent but expensive first-line immunosuppressant, thus solutions to reduce tacrolimus consumption while maintain therapeutic level are in urgent need. A two-phase prospective study was conducted to assess the efficacy of an ethanolic extraction preparation of *Schisandra sphenanthera* (Wuzhi tablet) as a tacrolimus-sparing agent in renal transplant recipients who were high-dose tacrolimus consumers (CYP3A5*1 allele carriers, CYP3A5 expressers). A total of 12 patients were included in the Part I study. After co-administration of Wuzhi tablet, the average individual increment (%) in dose-adjusted C_0 , C_{max} and $AUC_{0-12\text{ hour}}$ of tacrolimus were 198.8% (95% CI 149.2, 248.3), 111.0% (95% CI 63.4, 158.6) and 126.1% (95% CI 89.4, 162.8), respectively ($P < 0.01$), while the average individual reduction (%) in tacrolimus daily dose was 40.9% (95% CI 25.2, 56.6) ($P < 0.01$).

Subsequently, 32 patients were enrolled in a prospective, randomized, controlled study and randomly assigned to receive tacrolimus by CYP3A5 genotype plus Wuzhi tablet co-administration guided dosing (study group) or standard dosing (control group). Besides less tacrolimus dose requirement ($P < 0.01$), a more accurate tacrolimus initial dose characterized by lower incidence of out-of-range C_0 after initial dose ($P < 0.01$) and fewer dose changes ($P < 0.01$) was found in the study group. Moreover, no significant differences in acute rejection rate and serum creatinine levels were observed between two groups. Our results show that CYP3A5 genotype plus Wuzhi tablet co-administration guided tacrolimus dosing is a promising therapy for CYP3A5 expressers in the early post-transplant stage, while further study with a larger sample size is required to prove these findings.

Introduction

Tacrolimus (FK506), a macrolide isolated from the fermentation broth of *Streptomyces tsukubaensis* (Montini et al., 2006), is the first-line immunosuppressant used for the prevention of allograft rejection after solid organ transplantation. However, as an expensive drug, almost entire post-transplant life-long treatment with tacrolimus imposes a substantial financial burden on both patients and healthcare insurance systems. Therefore, during these years, both clinicians and pharmaceutical researchers have been trying to solve a problem, that is, how to

reduce tacrolimus consumption while maintain its therapeutic level (Jones and Morris, 2002; El-Dahshan et al., 2006; Liu et al., 2009).

Tacrolimus is predominantly metabolized in the liver and in the small intestine by cytochrome P450 3A (including CYP3A4 and CYP3A5), and its absorption is modulated through P-glycoprotein (P-gp) (Masuda and Inui, 2006). Thus, co-administration with inhibitors of CYP3A and/or P-gp to increase the blood level of tacrolimus, has been demonstrated to be an effective way for decreasing tacrolimus consumption (Hebert and Lam, 1999; Jones and Morris, 2002; El-Dahshan et al., 2006). Calcium channel blocker diltiazem, an inhibitor of both CYP3A4/5 (Jones et al., 1999) and P-gp (Cornwell et al., 1987), has been recommended as a tacrolimus-sparing agent by Kidney Disease: Improving Global Outcome (KDIGO) clinical practice guideline (Kidney Disease: Improving Global Outcomes Transplant Work, 2009).

Wuzhi tablet (registration number in China: Z20025766), an ethanolic extraction preparation of *Schisandra sphenanthera* (also called “wu-wei-zi” in China), is widely used for the treatment of viral and drug-induced hepatitis in China (Loo et al., 2007). Ethanolic extract of per gram of Wuzhi tablet powder contains 7.244 ± 0.777 mg Schisandrin A, 0.017 ± 0.001 mg Schisandrin B, 0.024 ± 0.003 mg Schisandrin C, 0.048 ± 0.002 mg Schisandrol A, 0.468 ± 0.020 mg Schisandrol B and

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ABBREVIATIONS: $AUC_{0-12\text{ h}}$, area under the concentration-time curve from 0 to 12 h; C_{max} , peak concentration; C_0 , trough concentration; LC-MS/MS, liquid chromatography–tandem mass spectrometry; PCR-RFLP, polymerase chain reaction–restriction fragment length polymorphism; P-gp, P-glycoprotein; TDM, therapeutic drug monitoring; t.i.d., ter in die; t_{max} , time to peak concentration.

10.089 $\pm$ 1.221 mg Schisantherin A, which was determined by a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis developed in our laboratory (Qin et al., 2013). Among these dibenzocyclooctene lignans, Schisandrin A, Schisandrin B, Schisandrol A, Schisandrol B and Schisantherin A were shown to be P-gp inhibitors (Pan et al., 2006; Wan et al., 2006; Fong et al., 2007), while Schisandrin A, Schisandrin B and Schisantherin A were reported to potently inhibit CYP3A4 (Iwata et al., 2004). Therefore, it is likely that Wuzhi tablet will interact with the substrates of CYP3A and/or P-gp, and suggesting that Wuzhi tablet could enhance the bioavailability of tacrolimus and used as a sparing agent.

Therefore, we conducted a two-phase prospective study to assess the efficacy of Wuzhi tablet as a tacrolimus-sparing agent in renal transplant recipients who were high-dose tacrolimus consumers. *CYP3A5**1 allele carriers (carriers of *CYP3A5**1/*1 genotype or *CYP3A5**1/*3 genotype, *CYP3A5* expressers), who have been proved to be associated with lower tacrolimus exposure by a number of reports including our previous studies (Li et al., 2011, 2015; Kurzwski et al., 2014), were enrolled in this study. Firstly, we investigated the tacrolimus-sparing effect of Wuzhi tablet by a pharmacokinetic study in which each patient served as his or her own control. Secondly, based on the tacrolimus-sparing effect of Wuzhi tablet, an individualized tacrolimus-sparing dosing regimen guided by *CYP3A5* genotype and Wuzhi tablet co-administration was established and used to guide tacrolimus initial dosing in renal transplant recipients by a prospective, randomized, controlled study.

Materials and Methods

Study Design and Patients. This work was performed in accordance with the current Declaration of Helsinki and Good Clinical Practice guidelines. The protocol was approved by the Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University.

The study consisted of two prospective studies. In the first part (Part I), adult (18–60 years) male and female patients receiving single primary living-donors renal transplantation in Kidney Transplant Department, the First Affiliated Hospital of Sun Yat-Sen University (KTD-SYSU) between June 2012 and October 2013 were eligible. The patients initiated tacrolimus-based triple immunosuppressive regimen according to the routine protocol of KTD-SYSU (Li et al., 2011). Specifically, tacrolimus (Prograf; Astellas, Killorglin, Ireland) was given 0.05–0.075 mg/kg twice daily, mycophenolate mofetil (Cellcept; Roche, Basel, Switzerland) was taken 1.0–1.5 g/d and prednisone (Guangdong Huanan Pharmacy Ltd., Dongguan, China) was given 30 mg/d. Those with abnormal hepatic function or receiving combined organ transplantations were excluded. The absence of medication known to affect tacrolimus blood levels (except for prednisone), such as diltiazem, verapamil, rifampicin, ketoconazole, itraconazole, erythromycin, clarithromycin or phenytoin, were checked for each patient. Patients were genotyped for *CYP3A5**3 (6986A > G) after renal transplantation. A total of twelve *CYP3A5* expressers (10 males and 2 females; mean age: 31.4 $\pm$ 11.0 years with a range of 18–53 years; one patient carried *CYP3A5**1/*1 genotype, and the others were *CYP3A5**1/*3 genotype carriers), who had took tacrolimus for 2 weeks and stable concentration level has reached, were enrolled and given Wuzhi tablet (0.62 g, t.i.d.; Guangxi Fanglue Pharmaceutical Co., Ltd., Nanning, China) for 14 days in addition to their normal triple immunosuppressive regimen. Tacrolimus pharmacokinetics was measured on the day before initiation of Wuzhi tablet and on day 14 of Wuzhi tablet supplement. Whole blood samples were taken before and at 0.5, 1, 1.5, 2, 3, 4, 6, 9 and 12 hours after tacrolimus administration. Trough levels (C_0) of tacrolimus were monitored every 3 days and dosages were subsequently adjusted to achieve therapeutic concentration between 5 and 10 ng/ml.

In the second part (Part II), 32 *CYP3A5* expressers were recruited before their primary living-donor renal transplantation in KTD-SYSU between June 2014 and June 2016. They were randomly and evenly assigned to “control” or “study” group. In control group, patients received routine initial tacrolimus-based triple immunosuppressive regimen as described in Part I study, without co-administration of Wuzhi tablet. In study group, Wuzhi tablet (0.62 g, t.i.d.) was co-administered, initial dose of tacrolimus was calculated basing on the

CYP3A5 genotype-guided algorithm developed from our previous study (Li et al., 2011) and the tacrolimus-sparing effect of Wuzhi tablet (results of Part I), while other immunosuppressants used were in accordance to the routine protocol of KTD-SYSU. The exclusion criteria were the same as those in Part I. The primary end points were the accuracy of the initial dose and the dose requirement of tacrolimus, including: 1) the C_0 after the initial dose, which was determined 3 days after the initiation of tacrolimus when steady-state concentration has been achieved, 2) the proportion of out-of-range C_0 (defined as $C_0 < 5$ or $C_0 > 10$ ng/ml) after initial dose, 3) the number of dose adjustments made to achieve therapeutic C_0 range of 5–10 ng/ml, and 4) the dose requirement to reach therapeutic C_0 range. The secondary endpoints included incidences of biopsy-proved acute rejection within 2 weeks after transplantation and renal allograft function represented by serum creatinine on 14 days after transplantation.

Quantitation of Tacrolimus in Whole Blood. Whole blood concentrations of tacrolimus were determined by our previously developed liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Li et al., 2008).

Pharmacokinetic Calculations. Trough concentration (C_0), peak concentration (C_{max}) and time to peak concentration (t_{max}) of tacrolimus were read directly from the observed whole blood concentration versus time profiles. The area under the whole blood concentration versus time from 0 to 12 hour ($AUC_{0-12 \text{ hour}}$) of tacrolimus was calculated using the linear trapezoidal rule by DRUG AND STATISTICS (DAS) software (version 2.1.1; Drug and Statistics, Mathematical Pharmacology Professional Committee of China). Correlations between C_0 with the $AUC_{0-12 \text{ hour}}$ before and after co-administration of Wuzhi tablet were assessed.

DNA Extraction and Genotyping. Total genomic DNA was extracted from peripheral blood leukocytes according to the sodium iodide method described previously (Loparev et al., 1991). The *CYP3A5**3 (6986 A > G) polymorphism was genotyped by using a previously published polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method (Zhang et al., 2013).

Statistical Analysis. Statistical analyses were performed in SPSS (Statistical Package for the Social Sciences) software (version 21; SPSS, IBM, NY). The data are expressed as mean $\pm$ S.D. The comparison of pharmacokinetic before and after co-administration of Wuzhi tablet was conducted using a Wilcoxon Signed-Rank Test. Mann-Whitney *U* test or χ^2 test was used for comparisons between continuous variables or discrete variables, respectively. Spearman's correlation was used to evaluate the correlation between C_0 and $AUC_{0-12 \text{ hour}}$.

A *P*-value less than 0.05 was considered as statistically significant. Statistical power of the sample size was calculated by using PASS (Power Analysis and Sample Size) software (version 11.0.7, PASS; NCSS, LLC, Atlanta, GA). All the results met the requirement to have more than 80% power to detect the difference between/among groups with two-side type one error at 5%.

Results

Part I. The mean whole blood concentration-time curve of tacrolimus before and after co-administration of Wuzhi tablet in 12 renal transplant recipients was shown in Fig. 1. Pharmacokinetics of tacrolimus before and after co-administration of Wuzhi tablet in 12 renal transplant recipients was presented in Table 1. Individual data on tacrolimus dose-adjusted C_0 , C_{max} and $AUC_{0-12 \text{ hour}}$ before and after co-administration of Wuzhi tablet were shown in Fig. 2.

After co-administration of Wuzhi tablet, the average individual increment (%) in dose-adjusted C_0 , C_{max} and $AUC_{0-12 \text{ hour}}$ of tacrolimus were 198.8% (95% CI 149.2, 248.3), 111.0% (95% CI 63.4, 158.6) and 126.1% (95% CI 89.4, 162.8), respectively ($P < 0.01$). The time to peak concentration (t_{max}) was significantly prolonged (2.4 ± 0.9 hour vs. 1.7 ± 1.0 hour, $P = 0.034$). The average individual reduction (%) in tacrolimus daily dose was 40.9% (95% CI 25.2, 56.6) ($P < 0.01$). Correlation between C_0 and $AUC_{0-12 \text{ hour}}$ were: 1) before co-administration of Wuzhi tablet, $r = 0.872$ ($P < 0.01$), and 2) after co-administration of Wuzhi tablet, $r = 0.921$ ($P < 0.01$).

Part II. As shown in Table 2, the distributions of age, gender and *CYP3A5* genotype were balanced in the control and study groups.

According to the genotype-guided algorithm: $C_0/\text{Dose} = 49.226 \times CYP3A5 + 76.053$, in which *CYP3A5* nonexpressers was coded as 1,

Fig. 1. Mean blood concentration-time curves of tacrolimus before and after co-administration of Wuzhi tablet (0.62 g, t.i.d.) in 12 renal transplant recipients who were CYP3A5 expressers.

CYP3A5 expressers was coded as 0 (Li et al., 2011). With targeting C_0 at 8 ng/ml, the calculated initial single dose was about 0.105 mg/kg in CYP3A5 expressers. From the results of Part I, co-administration of Wuzhi tablet could result in 2.26-fold increase in the dose-adjusted $AUC_{0-12\text{ hour}}$ of tacrolimus, we speculated that to achieve the same level of drug exposure, patients co-administered with Wuzhi tablet should require about 55% less tacrolimus than those without Wuzhi tablet co-administration. Therefore, the initial single dose of tacrolimus in the study group was set at 0.05 mg/kg.

Compared with the control group, the average initial tacrolimus single dose was 21.9% lower (0.050 ± 0.002 mg/kg vs. $0.064 \pm$

0.006 mg/kg, $P < 0.01$) while the average C_0 of tacrolimus after initial dose was 70.4% higher (7.31 ± 2.27 ng/ml vs. 4.29 ± 1.42 ng/ml, $P < 0.01$) in the study group. The proportion of out-of-range C_0 of tacrolimus after initial dose was significantly dropped from 68.8% to 12.5% ($P < 0.01$), and accordingly the dose adjustments were markedly decreased from 1.33 ± 0.78 times to 0.17 ± 0.39 times ($P < 0.01$) in the study group. A reduction of 37.5% in tacrolimus dose requirement (0.050 ± 0.006 mg/kg vs. 0.080 ± 0.012 mg/kg, $P < 0.01$) to reach therapeutic range was observed in study group in comparison with that in control group. The incidences of biopsy-proved acute rejection within 2 weeks after transplantation and serum

TABLE 1
Pharmacokinetic of tacrolimus before and after co-administration of Wuzhi tablet (0.62 g, t.i.d.) in 12 renal transplant recipients who were CYP3A5 expressers

	C_0/Dose (ng/ml)/(mg/kg)	$C_{\text{max}}/\text{Dose}$ (ng/ml)/(mg/kg)	$AUC_{0-12\text{ h}}/\text{Dose}$ (ng·ml·h)/(mg/kg)	t_{max} (h)
Before	96.9 ± 46.5	382.3 ± 192.2	2328.0 ± 1356.6	1.7 ± 1.0
After	$278.5 \pm 119.2^{**}$	$809.2 \pm 525.1^{**}$	$4892.4 \pm 2173.1^{**}$	$2.4 \pm 0.9^*$

$AUC_{0-12\text{ h}}$, area under the concentration-time curve from 0 to 12 h; t_{max} , time to peak concentration; C_{max} , peak concentration; C_0 , trough concentration.
* $P < 0.05$; ** $P < 0.01$ for comparison between before and after co-administration of Wuzhi tablet.

Fig. 2. Individual data on tacrolimus C_0 , C_{max} , $AUC_{0-12 \text{ hour}}$ and daily dose before and after co-administration of Wuzhi tablet in 12 renal transplant recipients who were CYP3A5 expressers. (A) Dose-adjusted C_0 before and after co-administration of Wuzhi tablet. (B) Dose-adjusted C_{max} before and after co-administration of Wuzhi tablet. (C) Dose-adjusted $AUC_{0-12 \text{ hour}}$ before and after co-administration of Wuzhi tablet. (D) Daily dose before and after co-administration of Wuzhi tablet. $AUC_{0-12 \text{ hour}}$, area under the concentration-time curve from 0 to 12 hour; C_{max} , peak concentration; C_0 , trough concentration.

creatinine on 14 days after transplantation were not different between two groups.

Discussion

The bioavailability of tacrolimus is primarily determined by CYP3A activity in the gut and liver and P-gp activity in the gut. Drug-drug interactions between tacrolimus and inducers or inhibitors of CYP3A and/or P-gp have been extensively reported, including diltiazem (Hebert and Lam, 1999; Jones and Morris, 2002) and rifampicin (Chenhsu et al., 2000), etc. With the wide spread use of herb drug, the herb-drug interactions of tacrolimus with herb drugs have become more and more common. St. John's Wort (*Hypericum perforatum*) was found to significantly enhance tacrolimus metabolism, through induction of both CYP3A and P-gp (Hebert et al., 2004). To the contrary, grapefruit juice could markedly increase bioavailability of tacrolimus with its active components potentially inhibiting CYP3A4 (Liu et al., 2009). In the

present study, we conducted a two-phase prospective study to investigate the influences of an ethanolic extraction preparation of *Schisandra sphenanthera* (Wuzhi tablet) on the pharmacokinetics and dose requirement of tacrolimus in renal transplant recipients carrying CYP3A5*1 allele. According to the increment of dose-adjusted $AUC_{0-12 \text{ hour}}$ of tacrolimus after co-administration of Wuzhi tablet from Part I study, we speculated that to achieve the same level of drug exposure, CYP3A5 expressers co-administered with Wuzhi tablet should require about 55% less tacrolimus than those without Wuzhi tablet co-administration. In the subsequent Part II study, when achieving the therapeutic range, a 37.5% decline in tacrolimus dose requirement was observed in the study group in comparison with that in control group, which was a little lower than our expectation. However, the mean C_0 was approximately 13% higher in the study group than that in the control group when achieving the therapeutic range ($7.26 \pm 1.84 \text{ ng/ml}$ vs. $6.42 \pm 1.00 \text{ ng/ml}$), suggesting that the dose requirement of tacrolimus in the study group could be further reduced by about 11.5% to obtain the same

TABLE 2
Patient's demographic details, primary and secondary end points in the Part II study

	Control group (n = 16)	Study group (n = 16)
Gender (male/female)	10/6	11/5
Age (yr)	37.7 ± 10.3	39.3 ± 12.9
CYP3A5 genotype		
CYP3A5*1/*1	2	4
CYP3A5*1/*3	14	12
Initial tacrolimus single dose (mg/kg)	0.064 ± 0.006	0.050 ± 0.002**
C ₀ of tacrolimus after initial dose (ng/ml)	4.29 ± 1.42	7.31 ± 2.27**
Percent of out-of-range C ₀ after initial dose	68.6%	12.5%**
Number of dose adjustment to reach therapeutic range	1.33 ± 0.78	0.17 ± 0.39**
Dose needed to achieve therapeutic range (mg/kg)	0.080 ± 0.012	0.050 ± 0.006**
Acute rejection rates within 2 wk after transplantation	6.25%	6.25%
Serum creatinine on day 14 after transplantation (μmol/l)	118.4 ± 29.1	124.3 ± 30.5

C₀, trough concentration.

**P < 0.01, compared with control group.

C₀ as that in the control group. These results indicated that Wuzhi tablet could be used as a tacrolimus-sparing agent.

Previous in vitro and in vivo experiments in our laboratories have elucidated the direct inhibitory effect of Wuzhi tablet on the activity of P-gp and CYP3A4, by using digoxin and midazolam (typical P-gp and CYP3A substrates) as probe substrates (Xue et al., 2013). Similarly, we demonstrated that Wuzhi tablet could significantly enhance the bioavailability of tacrolimus primarily through the inhibition of P-gp-mediated efflux and CYP3A-mediated metabolism in the intestine (Qin et al., 2010b). We further clearly clarified the involved mechanisms in the interaction between tacrolimus and the six major bioactive lignans in Wuzhi tablet, indicating that Schisandrol B was the most potent component contributing to the enhancement of the AUC, the oral bioavailability, the gut processes affecting availability, and the hepatic availability of tacrolimus (Qin et al., 2014).

In our previous study in rats, the AUC_{0-∞} value of tacrolimus was increased by 3.1 fold after co-administration of Wuzhi tablet at dosage extrapolated from the clinical practice in treating viral and drug-induced hepatitis (0.93 g, t.i.d.) (Qin et al., 2010b). Moreover, the inhibitory effect of Wuzhi tablet or the six lignans on tacrolimus metabolism was observed to be in a dose-dependent manner (Qin et al., 2014). Therefore, to avoid a sharp rise in tacrolimus concentration, the dosage of Wuzhi tablet used here was lower than that recommended for treating viral and drug-induced hepatitis. Moreover, tacrolimus trough concentrations were closely monitored and dosages were adjusted to achieve therapeutic concentration.

Several studies have indicated that tacrolimus C₀ was closely associated with the risk of rejection and toxicity during the crucial first week after transplantation when the patients were most likely to be vulnerable to early organ rejection (Laskow et al., 1996; Aidong et al., 2004). Therefore, the importance of prescribing the most appropriate dose of tacrolimus and achieving therapeutic trough concentration timely during the initial post-transplant period becomes clinically valuable. In the current study, under the CYP3A5 genotype plus Wuzhi tablet combination guided tacrolimus dosing regimen, the accuracy of tacrolimus initial dose for CYP3A5 expressers was significantly improved characterized by promising reductions in out-of-range C₀ after initial dose and dose changes, indicating that CYP3A5 expressers in study group may reach tacrolimus therapeutic level more promptly than those in control group.

Furthermore, acute rejection rate and serum creatinine levels in the study group was comparable to that in the control group, indicating that the immunosuppressive effect of tacrolimus or renal allograft function were not impaired by the supplement of Wuzhi tablet during the study period. Additionally, our previous studies in rats indicated that Wuzhi

tablet potentially increased tacrolimus oral bioavailability but not significantly influenced its tissue distribution (Qin et al., 2010a). Besides, drug-induced hepatitis is common in renal transplant recipients because many other kinds of drugs (e.g., anti-infectives, cardiovascular drugs, etc.) with hepatotoxicity are often co-administrated to prevent post-transplant side effects. These findings implied that Wuzhi tablet, also a hepatoprotective, may be a safe and promising tacrolimus-sparing agent.

Tacrolimus C₀ levels are commonly used for routine therapeutic drug monitoring (TDM) to guide concentration-controlled dosing, since they have been found to correlate well with AUC (Jusko et al., 1995; Venkataramanan et al., 1995). Whether such a good correlation would be influenced by the supplement of Wuzhi tablet was also taken into our consideration. An even better correlation was seen after co-administration of Wuzhi tablet compared with that before co-administration ($r = 0.921$ vs. 0.872), suggesting that C₀ is still an ideal drug exposure marker of tacrolimus when Wuzhi tablet is co-administered.

The influences of the extract of *Schisandra sphenanthera* on tacrolimus pharmacokinetics have been reported in healthy volunteers and liver transplant recipients (Xin et al., 2007; Jiang et al., 2010), while for renal transplant recipients, only the differences in C₀ between the groups with and without co-administration of the extract of *Schisandra sphenanthera* were investigated by two case-control studies (Xin et al., 2011; Wang et al., 2016). However, another preparation of extract of *Schisandra sphenanthera* called Wuzhi capsule was used in these studies, and the quality control constituent in Wuzhi capsule is Schisandrin A while in Wuzhi tablet is Schisantherin A probably due to the different pharmaceutical technologies. The content of major active constituents in Wuzhi tablet has been quantified and the underlying mechanism in the interaction between tacrolimus and the major bioactive constituents has been clearly clarified in our laboratory (Qin et al., 2013). Most importantly, an individualized tacrolimus dosing regimen by taking both of CYP3A5 genotype guided algorithm and the tacrolimus-sparing effect of Wuzhi tablet into consideration, has been successfully applied to guide individualized tacrolimus dosing in renal recipients, which has never been reported elsewhere.

The findings in the present study should be interpreted carefully. First, the relatively small sample size could not provide sufficient power to elucidate the safety and efficacy of Wuzhi tablet-tacrolimus combination therapy, especially for the impacts of Wuzhi tablet on the other co-administered drugs as well as the long-term outcome of co-administration. Second, this study included only the original twice-daily immediate-release formulation of tacrolimus (Prograf), and therefore the applicability of the co-administration of Wuzhi tablet and other formulations of tacrolimus (such as the once-daily

prolonged-release formulations) should be further evaluated, since it is well documented that various formulations of tacrolimus are not bioequivalent (Staatz and Tett, 2015).

In conclusion, co-administration of Wuzhi tablet could significantly reduce tacrolimus requirement but without impairing the immunosuppressive effect of tacrolimus and renal allograft function. The tacrolimus dosing regimen guided by CYP3A5 genotype plus Wuzhi tablet co-administration was not only tacrolimus-sparing but also more accurately predicting tacrolimus initial dose for CYP3A5 expressers, suggesting that it is a promising therapy in CYP3A5 expressers in the early post-transplant stage, while further study with a larger sample size is warranted to validate these findings.

Authorship Contributions

Participated in research design: Li, C. Wang, Huang.

Conducted experiments: Li, Chen, Qin, Fu, Zhang, Liu.

Performed data analysis: Li, Chen, Qin.

Wrote or contributed to the writing of the manuscript: Li, Bi, X. Wang.

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