Predictors of tacrolimus dose optimization when drug-drug interaction associated with voriconazole in heart transplant recipients

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Abstract

Aims: Voriconazole is the mainstay for the treatment for invasive fungal infections in heart transplant patients and significantly increase tacrolimus exposure because of drug-drug interaction (DDI). However, the magnitude of this DDI is highly variable and hard to predicted. The purpose of this study was to present the characteristics of DDI between tacrolimus and voriconazole, and further identify the predictors of tacrolimus dose modification. Methods: We retrospectively enrolled 69 heart transplant recipients without using voriconazole as the control and 68 patients received voriconazole treatment in voriconazole group. CYP3A4*1G, CYP3A5*3 and CYP2C19*2 or *3 were genotyped by Sanger sequencing. The requirement of tacrolimus dose for therapeutic concentrations and tacrolimus dose-corrected trough concentration (C0/D) before and after VRC administration were evaluated. Results: The DDI between tacrolimus and voriconazole was in a large inter-individual variability with more than ten-fold changes in tacrolimus dose (range 1.28–13.00) and C0/D (range 1.43–13.75). Besides, the fold changes of tacrolimus dose were associated with CYP2C19 genotype, which was significantly lower in CYP2C19 extensive metabolizers than that in CYP2C19 intermediate metabolizers or poor metabolizers (4.06±1.85 vs 5.49±2.47, p=0.0031). While no significant difference was found in both CYP3A4 and CYP3A5 genotypes. Moreover, CYP2C19 genotype and hematocrit were independent predicting factors for tacrolimus dose modification after voriconazole co-therapy. Conclusions: This study provided a potential basis for comprehensive factors to adjust tacrolimus dosage when co-administrated with voriconazole in individual patients. CYP2C19 genotype and hematocrit should be considered in tailoring tacrolimus dose.

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What is already known about this subject?

Drug-drug interaction between voriconazole and tacrolimus are inevitable after transplantation and usually result in tacrolimus overexposure and toxicities.

The extent and predictors of the drug-drug interaction between voriconazole and tacrolimus in heart transplant recipients are unknown, making tacrolimus dose modification a challenge.

What this study adds?

The magnitude of dug-drug interaction between voriconazole and tacrolimus is in large inter-individual variability with more than ten-fold change in tacrolimus modification.

CYP2C19 genotype and hematocrit were independent factors affecting tacrolimus dose adjustment after voriconazole combination.

Abstract

Aims : Voriconazole is the mainstay for the treatment for invasive fungal infections in heart transplant patients and significantly increase tacrolimus exposure because of drug-drug interaction (DDI). However, the magnitude of this DDI is highly variable and hard to predicted. The purpose of this study was to present the characteristics of DDI between tacrolimus and voriconazole, and further identify the predictors of tacrolimus dose modification.

Methods : We retrospectively enrolled 69 heart transplant recipients without using voriconazole as the control and 68 patients received voriconazole treatment in voriconazole group. *CYP3A4*1G* ,*CYP3A5* *3 and *CYP2C19*2 or *3* were genotyped by Sanger sequencing. The requirement of tacrolimus dose for therapeutic concentrations and tacrolimus dose-corrected trough concentration (C_0/D) before and after VRC administration were evaluated.

Results : The DDI between tacrolimus and voriconazole was in a large inter-individual variability with more than ten-fold changes in tacrolimus dose (range 1.28–13.00) and C_0/D (range 1.43–13.75). Besides, the fold changes of tacrolimus dose were associated with *CYP2C19* genotype, which was significantly lower in CYP2C19 extensive metabolizers than that in CYP2C19 intermediate metabolizers or poor metabolizers (4.06 ± 1.85 vs 5.49 ± 2.47,p =0.0031). While no significant difference was found in both *CYP3A4* and *CYP3A5* genotypes. Moreover, *CYP2C19*genotype and hematocrit were independent predicting factors for tacrolimus dose modification after voriconazole co-therapy.

Conclusions : This study provided a potential basis for comprehensive factors to adjust tacrolimus dosage when co-administrated with voriconazole in individual patients. *CYP2C19* genotype and hematocrit should be considered in tailoring tacrolimus dose.

Keywords

heart transplantation, tacrolimus, voriconazole, CYP2C19, CYP3A5

1. Introduction

Fungal infections (FIs), especially invasive aspergillosis (IA) is one of the common complications in heart transplant (HT) recipients and cause significant morbidity and mortality. The incidence of IA is 3.5% to 26.7% in HT patients,[1,2] and with risk factors including isolation of aspergillus fumigatus from bronchoalveolar lavage, re-operation, cytomegalovirus infection and post-transplant hemodialysis, et al.[3] Patients who receiving immunosuppressive therapy after solid organ transplantation are particularly vulnerable to severe infections. Triazole antifungal drugs such as voriconazole (VRC), posaconazole and itraconazole are the first or second line for IA treatment.[3,4] These agents are inhibitors of cytochrome P450 3A4 (CYP3A4), which plays an important role in metabolizing immunosuppressants such as cyclosporine A, tacrolimus (TAC) and sirolimus. Thus, drug-drug interactions (DDIs) are inevitable between azole antifungals and these immunosuppressants in patients received transplantation. Since changes in immunosuppressants exposure put patients at a high risk of rejection or toxicity, and the DDIs cannot be precisely predicted, it is really a complex task for clinicians to maintain immunosuppressants concentrations within the therapeutic window when concomitant with azole antifungals.

Among the triazole antifungal drugs, VRC is recommended as a first-line option for prophylaxis and treatment of IA and also commonly used after HT.[4,5] According to the VRC package insert, when initiation with VRC in patients already receiving TAC, the TAC dose should be reduced to one-third of the original dose and followed with frequent monitoring of TAC blood levels.[6] Despite this recommendation, the magnitude of DDI differs among patients and the pre-emptive dose modification can still result in TAC overexposure or even severe toxicity. In a retrospective analysis of renal and lung recipients, the change of TAC dose-adjusted concentration (C_0/D) between baseline and VRC co-therapy was 5.0±2.7 (range 1.0–20.2) and TAC dose decreased more than fourfold in 64% of patients.[7] These results suggested that VRC metabolism is complex *in vivo* and usually affected by clinical and genetic factors. Identification of predictors for TAC dose adjustment is extremely important for individualized therapy, however, little information is available for HT patients at present.

TAC is predominantly metabolized by hepatic and intestinal CYP3A4/5 enzymes.[8]Single-nucleotide polymorphism in *CYP3A5*3* (*rs776746; 6985A>G*) causes alternative splicing and protein truncation result in the absence of *CYP3A5* , contributing to inter-individual and inter-racial differences in CYP3A-dependent drug clearance.[9] It is well recognized that patients carrying at least one functional CYP3A5 allele (*CYP3A5*1*) require approximately two folds higher TAC dose than patients with*CYP3A5*3/*3* genotype.[10] Moreover,*CYP3A4*1G* (*rs2242480, 20239G>A*),*CYP3A4*1B* (*rs2740574, -392A>G*) and a novel*CYP3A4*22* (rs35599367, 15389C>T) are also significantly associated with TAC C_0/D.[11-13] While the frequencies of*CYP3A4*1B* and *CYP3A4*22* are less than 1% in Asian patients,[14] indicating a limited role in our study population. With regard to VRC, CYP2C19 is the primarily responsible for the metabolism of it into VRC N-oxide, though CYP3A4, CYP2C9 and members of the flavin containing mono-oxygenase (FMO) family also contribute to the metabolism.[15] Also, VRC has the potential to be both a substrate and an inhibitor of the CYP2C19, CYP3A4 and CYP2C9 enzymes. Of note, the *CYP2C19* genotype is a significant determinant of the wide pharmacokinetics variability for VRC. Therapeutic recommendations for the use of VRC should be based on*CYP2C19* genotype, subtherapeutic and supratherapeutic VRC concentrations could be avoided by choosing alternative agents in ultrarapid/rapid metabolizers (UMs **17/*17* or RMs, **1/*17*) and poor metabolizers (PMs, **2/*2* or **2/*3* or**3/*3*).[16]

Our previous study in HT patients using a population pharmacokinetic (PopPK) approach revealed that TAC clearance was reduced by 63% in*CYP3A5* expressers (**1/*1* or **1/*3*) and 81% in*CYP3A5* nonexpressers (**3/*3*) when co-administrated with VRC.[17] Another study showed that *CYP3A5*genotype and several clinical variables should be taken into considerations as modulators of the TAC–azole interaction.[7] A pharmacokinetic study from healthy volunteers demonstrated that the $AUC_{0\text{-}24}$ of TAC in CYP2C19 intermediate metabolizers (IMs, **1/*2, *1/*3, *2/*17*) and PMs were significantly higher than that in extensive metabolizers (EMs, **1/*1*) when co-administrated with VRC.[18] This finding indicated that *CYP2C19* genotypes may be a potential determinant for DDI between TAC and VRC. Previous study from hematopoietic

stem cell transplantation (HSCT) implied that the DDI between TAC and VRC is affected by the genetic polymorphisms in both*CYP3A5* and *CYP2C19 g* enes.[19] However, the influences of *CYP3A4/5* and *CYP2C19* genotypes on TAC dose adjustment with VRC are poorly understood. Therefore, the present study was conducted to evaluate the potential factors that can elucidate and predict the magnitude of DDIs between TAC and VRC in HT recipients so as to help TAC individualized therapy.

2. Methods

2.1 Study design and population

This is a retrospective study investigating the characteristics of DDI between TAC (Prograf, FK-506; Astellas Ireland Co., Ltd, Kerry, Ireland) and VRC (Pfizer, Karlsruhe, Germany) in HT patients. Patients were enrolled into the control group and the VRC group according to whether VRC was used or not. The study was carried out in accordance with the Declaration of Helsinki and with approval from the Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology (Ethical code: [2018]S331). None of the heart donors came from executed prisoners. Informed consent was obtained from the enrolled patients.

Eligible recipients were adults ([?]18 years) who received first HT at Union hospital, Tongji Medical College, Huazhong University of Science and Technology from February 2015 to November 2020. The exclusion criteria were as follows: (1) pregnancy or lactation; (2) hepatic or renal dysfunction; (3) incomplete dose information and clinical data; (4) receiving concomitant drugs that could interact with TAC (such as fluconazole, posaconazole, diltiazem, etc.) but except VRC. Moreover, the inclusion criteria of VRC group were the following: (1) concomitant administration of TAC and VRC for at least 7 days, (2) availability of at least 2 TAC trough concentrations (C_0) before and after VRC concomitant therapy. The demographic and clinical data were collected by reviewing electronic medical records.

2.2 Administration of immunosuppressants and VRC

All of patients received a triple immunosuppressive therapy, which included TAC, mycophenolate mofetil (CellCept, MMF; Roche, Shanghai, China) and corticosteroids according to the local clinical protocol as previously reported.[20] Briefly, oral TAC initiation was given approximately 48 h after transplantation surgery at a dose of 0.02–0.12 mg/kg/day and continued twice daily to achieve individualized target range (10–15 ng/mL, in the early postoperative days [0–60]) under standard of care of therapeutic drug monitoring (TDM).[21] MMF was initiated at a dose of 1 g every 12 h with dose adjustment for toxicity or allograft rejection. Oral prednisone was started 3 days after transplantation with an initial dose of 1 mg/kg/day (twice daily), and decreased 5 mg every 3 days to a maintenance dose of 10 mg/day. In Co-VRC group, patients with suspected or confirmed fungal infection was orally administrated VRC at the dose of 200 mg every 12 h.

2.3 TAC sampling and measurement

Blood samples were collected 2–3 times a week for C_0measurement during hospitalization and whenever deemed necessary by the attending physician (but not before day 3). The whole blood TAC C_0 was measured using an automated electrochemiluminescence immunoassay (ECLIA) by the Cobas e411 analyzer series (Roche, Switzerland), according to the manufacturer's protocol. Linearity was verified from 1.1 to 27.1 ng/mL for TAC, with total imprecision ranged from 3.9 to 9.4% for TAC.[22]

2.4 Genotyping of *CYP3A4 /5* and *CYP2C19*

Genomic DNA was extracted from 200 μL ethylenediaminetetraacetic acid-treated peripheral bloods using the QIAampR DNA Blood Mini Kit (Qiagen, Hilden, Germany). DNA was quantified using a spectrophotometer (Thermo, Inc., DE, USA) to determine the concentration and purity and stored at -80*C until detection. *CYP3A4*1G(rs2242480, 20239G>A)* , *CYP3A5*3 (rs776746, 6986A>G), CYP2C19*2 (rs4244285, 681G>A)* and*CYP2C19*3 (rs4986893, 636G>A)* genotyping were performed by Sanger sequencing using ABI3730xl analyzer (Applied Biosystems, CA, USA). All samples were analyzed in triplicate and both negative and positive controls were included to ensure authenticity of the results.

2.5 Statistical Analysis

All TAC concentrations were corrected for daily TAC dose (using the dose ingested the day before trough concentration measurement). Creatinine clearance (Ccr) was estimated using the Cockcroft-Gault formula.[23] All continuous variables were described as median (25th to 75th percentiles) or mean ± standard deviation. Continuous data were tested for normality using the Kolmogorov-Smirnov test. TAC C_0/D were not normally distributed. The nonparametric Mann–Whitney U and Fisher exact test or χ^2 test were used, respectively, to assess the quantitative and categorical differences between data from the 2 groups. These statistical analyses were carried out using GraphPad Prism 9 (version 9.0.0 121; GraphPad Software, Inc., La Jolla, CA). Univariate and multivariate logistic regression analyses were carried out using SPSS Statistics version 20.0 (IBM Corp., Armonk, NY, USA). All reported P values were two-tailed, and aPvalue <0.05 was considered statistically significant.

3. Results

3.1 Patient characteristics

A total number of 137 HT patients were enrolled for analysis, with 69 patients in the control group and the others in the VRC group (before VRC concomitant were named as Pre-VRC group, concomitant with VRC were named as Co-VRC group). Demographics of the study populations when reaching the TAC target concentration range were summarized in Table 1. There were no significant differences between the three groups with respect to age, sex, body weight, hematocrit, creatinine clearance, *CYP3A5* genotype and *CYP2C19* genotype. The frequencies of*CYP3A4*1G* , *CYP3A5*3* , *CYP2C19*2* and *CYP2C19*3*alleles were distributed in concordance with Hardy–Weinberg equilibrium. No patient was found to carry with *CYP2C19*3/*3*genotype.

3.2 Effects of VRC on the dose and C_0/D of TAC

As showed in Table 1 and Figure 1, to maintain therapeutic concentration level of TAC, the requirements of TAC dose were significantly lower and the C_0/D of TAC were significant higher in Co-VRC group than those in Pre-VRC and control groups. Compared to Pre-VRC group, the TAC dose reduced 4.75±2.42 folds and the TAC C_0/D increased 5.00±2.27 folds in Co-VRC group. The magnitude of DDI between TAC and VRC showed large individual variabilities with more than ten-fold changes in TAC dose (range 1.28–13.00) and TAC C_0/D (range 1.43–13.75). In detail, a fourfold reduction of TAC dose was needed for 63.24% patients (43/68) to obtain the same TAC concentration level before co-therapy with VRC (Figure 2).

3.3 Effects of *CYP3A4/5* genotypes on the dose and C_0/D of TAC

To investigate the influence of *CYP3A4/5* genotypes on this DDI, recipients were divided into different groups based on different genotypes. In the present study, the requirement of TAC dose was significantly lower in *CYP3A5* nonexpressers than that of*CYP3A5* expressers in control group (p<0.001), Pre-VRC group (p <0.001) and Co-VRC group (p =0.0028) (Figure 3A). Likewise, significant differences in TAC C_0/D between *CYP3A5* expressers and nonexpressers were observed in these three groups (Figure 3B). While there was no significant difference in TAC dose and C_0/D between *CYP3A4*1G*allele and *CYP3A4*1/*1* genotype in all groups (Figure 1S A and B). Besides, to reach similar target concentrations, no significant difference in fold changes of TAC dose and C_0/D were observed before and after VRC co-therapy both in different *CYP3A5*genotypes (Figure 3C and D) and*CYP3A4* genotypes (Figure 1S C and D). Moreover, according to*CYP3A4/5* genotypes, we divided CYP3A into CYP3A EMs (*CYP3A5*1* allele and *CYP3A4*1G* allele), CYP3A IMs (*CYP3A5*1* allele and *CYP3A4*1/*1* , or *CYP3A5*3/*3*and *CYP3A4*1G* allele) and CYP3A PMs (*CYP3A4*1/*1* and*CYP3A5*3/*3*). There was also no significant difference in fold changes of TAC dose and C_0/D before and after VRC co-therapy in all groups (Figure 2S).

3.4 Effect of *CYP2C19* genotype on the dose and C_0/D of TAC

Due to the small number of CYP2C19 PMs, we put CYP2C19 PMs together with IMs for further analysis. As showed in Figure 4, the requirement of TAC dose in CYP2C19 IMs/PMs were significant lower than that

of EMs in Co-VRC group (p =0.0395) (Figure 4A). The TAC C_0/D in CYP2C19 IMs/PMs were obviously higher than that of EMs (p =0.0417) (Figure 4B). *CYP2C19* genotypes were not associated with the changes of TAC dose and C_0/D in both control and Pre-VRC groups (Figure 4A and B). Furthermore, both the fold changes of TAC dose (4.06±1.85 *vs* 5.49±2.47, p =0.0031) and C_0/D (4.14±1.91 *vs* 5.91±2.59, p =0.0019) before and after VRC co-therapy in CYP2C19 EMs were significantly lower than these in CYP2C19 IMs/PMs (Figure 4C and D).

3.5 Combined effects of *CYP3A5* and *CYP2C19* genotypes on the dose and C_0/D of TAC concomitant with VRC

To further investigate the interpreted effects of *CYP2C19* and*CYP3A5* genotypes on the DDI between VRC and TAC, we subdivided the Co-VRC group into four groups based on genotypes. In CYP2C19 EMs, the TAC dosage needed to achieve target concentration were significantly lower in *CYP3A5* nonexpressers than those in*CYP3A5* expressers (p =0.0286). And TAC C_0/D was significantly higher in *CYP3A5* nonexpressers than expressers (p =0.0018). However, in CYP2C19 IMs/PMs, only TAC C_0/D showed significant difference between patients carried *CYP3A5*1* allele and *CYP3A5*3/*3* genotype (p =0.0341). Interestingly, both in *CYP3A5* expressers and nonexpressers, there were no significant difference in TAC dose and C_0/D between CYP2C19 EMs and IMs/PMs (Figure 5A and B). Moreover, we analyzed the fold changes of TAC dose and C_0/D before and after VRC initiation in different genotypes. Both in CYP2C19 EMs and IMs/PMs group, there were no significant difference in the fold changes of TAC dose and C_0/D between *CYP3A5* expressers and nonexpressers. Similarly, in CYP3A5 expressers group, there were no significant difference in the fold changes of TAC dose and C_0/D between CYP2C19 EMs and IMs/PMs. However, in *CYP3A5* nonexpressers group, the fold changes of TAC dose (4.02±1.44 *vs* 5.50±2.42,p =0.0173) and C_0/D (4.21±1.64 *vs*5.88±2.37, p =0.0076) in CYP2C19 EMs were significantly lower than those in CYP2C19 IMs/PMs (Figure 5C and D).

3.6 Predicted factors for the TAC dose adjustment with VRC co-therapy

Although *CYP2C19* genotype had an effect of on TAC dose adjustment in combination with VRC, we further investigated whether other factors from characteristics of HT recipients may also affect the TAC dose adjustment. The median fold change of TAC dose was 4.17, which defined as the cut-off value for further analysis. Risk factors for TAC dose adjustment, such as age, gender, steroid dose, days after transplantation, *CYP3A4* , *CYP3A5* and *CYP2C19*genotypes were first analyzed by univariate analysis. Covariates that showed significant correlations (p <0.10) were further evaluated in the multivariate analysis. CYP2C19 IM/PM (OR 4.592, 95% CI 1.417–14.879; p =0.011) and hematocrit (OR 1.192, 95% CI 1.053–1.35; p =0.006) were associated with the fold changes of TAC dose in both univariate and multivariate analysis, indicating that they were independent factors for TAC dose reduction in concomitant with VRC (Table 2). Moreover, the fold changes of TAC dose when co-treated with VRC were positively correlated with the levels of hematocrit (R=0.2681, p =0.027, Figure 6)

4. Discussion

Precise dose adjustment of TAC is crucial to avoid graft rejection and toxicities after organ transplantation.[24] The DDI between VRC and TAC is inevitable and highly variable in solid organ transplantation, which is a challenge for TAC dose modification. To the best of our knowledge, this is the first study to assess the magnitude of DDI between VRC and TAC in HT patients during the early stage of transplantation. The present study demonstrated that TAC dose adjustment was in a large inter-individual variability after VRC initiation and TAC dose could be approximately reduced to 1/4-1/5 of the original dose to maintain the same level. Moreover, it was *CYP2C19* but not*CYP3A4* and *CYP3A5* genotypes that significantly correlated with the adjustment of TAC dose. In multivariate analysis,*CYP2C19* genotype and hematocrit were independent factors affecting TAC dose modification after VRC combination.

Currently, the recommendation of TAC dose reduction in VRC package insert was arose from the results of pharmacokinetics study in healthy volunteers,[6] which didn't consider the potential factors from hematocrit, hepatic and renal function, DDIs as well as the genetic polymorphisms of metabolic enzymes and transporters.[25-28] Our results indicated that TAC dose adjustment due to DDI with VRC was complex and

largely variable in organ transplant patients, which was in line with some previous studies. A retrospective study in HSCT patients demonstrated that the TAC dose should be reduced to one-fifth after VRC co-administered.[29] Other studies from renal and liver transplant patients reported that the rule-of-thumb reduction of the TAC dose by one-third may not be satisfactory.[30,31]A reduction of 75% TAC dosage might be more appropriate for TAC dose when co-treated with VRC from a cohort of renal and lung recipients.[7] Therefore, TAC dose should not be reduced based on a fixed ratio when considering DDI, but should be individually modified according to patient characteristics.

As is commonly known, TAC is mainly metabolized by hepatic and intestinal CYP3A4/5 enzymes. Although CYP3A4 was the basis of DDI between TAC and VRC, the *CYP3A4*1G* was not found to be associated with the fold changes of TAC dose and C_0/D after VRC co-therapy. As expected, the requirements of TAC dose in patients carried *CYP3A5*1*allele were about 1.5–2 fold higher than those carried with *CYP3A5*3/*3*genotype in all groups, which was in line with previous studies.[10,32] Nevertheless, *CYP3A5*3* also didn't affect TAC dose modification and C_0/D changes in concomitant with VRC. Perhaps this could be explained by that VRC was a stronger suppressor of CYP3A enzyme and weakened the effect of*CYP3A5* genetic variants on TAC metabolism. Although *CYP3A5*genotype significantly affect TAC metabolism, genotype-based TAC dose adjustment may sometimes be inappropriate, especially in combination with drugs that strongly interact with TAC.

CYP2C19 is the primary enzyme responsible for VRC metabolism and the genetic polymorphism may affect the DDIs of VRC. Consistent with previous studies,[18,33] in Co-VRC group, the requirement of TAC dose was significant lower and TAC C_0/D was significant higher in CYP2C19 IMs/PMs compared to CYP2C19 EMs. More importantly, the fold changes of TAC dose to achieve therapeutic concentration range in CYP2C19 IMs/PMs were significantly higher than that of CYP2C19 EMs. In CYP2C19 EMs and CYP2C19 IMs/PMs, the TAC dose was reduced to approximately a quarter and one fifth of the initial dose, respectively. Besides, both univariate and multiple regression analysis revealed that CYP2C19 IMs/PMs was independent factors significantly contributing to the changes of TAC dose modification before and after VRC co-therapy. These illustrated that *CYP2C19* genotype played a more prominent role in TAC dose adjustment after VRC initiation, although TAC is mainly metabolized by CYP3A. When co-administered with VRC, *CYP2C19* genotype-dependent changes of TAC dose and C_0/D was a result of *CYP2C19* genotype-related VRC exposures. Previous study in liver transplant patients indicated that VRC trough levels were significantly higher in CYP2C19 IMs/PMs and lower in CYP2C19 EMs.[34,35] Experiments *in vitro* also demonstrated that the magnitude of inhibition of the metabolism of TAC by CYP3A was dependent on VRC concentration within a specific range.[31,36] However, this speculation needs to be further validated in HT patients and we will continue to pay attention to it.

Additionally, we also found that hematocrit was another determinant for dose adjustment of TAC when co-administrated with VRC. TAC almost extensively binds to red blood cells, as a consequence, the TAC concentrations are measured in whole blood instead of plasma and strongly affected by hematocrit.[8] Lower hematocrit level was previously identified as a covariate enhancing TAC clearance.[37] In renal transplant recipients, a 10% absolute decrease in hematocrit may increase TAC clearance by more than 50–100%.[38,39] Another study in liver transplant recipients showed a significant positive correlation between hematocrit and TAC ratio.[40] Of note, our study may be responsible for the fact that changes of TAC dose adjustment was positively correlated with levels of hematocrit. This indicated that TAC concentration fluctuation was insensitive in the low hematocrit level but more sensitive with the increased hematocrit level. Consequently, we need to realize that the combination of high whole-blood TAC concentrations with low hematocrit levels may result in extremely high unbound plasma concentrations and further lead to toxicity.

Although DDIs between VRC and TAC is inevitable, some strategies can be used to help dose adjustment. Many PopPK studies regarding TAC in organ transplantation had been published, TAC dose when concomitant with VRC can be recommended with model simulation or Bayesian estimation.[17,41,42] Besides, prolonged-release (PR) TAC formulations had been gradually applied in clinical practice, due to the advantages in improved medication compliance and less concentration fluctuations. Interestingly, the effects of

VRC on TAC exposure were substantially smaller and less variable after administrated with PR-TAC formulation than that in conventional formulation.[43]Because the absorption of PR-TAC is mainly in the distal small intestine or in the colon,[44] where CYP3A plays a minor role,[45,46] presumably decreasing the magnitude of DDI. Therefore, PR-TAC may be an optimal choice when considering the DDI between TAC and VRC, if the above findings could be further validated in organ transplant patients. Although these findings can provide some options, TDM of TAC is still essential and really an effective means to guide dose adjustment.

Some limitations exist in this study. Firstly, VRC trough concentrations were not routinely monitored, thus it's difficult to confirm whether DDI between TAC and VRC is associated with VRC metabolism. Secondly, TAC concentrations after eliminating VRC were not obtained so that we can't evaluate the TAC dose increment. Thirdly, the present study was performed in a single center with a relatively small sample size and the results should be further validated in a large population. In future, prospective studies with multi-center and larger scales should be encouraged to investigate DDIs between TAC and VRC in HT patients. Regardless of the limitations described above, our study demonstrated that *CYP2C19* genotype and hematocrit were predictors for TAC dose optimization after VRC combination. These results may potentially have clinical implications and will provide some references for TAC dose modification when combined with VRC.

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Figure 1. Comparison of the TAC dose (A) and C_0/D (B) in reaching the target levels after heart transplantation in different groups. *** $p < 0.001$. ** $p < 0.01$. * $p < 0.05$.

Figure 2. Proportional changes in TAC dose (A) and C_0/D (B) before and after VRC co-therapy.

Figure 3. Variations of TAC dose (A), TAC C_0/D (B), fold of TAC dose (C) and fold of TAC C_0/D (D) in recipients with different genotypes of *CYP3A5* . *** $p < 0.001$. **$p < 0.01$. * $p < 0.05$.

Figure 4. Variations of TAC dose (A), TAC C_0/D (B), fold of TAC dose (C) and fold of TAC C_0/D (D) in the recipients with different genotypes of *CYP2C19* . *** $p < 0.001$. ** $p < 0.01$. * $p < 0.05$.

Figure 5. Variations of TAC dose (A), TAC C_0/D (B), fold of TAC dose (C) and fold of TAC C_0/D (D) in Co-VRC group with different genotypes of *CYP3A5* and *CYP2C19* . ***$p < 0.001$. ** $p < 0.01$. * $p< 0.05$.

Figure 6. Relations of hematocrit associated with the changes of TAC dose after VRC co-therapy.

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