Pharmacokinetic Study of the Combination of Tacrolimus and Fluconazole in Renal Transplant Patients

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Background: *It was hypothesized that fluconazole in combination with tacrolimus can be used safely with an imitated area under curve (AUC) compared to tacrolimus. At every time point, this combination was presumed to correlate well with pre-intervention AUC, thus the dosage could be significantly reduced.*
Material and Method: *There were two groups of patients. Group I (n = 15) included patients who received tacrolimus at 0.1-0.3 mg/kg/day within one week after transplantation. These patients were studied for tacrolimus whole blood concentrations. The tacrolimus dosage was then reduced by 40% and given in combination with fluconazole at 100-200 mg/day for one week, tacrolimus whole blood concentrations were studied again. Group II (n = 8) included patients who had been transplanted for more than 3 months and had received a stable dosage of tacrolimus in combination with fluconazole for at least one month.*
Results: *In group I, before fluconazole combination, trough levels correlated well with AUC0-12. After fluconazole combination, trough levels still correlated well with AUC0-12. The after/before fluconazole-combination ratio of AUC0-12 and maximum tacrolimus concentration (Cmax) was 1.08 (90%CI; 0.98-1.19) and 1.17 (90%CI; 1.00-1.36), respectively. Correspondingly, the oral bioavailability, which was the after/before fluconazole combination ratio of AUC0-12/dose and absorption rate (Cmax/dose/body weight), was significantly increased [2.08 (90%CI; 1.80-2.40) and 2.24 (90%CI; 1.99-2.51), respectively]. Tacrolimus clearance after the fluconazole combination was significantly reduced, compared with before the combination (14.74 vs 38.79 L/h, p = 0.001). Mean tacrolimus dosage in this group could be reduced from 10.7 mg/day before fluconazole combination to 5.7 mg/day after it and to 3.7 mg/day at 3 months after transplantation (p = 0.001). In group II, trough levels correlated well with AUC0-12 and the mean tacrolimus dosage in this group was only 2.9 mg/day.*
Conclusion: *This present study showed a good correlation between tacrolimus trough levels and AUC, which occurred in monotherapy or in patients who received fluconazole. The tacrolimus trough levels could be trusted in monitoring patients who received a tacrolimus-based immunosuppressive regimen. The combination to fluconazole was ascertained and it was safe to reduce the dose of tacrolimus.*

Keywords: *Tacrolimus, Fluconazole, Renal transplant*

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Tacrolimus has potentially high intra-patient and inter-patient variability and, importantly, its therapeutic index is narrow. As a matter of safety and efficacy, therapeutic drug monitoring is therefore mandatory. Studies of liver[1], heart[2], and kidney[3] transplantation were found to correlate well between trough levels and area under the concentration curve (AUC). Meanwhile, recent studies by the Netherlands group[4]

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suggested that two points of drug levels were required to predict AUC.

A combination of fluconazole and tacrolimus augments tacrolimus blood levels[5]. It was found by Haihara et al that, after discontinuing fluconazole in liver transplant patients for four to nine days, the tacrolimus levels could be reduced by 12.9 to 80.8 percent[6]. In kidney transplantation, it was also reported that in a combination of fluconazole at 100 mg to tacrolimus, the dosage of tacrolimus could be reduced by forty percent without changing tacrolimus trough levels[7].

It was proposed from the previous study that fluconazole could increase the blood levels by decreasing the hepatic clearance of tacrolimus. Therefore, how azole would alter the tacrolimus AUC is questionable. The objectives of this present study were to evaluate the pharmacokinetics, correlations between drug concentrations and AUC, and safety of tacrolimus in combination with fluconazole.

Material and Method

The present study was approved by the ethical committee of the Faculty of Medicine, Chiang Mai University. The inclusion criteria comprised patients over 15 years of age with normal liver function. The authors divided the patients into 2 groups. Group I included patients with *de novo* kidney transplantation, and group II comprised patients who had been transplanted for more than three months and had received a stable dosage of tacrolimus in combination with fluconazole for at least one month. The exclusion criteria consisted of patients who received drugs known to alter tacrolimus pharmacokinetics, developed acute allograft rejection and suffered severe medical illness.

In group I, tacrolimus at 0.1 to 0.3 mg/kg divided into two doses was started within one week after transplantation. The tacrolimus dosage was adjusted to keep tacrolimus trough levels between 7 and 15 ng/ml. One week after reaching tacrolimus target levels, the blood at those levels was studied, and then fluconazole at 100 to 200 mg/day was started in combination with tacrolimus. The authors adjusted tacrolimus dosage to keep the tacrolimus trough levels between 7 and 15 ng/ml, and one week later, the authors studied the tacrolimus blood levels again. In group II, patients received tacrolimus in combination with 100 to 200 mg of fluconazole in two divided doses. The tacrolimus dosage was adjusted to keep trough levels between 5 and 15 ng/ml, and the tacrolimus blood levels were studied.

Tacrolimus blood levels were collected for 9 points; before tacrolimus administration, (drug concentration at time 0 (C0), and after tacrolimus administration for 1 (C1), 2 (C2), 3 (C3), 4 (C4), 6 (C6), 8 (C8), 10 (C10), and 12 hours (C12). Tacrolimus blood levels were measured by microparticle enzyme immunoassay using a monoclonal antibody (IMx Tacrolimus II Assay, Abbott Laboratories, Abbott Park, Illinois, USA)

There were 15 patients (12 male and 3 female) in group I, comprising 9 living-related kidney transplantations and 6 cadaveric kidney transplantations. The mean age was 42.6 $\pm$ 17.3 years and median time of transplantation 10 (range, 9-21) days. The mean serum creatinine was 3.2 $\pm$ 2.8 mg/dl. Prednisolone and fluconazole dosage were 15 $\pm$ 0 and 193 $\pm$ 47 mg/day, respectively. There were 8 patients (2 male and 6 female) in group II, comprising 5 living-related kidney transplantations and 3 cadaveric kidney transplantations. The mean age was 36.6 $\pm$ 13.5 years and median time of transplantation 22 (range, 5-82) months. The mean serum creatinine was 1.4 $\pm$ 0.8 mg/dl. Prednisolone and fluconazole dosage was 2 $\pm$ 4 and 225 $\pm$ 116 mg/day, respectively.

Maximum tacrolimus blood levels (Cmax) were measured in each patient; areas under the concentration curve 0 to 12 hours (AUC0-12) were calculated by the linear trapezoidal rule; AUC0-12 divided by tacrolimus dosage (AUC0-12/dose) was referred to oral bioavailability; maximum tacrolimus blood levels divided by dosage and body weight (Cmax/dose/body weight) were referred to absorption rate; and total body clearance of tacrolimus was calculated by using the TopFit version 2.0 program.

Statistical analysis

Correlations between tacrolimus blood levels and AUC0-12 were analyzed by the linear regression model; tacrolimus dosage, before and after fluconazole combination, were analyzed by the general linear model; and total body clearance of tacrolimus, before and after fluconazole combination, was analyzed by nonparametric analysis. The SPSS version 10.0 program (SPSS, Chicago, Illinois, USA) was used, and statistical significance was indicated with a p value < 0.05. Differences between AUC0-12, Cmax, AUC0-12/dose, and Cmax/dose/kg, after and before the fluconazole combination, were analyzed by the EquiTest program. The differences of these parameters were not significant in the after/before fluconazole combination ratio and the 90 percent confidence interval is in bioequivalence range.

Results

In group I, tacrolimus levels at each time point and AUC0-12 correlated well before the fluconazole combination (Table 1), however, after it, C1 did not correlate with AUC0-12. Tacrolimus trough levels correlated well with AUC0-12 before and after the fluconazole combination with a correlation coefficient of 0.82 and 0.80, respectively.

The after/before fluconazole combination ratio of AUC0-12 was 1.08 (90%CI, 0.98-1.19), which fell within the bioequivalence range, and meant that AUC0-12 before and after the fluconazole combination was no different. The after/before fluconazole combination ratio of Cmax was 1.17 (90%CI, 1.00-1.36), which also fell within the bioequivalence range (Table 2). Nevertheless, tacrolimus levels at each time point after the fluconazole combination were higher than those before it (Fig. 1). The after/before fluconazole combination ratio of AUC0-12/dose that referred to oral bioavailability, and Cmax/dose/body weight, which referred to absorption rate were 2.08 (90%CI, 1.80-2.40) and 2.24 (95%CI, 1.99-2.51), respectively (Table 2). This meant that after fluconazole combination, the oral bioavailability and absorption rate was increased about 2.08 and 2.24 times, respectively, compared to that before it. The total body clearance of tacrolimus decreased significantly after the fluconazole combination compared to that before it (14.7 vs 38.8 L/h, p = 0.001). The tacrolimus dosage in group I patients could be reduced from 10.7 mg/day on day 10 (range, 9-21) to 5.7 mg/d on day 20 (range, 14-35) after transplantation, and to 3.7 mg/day in month 3 (p = 0.001). There was no evidence of acute allograft rejection, tacrolimus toxicity, fungal infection or liver impairment during the 3-month follow up.

In group II patients, who had been transplanted for more than 3 months, the shape of the tacrolimus time-concentration curve was similar to that of group I patients (Fig. 1). Tacrolimus levels at each

Table 1. Correlation between tacrolimus levels and AUC0-12 in group I and II

Tacrolimus levels	Correlation coefficient					
	Group I (n = 15)				Group II (n = 8)	
	Before fluconazole combination		After fluconazole combination			
	r	p	r	p	r	p
Trough levels (C0)	0.82	<0.001	0.80	<0.001	0.98	<0.001
C1	0.67	0.006	0.22	0.414	0.72	0.047
C2	0.75	0.001	0.64	0.008	0.98	<0.001
C3	0.94	<0.001	0.87	<0.001	0.93	0.001
C4	0.92	<0.001	0.94	<0.001	0.87	0.005
C6	0.89	<0.001	0.96	<0.001	0.94	<0.001
C8	0.92	<0.001	0.87	<0.001	0.95	<0.001
C10	0.93	<0.001	0.89	<0.001	0.96	<0.001
C12	0.91	<0.001	0.81	<0.001	0.93	0.001

Table 2. Mean and 90% confidence interval of after/before fluconazole administration ratio of pharmacokinetic parameters in group I

Pharmacokinetic parameters	Mean	90% confidence interval	Bioequivalence range
AUC0-12 (ng.h/ml)	1.08	0.98-1.19	0.80-1.25
AUC0-12/dose (ng.h/ml/mg)	2.08	1.80-2.40	-
Cmax (ng/ml)	1.17	1.00-1.36	0.70-1.43
Cmax/dose/body weight (ng/ml/mg/kg)	2.24	1.99-2.51	-

AUC0-12, area under the concentration curve at 0 to 12 hours; Cmax, maximum tacrolimus concentration

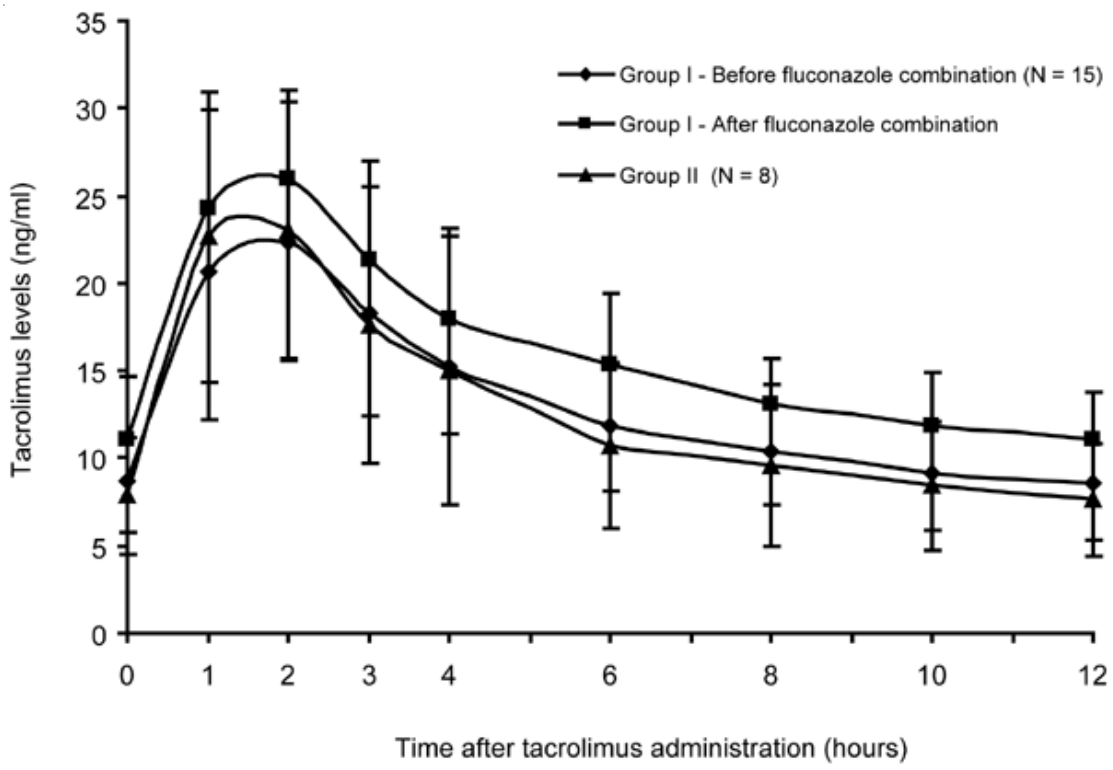

Fig. 1 Tacrolimus time-concentration curve in group I compared to group II

time point, including trough levels, correlated well with AUC0-12 (Table 1) and the tacrolimus dosage for these patients was only 2.9 mg/day.

Discussion

The present study clearly showed a contrasting result from the previous one which needed two or more time points of tacrolimus in correlation with AUC[6,7]. The result showed good correlation between trough levels and AUC0-12. This particular correlation was not dependent on either time of transplantation or fluconazole combination. There was one point at C1 that showed no statistically significant correlation to AUC0-12 in either group. It could be postulated that this variation in the first hour might be attributed to the rate of drug absorption. This absorption, however, did not detour the peak of Cmax at the second hour time point. As shown from the curve in both groups, the correlation of C2 to AUC0-12 was highly significant, and occurred either before or after the fluconazole combination. These data were interpreted as the absorption that might vary individually. Correspondingly, the rate of absorption might shift to the right or left of the curve, which means that there would be both a slow and quick absorber. Nevertheless, among these groups, all patients reached the Cmax at the second hour of the curve. The absorption rate at the first hour was postulated as attributable to the exact time of taking the drug, food ingested after taking the drug, and interaction of the drug absorption. It was also different in the enzyme kinetic, CYP450 3A5 and 3A4, as somehow controlled by pharmacogenetics[8].

The results showed a good correlation between C2, C3, C4, C6, C8, C10, and C12 and the AUC0-12. Each time point reached had high statistical significance. This correlation does not need two or more time points to achieve a reliable significance compared to a previous study[5]. This variation may be caused by the difference in race, as the subjects in most other studies were Caucasian people, and this study was performed with Asians who had a lower average body weight compared to Western people. The correlation between AUC and concentration at each time point was found through C12, which was close to the next dosage in the evening. The tacrolimus time-concentration curve was shown to gradually decline compared to that of cyclo-

sporine. This slow metabolism does not mean potential nephrotoxicity, but rather persistent extensive immunosuppressive effect, and it correlated to AUC0-12.

The tacrolimus profile after adding fluconazole did not change the shape of the curve. Pharmacokinetic study compared the bioequivalence between pre- and post-fluconazole administration that was in the ratio of 1.08 (90%CI, 0.98-1.19), which fell within the range of 0.80-1.25 for AUC0-12. This means that AUC0-12 of tacrolimus before the fluconazole combination is similar to that after it. The stable AUC0-12 was accrued, due to the decrease of total body clearance and increase of oral absorption rate of tacrolimus. This was confirmed by Cmax/dose/body weight that increased by 2.24 times (and the AUC0-12 increased by 2.08 times), which represented the oral bioavailability.

What happened to pharmacokinetics after three months posttransplantation? The dosage of tacrolimus was progressively reduced from the second week posttransplantation to the third week. At the time when the patient was discharged from hospital, the dosage of the drug had been reduced by nearly half. After three months follow up, the dosage reduction continued progressively. During this period, fluconazole was fixed at a dosage of 100 mg twice daily, but the required dose of tacrolimus was reduced to seventy percent to maintain the acceptable range of trough level. The correlation between trough level and AUC0-12 was still highly significant, and the time-concentration curve was similar to that of the third week. This observation of drug profile may imply either one of two ways. The first was more drug absorption during the onward time, or the second, further decline in total body clearance. In the fourth month posttransplantation, the patients took tacrolimus at an average dose of 3 to 4 mg per day to achieve the expected safety trough level.

Is nephrotoxicity questionable in an azole combination? During the three-month follow up period, the evidence of adverse reaction was not observed. Both groups of patients showed a stabilized serum creatinine level and any clue of nephrotoxicity was not detected. This safety profile might have derived from the time-concentration curve, which was determined by frequently adjusting trough levels. The time-concentration curve, which imitated that before the azole combination, showed that the tacrolimus level was above 15 mg/ml only during the first four hours after each dose. As the curve did not run abruptly higher than that before the fluconazole combination profile, it may avoid potential nephrotoxicity.

Would the trough level be more reliable as a practical point of counterbalance between safety and safe cost? The correlation found between the trough and other time points during each dose of tacrolimus and the AUC0-12, make the trough level more reliable in practical monitoring of immunosuppression. Taking the C2 level as a guide for immunosuppression may not be a practical point of view, because the exact time to draw the blood sample is obligatory. Drawing two blood samples for monitoring the AUC is also not a practical point, unless the patient is admitted to the hospital. The difference between the two drugs in the calcineurin inhibitor group may occur in the point of pharmacokinetics. However, the present study was performed in Asian people, who may have less reliable intestinal enzyme stability than Caucasians, and further investigation is needed.

Conclusion

In summary, tacrolimus pharmacokinetics has been clarified in the present study. It correlated well with AUC0-12 in both living and cadaveric kidney transplantations. After combining to oral fluconazole in a fixed dosage, the drug profile was shown as comparable in the bioequivalence range. The total dosage of tacrolimus can be decreased after the combination, and the data confirm that adding fluconazole contributes to increased oral absorption rates and decreased total body clearance. The relevance of C0 and the combination of tacrolimus and fluconazole may save costs while maintaining the safety of kidney transplant patients.

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การศึกษาเภสัชจลนศาสตร์การใช้ยาทาโครลิมัสร่วมกับฟลูโคนาโซลในผู้ป่วยปลูกถ่ายไต

ดุสิต ล้ำเลิศกุล, ขจรศักดิ์ นพคุณ, นพมาศ โรจนเสถียร, กิตติกา กาญจนารัตนากร, ศุภฤกษ์ จิตติกานนท์, อัมรา มโนยศ, ดิเรก บรรณจักร์, วุฒิเดช โอภาศเจริญสุข

วัตถุประสงค์*: เปรียบเทียบค่าทางเภสัชจลนศาสตร์คือ พื้นที่ใต้กร๊าฟจากการให้ยาทาโครลิมัสอย่างเดียว กับการให้ยาทาโครลิมัสร่วมกับฟลูโคนาโซล*

วัสดุและวิธีการ*: ผู้ป่วยกลุ่มแรกจำนวน 15 ราย ได้รับทาโครลิมัสขนาด 0.1-0.3 มก./กก./วัน ภายใน 1 สัปดาห์หลังการปลูกถ่ายไต ทำการลดขนาดยาลงร้อยละ 45 ร่วมกับการให้ฟลูโคนาโซลขนาด 100-200 มก./วัน เป็นเวลา 1 สัปดาห์*

ผู้ป่วยกลุ่มที่สองจำนวน 8 ราย ได้รับการปลูกถ่ายไตนานกว่า 3 เดือน ได้รับทาโครลิมัสขนาดคงที่ร่วมกับฟลูโคนาโซล

ผลการศึกษา*: ในผู้ป่วยกลุ่มแรกระดับยาก่อนรับประทานยา มีความสัมพันธ์อย่างดีกับพื้นที่ใต้กร๊าฟ ทั้งในช่วงรับประทานยาทาโครลิมัสอย่างเดียว หรือช่วงรับประทานยา 2 ตัว ฟลูโคนาโซลลดการขจัดยาทาโครลิมัสในผู้ป่วยกลุ่มที่สองพบผลการศึกษาในลักษณะเดียวกัน*

สรุป*: ระดับยาทาโครลิมัสก่อนรับประทานยา มีความสัมพันธ์อย่างดีกับพื้นที่ใต้กร๊าฟทั้งในกรณีรับประทานยาอย่างเดียว หรือรับประทานในขนาดลดลงร่วมกับฟลูโคนาโซล*