Pharmacogenetics of tacrolimus: ready for clinical translation?

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Tacrolimus (Tac) exhibits an interindividual pharmacokinetic variability that affects the dose required to reach the target concentration in blood. Tac is metabolized by two enzymes of the cytochrome P450 family, CYP3A5 and CYP3A4. The effect of the CYP3A5 genotype on Tac bioavailability has been demonstrated, and the main determinant of this pharmacogenetic effect is a single-nucleotide polymorphism (SNP) in intron 3 of CYP3A5 (6986 A>G; SNP rs776746; also known as CYP3A5*3). The mean dose-adjusted blood Tac concentration was significantly higher among CYP3A5*3 homozygotes than that of carriers of the wild-type allele (CYP3A5*1). In a recent prospective study, a group of kidney transplant patients received a Tac dose either according to the CYP3A5 genotype (the adapted group) or according to the standard regimen (the control group). All patients received induction therapy with mycophenolate mofetil, corticosteroids, and either basiliximab or intravenous clonal (anti-thymocyte globulin) antibodies. Patients in the adapted-dose group required 3–8 days (median 6 days) to reach the target range compared with 3–25 days (median 7 days) in the control group (P = 0.001). The total number of dose modifications was also lower in the adapted-dose group. This study also suggested that the CYP3A5 genotype might contribute minimally to the reduction of early acute rejection. However, additional studies are necessary to determine whether the pharmacogenetic approach could help reduce the necessity for induction therapy and co-immunosuppressors.

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The current immunosuppressive therapy in solid organ transplantation uses a combination of several drugs that function on multiple pathways of the immune response. These drugs are classified by their mechanism of action, such as calcineurin inhibitors (cyclosporine A, tacrolimus (Tac)), inhibitors of purine synthesis (mycophenolate mofetil), and mammalian target of rapamycin inhibitors (sirolimus, everolimus). These drugs are frequently combined with glucocorticoids (methylprednisolone, prednisone), monoclonal (muromonab, basiliximab, daclizumab), and polyclonal (anti-thymocyte globulin) antibodies.

Tacrolimus is an immunosuppressive drug used to prevent solid organ rejection, and also to treat autoimmune diseases. Tac, similar to cyclosporine, is a calcineurin inhibitor and suppresses the activation, proliferation, and differentiation of T cells. Calcineurin inhibitors prevents the transcription of several cytokine genes involved in immune responses.1–5 Tac has gradually replaced cyclosporine as the first-choice immunosuppressive drug, mainly because of its higher immunosuppressive activity and fewer adverse effects. However, Tac has also been associated with a higher risk of developing dyslipidemia, hypercholesterolemia, hypertension, post-transplant (PT) nephrotoxicity, and new-onset diabetes after transplantation.4–7

Tacrolimus is a macrolide antibiotic that inhibits calcineurin and thereby blocks the nuclear translocation of transcription factors. Tac binds to the FK-506-binding protein (FKBP-12), which is a necessary cochaperone for the immunosuppressive activity of Tac. Tac also inhibits the function of mammalian target of rapamycin (mTOR), which is part of the signaling pathway for cell cycle progression and proliferation. This dual action of Tac on calcineurin and mTOR explains its immunosuppressive and antiproliferative effects.

Tacrolimus is metabolized by two enzymes of the cytochrome P450 family, CYP3A5 and CYP3A4, whereas other P450 isoforms are much less effective.11–13 Most of the Tac biotransformation occurs in the liver, and to a lesser
extent in the small intestine. In vitro studies with human liver microsomes showed that CYP3A5 had high Tac catalytic efficiency, and its contribution was stronger in microsomes from individuals with low CYP3A4 concentrations. Several factors influence the blood concentration of Tac. Some factors are under the patients’ control, such as diet or the co-administration of drugs that share the same metabolic pathways with Tac (i.e., fluconazole and ketoconazole). However, some of the major determinants of Tac bioavailability reside in genes implicated in its absorption and metabolism. Several studies have reported that polymorphisms at the ABCB1/MDR-1, CYP3A4, and CYP3A5 affect Tac dose requirements, as discussed below.

**CYP3A5 IN Tac DOSE**

The effect of the CYP3A5 genotype on Tac bioavailability has been demonstrated by several laboratories. The main determinant of this pharmacogenetic effect is a single-nucleotide polymorphism (SNP) in intron 3 of CYP3A5 (6986 A>G; SNP rs776746), also known as CYP3A5*3 (for a complete list of the CYP variants, see the home page of the Human Cytochrome P450 Allele Nomenclature, http://www.cypalleles.ki.se). Most studies examined the effect of CYP3A5*3 on the twice-daily dose formulation of Tac (Prograf) at several PT times. The mean dose-adjusted blood Tac concentration was significantly higher among CYP3A5*3 homozygotes than that of carriers of the wild-type allele (CYP3A5*1). The CYP3A5*3 allele affects splicing of the pre-mRNA and greatly reduces P450-3A5 activity. The poor metabolizing phenotype of CYP3A5*3 homozygotes explains why they would require a lower Tac dose to reach the blood target concentration compared with carriers of the CYP3A5*1 allele.

We recently reported the results of a multicenter study of Tac-pharmacogenetics in Spanish patients who received a first cadaveric kidney graft (the REDinREN pharmacogenetic study). A total of 400 patients were treated with a standard triple immunosuppressive therapy with Tac (Prograf), prednisone, and mycophenolate mofetil. The initial oral dose of Tac was 0.2 mg/kg per day and was adjusted to reach a C0 of 10–15 ng/ml in the period from 0 to 3 months PT, and 5–10 ng/ml thereafter. Tac was measured in human whole blood with an automated chemiluminescent immunoassay and the Arquitect Tacrolimus assay (Abbott Laboratories, Chicago, IL). Compared with CYP3A5*1 carriers (n = 80), patients who were CYP3A5*3 homozygotes (n = 320) received lower median Tac (mg/kg per day) at 1 week (0.14 vs 0.12), at 6 months (0.10 vs 0.06), and at 1 year (0.08 vs 0.05) PT. These values were similar to those reported by others.

Assessing the impact of the CYP3A5*3 allele on Tac pharmacogenetics needs to consider the genotype frequencies among populations of various ethnic origins. Approximately 80% of Caucasians, but only 30% of African Americans, are CYP3A5*3 homozygotes (non-expressors). These differences in genotype frequencies could explain part of the observed variability in Tac dose requirements among different populations.

**CYP3A4 IN Tac DOSE**

A number of CYP3A4 SNPs have been identified. Most of the interindividual variability in CYP3A4 activity may be due to differences in transcript levels, and results from nucleotide changes in the promoter region. In particular, the CYP3A4*1B (−392 A>G; SNP rs2740574) is a common allele located in the promoter region, is associated with differences in transcriptional activity, and correlates with increased hepatic expression of CYP3A4.

Its expression varies in liver and other tissues, and its inherent concentration has a role on Tac metabolism in liver microsomes, particularly in microsomes from individuals who did not express CYP3A5. However, none of CYP3A4 SNPs has shown a clear influence on Tac pharmacokinetics. In our study, carriers of the −392 A>G variant had significantly higher Tac doses. A higher gene expression linked to this allele (compared with the wild type, CYP3A4*1) could explain the lower dose requirements among CYP3A4*1 homozygotes. Although our work confirmed the results from other studies, the significance of our study was limited by the low frequency of the CYP3A4*1B allele (only 6% of the patients were CYP3A4*1B carriers, and no patient was homozygous for this allele). However, CYP3A4 and CYP3A5 are closely linked, and the effect of the CYP3A4 polymorphisms on Tac pharmacokinetics could be due to linkage disequilibrium with CYP3A5*3. A way to solve this dilemma is to analyze the effect of CYP3A4 variation on patients with different CYP3A5 genotypes.

CYP3A4*1B carriers had significantly higher median Tac C0 values at 3 and 1 year PT, but not at 7 days PT than CYP3A4*1 homozygotes did. The same modifying effect of the CYP3A4 genotype was observed among CYP3A5*1 carriers. In contrast, Kuypers et al. reported similar Tac C0 values for the two CYP3A5*1 groups. However, no patient in their study was a CYP3A5*3 homozygote + CYP3A4*1B carrier. Because the conclusions of these studies are hampered by the low number of patients who were CYP3A5*1B carriers, additional studies with larger cohorts of patients are necessary to determine the value of genotyping CYP3A4 in addition to CYP3A5.

**ABCB1 POLYMORPHISMS IN Tac DOSE**

The ABCB1 gene (also known as the multidrug resistance-1 gene, MDR-1) encodes the P-glycoprotein (P-gp), which is a pump that drives the efflux of many drugs in the intestinal wall and other cell types. The amount of the drug that reaches the blood stream could depend on the P-gp activity, and ABCB1 polymorphisms linked to differences in P-gp expression/function could have an important role on dose requirements. The role of P-gp expression on Tac bioavailability was reported by Masuda et al., who found a strong correlation between ABCB1 mRNA levels in intestinal biopsies and the dose-adjusted Tac concentrations. The effect of several ABCB1 SNPs on Tac pharmacokinetics has been investigated, with conflicting results. We did not find a significant effect of the common c.3435 C/T
polymorphism (exon 26 SNP rs1045642) on Tac bioavailability. In addition, this SNP did not modify the effect of the CYP3A5 genotype.

However, the donor ABCB1 3435TT genotype was significantly associated with susceptibility to chronic allograft damage. The 3435 T homozygosity likely increased the renal expression of P-gp, which resulted in intrarenal accumulation of Tac. If this result is confirmed by others, the donor ABCB1 genotype could be a valuable tool to predict Tac-induced nephrotoxicity.

OTHER GENE VARIANTS IN Tac DOSE

Although the CYP3A5*3 is the main genetic determinant of Tac pharmacokinetics, this SNP explains ~50% of the total variability. Thus, other genetic variants could affect Tac metabolism and dose requirements. The effect of other nucleotide variants could also explain the variability between individuals with the same CYP3A5 genotype. For instance, 41% of our CYP3A5*3/*3 and 26% of the CYP3A5*1 carriers had C0 values in the target range (10–15 ng/ml) at 1 week PT. Although these frequencies diminished with time, 10% of the patients remained out of the target range (5–10 ng/ml) after 6 months PT. Data regarding the possible role of several polymorphisms on Tac pharmacogenetics have been recently presented. We assessed the effect of 96 DNA variants in 16 metabolizing enzymes on Tac dose requirements. In addition to CYP3A4, CYP3A5, and ABCB1, several P450, glutathione, and N-acetyl transferences, and thiopurine S-methyltransferase gene variants were studied. We did not detect any significant effects of these SNPs on Tac dose requirements. Moreover, none of these polymorphisms had a significant effect after correcting for the CYP3A5 genotype.

The CYP3A4 polymorphisms may also affect Tac pharmacokinetics. As discussed above, our data suggested an effect of the CYP3A4*1B allele on Tac metabolism. At 1 year PT, the patients who were CYP3A5*3/*3 + CYP3A4*1B carriers had Tac C0 values in the target range, whereas 6% of the CYP3A5*3/*3 + CYP3A4*1/*1 remained out of the target range. Most of the CYP3A4 variants found in the coding region have an allele frequency <1%. An exception was CYP3A4*2, a missense SNP (Ser222Pro) with a frequency of 5% among the Caucasians. This allele was linked to a lower clearance of the CYP3A4 substrate nifedipine, and carriers of this allele can thus be classified as ‘slow metabolizers’. The effect of this variant on Tac bioavailability has not been established. The sequencing of CYP3A4 may be very informative in patients whose C0 values cannot be explained by the CYP3A5 genotype, and the sequencing can help determine the overall contribution of CYP3A4 to Tac dose requirements. The same argument applies for the sequencing of CYP3A5 in those patients who are CYP3A5*1 carriers with C0 values that were above the target range. These patients could harbor one of several CYP3A5 variants that are linked to a reduced catalytic activity and a slow to null metabolizing phenotype.

READY FOR CLINICAL TRANSLATION?

The ultimate goal of the pharmacogenetics of Tac is to provide a tool to predict the dose for each patient before transplantation, and prevent the effects induced by an over/underdose. Haufloria et al. proposed a loading dose of 0.075 mg/kg and 0.150 mg/kg body weight twice a day among CYP3A5 non-expressors and expressors, respectively. These values were derived from a study of 19 volunteers (nine expressors, 10 non-expressors) who received a standard dose (0.1 mg/kg body weight twice a day). This and other studies paved the way toward clinical trials that evaluate the benefits of dosing according to the genotype.

The first prospective study has been recently reported by Thervet et al. A group of 280 patients received a Tac dose, either according to the CYP3A5 genotype (the adapted-dose group; n = 116) or to the standard regimen (the control group; n = 120). All patients received induction therapy with mycophenolate mofetil (Cell-Cept; Roche Farma, Basel, Switzerland), corticosteroids, and either basiliximab (Simulect; Novartis, Basel, Switzerland) or intravenous antithymocyte globulin (Thymoglobulin; Gensyme, Cambridge, MA). No drugs known to interact with CYP3A5 were administered. A limitation of this study was that Tac administration began on day 7 PT (a time required to determine the genotype), and the effect of the pharmacogenetic adaptation was thus not evaluated in patients treated with Tac from day 0. This delay in Tac dosing could affect the main clinical and analytical findings.

At day 7 PT, the patients in the adapted-dose group who were CYP3A5 expressors (n = 26) received an initial Prograf dose of 0.30 mg/kg per day, compared with 0.15 mg/kg per day among the CYP3A5 non-expressors (n = 90). The control group was treated with an initial dose of 0.20 mg/kg per day. The first measurement of the Tac C0 concentration was recorded after the sixth Tac dose (on day 10 PT). Patients in the adapted-dose group had Tac C0 values in the target range (10–15 ng/l) more frequently than the control group (43.3 vs 29.2%; P = 0.003). Moreover, the adapted-dose group required 3–8 days (median 6 days) to reach the target range compared with 3–25 days (median 7 days) in the control group (P = 0.001). The total number of dose modifications was also lower in the adapted-dose group (281 vs 420; P = 0.004). This study provided the first evidence that the genotyping-based dose adaptation reduces the time to reach the blood target concentration, but was this the only benefit? Tac blood levels are routinely monitored several times in the first few weeks PT to adjust the dose to the target concentration, which is achieved within the first 2 weeks in ~90% of the patients. Therefore, it should not be surprising that clinicians may consider that the adapted-dose method requires too much effort if the only benefit is to more rapidly (few days) determine the right dose. The significant delay in achieving the target blood concentration among CYP3A5 expressors has been linked to higher risk for early acute rejection. However, some authors failed to confirm the association between the CYP3A5*1 allele and acute
rejection.\textsuperscript{50,51} Thervet \textit{et al.}\textsuperscript{55} did not find significant differences in the incidence of delayed graft function, the number of PT dialysis sessions per patient, or the number of acute rejection episodes between the adapted and control groups. Their findings suggested that the Tac dose according to the CYP3A5 genotype might contribute minimally to the reduction of early acute rejection. However, the fact that their patients received biological induction therapy coupled with high dose of MMF during the first week could significantly reduce the incidence of acute rejection, affecting the results of the study. It is thus important to replicate this study on patients treated with an adapted dose from day 0, and also to determine whether the pharmacogenetic approach could help reduce the necessity for induction therapy and co-immunosuppressors.

Finally, recent studies have also demonstrated a significant effect of the CYP3A5 genotype on the pharmacokinetic and dose requirements for the once-daily Tac formulation (Advagraf, Astellas Pharma, Staines, UK).\textsuperscript{52,53} These results suggested that the pharmacogenetic approach for the twice-daily formulation could be also applied to this once-daily formulation, which may improve patient compliance.

In conclusion, genotyping of CYP3A5 may be useful to predict the Tac dose immediately after transplantation, and reduce the time required to reach the target concentration. However, the effect of the genotype-adapted dose on acute rejection and other clinical outcomes seems less clear. Therefore, trials to determine whether this pharmacogenetic approach could reduce the incidence of acute rejection and delayed graft function are necessary, particularly in patients without biological induction therapy.

DISCLOSURE
All the authors declared no competing interests.

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