Effects of Wuzhi Capsules on Blood Concentration of Tacrolimus in Renal Transplant Recipients

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Background: Tacrolimus is a widely used immunosuppressant in renal transplant recipients. It was demonstrated in rats and healthy volunteers that Wuzhi capsules could inhibit metabolism and maintain blood concentration of tacrolimus. However, there are no clinical studies of Wuzhi capsules in renal transplant recipients. This research aimed to assess the effect of Wuzhi capsules on the blood concentration of tacrolimus in renal transplant recipients.

Material/Methods: A total of 158 Chinese renal transplant recipients receiving tacrolimus with or without Wuzhi capsules were included in this retrospective study. The cohort study included 126 recipients, with 86 recipients receiving Wuzhi capsules (WZCs) and the other 40 recipients not receiving WZCs. Another 32 recipients were involved in a self-control study.

Results: Dose- and body weight-adjusted trough concentrations (C0/D/W) of tacrolimus in the WZC group were found to be significantly higher than that in the non-WZC group (P<0.05). The improvement of C0/D/W by administration of Wuzhi capsules was more significant in CYP3A5 expressers than in non-expressers following subgroup analysis. Furthermore, the WZC group had a remarkably higher proportion of subjects who reached target tacrolimus concentration than in the non-WZC group, both in CYP3A5 expressers (P=0.01) and non-expressers (P<0.001). Multiple linear regression analysis and self-control analysis confirmed the positive impact of Wuzhi capsules on tacrolimus concentration (P<0.001).

Conclusions: Wuzhi capsules can increase tacrolimus trough concentration without adverse effects on allograft function, especially in CYP3A5 expressers. Efficient and convenient immunosuppressive effects on renal transplant recipients can be achieved by treatment including administration of Wuzhi capsules.

MeSH Keywords: Cytochrome P-450 CYP3A • Drugs, Chinese Herbal • Kidney Transplantation • Tacrolimus

Abbreviations: WZCs – Wuzhi capsules; BW – body weight; C0/D/W – dose- and body weight-adjusted trough concentration of tacrolimus; Tac – tacrolimus; CYP – cytochrome P450; P-gp – P glycoprotein; BMI – body mass index; HBV – hepatitis B virus; eGFR – estimated glomerular filtration rate

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Background

Tacrolimus (Tac), one of the most effective and potent immunosuppressants, is a first-line medication widely used to prevent or treat graft rejection after solid organ transplantation [1–3]. However, Tac has a narrow therapeutic range and variable pharmacokinetics [4,5]. Underdose of Tac can result in insufficient suppression of recipients' immune reaction, causing allograft rejection, while overdose can cause nephrotoxicity and hepatotoxicity via Tac toxic reaction [6–8]. It is critical to keep the blood concentration of Tac within the target therapeutic concentration range.

Tac is used for long-term immunosuppression in renal transplant recipients, which imposes a financial burden on these recipients. In addition, it has been demonstrated that several adverse effects result from Tac, including nephrotoxicity, hepatotoxicity, promotion of tumor progression and diabetes occurrence [9–12]. Therefore, reasonable approaches to minimize Tac dose but maintain its therapeutic range are needed. Co-administrating agents that inhibit metabolism of Tac have been proposed and developed rapidly [13,14].

Metabolism of Tac occurs in the liver and small intestine, predominantly led by cytochrome P450 enzymes 3A4 and 3A5 (CYP3A4, CYP3A5) [15,16]. At the same time, P glycoprotein (P-gp) also limits its absorption through pumping Tac out of cells [17,18]. Both CYP enzymes and P-gp contribute to the reduction of oral bioavailability and blood concentration of Tac. Wuzhi capsules (WZCs) are preparations of extract of *Schisandra sphenanthera*, which is a traditional Chinese medicinal herb. WZCs are widely used in China to alleviate hepatotoxin-induced liver damage or virus-induced chronic hepatitis [19,20]. Its active components are schisandrins (A, B, C), schisandrols (A, B) and schisantherins (A, B), which have higher affinity to the CYP3A family than Tac [21–23]. Active lignans in Wuzhi and Tac are both substrates of CYP3A, but the affinity of lignans to CYP3A is much higher than Tac. Through *in vitro* and *in vivo* experiments in rats and healthy volunteers, it was demonstrated that WZCs could inhibit CYP3A-mediated metabolism and P-gp-mediated efflux of Tac, increase the oral bioavailability of Tac, and maintain its blood concentration [21,24,25].

WZCs were also frequently co-administered with Tac to treat drug-induced hepatitis or liver dysfunction in transplant recipients [23]. However, there are no clinical studies of Wuzhi capsules in renal transplant recipients. This research aimed to assess the effect of Wuzhi capsules on the blood concentration of Tac in renal transplant recipients. The influence of CYP3A5 (6986A>G) genetic polymorphisms on inter-individual variability of Tac concentration in kidney transplant recipients has been widely reported [26–28]. In this study, we also analyzed whether the effect of Wuzhi capsules on Tac interferes with different CYP3A5 (6986A>G) genotypes.

Material and Methods

Patients

A total of 158 recipients who received a first living donor kidney transplant in West China Hospital of Sichuan University from July 2017 to March 2018 were recruited. All donors were recipients' relatives and had kinship certificates. All recipients were given a calcineurin inhibitor-based triple immunosuppressive regimen (Tac, mycophenolate mofetil, and methylprednisolone). Oral administration of Tac was initiated at 2.0 or 3.0 mg per day on Day 2 after transplantation, and the daily dose was adjusted based on therapeutic drug monitoring. The target trough concentration (C0) range of tacrolimus was 5–8 ng/ml. Mycophenolate mofetil was initiated at 1.0 g bid on the transplant day, after which it was regulated to reach the area under the curve 45–75 mg.h/L with a maintenance dose of 5 mg or 10 mg qd after 2 weeks. The oral dose of Wuzhi capsules was 22.5 mg per day. The chemical structures of the active components from *Schisandra sphenanthera* extract were shown in Supplementary Figure 1.

This retrospective research consisted of 2 separate parts – one part was a cohort study with 126 recipients, and the other part was a self-control study with 32 recipients. In the cohort study of 126 recipients, recipients were divided into 2 groups based on whether they took Wuzhi capsules (WZC group, n=86) or not (non-WZC group, n=40) after transplantation. Wuzhi capsules were prescribed to recipients in Wuzhi capsules group on Day 2 at the same time when Tac was prescribed for the first time. In the self-control study, the 32 recipients did not take WZCs until 2 weeks after renal transplant, and the administration of WZCs lasted for the next 2 weeks. The research procedure is listed in Supplementary Figure 2.

All enrolled recipients were in a stable state. No diarrhea appeared in these recipients during the first month after transplantation. Recipients with acute rejection or who took medication (like omeprazole) that might influence the Tac concentration were excluded. Informed consent was obtained from all participants and this study was approved by the Ethics Committee of West China Hospital (2017–397). All experiments were performed in accordance with West China Hospital relevant guidelines and regulations.

Tacrolimus concentration and renal function

Tac trough concentration (C0, ng/mL) was evaluated by an automatic enzyme immunoassay analyzer (SIEMENS V-Twin, Germany) before tacrolimus was taken. Dose-adjusted C0 (C0/D, ng/ml per mg) was calculated by dividing the C0 with the 24-h Tac dose (mg). Dose- and body weight-adjusted C0 (C/D/W, ng/ml per mg/kg) was calculated by dividing the C0

Table 1. Demographic characteristics.

Parameters	Self-control (n=32)	WZC group (n=86)	Non-WZC group (n=40)	P-value (WZC *vs.* Non-WZC)
Recipient				
Gender (male, female)	28, 4	65, 21	31, 9	0.502
Age (years)	30.4±7.9	31.5±9.5	31.3±10.5	0.884
Body mass index	20.1±4.7	20.5±3.1	20.6±3.5	0.293
CYP3A5 genotype (expresser, non-expresser)	16, 16	47, 39	16, 24	0.127
Pre-transplant urine volume (ml/d)	424.8±477.9	422.8±494.0	330.3±383.7	0.620
No. of recipients on dialysis (hemodialysis/peritoneal/none)	30/0/2	81/1/4	35/3/2	0.319
Duration of dialysis before transplantation (months)	11.9±8.5	48.0±33.9	10.5±3.5	0.635
Total HLA mismatches	3.6±1.4	3.5±0.7	4.0±1.4	0.717
Panel reactive antibodies (%)	4.5±12.1	2.5±7.3	2.5±7.5	0.572
Co-infection with HBV	2	11	3	0.383
Donor				
Gender (Male, Female)	14, 18	30, 56	7, 33	**0.035**
Age (years)	47.7±8.9	49.7±8.2	48.1±8.8	0.322
Body mass index	22.7±5.0	23.4±3.0	23.1±3.0	0.676

The values were shown as mean±standard deviation or number. P<0.05 was shown in bold. WZC – Wuzhi capsules; HBV – hepatitis B virus.

with 24-h Tac dose per kilogram (mg/kg). Tac C0, C0/D, and C0/D/W on Day 7 (±2), Day 14 (±2), Day 21 (±2), and Day 28 (±2) after surgery were collected for further analysis. No alteration was made in drug dose before we assessed Tac C0 on Day 7 for the first time. Serum creatinine (Scr) was assessed with a 7-month median follow-up time (Roche Diagnostics, Roche, Switzerland). The definition of delayed creatinine recovery was Scr in males more than 140 μmol/l or Scr in females more than 110 μmol/l on Day 7 after transplantation. The estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) formula:

$$eGFR \text{ (ml/min/1.73 m}^2) = 186 \times \text{serum creatinine(mg/dl)}^{-1.154} \times \text{age}^{-0.203} \times (0.742 \text{ if female}) [29].$$

Genotyping

DNA was extracted from EDTA anticoagulated peripheral whole blood using the whole-blood DNA kit (Bioteke Corporation, Beijing, China) and stored at −80°C until analysis. CYP3A5 (6986A>G) genetic polymorphism was analyzed by polymerase chain reaction melting curve analyses (Roche Diagnostics, Penzberg, Bavaria, Germany). The results of control samples were identified by sequencing.

Statistical analysis

Genotype distribution was evaluated with Hardy-Weinberg equilibrium, whose concordance was assessed by the chi-square test. Continuous variables with the normal distribution or with skewed distribution were analyzed by the *t* test or Mann-Whitney U test, respectively. Categorical data were examined using Pearson's chi-square test or Fisher's exact test. Multiple linear regression analysis was performed to identify the most influential factors (P<0.3) in univariate analysis. Statistical analysis was carried out with SPSS 20.0 (SPSS, Inc., Chicago, IL, USA). A double-sided P-value <0.05 was considered statistically significant.

Results

### Demographic characteristics

The baseline characteristics of the 126 recipients in the cohort study and the 32 recipients in the self-control study were listed in Table 1. There were no significant differences between the WZC and non-WZC group in sex, recipients' and donors' age, or body mass index (BMI) (P>0.05). The distribution of donors' sex was significantly different between the WZC group

Table 2. The Tac blood concentration between WZC group and Non-WZC group.

	WZC group (n=86)	Non-WZC group (n=40)	P-value
C0 (ng/ml)			
Day 7	6.0±2.8	3.5±1.5	**<0.001**
Day 14	7.3±2.2	4.7±1.8	**<0.001**
Day 21	6.8±2.0	5.7±2.7	**0.008**
Day 28	7.3±2.5	6.4±3.0	0.062
Dose-adjusted C0 (ng/ml per mg)			
Day 7	2.1±0.9	1.6±0.7	**0.013**
Day 14	2.7±1.2	2.0±1.0	**0.001**
Day 21	2.8±1.1	2.3±1.2	**0.041**
Day 28	3.1±1.4	2.8±1.8	0.288
Dose and BW-adjusted C0 (ng/ml per mg/kg)			
Day 7	113.6±50.0	93.0±42.3	**0.025**
Day 14	154.1±76.6	117.8±64.8	**0.011**
Day 21	156.1±67.9	136.7±82.2	0.165
Day 28	175.7±88.4	170.2±135.9	0.814

The values were shown as mean±standard deviation. P-value <0.05 were shown in bold. Tac – tacrolimus; WZC – Wuzhi capsules; C0 – tacrolimus trough concentration; BW – body weight.
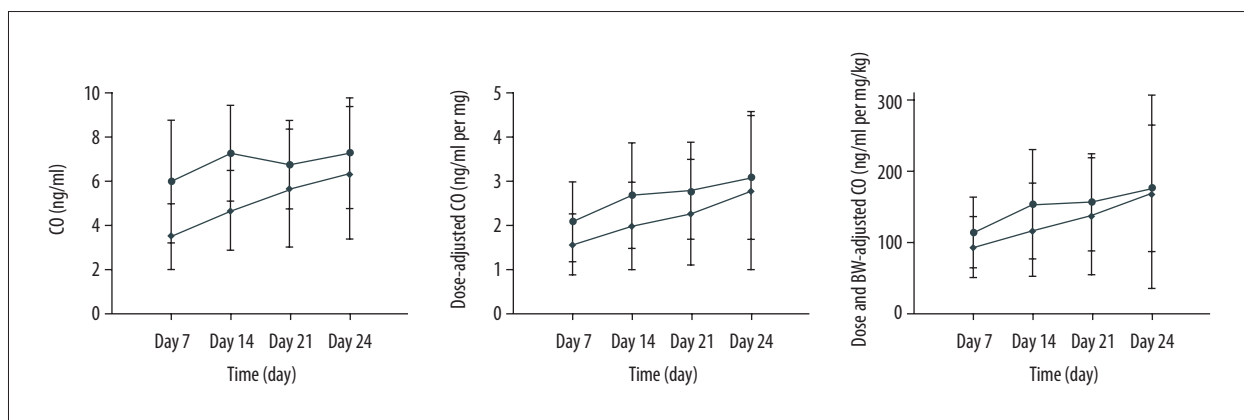
Figure 1. The Tac blood concentration of WZC group and Non-WZC group. Circles represent WZC group (n=86) and triangles represent Non-WZC group (n=41). Tac – tacrolimus; C0 – tacrolimus trough concentration; BW – body weight.

and non-WZC group (P=0.035). No significant differences were found in recipients' CYP3A5 genotype (CYP3A5 expressers: CYP3A5*1/*1 and CYP3A5*1/*3; CYP3A5 non-expressers: CYP3A5*3/*3), pre-transplant urine volume, numbers of recipients on dialysis, duration of dialysis before transplantation, total HLA mismatches, panel reactive antibodies, or co-infection with hepatitis B virus (HBV).

Tacrolimus concentration

In the WZC group, C0 (ng/ml) on Days 7, 14, and 21 were significantly higher than those in the non-WZC group (6.0±2.8 vs. 3.5±1.5, P<0.001; 7.3±2.2 vs. 4.7±1.8, P<0.001; 6.8±2.0 vs. 5.7±2.7, P=0.008, respectively), except on Day 28 (7.3±2.5 vs. 6.4±3.0, P=0.062). C0/D (ng/ml per mg) in the WZC group were still significantly higher than those in the non-WZC group on Days 7, 14, and 21 (2.1±0.9 vs. 1.6±0.7, P=0.013; 2.7±1.2 vs. 2.0±1.0, P=0.001; 2.8±1.1 vs. 2.3±1.2, P=0.041, respectively), but were not significantly different on Day 28 (3.1±1.4 vs. 2.8±1.8, P=0.288). C0/D/W (ng/ml per mg/kg) in the WZC group were remarkably greater than those in the non-WZC group on Days 7 and 14 (113.6±50.0 vs. 93.0±42.3, P=0.025; 154.1±76.6 vs. 117.8±64.8, P=0.011), but not on Days 21 and 28 (156.1±67.9, P=0.165; 175.7±88.4, P=0.814, respectively).

Table 3. The Tac blood concentration of WZC and Non-WZC group categorized by CYP3A5 genotype.

		CYP3A5 expressers		
Time	**Tacrolimus**	**WZC group (n=47)**	**Non-WZC group (n=16)**	**P-value**
Day 7	C0, ng/ml	5.2±2.1	3.0±1.5	**<0.001**
	C0/D, ng/ml per mg	1.8±0.7	1.4±0.7	0.096
	C0/D/W, ng/ml per mg/kg	101.3±41.8	75.7±37.5	**0.034**
Day 14	C0, ng/ml	6.8±1.9	3.7±1.1	**<0.001**
	C0/D, ng/ml per mg	2.2±0.7	1.5±0.5	**<0.001**
	C0/D/W, ng/ml per mg/kg	130.9±49.8	83.7±34.5	**0.001**
Day 21	C0, ng/ml	6.5±1.8	4.7±2.4	**0.003**
	C0/D, ng/ml per mg	2.3±0.8	1.9±1.0	0.064
	C0/D/W, ng/ml per mg/kg	133.7±46.9	101.1±56.8	**0.027**
Day 28	C0, ng/ml	6.9±2.7	4.5±1.7	**0.001**
	C0/D, ng/ml per mg	2.5±1.0	1.7±0.6	**0.005**
	C0/D/W, ng/ml per mg/kg	143.4±58.1	94.2±38.0	**0.002**
		CYP3A5 non-expressers		
Time	**Tacrolimus**	**WZC group (n=39)**	**Non-WZC group (n=24)**	**P-value**
Day 7	C0 (ng/ml)	7.0±3.1	3.8±1.4	**<0.001**
	C0/D (ng/ml per mg)	2.4±1.0	1.8±0.7	**0.011**
	C0/D/W (ng/ml per mg/kg)	128.5±55.3	104.3±42.1	0.071
Day 14	C0 (ng/ml)	7.8±2.5	5.4±1.8	**<0.001**
	C0/D (ng/ml per mg)	3.3±1.3	2.3±1.1	**0.004**
	C0/D/W (ng/ml per mg/kg)	182.1±93.0	140.6±70.7	0.065
Day 21	C0 (ng/ml)	7.3±2.1	6.4±2.6	0.122
	C0/D (ng/ml per mg)	3.4±1.1	2.7±1.3	**0.029**
	C0/D/W (ng/ml per mg/kg)	183.2±79.2	160.5±88.8	0.295
Day 28	C0 (ng/ml)	7.9±2.3	7.6±3.1	0.686
	C0/D (ng/ml per mg)	3.9±1.4	3.5±1.9	0.407
	C0/D/W (ng/ml per mg/kg)	214.6±102.7	220.8±153.9	0.848

The values were shown as mean±standard deviation. P-value<0.05 were shown in bold. Tac – tacrolimus; WZC – Wuzhi capsules; C0 – tacrolimus trough concentration; D – dose of Tac; W – body weight.

These statistical results were all presented in Table 2 and dynamic analysis results were shown in Figure 1.

Subgroup analysis based on CYP3A5 (6986A>G) genetic polymorphism was presented in Table 3. We divided the 126 recipients into 2 groups: CYP3A5 expressers (6986 AA + AG,*1/*1+*1/*3) and non-expressers (6986 GG, *3/*3). For CYP3A5 expressers,

C0, C0/D, and C0/D/W in the WZC group were significantly higher than in the non-WZC group (P<0.05), except for C0/D on Day 7 (P=0.096) and Day 21 (P=0.064). For CYP3A5 non-expressers, C0 in the WZC group was significantly greater than in the non-WZC group only on Day 7 (P<0.001) and Day 14 (P<0.001), and C0/D in the WZC group was significantly greater than the non-WZC group on Day 7 (P=0.011), Day 14 (P=0.004), and

Table 4. Multiple linear regression analysis.

		Coefficients	P-value	R Square	P-value (regression equation)
Dose-adjusted Tac C0 on Day 7	WZC	−0.291	**0.001**	0.197	**<0.001**
	CYP3A5 genotype	0.350	**<0.001**		
	Recipient BMI	−0.063	0.457		
	Donor gender	−0.176	**0.039**		
Dose-adjusted Tac C0 on Day 14	WZC	−0.384	**<0.001**	0.293	**<0.001**
	CYP3A5 genotype	0.429	**<0.001**		
	Recipient BMI	−0.054	0.494		
	Donor gender	−0.165	**0.039**		
Dose-adjusted Tac C0 on Day 21	WZC	−0.229	**0.008**	0.204	**<0.001**
	CYP3A5 genotype	0.421	**<0.001**		
	Recipient BMI	−0.051	0.547		
	Donor gender	−0.087	0.301		
Dose-adjusted Tac C0 on Day 28	WZC	−0.171	**0.038**	0.236	**<0.001**
	CYP3A5 genotype	0.505	**<0.001**		
	Recipient BMI	−0.002	0.981		
	Donor gender	−0.075	0.358		

P-value<0.05 were shown in bold. Tac – tacrolimus; WZC – Wuzhi capsules; C0 – tacrolimus trough concentration; BMI – body mass index.

Day 21 (P=0.029). Notably, the mean value of all concentration parameters in the WZC group was higher than those in the non-WZC group at all time points. Through multiple linear regression analysis after including parameters of WZC, CYP3A5 genotype, recipient BMI, and donor sex, it was identified that both WZC and CYP3A5 genotype were independent factors that affected Tac C0/D (P<0.05, shown in Table 4).

Therapeutic range

Regarding the proportion of subjects reaching the Tac target concentration range on Day 7, the achieved proportion in the WZC group was significantly higher than in the non-WZC group (shown in Table 5). The WZC group had 31 recipients with Tac C0 <5 ng/ml, 21 recipients with Tac C0 >8 ng/ml, and 34 recipients with Tac C0 between 5 to 8 ng/ml, making the target proportion 39.5%. The target proportion in the non-WZC group was 20.0%, which was significantly less than that in the WZC group (P<0.001). Noticeably, among CYP3A5 expressers, 44.7% of recipients in the WZC group reached the target range, which was more than twice that of the non-WZC group (18.7%) (P=0.01). Additionally, CYP3A5 non-expressers in the WZC group had a remarkably higher target proportion than in

the non-WZC group (30.8% *vs.* 20.8%, P <0.001). Although the use of WZCs also increased the proportion of subjects achieving Tac C0 more than 8 ng/ml (21% in WZC group; 0% in non-WZC group), it was not significant in CYP3A5 expressers (5%) compared to CYP3A5 non-expressers (17%).

Self-control analysis

Self-control analysis was carried out in the self-control study. Before co-administration of WZC, mean values of C0, C0/D, and C0/D/W in recipients were 3.2 (±1.2) ng/ml, 1.4 (±0.6) ng/ml per mg, and 83.0 (±37.2) ng/ml per mg/kg, respectively. However, after treatment with WZC, these parameters rose to 5.9 (±2.2) ng/ml, 2.6 (±1.1) ng/ml per mg, and 151.0 (±76.1) ng/ml per mg/kg, respectively (P<0.001 for all), which indicated a significant improvement of Tac concentration. Analysis results were presented in Table 6 and Figure 2. No significant differences in red blood cell count, hemoglobin, and hematocrit were found before and after the administration of WZC (median: 3.52×10⁹/L *vs.* 3.76×10⁹/L, P=0.127; median: 115.4 g/L *vs.* 121.7 g/L, P=0.201; median: 0.34 *vs.* 0.36, P=0,199, respectively).

Table 5. Proportion of recipients reached Tac target concentration categorized by WZC.

		C0 <5 ng/ml	C0 5–8 ng/ml	C0 >8 ng/ml	Rate	P-value
All recipients (n=126)	WZC group (n=86)	31	34	21	39.5%	**<0.001**
	Non-WZC group (n=40)	32	8	0	20.0%	
CYP3A5 expressers (n=63)	WZC group (n=47)	21	21	5	44.7%	**0.01**
	Non-WZC group (n=16)	13	3	0	18.7%	
CYP3A5 non-expressers (n=63)	WZC group (n=39)	10	12	17	30.8%	**<0.001**
	Non-WZC group (n=24)	19	5	0	20.8%	

P-value<0.05 were shown in bold; Rate refer to the proportion of recipients reached Tac target concentration (5–8 ng/ml).
Tac – tacrolimus; WZC – Wuzhi capsules; C0 – tacrolimus trough concentration.

Table 6. Self-control analysis of Tac concentration before using Wuzhi and after using Wuzhi.

	Before WZC	After WZC	P-value
C0 (ng/ml)	3.2±1.2	5.9±2.2	**<0.001**
Dose-adjusted C0 (ng/ml per mg)	1.4±0.6	2.6±1.1	**<0.001**
Dose and BW-adjusted C0 (ng/ml per mg/kg)	83.0±37.2	151.0±76.1	**<0.001**

The values were shown as mean±standard deviation. P-value<0.05 were shown in bold. Tac – tacrolimus; WZC – Wuzhi capsules; C0 – tacrolimus trough concentration; BW – body weight.
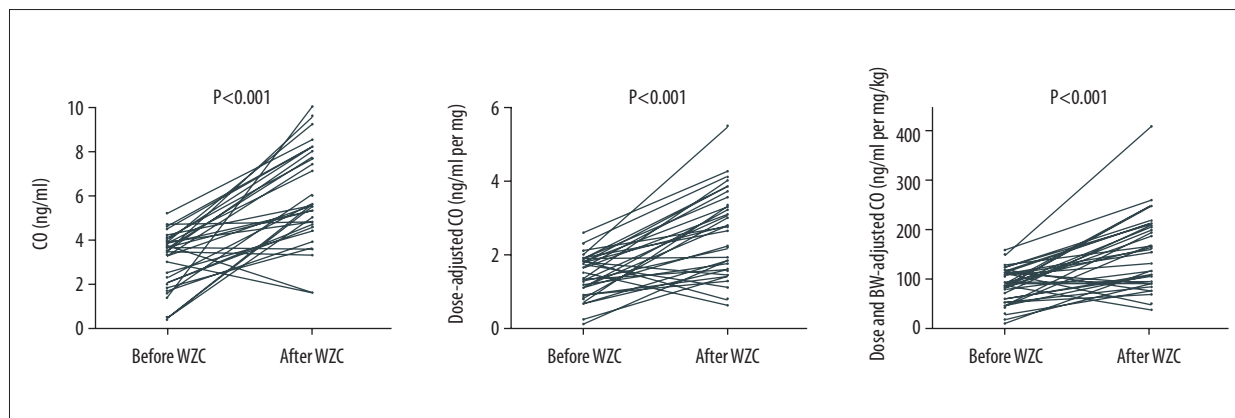
Figure 2. Self-control analysis of Tac blood concentration before using Wuzhi capsules and after using Wuzhi capsules. Tac – tacrolimus; C0 – tacrolimus trough concentration; BW – body weight.

Renal function

Non-delayed creatinine recovery was observed in 77 out of 86 recipients (89.5%) in the WZC group while 35 out of 40 (87.5%) recipients in the non-WZC group. Pearson chi-square test (χ^2=0.114) and P-value (P=0.765) suggested that there were no significant differences in creatinine recovery between the WZC group and non-WZC group (Supplementary Table 1). Within 6 months, eGFR (ml/min/1.73 m^2) in the WZC group declined from a preoperative level of 1043.9 (±363.8) to 109.1 (±63.9) on Day 7 and then eventually changed to 116.2 (±36.8).

A similar pattern was observed in the non-WZC group, whose eGFR shifted from an initial 1017.8 (±197.2) to 107.3 (±48.6) on Day 7 and 156.1 (±136.7) at the end. No significant differences were found between the WZC group and non-WZC group (P>0.05) (Supplementary Table 2). No significant differences were found in liver function between the WZC group and non-WZC group (P >0.05, data not shown).

Discussion

This present retrospective study analyzed the effects of Wuzhi capsules on the concentration of tacrolimus in renal transplant recipients. Relevant factors such as overall characteristics of recipients, pharmacological parameters, CYP3A5 genotypes, proportion of subjects who reached target Tac concentration, and renal function were taken into consideration. We found that Wuzhi capsules efficiently increased tacrolimus blood trough concentration in renal transplant recipients compared with recipients who did not take Wuzhi capsules, especially in CYP3A5 expressers.

Wuzhi capsules have been used to alleviate chemical or virus-induced liver damage and have been co-administered with Tac to treat drug-induced hepatitis in renal transplant recipients [19,20]. However, Wuzhi capsules co-administration with Tac is not the only form of medication-combined therapeutic regimen. Studies have investigated the relationship of herbal medicine like St. John's Wort or synthetic compounds like everolimus with tacrolimus metabolism, and corresponding effects were observed [30,31]. In recent years, studies through *in vivo* and *in vitro* experiments have provided clear evidence of Wuzhi capsules' positive impact on increasing Tac C0 [21]. Mechanistic analysis has revealed that Wuzhi capsules can inhibit CYP3A-mediated metabolism and P-gp-mediated efflux of Tac, as well as increase Tac oral bioavailability by reducing the intestinal first-pass effect [24]. Additional evidence has showed that the active lignans of Wuzhi capsules have higher affinity to CYP3A than Tac, which might explain why CYP3A-mediated Tac metabolism is inhibited by the non-competitive binding of lignans with CYP3A [23]. Generally, Tac accumulation in recipients' peripheral blood rose steadily during the first month. Tac concentration measured in the WZC group on Days 7, 14, and 21 were significantly higher than those in the non-WZC group, which indicated a remarkable improvement caused by Wuzhi capsules. However, it was noticed that this improvement was not significant on Day 28. We assumed that CYP3A might reach its saturated state of binding Tac or schisandrin with the increase of Tac concentration, which might offset Wuzhi capsules' effect.

It is widely known that CYP3A5 (6986A>G) genetic polymorphism is a predominant genetic factor affecting a person's Tac pharmacokinetics variability [18,32]. Such variability is likely to be a confounding factor in the effects of Wuzhi capsules. Therefore, subgroup analyses based on CYP3A5 expression were carried out. We found that in CYP3A5 expressers, Tac blood concentration in the WZC group was significantly higher than the non-WZC group, even on Day 28, but the differences in Tac blood concentration between the WZC group and non-WZC group in CYP3A5 non-expressers were not significant on Day 28. This finding confirmed that WZC affected

Tac concentration partially by inhibiting CYP3A5-mediated metabolism of Tac [33]. CYP3A5 expressers taking Wuzhi capsules had more significant improvement in Tac concentration. However, the effects of Wuzhi capsules had a trend of becoming less significant in CYP3A5 non-expressers, which suggested that those recipients might contribute to the poor effect of Wuzhi capsules on Tac blood concentration in the late stage of the first month.

Current clinical practice requires Tac blood concentration to achieve a stable state within the therapeutic range as soon as possible in case of graft rejection and organ toxicity [34]. A Tac C0 target range of 5–8 ng/ml is considered to be clinically effective and safe in our hospital; therefore, we performed the analysis on the proportion of recipients who reached target Tac concentration, showing that the WZC group had a significantly higher proportion of subjects achieving the target Tac concentration (39.5%) than in the non-WZC group (20.0%) (P<0.001). Subgroup analysis in CYP3A5 expressers (44.7% >18.7%, P=0.01) and non-expressers (30.8% >20.8%, P<0.001) revealed a similar outcome. Although the proportion of subjects in the WZC group achieving Tac C0 in the target range was also increased (21%), this was not obvious in CYP3A5 expressers (5%) compared to non-expressers. CYP3A5 expressers had relatively lower Tac concentrations than non-expressers. The use of WZC capsules might contribute to the increase of low level of Tac C0, but when the Tac level was high enough, the use of WZC might result in the excess of Tac concentration. Therefore, Wuzhi capsules could be of greater benefit in CYP3A5 expressers.

Some studies observed that recipient BMI and donor sex were associated with varied Tac pharmacokinetics [35]. In the present study, we carried out multiple linear regression analysis with variants including Wuzhi capsules intake, CYP3A5 genotype, recipient BMI, and donor sex. We compared the coefficients of these 4 factors and found that WZC intake was one of the most significant variants on Days 7, 14, 21, and 28 (P<0.001), as well as CYP3A5 genotype. Self-control analysis further confirmed this result. After treatment with Wuzhi capsules, the mean value of C0, dose-adjusted C0, and dose- and BW-adjusted C0 all increased significantly (shown in Table 5, P<0.001), which indicated a remarkable influence of Wuzhi capsules on Tac concentration.

Graft function after renal transplantation was monitored routinely. No significant differences in creatinine recovery were found between the WZC group and the non-WZC group (P=0.765). Furthermore, estimated glomerular filtration rates observed in the 6 months after transplantation had a similar variation trend in the WZC group and non-WZC group (P>0.05). Notably, eGFR in the WZC group was controlled within normal reference range, which promised a good recovery of renal

function, but not in the non-WZC group after 4 months from transplantation [36]. In conclusion, Wuzhi capsules did not cause additional harm to renal function recovery, but rather appear to facilitate graft survival.

Our study has certain limitations. Firstly, the sample size was not large enough and only Asians were included. Secondly, this study only recorded Tac concentration in the first month because most of the data on Tac dose after discharge is lost to follow-up. The fluctuation of Tac C0 in recipients after discharge from hospital should be followed up and further evaluated. Thirdly, the etiological factors of end-stage renal failure were not available. Finally, no more data about the pharmacokinetics of tacrolimus were available in this study.

Wuzhi capsules co-administration substantially increased the tacrolimus blood concentration and the proportion of subjects who reached the target concentration range. Large-scale and long-term studies are needed to further prove the validity of

these results. Tacrolimus blood concentration needs consistent monitoring and new formulations of co-administration drugs need to be developed to improve therapeutic outcomes.

Conclusions

The present study demonstrated that administration of Wuzhi capsules significantly increased Tac C0, with an improved proportion of subjects reaching the target concentration range, and we also found a potential protective effect on allograft survival in renal transplant recipients. Treatment with Wuzhi capsules might ease the economic burden caused by use of larger amounts of Tac. CYP3A5 expressers could benefit more from use of Wuzhi capsules than CYP3A5 non-expressers.
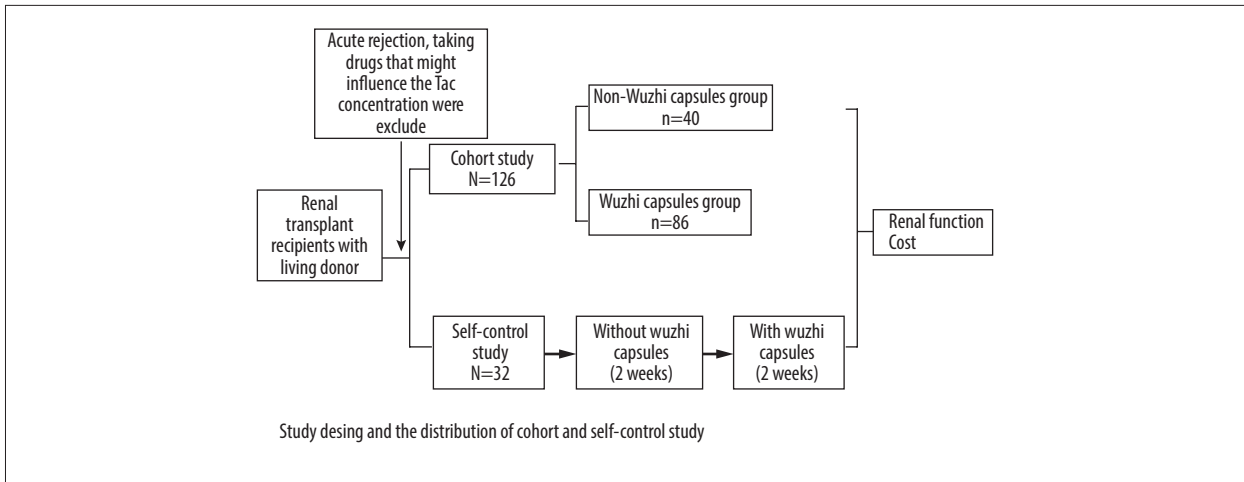
Conflict of interests

None.

Supplementary Data
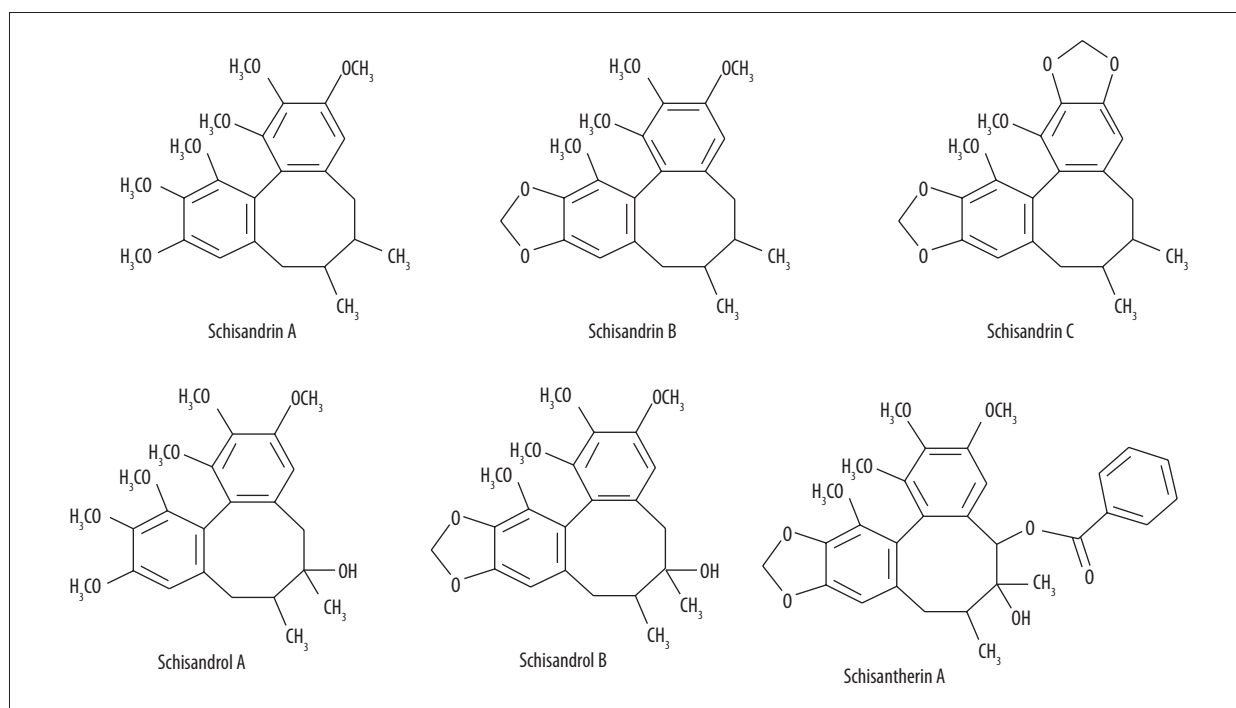
Supplementary Figure 1. The chemical structures of the active components from *Schisandra sphenanthera* extract.

Study design and the distribution of cohort and self-control study

Supplementary Figure 2. Study design and the distribution of cohort and self-control study.

Supplementary Table 1. Delayed creatinine recovery between WZC group and Non-WZC group.

	WZC group	Non-WZC group	Total recipients	χ^2	P-value
Non-delayed creatinine recovery	77	35	112	0.114	0.765
Delayed creatinine recovery	9	5	14		

The values are shown as number. WZC – Wuzhi capsules.

Supplementary Table 2. eGFR between WZC group and Non-WZC group.

	WZC group (n=86)	Non-WZC group (n=40)	P-value
Preoperative	1043.9±363.8	1017.8±197.2	0.671
Day 1	449.7±180.6	482.7±205.8	0.396
Day 2	219.6±135.4	224.8±108.1	0.837
Day 3	154.6±120.8	155.7±89.4	0.961
Day 7	109.1±63.9	107.3±48.6	0.862
Day 14	111.3±85.7	112.8±91.1	0.933
Day 21	120.9±89.2	111.7±47.0	0.450
1 month	126.0±112.7	124.0±85.1	0.911
2 month	125.4±89.2	128.9±103.9	0.861
3 month	135.7±173.3	124.4±82.4	0.646
4 month	113.0±30.3	157.1±167.6	0.179
5 month	119.0±80.1	137.9±122.1	0.521
6 month	116.2±36.8	156.1±136.7	0.386

The values are shown as mean±standard deviation. WZC – Wuzhi capsules; eGFR – estimated glomerular filtration rate.

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