

PHARMACOGENETICS AND CIRCADIAN EXPOSURE OF TACROLIMUS AND ITS IMPACT ON THE RENAL TRANSPLANT OUTCOME: A WAY TO A PERSONALIZED MEDICINE FOR TACROLIMUS

Shigeru Satoh^{1,2,5)}, Mitsuru Saito¹⁾, Kazuyuki Numakura¹⁾, Yoshio Miura¹⁾, Takamitsu Inoue¹⁾, Shinya Maita¹⁾, Takashi Obara¹⁾, Hiroshi Tsuruta¹⁾, Shintaro Narita¹⁾, Yohei Horikawa¹⁾, Norihiko Tsuchiya¹⁾, Tomonori Habuchi¹⁾, Yoko Mitobe^{1,2)}, Atsushi Komatsuda³⁾, Hideaki Kagaya⁴⁾ and Masatomo Miura⁴⁾

Abstract

Since January 2011, we have started to a personalized medicine for controlled-release tacrolimus, once-daily oral formulation of tacrolimus, based on the *CYP3A5* polymorphisms. Tacrolimus has a narrow therapeutic range, and its pharmacokinetics differs greatly among individuals. Many factors may affect the pharmacokinetics of tacrolimus, including *CYP3A5* genotypes. Patients with *CYP3A5*1* allele (*CYP3A5* expressers) require a higher daily tacrolimus dose than those with *CYP3A5*3/*3* genotype (non-expressers) in order to maintain the target trough level. Recently, we investigated the increase in renal cortical interstitial fibrosis (IF) from 0-hour to 1-year post-transplantation using an automated digital analysis of biopsy sections and assessed the relative risk of developing IF based on clinical characteristics, laboratory data, tacrolimus-based immunosuppressive regimens, and the *CYP3A5* polymorphism. In a multivariate analysis, *CYP3A5* non-expression correlated with the development of IF. The mean tacrolimus trough concentrations in the early stages after transplantation were unexpectedly higher among non-expressers than *CYP3A5* expressers, despite therapeutic drug monitoring. This unexpectedly high tacrolimus levels in non-expressers might influence the development of IF.

Before starting the personalized medicine for controlled-release tacrolimus, we analyzed circadian pharmacokinetics and pharmacogenetics of twice daily tacrolimus, and its association with transplant outcome. We briefly reviewed our clinical research into tacrolimus.

Hirosaki Med. J. 64, Supplement : S19—S27, 2013

Key words: tacrolimus; *CYP3A5* polymorphism; pharmacokinetics; circadian exposure; interstitial fibrosis

Introduction

Tacrolimus is widely used to prevent rejection following organ transplantation. Since a low blood concentration of this drug is one of the factors responsible for acute rejection, while a high blood concentration induces adverse effects such as hypertension, hyperglycemia, and nephropathy, it is important to determine

the appropriate tacrolimus dose, particularly in the early stages of transplantation¹⁾. However, tacrolimus has a narrow therapeutic range, and its pharmacokinetics differs greatly among individuals¹⁻³⁾. Therefore, daily doses must be adjusted according to whole-blood trough concentrations.

Many factors may affect the pharmacokinetics of tacrolimus, including genetic factors.

¹⁾Department of Urology and ²⁾Division of Renal Replacement Therapeutic Science, Akita University School of Medicine, Akita, Japan

³⁾Department of Nephrology and Rheumatology, Akita University School of Medicine, Akita, Japan

⁴⁾Department of Pharmacy, Akita University Hospital, Akita, Japan

Address correspondence to: Shigeru Satoh, M.D., Ph.D., Division of Renal Replacement Therapeutic Science, Department of Urology, Akita University School of Medicine, 1-1-1 Hondo, Akita 010-8543, Japan.

Fax: 81-18-836-2619, Phone: 81-18-884-6239

E-mail: shigerus@doc.med.akita-u.ac.jp

Tacrolimus is a substrate of cytochrome P450 3A (CYP3A), and much of the inter-individual variability in its pharmacokinetics is explained by the presence of a single nucleotide polymorphism in intron 3 of the *CYP3A5* 6986A>G, resulting in the absence of a functional CYP3A5 protein in homozygous carriers (*CYP3A5**3/*3)^{1, 4)}. Studies have shown that the dose-adjusted trough level and area under the blood concentration-time curve (AUC) were lower in carriers of the *CYP3A5**1 allele (CYP3A5 expressers) than in individuals with the *CYP3A5**3/*3 genotype (non-expressers)^{1, 5-8)}. Therefore, CYP3A5 expressers need a larger dose of tacrolimus to reach target trough levels than non-expressers. However, impact of *CYP3A5* pharmacogenetics on the transplant outcome has not yet been clarified.

Furthermore, circadian variations in the pharmacokinetics of tacrolimus are controversial⁹⁻¹²⁾.

Herein, we briefly reviewed our clinical research into tacrolimus pharmacogenetics^{1, 7, 13)}, circadian pharmacokinetics^{12, 14)}, and its association with transplant outcome^{15, 16)}.

***CYP3A5* polymorphism**

The *CYP3A5* gene located at 7p21 harbors an important single nucleotide polymorphism (A6986G) in intron 3, of which A and G alleles are designated as *CYP3A5**1 and *CYP3A5**3, respectively. The *CYP3A5**3 created a cryptic acceptor splice site and transcribes the variant mRNA (*SVI-CYP3A5*) having an excess of 131bp between exon 3 and exon 4^{1, 17)}. The protein translated from the *SVI-CYP3A5* mRNA is truncated at amino acid 102 due to a premature stop codon and only a small amount of complete CYP3A5 protein is translated from the wild type *CYP3A5* (*wt-CYP3A5*) mRNA¹⁷⁾. Thus, livers with *CYP3A5**1/*3 express 4 times higher and 10 times lower *wt-CYP3A5* mRNA than livers with *CYP3A5**3/*3 and *CYP3A5**1/*1, respectively¹⁸⁾.

A higher CYP3A5 protein concentration in the liver was also reportedly associated with the *CYP3A5**1 allele¹⁷⁾. CYP3A5 is also the major enzyme for tacrolimus in the small intestine and its expression is believed to be responsible for the decreased tacrolimus bioavailability¹⁹⁾.

***CYP3A5* pharmacogenetics of tacrolimus**

Previous studies had mentioned the effect of the gene polymorphism on the blood concentration of tacrolimus, but not other pharmacokinetic parameters, except for the trough levels, have been analyzed^{5, 20, 21)}. In 2004, we reported that the association of *CYP3A5* polymorphisms with the pharmacokinetics of tacrolimus in renal transplant recipients¹⁾. On 28 days after transplantation, we observed more than a 1.5-fold difference of the body weight-adjusted daily tacrolimus dose in Japanese renal allograft recipients. Pharmacokinetic analysis demonstrated that CYP3A5 expressers required an increased dose of tacrolimus to achieve the optimal trough levels and AUC₀₋₁₂ compared with non-expressers. CYP3A5 expressers showed two thirds of the dose-adjusted trough levels, C_{max} and AUC₀₋₁₂ compared with non-expressers. Our data also demonstrated that there was no significant difference in AUC₀₋₁₂ when targeting the same trough levels in the two groups. These findings suggested that the tacrolimus dose should be optimized according to the *CYP3A5* genotype of each recipient, but the same target trough level can be used as an index of drug exposure despite the different genotype.

Other polymorphisms and tacrolimus pharmacokinetics

Tacrolimus is a substrate of CYP3A4, CYP3A5, p-glycoprotein, which are encoded by *CYP3A4*, *CYP3A5*, and multidrug resistance

1 (*MDR1*) genes, respectively. However, our studies showed that the *MDR1* (*ABCB1*) *C3435T* polymorphism was not an important factor in tacrolimus pharmacokinetics^{1,7}.

Recently, we investigated the impact of the *CYP3A4**1/**IG* polymorphism compared with *CYP3A5* genotypes on the dose-adjusted pharmacokinetics of tacrolimus¹³. Hessenlink et al.⁵ reported that dose-adjusted trough levels of tacrolimus were lower in patients with the *CYP3A4***IB* (-290A>G) allele than those with the *CYP3A4**1/*1 genotype (wild-type). However, the frequency of polymorphisms in *CYP3A4**1/**IB* is quite low in Asian populations²² and was zero among our subjects¹⁵.

To date, more than 40 SNPs of the *CYP3A4* gene have been published on the Human CYP Allele Nomenclature Committee's homepage. In a study of SNPs and haplotype frequencies of *CYP3A4* in a Japanese population, Fukushima-Uesaka et al.²³ found 24 SNPs including 17 novel ones, and that the most common SNP was the *CYP3A4**1/**IG* polymorphism, 20230G>A, within intron 10 of the *CYP3A4* gene. They also found that the *CYP3A4***IG* haplotype was strongly, but not completely, linked to the *CYP3A5**1 haplotype²³. Therefore, we hypothesized that the *CYP3A4***IG* allele might be associated with the pharmacokinetics of tacrolimus and affect inter-individual differences in combination with the *CYP3A5**1/*3 polymorphism. We found that there were significant differences in the dose-adjusted AUC_{0-12} and C_0 of tacrolimus between patients with the *CYP3A4**1/*1 genotype and those with the **IG* allele, however, in a multivariate analysis, the contribution of *CYP3A4**1/**IG* to the pharmacokinetics of tacrolimus was about 2-fold less than that of the *CYP3A5**1/*3 polymorphism. The dose-adjusted AUC_{0-12} of tacrolimus was lower in *CYP3A5* expresser with the *CYP3A4***IG* allele than those with the *CYP3A4**1/*1 genotype, but did not differ among the non-expressers. Although its effect on *CYP3A4* activity is not

clear, *CYP3A4**1/**IG* might contribute the inter-individual difference in the pharmacokinetics of tacrolimus, especially among *CYP3A5* expressers¹³.

Circadian pharmacokinetics of tacrolimus and *CYP3A5* genotype

Twice daily tacrolimus is generally administered in two equally divided doses every 12 hr, and the concentrations of tacrolimus are routinely measured and the administered doses are adjusted according to the target trough level^{12, 24-26}. Transplant clinicians generally assume that the equivalent peak concentrations and AUCs are obtained after each dose of tacrolimus²⁴. However, circadian variations in the pharmacokinetics of tacrolimus are controversial^{12, 24-26}. Moreover, there had been no available reports regarding differences in the circadian pharmacokinetics of tacrolimus between the early stage and maintenance stage beyond 1-yr after transplantation with the same designated-time administration strategy. Therefore, we investigated whether the pharmacokinetics of tacrolimus shows circadian variation with the same designated-time administration strategy and also compared the influences of *CYP3A5* polymorphisms on the pharmacokinetics in the maintenance stage (beyond 1-yr) to those in the early stage (day 28)¹⁴.

The daily dose of tacrolimus was equally divided into two fractions given every 12 hr at a designated time (9:00 and 21:00 hrs). Most of the pharmacokinetic parameters did not differ significantly between daytime and nighttime in the early or maintenance stage. Since the dose of tacrolimus in the maintenance stage was significantly decreased compared to that in the early stage, both daytime and nighttime AUC_{0-12} were smaller in the maintenance than early stage. There were no significant differences

between the daytime and nighttime AUC_{0-12} of each *CYP3A5* genotype group in either the early or maintenance stage.

A few studies have reported that tacrolimus pharmacokinetics showed circadian variation^{24,26)}. Min *et al.*²⁵⁾ and Iwahori *et al.*²⁶⁾ reported that the AUC_{0-12} of tacrolimus was significantly greater, C_{max} was higher, and t_{max} was shorter after the morning dose than after the evening dose in 12 stable liver and 11 kidney allograft recipients, respectively, in the early stage after transplantation. In the maintenance state, Hardinger *et al.*²⁴⁾ also showed a greater tacrolimus AUC_{0-12} (117 vs. 97 ng·hr/mL) and two-fold higher C_{max} (17.8 vs. 8.4 ng/mL) after the morning dose than after the evening dose. However, our study showed that tacrolimus concentration-time profiles in the nighttime closely resembled those in the daytime¹⁴⁾.

These circadian pharmacokinetic differences in each study might result from the interval between tacrolimus administration and meal consumption because the tacrolimus AUC was smaller after meals than during fasting. Hardinger *et al.*²⁴⁾ designed their study so that food was available from 2.5 to 3 hours prior to the evening dose and fasting occurred for 10 hours prior to the morning dose. In that study, breakfast was provided 2 hr after the morning dose of tacrolimus at 10:00 hr, lunch at noon, and dinner at 17:00 hr. In our study, morning doses were given 1.5 hours after breakfast, whereas nighttime doses were given 3 hours after the evening meal during both the early and maintenance stages after transplantation. Breakfast was provided at 7:30, lunch at noon, and dinner at 18:00 hr. Based on previous findings as well as our own study, the interval between the consumption of food and administration of tacrolimus may play a role in the circadian variation of tacrolimus pharmacokinetics^{12, 14, 24)}.

***CYP3A5* genotypes and early and late transplant outcome**

With regard to the impact of *CYP3A5* polymorphisms on tacrolimus trough concentrations and transplant outcome, interesting studies have been reported. MacPhee *et al.*²⁷⁾ assessed the time taken to achieve tacrolimus target concentrations in 178 renal transplant recipients. Although the immunosuppressive regimen in the 178 recipients was not identical, the target concentrations were 15-20 µg/L (the same as to ng/mL in our study) during the first 7 days, then 10-15 µg/L up to 3 months after transplantation. Their standard protocol for tacrolimus-dosing was to give an initial oral dose of 0.1 mg/kg twice daily. In their study, despite the use of therapeutic drug monitoring (TDM), *CYP3A5* expressers had significantly lower mean tacrolimus trough concentrations during the first 2 weeks after transplantation and experienced a delay in achieving target concentrations. Although the overall rate of biopsy-confirmed acute rejection (AR) did not differ, AR episodes occurred earlier in *CYP3A5* expressers compared with nonexpressers²⁷⁾.

Furthermore, with regard to the impact of *CYP3A* polymorphisms on long-term tacrolimus disposition and drug-related toxicity, Kuypers *et al.*²⁸⁾ recently reported that the *CYP3A4*1/CYP3A5*1* and *CYP3A4*1B/CYP3A5*1* genotypes were significantly more frequently associated with the development of biopsy-confirmed tacrolimus-related nephrotoxicity than the *CYP3A4*1/CYP3A5*3* genotype. However, the association between *CYP3A5*1* allele and chronic allograft nephropathy (CAN) is poorly documented.

These reported associations of *CYP3A5* polymorphisms with tacrolimus trough concentrations and the frequency of biopsy-confirmed AR or CAN had not been confirmed with a different targeting concentration strategy

and/or administration route of tacrolimus, or different ethnics yet. We retrospectively assessed whether *CYP3A5* polymorphisms influence tacrolimus trough concentrations adjusted with TDM and the frequency of biopsy-confirmed AR at 1 month and biopsy-confirmed CAN at 1 year after renal transplantation in Japanese recipients under our targeting tacrolimus trough concentration strategy, comparing results with previous reports²⁷⁻³⁰.

The previously reported results showing the association of the *CYP3A5 *1* allele with the early occurrence of AR episodes²⁷ was not found in our study¹⁵. The frequencies of biopsy-confirmed subclinical AR were 15.8% and 36.4% in *CYP3A5* expressers and nonexpressers, respectively, which were lower than the previous report showing over 40%^{15, 27}. Our initial dosing and targeting concentration strategy for tacrolimus might reduce the frequency of AR.

MacPhee et al.²⁷ indicated that AR episodes occurred earlier in *CYP3A5* expressers. They suggested that lower tacrolimus blood concentrations early after transplantation were associated with episodes of AR occurring earlier in *CYP3A5* expressers. However, their study involved 44 cases with AZA, 26 with MMF, and the remainder without AZA or MMF. To assess the association between *CYP3A5* polymorphism and the occurrence of AR, an identical immunosuppressive regimen with same drugs and same targeting blood concentration of tacrolimus should be adopted. Indeed, comparing AZA, MMF 1g/day, and MMF 2g/day groups, the incidence rates of biopsy-confirmed AR at 1 year were 32.2%, 32.2 %, and 8.6%, respectively³¹. From this point of view, although the number of subjects was small, our study design involving highly selected patients may have been adequate to assess the association of *CYP3A5* polymorphism with the frequency of AR episodes.

Interestingly, the prevalence of recipients with

subclinical progressive CAN was significantly higher in *CYP3A5* nonexpressers (45.5%) compared to that in *CYP3A5* expressers (10.5%) in our (p=0.019). Although the incidence of CAN is related to the timing of the protocol biopsy, varying from 25 to 50% at 1 yr, the progression from normal histology to CAN or worsening of CAN grade occurs mainly within the first year after transplantation³². A number of immune and non-immune risk factors have been identified that appear to predispose patients to the development of CAN. With regard to the immunosuppressive protocol, calcineurin inhibitor-based immunosuppressive regimens correlated with the development of CAN³³. However, there had been no available studies indicating whether tacrolimus exposure and *CYP3A5* polymorphisms were associated with the development of CAN.

Kuypers et al.²⁸ reported that *CYP3A4*1/CYP3A5*1* and *CYP3A4*1B/CYP3A5*1* genotypes were significantly more frequently associated with the development of biopsy-confirmed tacrolimus-related nephrotoxicity than the *CYP3A4*1/CYP3A5*3* genotype. Tacrolimus dose requirements and apparent oral clearance in recipients with the *CYP3A4*1/CYP3A5*1* and *CYP3A4*1B/CYP3A5*1* genotypes were associated with persistent significantly lower dose-corrected exposure and more frequent development of biopsy-proven tacrolimus-related nephrotoxicity within 5 years after transplantation in their study²⁸. They speculated that tacrolimus nephrotoxicity could be the result of higher systemic or tissue concentrations of toxic metabolites produced by these *CYP3A* enzymes²⁸.

While tacrolimus is a substrate of 3A4 and 3A5, the frequency of polymorphisms in the *CYP3A4* is quite low in Asian populations²² was not found in our subjects. Accordingly, we could not discuss the impact of *CYP3A4* polymorphism on the frequency of subclinical

CAN. Although tacrolimus-related nephrotoxicity is a cause of CAN, it is difficult to distinguish the causes of advanced interstitial fibrosis (IF) /tubular atrophy (TA). At least our results suggested that the *CYP3A5* *1 allele was not associated with the development of subclinical advanced CAN. In our study, the *CYP3A5* *3/*3 genotype was associated with biopsy-confirmed subclinical CAN. The mean tacrolimus trough concentrations of *CYP3A5* non-expressers in the maintenance stage after transplantation were unexpectedly higher than those of *CYP3A5* expressers, despite TDM between 5 and 10 ng/mL. This unexpected results and our higher blood concentrations strategy of tacrolimus might have been associated with the development of advanced CAN in *CYP3A5* nonexpressers in our study¹⁵.

Interstitial fibrosis and *CYP3A5* pharmacogenetics

IF is the main histopathological feature of chronic allograft injury (CAI)³⁴. Although IF, TA, fibrointimal hyperplasia of vessels, and glomerulosclerosis can all occur during CAI³⁵, the degree of IF has shown the best correlation with clinical outcome³⁶.

Histopathologic findings are usually graded using the Banff 05 and 07 classifications^{37, 38}. However, IF/TA is scored semiquantitatively using the Banff system making a precise quantification difficult. The automated computerized digital analysis of biopsy sections, stained by various methodologies to reveal fibrotic tissue, has been reported by a number of groups^{34, 36, 39, 40}. Although these studies measured the extent of IF in the cortex at several time-points after transplantation, they didn't report quantitative measurements of IF in donor kidney at the cold preservation time immediately before transplantation (0-hour or time-zero biopsy). Mancilla *et al.*⁴¹ reported that

there were significant correlations between time-zero biopsy and clinical pre-donation parameters such as the age and serum creatinine (SCr) level of donors before transplantation. Percent IF (%IF), as a measure of the allograft cortical area affected by IF, at 0-hour may influence %IF at 1-month and 1-year posttransplantation. Therefore, we postulated that increases in %IF from the 0-hour to the 1-month and 1-year biopsy might reflect the actual rate of increase in IF in the allograft after transplantation.

CAI is a multifactorial process based on immunologic and nonimmunologic factors, such as elderly donors, delayed graft function, AR, cytomegalovirus infection and BK nephropathy, cardiovascular disease, metabolic disorders including hypertension, hyperlipidemia, and diabetes mellitus, no use of angiotensin II receptor blockers, and immunosuppressive regimens with calcineurin inhibitors (CNIs)^{34, 42-52}. However, the association of the *CYP3A5* genotype with renal transplant outcome had not been clarified.

We study investigated the increase in renal cortical IF from 0-hour to 1-year posttransplantation (%IF) using an automated digital analysis of biopsy sections in living renal transplant recipients and assessed the relative risk of developing IF based on clinical characteristics, laboratory data, tacrolimus-based immunosuppressive regimens, and the *CYP3A5* polymorphism. We found that %IF increased about 1.7 and 2.2-fold from 0-hour to 1-month and 1-year posttransplantation, respectively. In a multivariate analysis, *CYP3A5* non-expression correlated with the development of IF. The mean tacrolimus trough concentrations in the early stages after transplantation were unexpectedly higher among non-expressers than *CYP3A5* expressers, despite TDM. This unexpectedly high tacrolimus levels in non-expressers might influence the development of IF, because CNIs have a significant adverse impact on renal

function and induce a fibrogenic response that may lead to scarring of the renal allograft¹⁶⁾.

Conclusions

Our studies suggested that a new regimen with lower and narrow target trough levels of tacrolimus or a dosing strategy based on the *CYP3A5* genotype is needed to assess the association between the *CYP3A5* polymorphism, exposure to tacrolimus, and the development of IF.

REFERENCES

- 1) Tsuchiya N, Satoh S, Tada H, et al. Influence of CYP3A5 and MDR1 (ABCB1) polymorphisms on the pharmacokinetics of tacrolimus in renal transplant recipients. *Transplantation* 2004;78:1182-7.
- 2) Venkataramanan R, Swaminathan A, Prasad T et al. Clinical pharmacokinetics of tacrolimus. *Clin Pharmacokine*. 1995;29:404-30.
- 3) Staatz CE, Tett SE. Clinical pharmacokinetics and pharmacodynamics of tacrolimus in solid organ transplantation. *Clin Pharmacokinet* 2004;43:623-53.
- 4) Haufroid V, Wallemacq P, VanKerckhove V, et al. CYP3A5 and ABCB1 polymorphisms and tacrolimus pharmacokinetics in renal transplant candidates: guidelines from an experimental study. *Am J Transplant* 2006;6:2706-13.
- 5) Hesselink DA, van Schaik RH, van der Heiden IP, et al. Genetic polymorphisms of the CYP3A4, CYP3A5, and MDR-1 genes and pharmacokinetics of the calcineurin inhibitors cyclosporine and tacrolimus. *Clin Pharmacol Ther* 2003;74:245-54.
- 6) Roy JN, Barama A, Poirier C, Vinet B, Roger M. Cyp3A4, Cyp3A5, and MDR-1 genetic influences on tacrolimus pharmacokinetics in renal transplant recipients. *Pharmacogenet Genomics* 2006;16:659-65.
- 7) Tada H, Tsuchiya N, Satoh S, et al. Impact of CYP3A5 and MDR1 (ABCB1) C3435T polymorphisms on the pharmacokinetics of tacrolimus in renal transplant recipients. *Transplant Proc* 2005;37:1730-2.
- 8) Haufroid V, Mourad M, Van Kerckhove V, et al. The effect of CYP3A5 and MDR1 (ABCB1) polymorphisms on cyclosporine and tacrolimus dose requirements and trough blood levels in stable renal transplant patients. *Pharmacogenetics* 2004;14:147-54.
- 9) Hardinger KL, Park JM, Schnitzler MA, Koch MJ, Miller BW, Brennan DC. Pharmacokinetics of tacrolimus in kidney transplant recipients: twice daily versus once daily dosing. *Am J Transplant* 2004;4:621-5.
- 10) Min DI, Chen HY, Lee MK, Ashton K, Martin MF. Time-dependent disposition of tacrolimus and its effect on endothelin-1 in liver allograft recipients. *Pharmacotherapy* 1997;17:457-63.
- 11) Iwahori T, Takeuchi H, Matsuno N, et al. Pharmacokinetic differences between morning and evening administration of cyclosporine and tacrolimus therapy. *Transplant Proc* 2005;37:1739-40.
- 12) Tada H, Satoh S, Iinuma M, et al. Chronopharmacokinetics of tacrolimus in kidney transplant recipients: occurrence of acute rejection. *J Clin Pharmacol* 2003;43:859-65.
- 13) Miura M, Satoh S, Kagaya H, et al. Impact of the CYP3A4*1G polymorphism and its combination with CYP3A5 genotypes on tacrolimus pharmacokinetics in renal transplant patients. *Pharmacogenetics* 2011;12:977-84.
- 14) Satoh S, Kagaya H, Saito M et al. Lack of tacrolimus circadian pharmacokinetics and CYP3A5 pharmacogenetics in the early and maintenance stages in Japanese renal transplant recipients. *Br J Clin Pharmacol* 2008;66:207-14.
- 15) Satoh S, Saito M, Inoue T, et al. CYP3A5*1 allele associated with tacrolimus trough concentrations but not subclinical acute rejection or chronic allograft nephropathy in Japanese renal transplant recipients. *Eur J Clin Pharmacol* 2009;65:473-81.
- 16) Miura Y, Satoh S, Saito M, et al. Factors increasing quantitative interstitial fibrosis from 0 hr to 1 year in living kidney transplant patients receiving

- tacrolimus. *Transplantation* 2011;91:78-85.
- 17) Kuehl P, Zhang J, Lin Y, et al. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nat Genet* 2001;27:383-91.
 - 18) Lin YS, Dowling AL, Quigley SD, et al. Co-regulation of CYP3A4 and CYP3A5 and contribution to hepatic and intestinal midazolam metabolism. *Mol Pharmacol* 2002;62:162-72.
 - 19) Lampen A, Christians U, Guengerich FP, et al. Metabolism of the immunosuppressant tacrolimus in the small intestine: cytochrome P450, drug interactions, and interindividual variability. *Drug Metab Dispos* 1995;23:1315-24.
 - 20) Thervet E, Anglicheau D, King B, et al. Impact of cytochrome P450 3A5 genetic polymorphism on tacrolimus doses and concentration-to-dose ratio in renal transplant recipients. *Transplantation* 2003;76:1233-5.
 - 21) Macphee IA, Fredericks S, Tai T, et al. Tacrolimus pharmacogenetics: polymorphisms associated with expression of cytochrome p4503A5 and P-glycoprotein correlate with dose requirement. *Transplantation* 2002;74:1486-9.
 - 22) Lamba JK, Lin YS, Schuetz EG, Thummel KE. Genetic contribution to variable human CYP3A-mediated metabolism. *Adv Drug Deliv Rev* 2002;54:1271-94.
 - 23) Fukushima-Uesaka H, Saito Y, Watanabe H et al. Haplotypes of CYP3A4 and their close linkage with CYP3A5 haplotypes in a Japanese population. *Hum Mutat* 2004;23:100-8.
 - 24) Hardinger KL, Park JM, Schnitzler MA, Koch MJ, Miller BW, Brennan DC. Pharmacokinetics of tacrolimus in kidney transplant recipients: twice daily versus once daily dosing. *Am J Transplant* 2004;4:621-5.
 - 25) Min DI, Chen HY, Lee MK, Ashton K, Martin MF. Time-dependent disposition of tacrolimus and its effect on endothelin-1 in liver allograft recipients. *Pharmacotherapy* 1997;17:457-63.
 - 26) Iwahori T, Takeuchi H, Matsuno N, et al. Pharmacokinetic differences between morning and evening administration of cyclosporine and tacrolimus therapy. *Transplant Proc* 2005;37:1739-40.
 - 27) MacPhee IA, Fredericks S, Tai T, et al. The influence of pharmacogenetics on the time to achieve target tacrolimus concentrations after kidney transplantation. *Am J Transplant* 2004;4:914-9.
 - 28) Kuypers DR, de Jonge H, Naesens M, Lerut E, Verbeke K, Vanrenterghem Y. CYP3A5 and CYP3A4 but not MDR1 single-nucleotide polymorphisms determine long-term tacrolimus disposition and drug-related nephrotoxicity in renal recipients. *Clin Pharmacol Ther* 2007;82:711-25.
 - 29) Undre NA, van Hooff J, Christiaans M, et al. Low systemic exposure to tacrolimus correlates with acute rejection. *Transplant Proc* 1999;31:296-8.
 - 30) Staatz C, Taylor P, Tett S. Low tacrolimus concentrations and increased risk of early acute rejection in adult renal transplantation. *Nephrol Dial Transplant* 2001;16:1905-9.
 - 31) Miller J, Mendez R, Pirsch JD, Jensik SC. Safety and efficacy of tacrolimus in combination with mycophenolate mofetil (MMF) in cadaveric renal transplant recipients. FK506/MMF dose-ranging kidney transplant study group. *Transplantation* 2000;69:875-80.
 - 32) Ortiz F, Paavonen T, Tornroth T, et al. Predictors of renal allograft histologic damage progression. *J Am Soc Nephrol* 2005;16:817-24.
 - 33) Bakker RC, Hollander AA, Mallat MJ, et al. Conversion from cyclosporine to azathioprine at three months reduces the incidence of chronic allograft nephropathy. *Kidney Int* 2003;64:1027-34.
 - 34) Servais A, Meas-Yedid V, Buchler M, et al. Quantification of interstitial fibrosis by image analysis on routine renal biopsy in patients receiving cyclosporine. *Transplantation* 2007;84:1595-601.
 - 35) Nankivell BJ, Borrows RJ, Fung CL, O'Connell PJ, Allen RD, Chapman JR. The natural history of chronic allograft nephropathy. *N Eng J Med* 2003;349:2326-33.
 - 36) Pape L, Henne T, Offner G, et al. Computer-assisted quantification of fibrosis in chronic

- allograft nephropathy by picosirius red-staining: a new tool for predicting long-term graft function. *Transplantation* 2003;76:955-8.
- 37) Solez K, Colvin RB, Racusen LC, et al. Banff '05 meeting report: differential diagnosis of chronic allograft injury and elimination of chronic allograft nephropathy ('CAN'). *Am J Transplant* 2007;7:518-26.
- 38) Solez K, Colvin RB, Racusen LC, et al. Banff 07 classification of renal allograft pathology: updates and future directions. *Am J Transplant* 2008;8:753-60.
- 39) Diaz Encarnacion MM, Griffin MD, Slezak JM, et al. Correlation of quantitative digital image analysis with the glomerular filtration rate in chronic allograft nephropathy. *Am J Transplant* 2003;4:248-56.
- 40) Grimm PC, Nickerson P, Gough J, et al. Computerized image analysis of Sirius red-stained renal allograft biopsies as a surrogate marker to predict long-term allograft function. *J Am Soc Nephrol* 2003;14:1662-8.
- 41) Mancilla E, Avila-Casado C, Uribe-Urbe N, et al. Time-zero renal biopsy in living kidney transplantation: a valuable opportunity to correlate predonation clinical data with histological abnormalities. *Transplantation* 2008;86:1684-8.
- 42) Oppenheimer F, Aljama P, Asensio Peinado C, et al. The impact of donor age on the results of renal transplantation. *Nephrol Dial Transplant* 2004;19 (Suppl 3): iii 11-5.
- 43) Scolari MP, Cappuccilli ML, Lanci N, et al. Predictive factors in chronic allograft nephropathy. *Transplant Proc* 2005;37:2482-4.
- 44) Nankivell BJ, Borrows RJ, Fung CLS, O'Connell PJ, Chapman JR, Allen RDM. Delta analysis of posttransplantation tubulointerstitial damage. *Transplantation* 2004;78:434-41.
- 45) Heilman RL, Devarapalli Y, Chakkera HA, et al. Impact of subclinical inflammation on the development of interstitial fibrosis and tubular atrophy in kidney transplant recipients. *Am J Transplant* 2010;10:563-70.
- 46) Reischig T, Jindra P, Hes O, Bouda M, Kormunda S, Treska V. Effect of cytomegalovirus viremia on subclinical rejection or interstitial fibrosis and tubular atrophy in protocol biopsy at 3 months in renal allograft recipients managed by preemptive therapy or antiviral prophylaxis. *Transplantation* 2009;87:436-44.
- 47) Heilman RL, Chakkera HA, Reddy KS, et al. Clinical factors associated with graft fibrosis in kidney-transplant recipients on steroid-avoidance immunosuppression. *Clin Transplant* 2008;22:309-15.
- 48) Micozkadioglu H, Ozdemir FN, Sezer S, Arat Z, Haberal M. Weight gain after living-related renal transplantation affects long-term graft function. *Transplant Proc* 2005;37:1029-32.
- 49) Arias M, Fernandez-Fresnedo G, Rodrigo E, Ruiz JC, Gonzalez-Cotruello J, Gomez-Alamillo C. Non-immunologic intervention in chronic allograft nephropathy. *Kidney Int Suppl* 2005;37:S118-20.
- 50) Ortiz F, Paavonen T, Tornroth T, et al. Predictors of renal allograft histologic damage progression. *J Am Soc Nephrol* 2005;16:817-24.
- 51) Rush DN, Cockfield SM, Nickerson PW, et al. Factors associated with progression of interstitial fibrosis in renal transplant patients receiving tacrolimus and mycophenolate mofetil. *Transplantation* 2009;88:897-903.
- 52) Bakker RC, Hollander AA, Mallat MJ, Bruijn JA, Paul LC, de Fijter JW. Conversion from cyclosporine to azathioprine at three months reduces the incidence of chronic allograft nephropathy. *Kidney Int* 2003;64:1027-34.