Pharmacodynamic monitoring of immunosuppressive drugs

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Pharmacodynamic (PD) monitoring measures the biological response to a drug, which alone—or coupled with pharmacokinetics—provides a novel method for optimization of drug dosing. PD monitoring has been investigated by us and other investigators primarily for four immunosuppressive drugs: cyclosporine (CsA), azathioprine (AZA), mycophenolate mofetil (MMF), and rapamycin (RAPA). PD monitoring of CsA and MMF involves measuring the activity of the enzymes calcineurin and inosine monophosphate dehydrogenase, respectively. The PD of AZA is assessed by measuring the activity of thiopurine methyltransferase, which is induced by a metabolite of AZA, 6-mercaptopurine. The PD for RAPA involves measuring the activity of a P70 S6 kinase in lymphocytes. To date, the most detailed studies have been performed with PD monitoring of CsA and MMF. Similarities exist in the PD responses to CsA and MMF in renal-transplant patients. At trough concentrations in blood, both drugs reduce the activity of their target enzymes by only 50%; however, considerable interpatient variability is evident. Throughout the dosing interval, the enzyme activities parallel the respective drug concentrations. AZA treatment of renal-transplant patients who exhibited an increase in thiopurine methyltransferase activity from time of transplantation resulted in fewer episodes of active rejection. Additional clinical trials are currently underway to relate various pharmacokinetics and PD parameters to clinical response, to ascertain which provides the best guide for dosing. PD monitoring may provide an alternative approach to additional measurements of drug concentrations.

Several approaches can be used to assess the appropriateness of the dosing regimen for an immunosuppressive drug. The first, assessment of clinical response, has serious limitations because signs of rejection or toxicity may be difficult to recognize clinically. The second involves assessment of the pharmacokinetic properties of the drug and relating various parameters (e.g., trough concentration, area under the time–concentration curve) to immunosuppressive efficacy or toxicity. This approach also has limitations in that the apparent concentrations of the drug measured may not accurately reflect the concentrations of the pharmacologically active moieties because of cross-reactivity of inactive metabolites in the assays [1, 2]. In addition, therapeutic ranges for the drugs have been difficult to establish, given the dependency of such values on the type of transplant, the time posttransplant, and what regime of immunosuppressive drugs (if any) is used in combination with the drug of interest [1, 2]. The third approach, pharmacodynamic (PD) monitoring, involves measuring the biological effect of the drug at its target site. If measurement at its target site is not possible, the enzyme activity should be measured in a matrix that provides a good surrogate marker for the enzyme activity at the target site. This approach has been reported for the monitoring of cyclosporine (CsA) [3, 4], azathioprine (AZA) [5], mycophenolate mofetil (MMF) [6, 7], and rapamycin (RAPA) [8] induced immunosuppression.

**Cyclosporine**

The immunosuppressive action of CsA is currently thought to be initiated subsequent to uptake by lymphocytes and intracellular binding to cyclophillin, a peptidyl-propyl isomerase. The CsA–cyclophillin complex selectively inhibits calcineurin (CN), a serine–threonine phosphatase with an essential role in calcium-dependent signal transduction [9, 10] (as shown in Fig. 1). In activated T cells, inhibition of this phosphatase reduces translocation of the cytoplasmic subunit of the nuclear

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Nonstandard abbreviations: PD, pharmacodynamics; CsA, cyclosporine; AZA, azathioprine; MMF, mycophenolate mofetil; RAPA, rapamycin; CN, calcineurin; PBL, peripheral blood lymphocytes; PPI, PPIA, PP2C, protein phosphatases 1, 2A, and 2C; GVHD, graft vs host disease; 6-MP, 6-mercaptopurine; TPMT, thiopurine methyltransferase; MPA, mycophenolic acid; IMPDH, inosine monophosphate dehydrogenase.
factor to the nuclear subunit, with subsequent impairment of transcription of the genes for interleukin-2 and other lymphokines [9, 10]. PD approaches for monitoring CsA have therefore been based on measuring CN inhibition in peripheral blood lymphocytes (PBL). Synthetic phosphorylated polypeptides (used as substrates [3, 4]) can be dephosphorylated by several other phosphatases besides CN. The most frequently used substrate is a 19-amino acid peptide, which mimicks the N-terminal region of nuclear factor for activated T cells (NF-AT), the natural substrate for CN. CN activity, which is measured by release of $^{32}$P from the phosphorylated substrate, is therefore determined in the presence of okadaic acid, which inhibits protein phosphatases 1 and 2A (PP1 and PP2A). The remaining phosphatase activity can then be attributed to CN and PP2C, the latter being CsA-resistant. The PP2C component of the measured phosphatase activity is determined by assaying an aliquot of PBL after exposure to a concentration of CsA that completely inhibits CN activity (Table 1).

The first clinical report of PD monitoring of CsA by measurement of CN activity involved adult bone-marrow transplant patients [3]. The CN activities in PBL isolated from 62 bone-marrow-transplant recipients and 12 normal volunteers were analyzed with respect to administration of CsA, the concentration of CsA in plasma, and the presence or absence of acute graft vs host disease (GVHD).

In all instances, CN activity in patients receiving CsA was significantly less than that in normal subjects ($P < 0.05$) [3]. The lower CN activity in patients with CsA-resistant GVHD suggests that GVHD is not the result of inadequate suppression of CN activity. If inhibition of CN were the only physiological target of CsA, increasing doses or treating with other inhibitors of CN, such as FK506, would not be expected to ameliorate GVHD.

More recently, the degrees of CN blockade and cytokine expression (interleukin-2 and interferon-γ) was measured in PBL from stable CsA-treated renal-transplant recipients [4]. CsA in vitro inhibited both CN selectivity and cytokine induction by 50% at a concentration of 10−20 μg/L [4]. In CsA-treated patients with therapeutic trough concentrations of CsA, CN activity was 50% less than in the controls [4]. A significant negative correlation with CsA trough concentrations ($P < 0.05$) was observed [4, 5]. In addition, CsA concentrations and CN activity were inversely related throughout the dosing interval [4]. The induction of cytokine mRNA was not totally blocked, which corresponded with the reduced CN activity [4]. The differential sensitivity of CN inhibition in vivo vs in vitro most probably results from the difference between the free fraction of CsA in biological fluids and that in culture medium. The partial CN inhibition in vivo may explain why the immune responsiveness of patients receiving CsA is reduced but still sufficient for host defense.

Tacrolimus (FK506) also mediates its immunosuppressive activity in a manner similar to CsA, through inhibition of CN activity; hence, PD monitoring of this drug is also possible. However, detailed studies on the PD monitoring of this drug have not been reported. Further studies are required to determine whether CN activity or drug concentrations better correlate with clinical response to FK506.

AZATHIOPRINE
Although AZA has been used extensively for >25 years in transplantation, accurate monitoring of its immunosuppressive efficacy and toxicity has not occurred. AZA is metabolized in the liver to a pharmacologically inactive intermediate of the drug, 6-mercaptopurine (6-MP), by the enzyme glutathione S-transferase—a conversion that also depends on the concentration of glutathione. 6-MP is further converted in the liver and gut to 6-thioguanine nucleotides, the pharmacologically active metabolites, by several enzymes, including hypoxanthine guanine phosphoribosyltransferase. These nucleotides act as metabolic...
analogs and are thought to be responsible for both the immunosuppressive activity and toxicity of AZA. 6-MP is also metabolized by the enzyme thiopurine methyltransferase (TPMT) to 6-methylmercaptopurine [5]. TPMT, the expression of which is inherited in an autosomal codominant fashion, exhibits a wide range of activity in the normal population; this variation might account for the variable responses of individuals to AZA [11].

In renal-transplant recipients, a very low TPMT activity precludes the use of AZA because of severe myelotoxicity [12, 13]. More recently, a prospective evaluation of erythrocyte TPMT activity in kidney-transplant recipients with regard to AZA efficacy found that those patients who exhibited an increase in TPMT activity from the time of transplantation experienced fewer episodes of acute rejection and therefore had better immunosuppression than the patients whose TPMT activity did not increase [5]. The investigators postulated that the AZA-induced increase in TPMT activity reflects appropriate conversion of AZA to 6-MP by glutathione S-transferases and therefore reflects the immunosuppressive potency of the drug [5]. Therefore, TPMT activity appears to be an interesting marker for AZA-induced immunosuppression and deserves further clinical laboratory investigation.

MYCOPHENolate mofetil
MMF is a morpholinoethyl ester of mycophenolic acid (MPA). MMF can be considered a prodrug, because its immunosuppressive activity is expressed only after hydrolysis to MPA. MPA mediates its effect by inhibiting inosine monophosphate dehydrogenase (IMPDH), an enzyme involved in the de novo biosynthesis of purine nucleotides [14, 15]. Lymphocytes have a greater dependence on the de novo pathway than on the salvage pathway. The majority of other cells rely more heavily on the salvage pathway, allowing therapeutic immunosuppression with minimal toxicity.

Multicenter trials evaluating the efficacy of MMF in conjunction with CsA and steroids have shown a considerable reduction in rejection episodes in groups randomized to receive MMF, in comparison with control groups who received AZA [16]. The requirement of monitoring MPA to optimize immunosuppressive efficacy has not been firmly established. However, there is consensus that PD or pharmacokinetics monitoring may be required in certain patient populations with an expected high variability in pharmacokinetics behavior (liver-transplant patients early posttransplant, pediatric patients, patients with altered absorption), in situations where drug–drug interactions are suspected, or in assessing patient compliance [17]. We have recently reported the development of an assay to measure IMPDH activity in whole blood as a means to assess MPA-induced immunosuppression [6] (see Fig. 2). This assay is based on the conversion of [3H]inosine to xanthine, which is measured by the release of tritium. Because almost all the IMPDH activity in whole blood is found in lymphocytes [6, 7], measurement of the enzyme activity in this medium provides a good indication of MPA at its primary target. Animal studies in both rabbits and dogs have shown an inverse relationship between IMPDH activity and drug concentration [6, 7].

More recently, a prospective study involving PD assessment of MPA-induced immunosuppression has been performed in renal-transplant recipients. The relationship between MPA concentrations in plasma and IMPDH activity in whole blood was investigated in renal-transplant recipients randomized to receive either MMF or AZA in combination with CsA and prednisone. An inverse relationship was found between MPA concentrations and IMPDH activity in whole blood. The peak concentration of MPA, achieved 1-h postdose, inhibited 40% of IMPDH activity. As the MPA concentration is decreased throughout the dosing interval, the IMPDH activity is gradually restored.

The significant inhibition of IMPDH activity (P <0.05) in MMF-treated patients, as compared with the AZA-treated “controls”, is maintained for ~8-h postdose. No significant (P >0.05) difference between the predose and the 12-h postdose activity of IMPDH was observed. In a recent study (unpublished observations, R. Yatscoff), the...
The relationship between inhibition of IMPDH activity, MPA concentrations, and acute rejection episodes was investigated in 25 renal-transplant patients during the first 3 months posttransplant. Inhibition of IMPDH activity was measured 2-h postdose, with the predose activity (trough) considered as baseline. Measurement of drug concentration at this time approximates the point at which peak concentrations of MPA were attained. Both the drug concentrations and the enzyme activity demonstrated considerable intra- and interpatient variability, and no correlation between the two was observed. Biopsy-proven rejection episodes occurred in three patients. At the time of rejection, no inhibition of IMPDH activity was observed, despite adequate drug concentrations. Moreover, many patients who had no inhibition of IMPDH at certain times throughout the monitoring period did not experience rejection episodes. Further studies are underway to investigate the cause for the considerable variability observed, as well as to further investigate whether IMPDH activity measurement or MPA concentrations better correlate with immunosuppressive efficacy and graft outcome.

**RAPAMYCIN**

RAPA (Sirolimus) is currently undergoing phase III clinical evaluation. The drug has been shown to be efficacious alone or in combination with other immunosuppressive drugs, such as CsA, in prolongation of renal-allograft survival [18, 19]. Both animal and clinical studies have shown a relationship between trough concentrations of RAPA and graft survival [20]. RAPA mediates its immunosuppressive activity by inhibiting the P70 S6 kinase involved in the regulation of translation and by modulating nuclear events that may involve inhibition of phosphorylation of retinoblastoma protein through a cyclin-dependent process [21] (see Fig. 1). We have developed a PD assay in whole blood based on measurement of P70 S6 kinase activity [8] (see Fig. 3). Almost all of the activity of this enzyme in whole blood resides in lymphocytes. The threshold for inhibition of this enzyme is a RAPA concentration of 10 μg/L [8], the drug concentration shown to result in optimal graft survival in clinical and animal studies [22]. At greater concentrations, a proportional relationship exists between inhibition of the enzyme and RAPA concentrations. Studies are underway to investigate the relationship between the inhibition of the enzyme activity, RAPA concentrations, and graft survival.

In conclusion, PD monitoring has the potential to augment pharmacokinetics and trough concentration monitoring to optimize the dosing regimen of immunosuppressive drugs. The PD assessment of drug effect may not always reflect the pharmacokinetics status of the drug. However, its use challenges investigators to determine which approach best correlates with the clinical status/outcome of the patient. A summary of the recent advances in PD monitoring of immunosuppressive drugs is shown in Table 2. Of course, further clinical studies are required to confirm these findings. However, because many of the assays are time-consuming and not amenable for use in routine clinical laboratories, the challenge is for clinical scientists to develop robust PD assays to facilitate the further evaluation of this approach.

### Table 2. Pharmacodynamic monitoring of immunosuppressive drugs: overview/summary.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Assay robustness</th>
<th>Relationship to pharmacokinetics</th>
<th>Clinical validation data</th>
</tr>
</thead>
<tbody>
<tr>
<td>CsA</td>
<td>Fair</td>
<td>Good</td>
<td>Minimal</td>
</tr>
<tr>
<td>AZA</td>
<td>Good</td>
<td>Good</td>
<td>Minimal</td>
</tr>
<tr>
<td>MMF</td>
<td>Good</td>
<td>Good</td>
<td>Minimal</td>
</tr>
<tr>
<td>RAPA</td>
<td>Poor</td>
<td>?</td>
<td>?</td>
</tr>
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