Research Program

(Version number: V2.0 Version date: 2023.01.28)

Project Title: Effect of Azole/Echinocandin Use on Tacrolimus Pharmacokinetics in Kidney Transplant Recipients

Project origin: Investigator-initiated

Department:

Principal Investigator:

Investigator statement and protocol signature page

As the main person in charge of this research project, I will follow the ethical principles of the Ministry of Health's Measures for Ethical Review of Biomedical Research Involving Human Beings (2016), the WMA Declaration of Helsinki (2013) and the CIOMS International Ethical Guidelines for Biomedical Research on Human Beings (2002), and the GCP, and will conduct the study according to the requirements of the present protocol under the guidance of the Code of Practice for Quality Management of Pharmaceutical Clinical Trials using the protocols approved by the Ethics Committee in order to ensure the scientific validity of the study and to protect the health and rights of the subjects.

Name：

Date：January 28, 2023

Program Summary

Program Title	Effect of Azole/Echinocandin Use on Tacrolimus Pharmacokinetics in Kidney Transplant Recipients
Version No./Version Date	V2.0/2023.01.28
Principal Investigator	
Nature of Research	A retrospective cohort study
Study Objective	To investigate the effect of azoles/echinocandins on tacrolimus C_0, D and C_0/D
Sample Size	Approximately 521 inpatients using target medications from January 1, 2015 to April 1, 2023 to be included
Research Population	Inpatients on target medications from January 1, 2015 to April 1, 2023
Research Method	Non-interventional, retrospective methods of data analysis
Inclusion Criteria	1. First time kidney transplantation; 2. Taking a tacrolimus-based triple immunosuppressive regimen (tacrolimus + sodium mescaline enteric tablets + glucocorticoids) after renal transplantation; 3. Age ≥ 18 years.
Exclusion criteria	1. Combined multi-organ transplants; 2. Pregnant and lactating women; 3. Severe impairment of liver function or severe gastrointestinal disease, gastrointestinal resection, malabsorption syndrome; 4. Allergy or intolerance to macrolides, mycophenolic acid or glucocorticoids; 5. Missing or incomplete clinical information and postoperative follow-up data (e.g., missing target drug concentration, dose, etc.); 6. Recipients who had their grafts removed or died due to rejection or serious complications within 1~2 months after surgery; 7. Poor compliance and accuracy of results (e.g. irregularity in blood collection time); 8. Postoperative co-application of other drugs that affect the blood concentration of target drugs such as tacrolimus or voriconazole or caspofungin (e.g. pentothal capsules, rifampicin, phenytoin sodium, carbamazepine, etc.) at the same time.
Key Efficacy Indicators	The C_0/D of tacrolimus
Secondary Efficacy Indicators	The C_0 and D of tacrolimus

Security Indicators	Acute rejection, delayed recovery of transplanted kidney function, death 30 days after surgery, postoperative infection
Statistical Analysis Method	chi-square test, independent samples t-test, paired samples t-test, two-sample Wilcoxon rank sum test, Mann-Whitney U test
Forms of publication of research results	academic paper

1. Objective

This study investigated the effect of combined antifungal drugs (voriconazole/posaconazole/caspofungin/micafungin) on the trough concentration of tacrolimus in renal transplant recipients and analyzed the effects of genetic factors (CYP3A5, CYP2C19) and clinical factors on the drug-drug interactions between antifungal drugs (voriconazole/posaconazole/caspofungin/micafungin) and tacrolimus with a view to providing a reference basis for the rational use of tacrolimus and antifungal drugs in the rational use of clinical medication to provide a reference basis.

2. Background

Chronic kidney disease (CKD) has become a global public health problem. According to statistics, the prevalence of CKD is 11%~13% worldwide, and about 700 million people suffer from CKD in varying degrees [1]. 2022 Meta-analysis showed that the prevalence of CKD among Chinese adults is 13.1%. With the progression of CKD, the function and structure of the kidney are damaged, manifested by renal hypoplasia, and even renal failure in the end stage [2], and all organs of the patient's body may be involved, which not only causes great physical and mental damage, but also leads to the loss of personal and social medical costs. Kidney transplantation is the most effective clinical treatment for patients with end-stage renal failure [3], providing better survival and quality of life compared with long-term dialysis [4].

For renal transplant recipients, immunosuppressive drugs should be administered for a long time after surgery to prevent auto rejection, and the choice of immunosuppression is often based on the calcineurin inhibitor (CNI) as a two-combination (CNI + mycophenolate mofetil/glucocorticoid) or three-combination (CNI

+ mycophenolate mofetil + glucocorticoid) immunosuppressive regimen. Since renal transplant patients need to receive immunosuppressive therapy for a long period of time, how to apply immunosuppressive drugs safely and effectively is the key to whether the transplanted kidneys can maintain a long enough survival time and whether the patients can achieve a high quality of life. Tacrolimus (TAC) and cyclosporin are the calcineurin inhibitor (CNI) [5], and compared with cyclosporin, TAC can reduce the incidence of rejection (42.1% vs. 18.4%, P=0.03) [6] and improve the graft survival. TAC can reduce the incidence of rejection (42.1% vs. 18.4%, P=0.03) [6], and increase the survival rate of grafts [7], and has been gradually replacing cyclosporine as the first-line immunosuppressant because of its significant therapeutic efficacy and low adverse effects.

These immunocompromised patients are more susceptible to infections due to prolonged immune system suppression, which is currently the leading cause of death in solid organ transplantation (SOT) recipients. Currently, infection is the leading cause of death in solid organ transplantation (SOT) recipients, and invasive fungal disease (IFD) remains the leading cause of morbidity and mortality in solid organ transplant recipients after SOT. Epidemiologic surveys abroad have shown that the pathogens of postoperative IFD in SOT recipients are mainly Candida (53.0%-59.0%) and Aspergillus (19.0%-24.8%). In clinical treatment, the commonly used antifungal agents are triazoles and echinocandins.

Most antifungal drugs in vivo are metabolized by cytochrome P450 (CYP450) enzymes in the liver, e.g., voriconazole is metabolized by CYP2C19, CYP2C9, and CYP3A4 enzymes, and micafungin is metabolized by CYP1A2, CYP2B6, CYP2C, and CYP3A. In contrast, SOT recipients require long-term use of TAC, which is metabolized mainly by CYP450 3A enzymes in the liver and small intestine, with the CYP3A4 and CYP3A5 isoenzymes contributing significantly [8]. Studies have shown that there are drug-drug interactions between antifungal drugs and TAC, which can cause changes in TAC blood levels. Co-administration of caspofungin reduced the peak concentration (Cmax) of TAC by approximately 20% [9]. Another study [10] found that VRC affects TAC metabolism mainly related to polymorphisms in the CYP2C19 gene.

Guidelines suggest that when VRC is initiated in SOT recipients already receiving TAC, the dose of tacrolimus should be reduced to one-third of the usual dose. However, drug-drug interactions between VRC and TAC have significant inter-individual variability among recipients, and empirically based dose adjustments can still result in significant TAC overdose [11], so dose adjustments for TAC at the time of initiating or discontinuing VRC are not uniform.

There is a drug-drug interaction between VRC and TAC that leads to an increase in TAC trough concentrations; however, there is significant inter-individual variability in this effect, and a reduction in the dose of TAC to one-third of the usual dose of VRC in combination with TAC, as recommended by the guideline, is not sufficient to meet the clinical need [11]. Studies of VRC in combination with TAC have focused on bone marrow and lung transplant recipients, with fewer studies in renal transplant recipients [11-13]. Caspofungin and micafungin, both of which are metabolized by the liver, may have drug-drug interactions with TAC, which may affect the TAC trough concentration, whereas there are fewer reports of drug-drug interactions between micafungin and TAC.

Base on the previous studies, this study was conducted to investigate the effects of CYP3A5 and CYP2C19 gene polymorphisms on the trough concentration of TAC after renal transplantation, to investigate the effects of antifungals on the trough concentration of TAC, and to analyze the effects of genetic factors on drug interactions between antifungals and TAC, in order to provide a reference basis for rational use of TAC and antifungals in clinical practice. The effects of genetic factors on the interaction between antifungal drugs and TAC were also analyzed, with a view to providing a reference basis for the rational use of TAC and antifungal drugs in clinical practice. It is of great significance to prevent and control fungal infections after renal transplantation and at the same time make TAC reach the therapeutic target concentration as early as possible, in order to minimize the occurrence of immune rejection after renal transplantation, to reduce the adverse reactions and toxic effects of TAC, to improve the safety and effectiveness of TAC, and to achieve the optimal therapeutic effect of TAC.

References:

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3. Content of the Study

3.1 Trial population

All hospitalized patients who meet the inclusion criteria from January 1, 2015 to April 1, 2023 will be included in this study as the study population, and the data will be obtained from the Lianzhong Digital Case Browser, LIS Result View and HIS system of the First Affiliated Hospital of Shandong First Medical University.

3.2 Quality control measures

Quality control was carried out throughout the study.

(1) Collecting data during the study time period to ensure that the entered data are consistent with the original data.

(2) Verify the data and organize the data.

(3) Data analysis and verification by another person.

3.3 Sample size calculation

In the part of the study on the interaction of tacrolimus with triazoles, tacrolimus C_0/D was used as an index of outcome based on the results of the pre-test of the clinical data of the patients with co-administration of voriconazole/posaconazole. Tacrolimus C_0/D was 753.11±562.35 ng/ml/(mg/kg/d) in patients in the group with co-administered voriconazole and 143.26±99.78 ng/ml/(mg/kg/d) in the group without co-administered triazoles. To obtain 90% statistical validity, a two-sided alpha value of 0.05 was set to detect differences between groups. The total sample size for this study was calculated using the Performance Analysis and Sample Size (PASS) software (version 15.0), and a t-test was selected to obtain a sample size of at least 12 cases in each of the combined voriconazole group and the non-combination group. Tacrolimus C_0/D was 540.51±347.88 ng/ml/(mg/kg/d) in patients in the co-voriconazole group and tacrolimus C_0/D was 143.26±99.78 ng/ml/(mg/kg/d) in the non-combination group. To obtain 80% statistical validity, a two-sided alpha value of 0.05 was set to detect differences between groups. The total sample size for this study was calculated using the Performance Analysis and Sample Size (PASS) software (version 15.0), and a t-test was selected to obtain a sample size of at least 9 cases in each of the combined posaconazole group and the non-combination group.

In the part of the study on the interaction between tacrolimus and echinocandins, recipients who underwent kidney transplantation at the First Affiliated Hospital of Shandong First Medical University (Qianfoshan Hospital, Shandong Province, China) and were taking the antifungal medication caspofungin/micafungin for tacrolimus blood concentration monitoring after the operation between January 1, 2015, and April 1, 2023, were selected, and tacrolimus combined with caspofungin medication group was collected from 171 recipients, 24 recipients in the tacrolimus combined with

micafungin drug group, and 305 recipients in the no antifungal drug group. In summary, at least 521 kidney transplant recipients are needed.

3.4 Treatment of missing values

Missing data or illogical data are handled by deleting cases containing missing values or by using missing value filling methods (mean value interpolation, etc.). The appropriate processing method is selected later on a case-by-case basis.

3.5 Specific research content

To study the effect of co-administration of azoles/echinocandins on tacrolimus C_0, D and C_0/D; before and after co-administration of tacrolimus C_0/D according to CYP3A5 genotype stratification analysis. To study the effect of CYP2C19 and CYP3A5 genotypes on tacrolimus in combination with voriconazole.

4. Method

4.1 Enrollment criteria

4.1.1 Inclusion criteria

(1) First time kidney transplantation;

(2) Taking a tacrolimus-based triple immunosuppressive regimen (tacrolimus + sodium mescaline enteric tablets + glucocorticoids) after renal transplantation;

(3) Age ≥ 18 years

4.1.2 Exclusion criteria

(1) Combined multi-organ transplants;

(2) Pregnant and lactating women;

(3) Severe impairment of liver function or severe gastrointestinal disease, gastrointestinal resection, malabsorption syndrome;

(4) Allergy or intolerance to macrolides, mycophenolic acid or glucocorticoids;

(5) Missing or incomplete clinical information and postoperative follow-up data (e.g., missing target drug concentration, dose, etc.);

(6) Recipients who had their grafts removed or died due to rejection or serious complications within 1~2 months after surgery;

(7) Poor compliance and accuracy of results (e.g. irregularity in blood collection time);

(8) Postoperative co-application of other drugs that affect the blood concentration of target drugs such as tacrolimus or voriconazole or caspofungin (e.g. pentothal capsules, rifampicin, phenytoin sodium, carbamazepine, etc.) at the same time.

4.2 Data collection

Kidney transplant patients were strictly screened according to the NaCl criteria, and the patients' basic information (name, hospitalization number, contact information, gender, age, height, weight), medical history (diabetes mellitus and hypertension), the time of kidney transplantation, tacrolimus medication (time of medication and daily dosage), and its trough concentration (C_0), co-medication (time of medication and daily dosage, and the types of co-medications including posaconazole, voriconazole, caspofungin and micafungin), and the patients' liver and biliary function indexes, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), glutamyltransferase (GGT), alkaline phosphatase (ALP), total bilirubin (TBIL), and direct bilirubin (DBIL), etc., and the calculation of tacrolimus trough blood concentration/daily dose ratio (C_0/D) [(ng/ml/)/(mg/kg/d)].

4.3 Methods of measuring the study indicators

The required C_0 and D values for tacrolimus were filtered from the United Digital Case Browser, and the tacrolimus C_0/D values were entered and calculated in Excel.

4.4 Data management method

All data were uniformly entered and saved in Excel, statistically analyzed in SPSS 22.0, and the results of the analysis were also uniformly presented by Excel, and plots were made with GraphPad Prism 8.0.

5. Trial Procedures

5.1 Subject management

1) Recruitment method of subjects

This is a retrospective study with data from Lianzhong Digital Case Browser, LIS Result View and HIS system of the First Affiliated Hospital of Shandong First Medical University, free of subject recruitment.

2) Informed consent process

As this study was a retrospective study, the informed consent process was exempted.

6. Start and End of the Trial

The time period of the extracted EHR data is from January 1, 2015 to April 1, 2023; The study period for this project is from February 1, 2023 to June 1, 2024.

7. Ethical Principles and Compliance with Relevant Regulations

The study strictly followed the current Declaration of Helsinki (2013) as well as relevant laws and regulations applicable within the People's Republic of China and the review opinions of the Ethics Committee.

8. Statistical Analysis

SPSS 22.0 software was used to statistically analyze the data; propensity score matching was taken to match the baseline data of kidney transplant patients in the combination and non-combination groups, and a new dataset with successful matching was obtained, and normality test was performed by Shapiro-Wilk method. Measures conforming to normal distribution were expressed as (mean±sd), non-normal measures as median (interquartile spacing), and counts as frequency (rate). Between groups: independent samples t-test was used for measures that met normal distribution, Mann-Whitney U test was used for measures that did not meet normal distribution, and chi-square test was used for count data to analyze the effects of genetic polymorphisms, azoles/echinocandins on the trough concentration of tacrolimus in renal transplantation recipients; within groups: paired samples t-test was used for measures that met normal distribution, Wilson's t-test was used for measures that did not meet normal distribution, and Wilson's t-test was used for measures that were not distributed. Wilcoxon test was used to analyze the changes in tacrolimus trough concentration in renal transplant recipients before and after co-administration of azoles or echinocandins. $p<0.05$ indicates that the difference is statistically significant. The chi-square test was used to analyze whether the genotype distribution conformed to Hardy-Weinberg equilibrium, and $P>0.05$ indicated that it conformed to population genetic balance.

9. Form of Publication of Research Results

The research results will be published in the form of academic papers.