Research Highlights

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calcineurin inhibitors in solid organ transplantation

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Genetic determinants of calcineurin inhibitor concentration at the pharmacodynamic target

Evaluation of: Crettol S, Venetz JP, Fontana M *et al.*: Influence of *ABCB1* genetic polymorphisms on cyclosporine intracellular concentration in transplant recipients. *Pharmacogenet. Genomics* 18, 307–315 (2008).

Calcineurin inhibitors (CNIs), mainly represented by ciclosporin (CsA) and tacrolimus (Tac), are drugs with potent immunosuppressive properties largely used to prevent graft rejection after solid organ transplantation. Both drugs prevent cellular rejection through the selective inhibition of interleukin-2 production by T cells. Their clinical use is, however, complicated by a narrow therapeutic index, a significant inter- and intra-patient pharmacokinetic variability, and the potential for serious drug interactions. Therapeutic drug monitoring (TDM) and, therefore, dosage individualization, are highly recommended to optimize efficacy and reduce toxicity, mainly on the kidney. In daily practice, conventional TDM is based on the measurement of trough whole-blood concentrations just before the next dose. While the relationship between high trough concentrations and toxicity has been demonstrated in various studies, its relationship with graft rejection (incidence and severity) appears more controversial. A significant relationship between low trough concentrations and acute rejection has been shown in some studies, but not consistently. It should be noted that whole-blood CNI concentrations, depending on individual pharmacokinetics and pharmacogenetics, do not necessarily reflect their intracellular concentration, and hence their ability to interact with the ultimate target, calcineurin. Since T cells are the

pharmacodynamic target of CNIs, a direct quantification within this cells population would appear meaningful for TDM purposes. Hence, specialized analytical methods based on liquid chromatography tandem mass spectrometry (LC-MS/MS) technology have been validated for the quantification of CNIs in peripheral blood mononuclear cells, representing a blood fraction enriched in lymphocytes [1]. Such methods are difficult to implement in routine practice (rapid processing to avoid *in vitro* drug redistribution), and alternative ways to evaluate intracellular concentrations would be largely welcome.

In the case of CsA, Crettol *et al.* recently showed that blood concentrations only partially reflected the highly variable intracellular concentrations, and that for a given CsA blood concentration, intracellular concentration varied over a tenfold factor among patients [2]. More interestingly, they showed that CsA intracellular concentrations, and to a lesser extent intracellular versus blood concentration ratios, were determined by genetic polymorphisms in *ABCB1*, a gene coding for the efflux protein P-glycoprotein (P-gp) expressed at the cellular membrane of T cells. The 1199A allele (*ABCB1* 1199G>A) was associated with decreased intracellular concentrations probably caused by a higher P-gp activity towards CsA, while the 3435T allele (*ABCB1* 3435C>T) led to increased intracellular concentrations, most probably owing to a lower P-gp activity towards the drug [2]. The authors also showed that *ABCB1* polymorphisms influenced CsA intracellular concentrations to a significantly higher extent than blood concentrations. This work is important in that it provides physicians with genetic determinants of CNI concentration at the

Vincent Haufroid^{1,2†}

[†]Author for correspondence:

¹Department of Clinical Chemistry, Université catholique de Louvain, Saint-Luc University Hospital, Brussels, Belgium

²Industrial Toxicology and Occupational Medicine Unit, Université catholique de Louvain, Saint-Luc University Hospital, Brussels, Belgium

Tel.: +32 2764 6725

Fax: +32 2764 6942

vincent.haufroid@uclouvain.be

Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

pharmacodynamic target. Integrating such data (with others still to be elucidated) in a physiologically-based pharmacokinetic model might, indeed, facilitate simulation of intracellular versus blood concentration relationships, and contribute to extrapolate intracellular concentrations from whole-blood measurements, performed routinely in all laboratories involved in solid organ transplantation. Such an approach could therefore offer an elegant alternative to the laborious LC-MS/MS methods to measure intracellular CNI concentrations.

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Tacrolimus dose adjustment according to *CYP3A5* genotype

Evaluation of: Fukudo M, Yano I, Yoshimura A *et al.*: Impact of *MDR1* and *CYP3A5* on the oral clearance of tacrolimus and tacrolimus-related renal dysfunction in adult living-donor liver transplant patients. *Pharmacogenet. Genomics* 18, 413–423 (2008).

The daily practice of therapeutic drug monitoring (TDM) reveals a large inter-individual variability in Tac pharmacokinetics, and particularly in the dose required to achieve target blood concentrations [1]. Among several factors investigated, polymorphisms in genes coding for biotransformation enzymes (CYP isoenzymes 3A4 and 3A5) have received much attention to date. Contrary to CYP3A4, expression of CYP3A5 has been found to be largely determined by genetic polymorphisms, with between 15–25% of Caucasians expressing CYP3A5 (*CYP3A5*1/*1* or *CYP3A5*1/*3*) at a detectable level [2]. The presence of the *CYP3A5*3* allele (the most frequent in Caucasians) results in a splicing defect and in the absence of protein activity, and only individuals with at least one *CYP3A5*1* allele are, therefore, classified as CYP3A5 expressors. It is now well established that whole-blood Tac concentrations and dose requirements are strongly associated with the *CYP3A5* genotype [3]. Indeed, several reports confirmed that patients carrying at least one *CYP3A5*1* allele have a lower Tac concentration:dose (C:D)

ratio, at the steady state, when compared with nonexpressors (*CYP3A5*3/*3*) in kidney, liver, lung and heart transplant recipients [3]. The impact of *CYP3A5* genotype on Tac pharmacokinetics was also studied in the early phase post-transplantation, and a significant delay in reaching target trough concentrations has been observed in CYP3A5 expressors during the first 2 weeks after kidney transplantation [4]. In practice, for kidney transplantation, guidelines for Tac loading dose have been proposed based on *CYP3A5* genotype, with a doubling of the loading dose in CYP3A5 expressors [5]. In adult liver transplantation, Fukudo *et al.* recently showed that the intestinal *CYP3A5* genotype was an important determinant for the variability of Tac oral clearance (CL/F) during the first month after transplantation [6]. Based on their population pharmacokinetic analysis, these authors also proposed to consider a higher dose of Tac for adult recipients with the *CYP3A5*1* allele in order to maintain trough concentrations similar to those in patients with the intestinal *CYP3A5*3/*3* genotype. The true benefit of such a genetically-based Tac loading dose adjustment should, however, be evaluated on the basis of a prospective randomized, controlled study analyzing the incidence of acute rejection as an end point. Such multicenter studies are still in progress and we are looking forward to the results.

The results found by Fukudo *et al.* were also important in term of chronic toxicity [6]. Interestingly, they demonstrated that the cumulative incidence of renal dysfunction over the first year after liver transplantation was significantly related to the recipient's, but not donor's, *CYP3A5* genotype, in spite of dose adjustments. Their finding suggests that CYP3A5 in the kidney plays a protective role in the development of renal dysfunction, probably by reducing drug accumulation within renal cells. Therefore, a genetically-based Tac dose adjustment would also be important several weeks after the transplantation (not only for the loading dose). In patients with a renal *CYP3A5*3/*3* genotype, lower target Tac concentrations could prevent nephrotoxicity, possibly compensated by other immunosuppressive drugs such as mycophenolate mofetil in order to maintain an effective immunosuppression.

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CYP3A5 as a genetic determinant of the clinical variability of CYP3A-mediated drug interactions involving tacrolimus

Evaluation of: Kuypers DR, De Jonge H, Naesens M *et al.*: Effects of *CYP3A5* and *MDR1* single nucleotide polymorphisms on drug interactions between tacrolimus and fluconazole in renal allograft recipients. *Pharmacogenet. Genomics* 18(10), 861–868 (2008).

Clinically significant drug interactions between calcineurin inhibitors (in particular Tac) and antifungal azoles are relatively common in solid organ transplantation. The magnitude of such interactions is drug- and patient-specific, and depends mainly on the potency of the azole involved (inhibition constant towards CYP3A4 and P-gp, lipophilicity), the resulting plasma concentrations of each drug, the drug formulation, and interpatient variability [1]. These interactions can cause either Tac-related toxicity owing to CYP3A or P-gp inhibition during antifungal therapy or, at the opposite, Tac underexposure (with acute rejection risk) if initial-dose adjustments are not corrected in a timely fashion after discontinuation of the antifungal therapy. To avoid complex drug interactions in their design, most of the studies published so far in the field of the pharmacogenomics of calcineurin

inhibitors clearly mentioned in the materials and methods section the absence of concomitant medications known to interact with the pharmacokinetics of immunosuppressants, such as calcium channel blockers (diltiazem, nifedipine and verapamil), antiepileptics (phenytoin and carbamazepine), antifungals (fluconazole and ketoconazole), and macrolide antibiotics (erythromycin and clarithromycin). However, in clinical practice, transplant patients are at risk for different types of opportunistic infections and polymedication with antifungals or antibiotics are not so rare. In an observational study in renal allograft recipients, Kuypers *et al.* [2] recently evaluated the impact of *CYP3A4*, *CYP3A5* and *ABCB1* genetic polymorphisms on Tac exposure (predose trough concentrations) and dose requirements before, during and after administration of the antifungal triazole fluconazole [2]. Interestingly, they demonstrated that, although Tac dose requirements were persistently higher in *CYP3A5* expressors (patients with at least one *CYP3A5*1* allele) compared with nonexpressors (a consistent finding throughout several studies), a significant decrease in Tac dose following exposure to fluconazole was only triggered in

nonexpressors (*CYP3A5*3/*3*), suggesting that *CYP3A5* expressors are less susceptible for the inhibitory effects of fluconazole on Tac metabolism through intestinal and hepatic CYP3A4 and CYP3A5. In addition, *CYP3A5* nonexpressors were more frequently exposed to supratherapeutic trough Tac concentrations during treatment with fluconazole, leading them more susceptible to Tac-related toxicity such as chronic nephrotoxicity. With this study, Kuypers *et al.* identified a genetic determinant of the clinical variability of CYP3A-mediated drug interactions and opened a new field of investigation for future pharmacogenomics studies.

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