Pharmacogenetics of tacrolimus: ready for clinical translation?

Eliecer Coto[1,2,3,4], Beatriz Tavira[1], Beatriz Suárez-Álvarez[4,5], Carlos López-Larrea[3,4,5], Carmen Díaz-Corte[2,4,6], Francisco Ortega[2,3,4,6] and Victoria Álvarez[1]

[1]Genética Molecular, Hospital Universitario Central de Asturias, Oviedo, Spain; [2]Departamento de Medicina, Universidad de Oviedo, Oviedo, Spain; [3]Fundación Renal 'Iñigo Alvarez de Toledo', Madrid, Spain; [4]Red de Investigación Renal, Redinren, Spain; [5]Inmunología, Hospital Universitario Central de Asturias, Oviedo, Spain and [6]Nefrología, Hospital Universitario Central de Asturias, Oviedo, Spain

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 anti-thymocyte globulin. 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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Correspondence: *Eliecer Coto, Genética Molecular, Hospital Universitario Central de Asturias, 33006 Oviedo, Spain.*
E-mail: eliecer.coto@sespa.princast.es

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.[1–3] 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]

Tac shows an interindividual pharmacokinetic variability that affects the dose required to reach the target concentration in blood.[8,9] The current therapeutic approach is based on an initial daily dose of 0.2 mg/kg (given in two equal 12-h doses in the case of Prograf (Astellas Pharma, Deerfield, IL)). The blood level is measured 12 h after dose (immediately before receiving the next dose) and is known as the trough or C_0 level. The clinician uses the C_0 level in each individual patient to decide whether to maintain, increase, or reduce the dose.[9,10] The target C_0, which is 10–15 ng/ml in the period 0 to 3 months PT, and 5–10 ng/ml thereafter, is considered as the optimal concentration to avoid rejection (a concentration too low) and toxicity (a concentration too high).[10]

Mainly, Tac 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.[11] 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).[14,15] However, some of the major determinants of Tac bioavailability reside in genes implicated in its absorption and metabolization. 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 *CYP3A*5 genotype on Tac bioavailability has been demonstrated by several laboratories.[16–24] 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).[25,26] 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.[11,12] The poor metabolizing phenotype of *CYP3A5*3/*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).[24] 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 C_0 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).[27] 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).[28] These differences in genotype frequencies could explain part of the observed variability in Tac dose requirements among different populations.[29]

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.[30] In particular, the *CYP3A4*1B* (−392A>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.[31,32]

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.[11] However, none of *CYP3A4* SNPs has shown a clear influence on Tac pharmacokinetics.[33] In our study, carriers of the −392 A>G variant had significantly higher Tac doses.[24] 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,[24] 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 C_0 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.[24] In contrast, Kuypers *et al.*[23] reported similar Tac C_0 values for the two *CYP3A5*1* groups. However, no patient in their study was a *CYP3A5*3* homozygote + *CYP4A4*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*.[23,24]

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.[33–38] The role of P-gp expression on Tac bioavailability was reported by Masuda *et al.*,[39] 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.[17–23] We did not find a significant effect of the common c.3435 C/T

polymorphism (exon 26 SNP rs1045642) on Tac bioavailability.[24] 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.[40] The 3435 T homozygosity likely increased the renal expression of P-gp, which resulted in intrarenal accumulation of Tac.[40] 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.[20] 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 C_0 values in the target range (10–15 ng/μl) at 1 week PT. Although these frequencies diminished with time, 10% of the patients remained out of the target range (5–10 ng/μl) after 6 months PT. Data regarding the possible role of several polymorphisms on Tac pharmacogenetics have been recently presented.[41] We assessed the effect of 96 DNA variants in 16 metabolizing enzymes on Tac dose requirements.[24] In addition to *CYP3A4*, *CYP3A5*, and *ABCB1*, several P450, glutathione and *N*-acetyl transferases, 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 C_0 values in the target range, whereas 6% of the CYP3A5*3/*3 + CYP3A4*1/*1 remained out of the target range.[24] 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'.[42,43] The effect of this variant on Tac bioavailability has not been established. The sequencing of *CYP3A4* may be very informative in patients whose C_0 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 C_0 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. Haufroid *et al.*[20] 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 benefit of dosing according to the genotype.[44]

The first prospective study has been recently reported by Thervet *et al.*[45] 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; Genzyme, 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 C_0 concentration was recorded after the sixth Tac dose (on day 10 PT). Patients in the adapted-dose group had Tac C_0 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.[46] The significant delay in achieving the target blood concentration among CYP3A5 expressors has been linked to higher risk for early acute rejection.[47–49] However, some authors failed to confirm the association between the *CYP3A5*1* allele and acute

rejection.[50,51] Thervet *et al.*[45] 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).[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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