Hemoglobin Levels Influence Pharmacokinetics of Tacrolimus in Kidney Transplant Patients

นิพนธ์ต้นฉบับ

สาวิตรี ไพรสุวรรณ[1], สมฤทัย วัชราวิวัฒน์[1*] และ ยิ่งยศ อวิหิงสานนท์[2]

[1] ภาควิชาเภสัชกรรมปฏิบัติ คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพมหานคร
[2] สาขาวิชาโรคไต ภาควิชาอายุรศาสตร์ คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพมหานคร

* **ติดต่อผู้นิพนธ์:** Somratai.r@chula.ac.th

วารสารไทยเภสัชศาสตร์และวิทยาการสุขภาพ 2555;7(1):1-6

Original Article

Sawitree Praisuwan[1], Somratai Vadcharavivad[1*] and Yingyos Avihingsanon[2]

[1] Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand
[2] Renal Division, Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

* **Corresponding author:** Somratai.r@chula.ac.th

Thai Pharmaceutical and Health Science Journal 2012;7(1):1-6

บทคัดย่อ

วัตถุประสงค์: เพื่อศึกษาหาความสัมพันธ์ระหว่างระดับ hemoglobin และเภสัชจลนศาสตร์ของยา tacrolimus ในผู้ป่วยไทยที่ได้รับการปลูกถ่ายไต **วิธีการศึกษา:** ทำการศึกษาแบบย้อนหลังในผู้ป่วยปลูกถ่ายไต จำนวน 71 คน ที่มาติดตามการรักษา ณ โรงพยาบาลจุฬาลงกรณ์ กรุงเทพ โดยเก็บข้อมูลระดับความเข้มข้นยาจากเวชระเบียน ในระยะ 1 ถึง 6 เดือนหลังได้รับยา tacrolimus คำนวณหาค่าสัดส่วนขนาดยาต่อความเข้มข้นของยา tacrolimus ในเลือดที่เวลาก่อนให้ยามื้อถัดไป (D/C_{trough}) และหาความสัมพันธ์ระหว่างระดับ hemoglobin และ D/C_{trough} โดยการวิเคราะห์ความถดถอยเชิงเส้น **ผลการศึกษา:** จากการศึกษาพบว่า ระดับ hemoglobin มีความสัมพันธ์เชิงลบกับค่า D/C_{trough} ($r = -0.41$, $P < 0.01$) และได้สมการทำนายค่าสัดส่วนขนาดยาต่อความเข้มข้นยาคือ D/C_{trough} (L/kg) = 26.38 - 1.44 Hemoglobin (g/dl) นอกจากนั้นค่า D/C_{trough} ในกลุ่มผู้ป่วยที่มีระดับ hemoglobin ต่ำกว่า 12 กรัม/ดล. มีค่าสูงกว่ากลุ่มผู้ป่วยที่มีระดับ hemoglobin ปกติอย่างมีนัยสำคัญทางสถิติ (11.10 ± 8.73 และ 7.31 ± 4.05 L/kg ตามลำดับ). **สรุป:** ค่าสัดส่วนขนาดยาต่อความเข้มข้นของยา tacrolimus ที่เวลาก่อนให้ยามีความสัมพันธ์กับระดับ hemoglobin การเปลี่ยนแปลงของระดับ hemoglobin อาจช่วยในการปรับขนาดใช้ยา tacrolimus ให้มีความปลอดภัยและประสิทธิผลดีในผู้ป่วยปลูกถ่ายไต

คำสำคัญ: hemoglobin, kidney transplant, pharmacokinetics, tacrolimus

Abstract

Objective: To determine the relationship between hemoglobin levels and pharmacokinetics of tacrolimus in Thai kidney transplant patients. **Methods:** The clinical data of 71 kidney transplant recipients at King Chulalongkorn Memorial Hospital, Bangkok were retrospectively collected during 1 to 6 months after initiation of tacrolimus treatment. The ratio of dose to trough whole-blood concentrations of tacrolimus (D/C_{trough}) was calculated. Linear regression was used to determine the relationship between hemoglobin levels and D/C_{trough}. **Results:** Hemoglobin levels were inversely associated with D/C_{trough} ($r = -0.41$, $P < 0.01$). The relationship could be described as an equation (D/C_{trough} (L/kg) = 26.38 - 1.44 Hemoglobin (g/dl)). Furthermore, D/C_{trough} was significantly higher in the patients with low hemoglobin levels (<12 g/dL) than those with normal hemoglobin levels (11.10 ± 8.73 vs 7.31 ± 4.05 L/kg, respectively). **Conclusion:** The ratio of dose to trough concentrations of tacrolimus significantly correlates with hemoglobin levels. We should consider hemoglobin levels of kidney transplant patients whenever modifying their tacrolimus dosage.

Keywords: hemoglobin, kidney transplant, pharmacokinetics, tacrolimus

Introduction

Tacrolimus is a potent calcineurin-inhibitor immune-suppressant indicated for prophylaxis of kidney rejection in patients receiving allogenic kidney transplant and improves graft survival.[1-3] The pharmacokinetic parameters of tacrolimus are quite variable among individuals.[4,5] With a narrow therapeutic index, therapeutic use of tacrolimus is quite complicated. Adequate immunosuppression is crucial to prevent acute rejection, while overimmunosuppression can lead to nephrotoxicity, infections and increased risk of other complications. There is a poor correlation between blood concentrations of tacrolimus and drug dosage.[5,6] Therapeutic drug monitoring is necessary to ensure appropriate immunosuppression and to avoid adverse drug reactions.

Trough whole blood concentration of tacrolimus is a good indicator of the total body exposure of tacrolimus. A strong correlation existed between the trough concentrations (C_0) of tacrolimus in whole blood and area under the concentration-time curve from time 0 to 12 hours (AUC_{0-12}).[7] It has been shown that trough whole blood concentrations of tacrolimus are significantly related to clinical endpoints, i.e. there was a significant correlation between increased trough concentrations and decreased rejection episodes.[8,9] Several studies have been reported a correlation between high trough whole blood concentrations of tacrolimus and toxicity, particularly nephrotoxicity and neurotoxicity.[10-14] In clinical practice, target trough concentration is determined according to individual patient's risks of rejection and toxicity,

tacrolimus dosage is then adjusted to achieve the predetermined target concentration.[3,15]

In systemic circulation, tacrolimus is primarily distributed to erythrocytes.[16] Since tacrolimus is a low hepatic extraction ratio drug, its hepatic clearance might be influenced by changes in erythrocyte binding, resulting in interindividual variations of the tacrolimus dosage to achieve target concentration.[17] Anemia is one of the common clinical conditions found in pre-transplant chronic kidney disease and post-kidney transplant patients. Hematocrit or hemoglobin level is one of the plausible factors affecting interindividual variability of tacrolimus pharmacokinetics. The inverse relationship between tacrolimus clearance and hematocrit has been reported in some studies in kidney [18-20] and liver [21, 22] transplant recipients. However, other studies observed no significant relationship.[23,24] The coefficient of variation for same-sample hematocrit is greater than that for hemoglobin.[25] A change in plasma volume affects hematocrit level. Severity of anemia is, therefore, assessed best by measuring hemoglobin levels rather than hematocrit levels.

The purpose of this study was to determine whether hemoglobin levels affect the ratio of dose to trough whole-blood concentrations of tacrolimus (D/C_{trough}) of tacrolimus in Thai kidney transplant patients.

Materials and Methods

Patients

A retrospective analysis of data from 71 adult kidney transplant patients who came to follow up at King Chulalongkorn Memorial Hospital, Bangkok, Thailand during January 2009 to September 2011 was performed. Drug concentration data and relevant patient information were obtained at steady-state (between 1 - 6 months after initiation of tacrolimus) from clinical patient medication profiles and therapeutic drug monitoring records. A same daily dose of tacrolimus must be continued for at least 8 days before blood sample data were gathered to ensure that tacrolimus blood concentrations were under steady-state conditions. Patients with history of cirrhosis, patients with hepatocellular carcinoma or patients with the last hepatic enzyme greater than 3 times of upper normal limit value (AST > 120 U/L and ALT > 150 U/L) were excluded from this study. Additional exclusion criteria were patients who were receiving co-medications that could interfere the pharmacokinetics of tacrolimus (excepted prednisolone) i.e., rifampicin, amobarbital, phenobarbital, phenytoin, diltiazem, nifedipine, clarithromycin, erythromycin, telithromycin, clotrimazole, fluconazole, itraconazole, ketoconazole, voriconazole, protease inhibitors or sirolimus.

The study was obtained the clinical clearance by the Institutional Review Board of Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (COA No. 186/2011 IRB No. 042/54, Date of approval: April 7, 2011).

Drug Administration

Initially, all patients received oral tacrolimus (Prograf®) therapy as part of their triple immunosuppressive regimens comprising tacrolimus and prednisolone with either mycophenolate or azathioprine. During 1 to 6 months after initiation of tacrolimus therapy, mycophenolate or prednisolone were withdrawn in 5 patients because of the adverse events or coexisting illness. The recommended initial dose of tacrolimus was 0.05 mg/kg twice daily. Subsequent dose were adjusted on the basis of clinical evidence of efficacy and toxicity and to maintain tacrolimus trough blood concentrations between 4 and 10 ng/ml during the first year following transplantation. All patients were educated by a pharmacist in kidney transplant team to take tacrolimus at 8 am and 8 pm and avoid using any drugs that may interact with tacrolimus.

Analytical Methods

Whole blood tacrolimus concentrations were determined by chemiluminescent microparticle immunoassay (CMIA) method with the ARCHITECT system® I1000SR analyzer. According to manufacturer's information, a limit of quantification of assay was 0.8 ng/ml. The linear range of the assay was up to 30 ng/ml. Blood concentrations higher than 30 ng/ml were diluted according to the manufacturer's protocol. The intra and interassay coefficient of variation for tacrolimus concentrations at 5, 11, and 22 ng/ml were less than 10%. The mean recovery with tacrolimus concentrations at 6.9, 9.3, 15.2, and 18.8 ng/ml was 102% (98 to107%).

Statistical Analysis

The correlation between the ratio of dose to trough concentrations of tacrolimus and hemoglobin levels was analyzed using simple linear regression. The means of data

were compared by student's t-tests. The differences at p-value less than 0.05 were considered statistical significant.

Results

Drug concentration data and relevant patient information were gathered from 30 living and 41 cadaveric kidney transplant recipients. Their mean age was 45 years and their mean body weight was 59 kg. Forty-four (62%) patients were male. The characteristics of the patients are shown in Table 1.

All patients were administered oral tacrolimus every 12 hours. Data of tacrolimus blood concentration were collected only if the patients did not receive any operation, dialysis nor plasma exchange. The same daily doses of tacrolimus were continued for 37.72 ± 24.72 days (ranging from 8 to 123 days). The medication records were checked to ensure that the patients did not receive any medications that can interfere tacrolimus pharmacokinetics except prednisolone.

Table 1 Characteristics of patients and immunosuppressive treat-ments.

Characteristics	Mean ± SD (Range)
Age (years)	44.88 ± 10.09 (18.40-65.56)
Body weight (kg)	59.05 ± 11.88 (37.00-91.20)
Body mass index (kg/m^2)	21.99 ± 3.53 (16.01-31.63)
Number of renal transplant, n (%)	
First renal transplant	66 (93.0)
Second real transplant	5 (7.0)
Type of donor, n (%)	
Deceased donors	41 (57.7)
Living donors	30 (42.2)
Indication of tacrolimus therapy, n (%)	
Primary therapy	44 (62.0)
Rescue therapy	26 (36.6)
Intolerance therapy	1 (1.4)
Days postoperation (days)	98.00 (33.00-6,140.00)*
Duration of tacrolimus therapy (days)	91.72 ± 30.14 (34.00-194.00)
Biological and clinical data	
Hemoglobin (g/dl)	11.93 ± 2.01 (7.60-16.70)
Hematocrit (%)	37.30 ± 6.63 (22.90-55.70)
Albumin (n = 49 patients) (g/dl)	4.16 ± 0.31 (3.20-4.70)
Serum creatinine (mg/dl)	1.63 ± 0.69 (0.69-4.43)
Thai eGFR (mg/min/1.73 m^2)	61.73 ± 18.47 (19.75-114.75)
Associated treatments	
Mycophenolate mofetil (n = 46 patients) (mg/day)	1,239.13 ± 311.61 (750.00-2,000.00)
Mycophenolate sodium (n = 17 patients) (mg/day)	974.12 ± 169.08 (720.00-1,080.00)
Azathioprine (n = 4 patients) (mg/day)	75.00 ± 28.87 (50.00-100.00)
Prednisolone (n = 69 patients) (mg/day)	10.42 ± 5.45 (1.25-20.00)

* Days postoperation value are median (range).

Thai eGFR = 375.5 x Scr $^{(-0.848)}$ x Age $^{(-0.364)}$ x 0.712 (if female), where Thai eGFR is Thai estimated glomerular filtration rate (mg/min/1.73 m^2), Scr is serum creatinine (mg/dl) and Age is age of patient (years). [29]

Table 2 Characteristics of tacrolimus treatment.

Tacrolimus treatment	Mean ± SD (Range)
Maintenance dose of tacrolimus (mg/kg)	0.06 ± 0.37 (0.01-0.22)
Trough blood concentration (ng/ml)	7.87 ± 3.84 (1.90-19.45)
BW-adjusted D/C_{trough} (L/kg)	9.23 ± 7.05 (1.38-38.96)
Patients with low hemoglobin level (Hb < 12 g/dl) (n = 36)	11.10 ± 8.73**
Patients with normal hemoglobin level (Hb ≥ 12 /dl) (n = 35)	7.31 ± 4.05

D is the oral dose of tacrolimus (mg/kg), C_{trough} is the trough blood concentrations of tacrolimus (ng/ml) and BW is the body weight.

** *P*-value = 0.02, comparing with patients whose hemoglobin levels ≥ 12 g/dl

The mean dose of tacrolimus was 0.06 ± 0.37 mg/kg and the corresponding mean trough concentration was 7.87 ± 3.84 ng/ml as presented in Table 2. All blood concentrations were obtained near (less than 15 minutes before or after) to the trough (12 hours after previous dose).

Figures 1 and 2 show plots of observed trough blood concentrations against maintenance doses of tacrolimus and the relationship between hemoglobin levels and D/C_{trough} of tacrolimus, respectively. Statistical evaluation of the data indicated a negative significant correlations between D/C_{trough} of tacrolimus and hemoglobin levels (r = -0.41, P < 0.01). The relationship describing D/C_{trough} was body weight-adjusted D/C_{trough} (L/kg) = 26.378 – 1.437 Hgb while Hgb is hemoglobin levels in g/dl.

The ratio of dose to trough concentrations of tacrolimus in the patients with low hemoglobin levels (<12 g/dl) and those with normal hemoglobin levels (≥12 g/dl) were 11.10 ± 8.73 and 7.31 ± 4.05 L/kg, respectively with P = 0.02 as shown in Table 2.

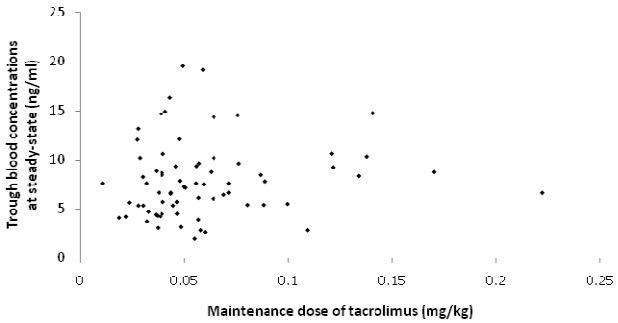

Figure 1 Scatter plot of observed trough blood concentrations at steady-state (ng/ml) and maintenances dose of tacrolimus (mg/kg) in 71 clinical kidney transplant patients.

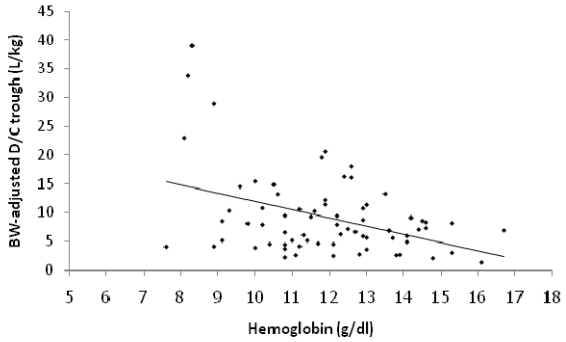

Figure 2 Relationship between hemoglobin levels (g/dl) and BW-adjusted D/C_{trough} of tacrolimus (L/kg) (r = -0.41, P < 0.01).

Discussions and Conclusions

The relationship between hemoglobin and tacrolimus clearance has not been previously reported. Effect of hematocrit on pharmacokinetics of tacrolimus has been demonstrated in adult kidney transplant patients.[18, 19] There was a negative correlation between hematocrit levels and the ratio of dose to trough concentrations of tacrolimus (r = -0.84, P = 0.02).[18] An inverse association between hematocrit levels and the drug apparent clearance were also found (r^2 = 0.24).[19] The relationship between hematocrit and clearance was as well reported in pediatric patients. The pediatric kidney transplant recipients with low-hematocrit level (< 33%) had higher tacrolimus clearance than those with high-hematocrit levels (≥ 33%) with statistical significance (p = 0.007).[20]

There are some concerns regarding the use of hematocrit as a monitoring parameter of anemia.[25] First, severity of anemia is assessed best by measuring hemoglobin levels rather than hematocrit levels. A low hematocrit reflects a low number of circulating red blood cells and is an indicator of a decrease in the oxygen-carrying capacity or of overhydration. A high hematocrit may reflect an absolute increase in the number of erythrocytes, or a decrease in plasma volume. Second, the coefficient of variation for same-sample hematocrit is greater than that for hemoglobin. Within-run and between-run coefficients of variation in automated analyzer measurement of hemoglobin are one half and one third those for hematocrit, respectively. The variability in this hematocrit measurement is greater because blood-sample storage conditions have no effect on hemoglobin measurement but hematocrit increases with storage temperature and duration. Third, the hematocrit is typically measured from a blood sample by an automated machine. Most of the machines, instead of directly measure, calculate hematocrit level based on the determination of the amount of hemoglobin and the average volume of the red blood cells. Moreover, hyperglycemia is related to an increase in MCV and elevates the hematocrit result.[25]

During 1-6 months (early stage) after transplantation, risks of rejection and infection are very high. Optimization of immunosuppressive therapy is, therefore, crucial. This study was conducted to determine a correlation between hemoglobin levels and ratio of dose to trough whole-blood concentrations of tacrolimus during the early stage of post-transplantation. The routine therapeutic drug monitoring data during 1-6 months after initiation of tacrolimus treatment from 71 Thai kidney transplant patients were collected, reviewed, and analyzed.

We observed the inverse relationship between hemoglobin levels and D/C_{trough} of tacrolimus (r = -0.41, P < 0.01). The association could be described as D/C_{trough} (L/kg) = 26.378 – 1.437 Hgb while Hgb is hemoglobin levels in g/dl (r^2 = 0.17). It was also found that the ratio of D/C_{trough} of tacrolimus was significantly higher in the patients with hemoglobin <12 g/ml than those with normal hemoglobin ≥ 12 g/ml (P = 0.02).

The ratio of dose to trough whole-blood concentrations of tacrolimus was considered to be proportional to the total body clearance and was referred to as relative clearance at steady state in several studies.[18,21,26] One possible explanation for the higher ratio of dose to trough concentrations of tacrolimus among patients with decreased hemoglobin could be changed in unbound fraction. Since tacrolimus is extensively bound to erythrocytes, resulting in the distribution ratio of 35 (range 12 to 67) for tacrolimus whole blood concentrations to plasma concentrations.[16] And tacrolimus is a low hepatic clearance drug with the extraction ratio about 3% of hepatic blood flow.[17,18] Tacrolimus clearance is influenced by changes in its unbound fraction and hepatic enzyme activity (intrinsic clearance).[17,27] Low hemoglobin level probably results in a reduced fraction of tacrolimus bound to erythrocytes and an increased plasma unbound fraction, which was more metabolized by the liver.

The drug's clearance is an important factor determining the oral maintenance dosage which produce a desired average plasma concentration at steady state.[28] Knowing the influence of hemoglobin on D/C_{trough} may be used as a guide in the adjustment of tacrolimus dose. Patients with lower

levels of hemoglobin would need higher daily tacrolimus dosage to achieve the target level for prophylaxis of renal allograft rejection. When hemoglobin levels decreased, the ratio of dose to trough concentrations was, thereby, increased, higher tacrolimus doses might be required to maintain the target blood concentrations. In patients whose hemoglobin level is changing, their tacrolimus levels should be monitored and dosage adjustments may be required to maintain steady-state blood concentrations within their target ranges.

A plot of observed steady-state trough blood concentrations against maintenance doses of tacrolimus (Figure 1) shows non-linear relationship between doses and tacrolimus steady-state trough concentrations (r = 0.10, *P* = 0.40). This may partially explained that tacrolimus exhibits highly interpatient variability in its pharmacokinetics. Therapeutic drug monitoring is necessary for optimal prevention of renal transplant rejection.

It should be emphasized the clearance we discussed here are the clearance of total drug (both bound and unbound forms); however, the unbound clearance of the low hepatic extraction drug is not affected by the change of unbound fraction, thus, careful interpretation of this results would be prudent. The total concentration of tacrolimus may be observed to be lower; however, with the lower concentration of hemoglobin resulting in the higher unbound fraction, thus, the unbound concentration may not be as low as we estimate from the total concentration of tacrolimus.

D/C_{trough} of tacrolimus, though is significantly correlated with hemoglobin levels, might be influenced by different factors. In order to describe other factors, such as various demographic, hematological, biochemical parameters and genetic polymorphisms of the drug metabolizing enzymes and drug transporters that affect the variability in tacrolimus pharmacokinetics in Thai kidney transplant patients, the population pharmacokinetics study of tacrolimus including these factors is needed in future studies.

In conclusion, we demonstrated the relationship between hemoglobin levels and the ratio of dose to trough concentrations of tacrolimus by using clinical data from Thai kidney transplant patients. To modify tacrolimus dosage in renal transplant patients, we should consider hemoglobin levels as one factor influencing drug levels.

Acknowledgements

This study was financially supported by CU graduate School Thesis Grant from Graduate School, Chulalongkorn University, Bangkok, Thailand. We also would like to thank Sarin Watanatorn, RN and Napaporn Boonnaj, MS of the Division of Nephrology for their helpful cooperation. The authors have no conflict of interest to declare.

References

1. Webster AC, Woodroffe RC, Taylor RS, et al. Tacrolimus versus ciclosporin as primary immunosuppression for kidney transplant recipients: meta-analysis and meta-regression of randomised trial data. *BMJ* 2005;331(7520):810.
2. Ekberg H, Tedesco-Silva H, Demirbas A, et al. Reduced exposure to calcineurin inhibitors in renal transplantation. *N Engl J Med* 2007 20;357(25):2562-2575.
3. Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group. KDIGO Clinical practice guideline for the care of kidney transplant recipients. *Am J Transplant* 2009;9(Suppl 3):S1-S155.
4. Venkataramanan R, Swaminathan A, Prasad T, et al. Clinical pharmacokinetics of tacrolimus. *Clin Pharmacokinet* 1995; 29(6):404-430.
5. Christians U, Pokaiyavanichkul T, Chan L. Tacrolimus. In: Burton ME, Schentag JJ, Shaw LM, et al. (ed.). Applied pharmacokinetics and pharmacodynamics: Principles of therapeutic drug monitoring. 4th ed. United States of America. Lippincott Williams & Wilkins, 2006: pp.529-561.
6. Staatz CE, Willis C, Taylor PJ, et al. Population pharmacokinetics of tacrolimus in adult kidney transplant recipients. *Clin Pharmacol Ther* 2002;72(6):660-669.
7. Jusko WJ, Piekoszewski W, Klintmalm GB, et al. Pharmacokinetics of tacrolimus in liver transplant patients. *Clin Pharmacol Ther* 1995; 57(3):281-290.
8. Ringe B, Braun F, Schutz E, et al. A novel management strategy of steroid-free immunosuppression after liver transplantation: efficacy and safety of tacrolimus and mycophenolate mofetil. *Transplantation* 2001;71(4):508-515.
9. Staatz C, Taylor P and Tett S. Low tacrolimus concentrations and increased risk of early acute rejection in adult renal transplantation. *Nephrol Dial Transplant* 2001;16:1905-1909.
10. Backman L, Nicar M, Levy M, et al. FK506 trough levels in whole blood and plasma in liver transplant recipients: correlation with clinical events and side effects. *Transplantation* 1994;57(4): 519-525.
11. Kershner R, Fitzsimmons W. Relationship of FK506 whole blood concentrations and efficacy and toxicity after liver and kidney transplantation. *Transplantation* 1996;62(7):920-926.

12. Laskow D, Vincenti F, Neylan J, et al. An open-label, concentration-ranging trial of FK506 in primary kidney transplantation: a report of the United States Multicenter FK506 Kidney Transplant Group. *Transplantation* 1996;62(7):900-905.
13. Przepiorka D, Nash RA, Wingard JR, et al. Relationship of tacrolimus whole blood levels to efficacy and safety outcomes after unrelated donor marrow transplantation. *Biol Blood Marrow Transplant* 1999;5(2):94-97.
14. Winkler M, Pichlmayr R, Neuhaus P, et al. Optimal FK 506 dosage in patients under primary immunosuppression following liver transplantation. *Transpl Int* 1994;7(Suppl 1):S58-S63.
15. Micromedex® 2.0. Tacrolimus. [Online]: Thomson Reuters 1974-2012 (Acessed on March 11, 2012 at http://www.thomsonhc.com)
16. Quan DJ, Winter ME. Immuosuppressants: cyclosporine, tacrolimus and sirolimus. In: Winter ME (ed.). Basic clinical pharmacokinetics. 5th ed. China. Lippincott Williams & Wilkins, 2010: pp.250-276.
17. Bauer LA. Applied clinical pharmacokinetics. 2nd ed. United States of America. The McGraw-Hill Companies, 2008.
18. Undre NA, Schafer A. Factors affecting the pharmacokinetics of tacrolimus in the first year after renal transplantation. *Transplant Proc* 1998;30(4):1261-1263.
19. Benkali K, Premaud A, Picard N, et al. Tacrolimus population pharmacokinetic-pharmacogenetic analysis and Bayesian estimation in renal transplant recipients. *Clin Pharmacokinet* 2009;48(12):805-816.
20. Zhao W, Elie V, Rousset G, et al. Population pharmacokinetics and pharmacogenetics of tacrolimus in de novo pediatric kidney transplant recipients. *Clin Pharmacol Ther* 2009;86(6):609-618.
21. Minematsu T, Sugiyama E, Kusama M, et al. Effect of hematocrit on pharmacokinetics of tacrolimus in adult living donor liver transplant recipients. *Transplant Proc* 2004;38:1506-1511.
22. Zahir H, McLachlan AJ, Nelson A, et al. Population pharmacokinetic estimation of tacrolimus apparent clearance in adult liver transplant recipients. *Ther Drug Monit* 2005;27:422-430.
23. Sam WJ, AW M, Quak SH, et al. Population pharmacokinetics of tacrolimus in Asian paediatric liver transplant patients. *Br J Clin Pharmacol* 2000;50:531-541.
24. Antignac M, Hulot JS, Boleslawski E, et al. Population pharmaco-kinetics of tacrolimus in full liver transplant patients: modelling of post-operative clearance. *Eur J Clin Pharmacol* 2005;61:409-416.
25. National Kidney Foundation. KDOQI Clinical practice guidelines and clinical practice recommendations for anemia in chronic kidney disease. Am *J Kidney Dis* 2006;47(Suppl 3):s1-s145.
26. Hu RH, Lee PH, Tsai MK. Clinical influencing factors for daily dose, trough level, and relative clearance of tacrolimus in renal transplant recipients. *Transplant Proc* 2000;32:1689-1692.
27. Shargel L, Wu-Pong S, Yu ABC. Applied biopharmaceutics and pharmacokinetics. 5th ed. New York. McGraw-Hill; 2005.
28. Winter ME. Basic clinical pharmacokinetics. 5th ed. China. Lippincott Williams & Wilkis, 2010.
29. Praditpornsilpa K, Townamchai N, Chawatanarat T, et al. The need for robust validation for MDRD-based glomerular filtration rate estimation in various CKD populations. *Nephrol Dial Transplant* 2011;26(9):2780-2785.

Editorial note
Manuscript received in original form on March 1, 2012;
accepted in final form on July 2, 2012