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# Analysis of tacrolimus blood concentrations in renal transplant patients

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**ABSTRACT.** This study aimed to 1) analyze the results of tacrolimus blood concentration monitored in patients after renal transplantation, 2) observe and establish an optimal therapeutic window for patients, and 3) provide evidence for the clinical and rational use of drugs. Tacrolimus blood concentration was determined by enzyme-linked immunosorbent assay. A total of 1824 cases were obtained from the monitoring of 74 patients after renal transplantation. These cases were then retrospectively analyzed. Over time, the mean whole blood tacrolimus trough concentration after transplantation gradually decreased. This result suggests that the optimal therapeutic windows for patients with renal transplants are as follows: 5 to 20 µg/L at 1 month after surgery; 5 to 15 µg/L at 1-3 months after surgery; 4 to 12 µg/L at 3-6 months after surgery; 4 to 10 µg/L at 6-12 months after surgery; and 3 to 8 µg/L at >12 months after surgery. The absorption of tacrolimus is highly variable. Therefore, tacrolimus concentration in the blood and the recommended clinical therapeutic window should be routinely monitored to adjust the treatment regimen and reduce adverse

 

reactions. In this way, treatment can be optimized.

**Key words:** Tacrolimus; Monitoring of blood concentration; ELISA; Renal transplantation

## INTRODUCTION

Tacrolimus (FK506) is a potent macrolide immunosuppressant that exhibits immune inhibitory functions by blocking T-cell activation, gene transcription, and cytokine secretion. It also blocks cytokine receptor expression by specifically inhibiting calcineurin activity (Fung, 2004). Tacrolimus is mainly applied in immunosuppressive therapy after transplantation of liver, kidney, heart, and other organs, where it improves the survival of transplanted organs and reverses organ rejection. Tacrolimus can be used as a therapeutic alternative to resolve refractory chronic rejection or intolerable side effects after treatment with cyclosporine (Plosker and Foster, 2000; Webster et al., 2005). However, tacrolimus has several serious side effects, including renal toxicity, neurotoxicity, diabetes, and hypertension. In clinical applications, tacrolimus often exhibits toxicity or rejection within the specified treatment concentration range for the following reasons: it covers a narrow therapeutic window, its pharmacokinetics and pharmacodynamics differ among individuals, and it lacks a close correlation between dosage and serum concentration; as a result, clinical treatment becomes difficult (Henry, 1999; Sperschneider et al., 2001; Margreiter et al., 2002; Ghio et al., 2004). For the most efficient immunosuppressant treatment and to reduce adverse reactions, patients who take tacrolimus after receiving organ transplants should have routine therapeutic drug monitoring. An optimal therapeutic window concentration should be established, and medications should be adjusted accordingly (Kahan et al., 2002). The present study statistically analyzed cases of 41 patients in whom cyclosporine treatment was switched to tacrolimus after the patients underwent renal transplantation in our hospital. On the basis of our results, we determined the optimal therapeutic window for tacrolimus in patients with organ transplants to provide a reference for the clinical and rational use of the drug.

## MATERIAL AND METHODS

### Subjects

We obtained the results of 1824 tacrolimus blood concentrations monitored in 74 renal transplant recipients (51 male and 23 female patients) aged 13-72 years (mean age = 38.93 ± 10.87 years) admitted to our hospital. Donor characteristics were as follows: age 23-60 years (mean age = 43.76 ± 10.27 years); 59 male and 15 female patients; 65 deceased and 9 living. We reviewed follow-up data to determine the adequacy of treatment. This study was conducted in accordance with the Declaration of Helsinki and with approval from the Ethics Committee of the 401st Hospital of Chinese People's Liberation Army. Written informed consent was obtained from all participants.

### Immunosuppressive therapy

A total of 41 patients with renal transplants received the cyclosporin A + mycophenolate

+ prednisone (CsA + MMF + pred) scheme. This treatment was later replaced with the tacrolimus + mycophenolate + prednisone (FK506 + MMF + pred) scheme (CsA, cyclosporin soft capsules, Novartis Pharma GmbH, Eberbach, Gemany; MMF, mycophenolate mofetil capsules, Roche Registration Ltd., Welwyn Garden City, UK; FK506, Astellas Ireland Co., Ltd., Killorglin, Co., Kerry, Ireland; and pred, P Zhejiang Xianju Pharmaceutical Co., Ltd., Taizhou City, China). The former treatment was replaced with the latter within 1 month to 5 years after renal transplantation. The replacement dosages ranged from 0.05 to 0.20 $mg \cdot kg^{-1} \cdot day^{-1}$. A total of 33 patients received the FK506 + MMF + pred scheme after renal transplantation. Tacrolimus was orally administered at 24 h after surgery with an initial dose of 0.10 to 0.25 $mg \cdot kg^{-1} \cdot day^{-1}$ and an interval of 12 h. The dosage was subsequently adjusted according to the results of tacrolimus trough concentrations and clinical status.

## Sample collection

Blood tacrolimus trough concentrations were monitored at the following frequencies and intervals: immediately, 1 week after surgery, once or twice per week for 1 month, once every 1-2 weeks during months 1-3, and at least once a month for 3-6 months. Blood was drawn at irregular intervals for more than 6 months. Liver and renal function tests, as well as blood concentrations and other clinical indicators, were determined to verify the results. All parameters were monitored as necessary, particularly if abnormal clinical symptoms were observed. All patients were monitored each morning with a 1-mL blood sample drawn 30 min before the administration of medication. Samples were placed in EDTA-$K_2$ anticoagulant tubes and examined the same day.

## Monitoring of tacrolimus

Enzyme-linked immunosorbent assay (ELISA; PRO-Trac™ II Tacrolimus, USA) was conducted with the following procedure: approximately 25 µL blood was added to 150 µL digestion mixture and vortexed for 15 to 30 s. The resulting mixture was allowed to stand at room temperature for 15 min and then was placed in a water bath at 75°C for 15 min. The scroll was then removed from the water bath and mixed manually for 15 to 30 s. Afterwards, the scroll was centrifuged at 1800 rpm for 10 min at room temperature. Approximately 100 µL supernatant was carefully added to the enzyme-labeled plate. Enzyme-labeled diluent (50 µL) was then placed in the blank control hole, and tacrolimus monoclonal antibody (50 µL) was placed in the other holes. The plates were sealed and placed in an oscillator (Stat Fax 2200 Microplate Incubator + Shaker, Awareness Technology, Inc., USA) at 700 ± 50 rpm for 30 ± 2 min at room temperature. The 5X enzyme-labeled antibody was diluted to 1X by adding enzyme-labeled diluent. In each hole, 50 µL diluted enzyme-labeled antibody was added. The plate was then sealed at room temperature and oscillated at 700 ± 50 rpm for 60 ± 2 min. Afterwards, the plate was washed 3 times. After 5 min, 300 µL lubricant was added to each hole. Next, 200 µL substrate solution was added, and the board was then sealed. The resulting solution was oscillated at 700 ± 50 rpm at room temperature. To terminate the reaction, we added 100 µL stop buffer to each hole. We observed that the color of the solution changed from blue to yellow. At 5 min, absorbance was determined at 450 nm/630 nm dual wavelength by using a microplate reader (ELx800 Absorbance Microplate Reader, BioTek Instruments Inc., USA). The experimental results were then recorded. Complete determination required approximately 3.5 to 4 h.

 

## Quality control

The samples were evaluated by ELISA using a blank control sample and 0, 0.3, 1, 3, 10, and 30 µg/L of the treatment. A total of 17 standard concentrations were then used to draw the standard curve. The results were subjected to quality assurance by using low- and high-quality control samples. If the determined value was within the allowable range, the sample results were recorded to ensure that the monitoring results were accurate and reliable.

# RESULTS

## Whole blood tacrolimus trough concentrations

A total of 1824 blood tacrolimus trough concentrations from 74 patients with renal transplants were grouped and compared in terms of the time after the surgery. The results are shown in Table 1. The mean whole blood tacrolimus trough concentration decreased gradually after transplantation: 4.92 to 15.54 µg/L after 1 month; 5.29 to 11.15 µg/L after 1-3 months; 4.09 to 10.37 µg/L after 3-6 months; 3.67 to 8.77 µg/L after 6-12 months; 3.31 to 8.67 µg/L after 12-24 months; 2.70 to 8.06 µg/L after 24-60 months; and 2.35 to 7.77 µg/L for >60 months.

**Table 1.** Whole blood tacrolimus trough concentrations at different times after surgery (means ± SD, µg/L).

| Time after surgery (months) | Cases (times) | Concentration (µg/L) | Percentage (%) |
|---|---|---|---|
| <1 | 69 | 10.23 ± 5.31 | 3.78 |
| 1-3 | 152 | 8.22 ± 2.93 | 8.33 |
| 3-6 | 172 | 7.23 ± 3.14 | 9.43 |
| 6-12 | 208 | 6.22 ± 2.55 | 11.40 |
| 12-24 | 245 | 5.99 ± 2.68 | 13.43 |
| 24-60 | 373 | 5.38 ± 2.68 | 20.45 |
| >60 | 605 | 5.06 ± 2.71 | 33.18 |

## Distribution of blood tacrolimus trough concentrations

Results were statistically analyzed. Distribution and frequency are shown in Table 2. Approximately 86.13% of the blood tacrolimus concentrations were in the range of 3 to 15 µg/L.

## Distribution of blood tacrolimus trough concentrations at different time periods

Table 3 shows the distribution and frequency of whole blood tacrolimus trough concentrations at different time periods after renal transplantation. The range of effective tacrolimus blood concentration was 10 to 15 µg/L at 3 months after surgery. This result was maintained at a trough concentration of 5 to 10 µg/L which is consistent with results of previous studies (Armstrong and Oellerich, 2001; Bekersky et al., 2001; Napoli, 2006). Triple immunosuppressive therapy was applied after renal transplantation, and ELISA was used to monitor

our findings. The results summarized in Tables 1, 2, and 3, combined with clinical findings, revealed the following therapeutic windows for tacrolimus in patients with renal transplants: 5 to 20 µg/L at 1 month after surgery; 5 to 15 µg/L at 1-3 months; 4 to 12 µg/L at 3-6 months; 4 to 10 µg/L at 6-12 months; and 3 to 8 µg/L at >12 months.

**Table 2.** Distribution of whole blood tacrolimus trough concentrations.

| Range of whole blood trough concentration (µg/L) | Cases (times) | Percentage (%) |
|---|---|---|
| <3 | 219 | 12.01 |
| 3-5 | 588 | 32.24 |
| 5-10 | 819 | 44.90 |
| 10-15 | 164 | 8.99 |
| 15-20 | 29 | 1.59 |
| >20 | 5 | 0.27 |

**Table 3.** Distribution of whole blood tacrolimus trough concentrations at different times after surgery (µg/L).

| Time (months) | <3 | | 3-5 | | 5-10 | | 10-15 | | 15-20 | | >20 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | % | N | % | N | % | N | % | N | % | N | % |
| <1 | 0 | 0 | 8 | 11.60 | 32 | 46.38 | 18 | 26.09 | 7 | 10.14 | 4 | 5.79 |
| 1-3 | 3 | 1.97 | 16 | 10.53 | 96 | 63.16 | 32 | 21.05 | 5 | 3.29 | 0 | 0 |
| 3-6 | 6 | 3.49 | 44 | 25.58 | 87 | 50.58 | 30 | 17.44 | 5 | 2.91 | 0 | 0 |
| 6-12 | 12 | 5.77 | 44 | 21.15 | 131 | 62.98 | 20 | 9.62 | 1 | 0.48 | 0 | 0 |
| 12-24 | 19 | 7.76 | 81 | 33.06 | 123 | 50.20 | 20 | 8.16 | 2 | 0.82 | 0 | 0 |
| 24-60 | 49 | 13.14 | 160 | 42.89 | 146 | 39.14 | 13 | 3.49 | 4 | 1.07 | 1 | 0.27 |
| >60 | 130 | 21.49 | 235 | 38.84 | 204 | 33.72 | 31 | 5.12 | 5 | 0.83 | 0 | 0 |

## DISCUSSION

Different therapeutic windows for tacrolimus have been obtained in previous studies and in this experiment. Such differences may be associated with individual characteristics, race, determination method, and clinical factors in patients with renal transplants, but the result of treatment was relatively good. Tacrolimus concentrations differed greatly at 1 month after renal transplantation (Table 3) because of individual differences and the short time period after surgery when functional indices of the body were not yet restored to normal. Furthermore, multiple combined medications influenced one another. In this experiment, tacrolimus concentrations in some patients fluctuated irregularly or were maintained high or low with large individual differences during long-term treatment. Therefore, tacrolimus blood concentration should be monitored after surgery, and an optimal therapeutic window concentration should be established to guide its clinical and rational use.

Tacrolimus blood concentration showed a “wavy” fluctuation because of different tacrolimus pharmacokinetics in various patients and in the same individual at different periods. Pathological and physiological factors, genetic polymorphisms, diet, medication, and other patient factors also affect such differences (Bazin et al., 2010; Hirano et al., 2012). For example, one patient was given diltiazem hydrochloride spansule 60 mg twice a day because of illness, and the tacrolimus concentration increased 1.08 times. Two patients were given five ester capsules twice a day, and the tacrolimus concentrations rose to 1.95 and 1.43 times higher than the initial dose, respectively. Another patient consumed a grapefruit as the drug

was being administered, and as a result, the tacrolimus concentration in the blood increased significantly. The preceding findings could be attributed to cytochrome P450 3A5 (CYP3A5), the main enzyme involved in drug metabolism. Current clinical data reveal that Itsumi Kokosu (the main component of a five-ester capsule), grapefruit juice (grapefruit), diltiazem, and imidazoles combined with tacrolimus can increase the blood concentration of tacrolimus to different degrees; the inhibitory mechanism of CYP3A5 activity on tacrolimus involves CYP3A substrate (Chang et al., 2010; Press et al., 2010; Page et al., 2011; Pavan et al., 2011). If patients are taking medications or other substances that interact with CYP3A5, blood tacrolimus concentrations may be maintained within the normal range by appropriately reducing the tacrolimus dose and determining its concentration in the blood. To avoid rejection or side effects, we cannot arbitrarily stop or change the dose of tacrolimus. In one patient, the actual concentration of tacrolimus in the blood could not be accurately assessed, possibly because of several factors, including insufficient medication dose, drawing blood after more than 12 h of taking tacrolimus, and irregular medication absorption before or after meals. Such factors, combined with clinical status and individual differences in the pharmacokinetics of tacrolimus and clinical indices, can provide a comprehensive evaluation of the drug concentration in the blood and help reduce adverse reactions. Thus, the survival rate and quality of life of patients undergoing organ transplantation could be improved.

Tacrolimus has been used in clinical application for years; many clinical trials have confirmed that tacrolimus can be used efficiently and safely to prevent acute rejection after renal transplantation (Webster et al., 2005). With tacrolimus commercially developed and distributed by local companies and with an adjusted ratio of medical insurance reimbursement, numerous patients prefer the oral administration of tacrolimus over cyclosporin A. Introduction of the sustained-release capsule can further increase compliance and improve patients' quality of life (Hatakeyama et al., 2012; Nakamura et al., 2012; Sessa et al., 2012). Among the 74 patients included in our study, 41 who were treated with the CsA + MMF + pred scheme decided to replace this treatment with the FK506 + MMF + pred scheme within 1 month to 5 years after renal transplantation. The following reasons were given for changing treatment schemes: to reverse acute and chronic rejection, to alleviate side effects of hirsutism and gingival hyperplasia, and to improve graft survival time and quality.

Tacrolimus has gradually replaced cyclosporin A as the preferred immunosuppressive drug for organ transplantation. Because of differences among individuals taking tacrolimus, narrow therapeutic windows, and lack of close correspondence between dosage and serum concentration, it is important to monitor whole blood tacrolimus trough concentrations during treatment, establish optimal therapeutic window concentrations, and adjust medication accordingly. Moreover, various pathological and physiological factors, diet, medication, gene polymorphism, and other parameters influencing patients after renal transplantation can affect whole blood tacrolimus trough concentrations (Haufroid et al., 2004; Loh et al., 2008). Therefore, we must consider the various influencing factors and develop individualized medication dosing to obtain optimal treatment effects.

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