Review

Pharmacodynamic Monitoring of Calcineurin Phosphatase Activity in Transplant Patients Treated with Calcineurin Inhibitors

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Summary: Calcineurin inhibitors, tacrolimus and cyclosporine, have been widely used to prevent the rejection or graft-versus-host disease after transplantations. Since these drugs have a narrow therapeutic range and show large inter- and intraindividual pharmacokinetic variability, frequent therapeutic drug monitoring is required to control their blood concentrations. Even with blood concentrations within the therapeutic range, some patients still experience acute rejection or infections. Tacrolimus and cyclosporine form a complex with their respective binding proteins, immunophilins, which in turn inhibit the phosphatase activity of calcineurin, a key enzyme in the activation of T lymphocytes. Pharmacodynamic assessment of calcineurin phosphatase activity in combination with the monitoring of blood concentrations has been studied. The inhibitory effects on calcineurin activity in peripheral blood mononuclear cells differed between tacrolimus and cyclosporine in transplant patients. The pharmacodynamics of both drugs shows great inter- as well as intraindividual variation, and acute rejection was associated with calcineurin activity. Calcineurin activity at trough time points was suggested as a single surrogate predictor for overall calcineurin activity throughout dosing periods. Monitoring of calcineurin phosphatase activity might be useful to determine the therapeutic range of tacrolimus and cyclosporine concentrations for an individual patient treated with a calcineurin inhibitor.

Keywords: tacrolimus; cyclosporine; calcineurin; pharmacodynamics; therapeutic drug monitoring; transplantation

Introduction

Transplantation is now acknowledged as a life saving therapy for patients with end-stage organ failure. Calcineurin inhibitors, tacrolimus and cyclosporine, have been cornerstone immunosuppressants to prevent acute rejections after transplantations.1,2) Although two drugs are quite different in chemical structure, they have a similar mechanism of action involving the formation of a complex with their respective binding proteins, immunophilins; FK506-binding protein (FKBP12) for tacrolimus and cyclophilin for cyclosporine.3) Subsequently, the drug-immunophilin complexes bind to and inhibit the activity of the Ca\(^{2+}\)- and calmodulin-dependent protein phosphatase calcineurin, which is a key enzyme of the rate-limiting step in the activation of T lymphocytes, and an important regulator of nuclear factor of activated T cells (NFAT), which activates the transcription of genes for cytokines such as interleukin-2 and interferon-\(\gamma\) (Fig. 1).3–5) Therefore, tacrolimus and cyclosporine inhibit the activation of T lymphocytes, and have been widely used to prevent the rejection or graft-versus-host disease (GVHD) after transplantations. Moreover, calcineurin inhibitors have severe toxicity in the kidney, central nervous system and pancreas, and narrow therapeutic windows.6–8)

The bioavailability of tacrolimus and cyclosporine (classical formulation) after oral administration is poor and varies from 4% to 89% and from <5% to 89%, respectively.7,9) It is now acknowledged that both active secretion by the drug transporter P-glycoprotein (alternatively called MDR1 or ABCB1) from enterocytes into the lumen and intestinal metabolism by the cytochrome P450 (CYP) 3A subfamily play an important role in the bioavailability of calcineurin inhibitors.10,11) The intestinal MDR1 expression level was demonstrated to affect the pharmacokinetics of tacrolimus and cyclosporine in recipients with living-donor liver transplants.
The strategy to evaluate the pharmacological effects of cyclosporine and tacrolimus includes the measurement of calcineurin phosphatase activity in circulating blood.14-31 Table 1 shows a summary of reports assessing calcineurin phosphatase activity in blood or peripheral blood mononuclear cells (PBMCs) of transplant patients.20,21 Despite having blood concentrations within therapeutic range, some patients still experience an acute rejection or infections.20 Therefore, pharmacodynamic assessment in combination with the monitoring of blood concentrations may help to define an effective and safe therapeutic range for individuals treated with a calcineurin inhibitor. In this review, we describe recent topics regarding the relationship between the pharmacokinetics and narrow therapeutic window, frequent therapeutic drug monitoring (TDM) is required to control the blood concentrations of these drugs.

Despite having blood concentrations within therapeutic range, some patients still experience an acute rejection or infections.20 Therefore, pharmacodynamic assessment in combination with the monitoring of blood concentrations may help to define an effective and safe therapeutic range for individuals treated with a calcineurin inhibitor. In this review, we describe recent topics regarding the relationship between the pharmacokinetics and pharmacodynamics of calcineurin inhibitors based on measurements of calcineurin phosphatase activity in blood or peripheral blood mononuclear cells (PBMCs) of transplant patients.

### Therapeutic Drug Monitoring of Calcineurin Inhibitors

Despite the large interindividual variation in the pharmacokinetics of tacrolimus, the area under the concentration-time curve (AUC) correlated well with the trough level (C0).9 Therefore, monitoring of C0 has usually been performed in patients receiving tacrolimus. In our early experience in 55 pediatric patients receiving a LDLT, the C0 of tacrolimus was associated with the onset of rejection or adverse effects such as hyperkalemia, renal dysfunction, and hyperglycemia.8 Patients with a steady C0 level of 10–20 ng/mL were safely discharged from hospital without complications, and this range was suggested to be the therapeutic window. In a recent report, a target trough tacrolimus concentration of 6 ng/mL was proposed to minimize toxicity in the late post-transplant period.21 On the other hand, there are conflicting reports on the correlation between tacrolimus C0 and systemic drug exposure in some transplant patients.6,22 The clinical significance of the C0-based adjustment of the dosage of tacrolimus needs to be re-evaluated.

Cyclosporine also has a narrow therapeutic range and shows large inter- and intra-individual variability, and therapeutic drug monitoring of C0 has been classically performed to avoid adverse events such as nephrotoxicity and neurotoxicity.27 However, the C0 level does not correlate well with systemic drug exposure and clinical outcome.23,24 Since the introduction of a microemulsion of cyclosporine (Neoral®) into organ transplantation in the mid-1990s, consistent and reliable absorption with low interindividual variability has been achieved.25 Studies in renal transplant patients have shown that exposure to cyclosporine in the first 4 hours (hr) post-dose (AUC0–4) is a good predictor for outcome, as is the full pharmacokinetic profile (AUC0–12).26,27 In addition, blood cyclosporine concentrations at 2 hr post-dose (C2) have consistently been found to be the most sensitive marker for the absorption profile (AUC0–4) in several organ types.27,28 A new monitoring strategy based on C2 levels was demonstrated to be superior to traditional C0 monitoring for liver transplant recipients in reducing the incidence and severity of acute rejections.29,30 In addition, a large, multicenter, prospective trial of cyclosporine C2 monitoring in de novo kidney recipients (MO2ART; monitoring of 2-hr absorption in renal transplantation) showed the efficacy as well as safety of C2 monitoring.31 The LIS2T study (Liver International Study of Neoral C2 monitoring versus Tacrolimus C0 monitoring) demonstrated that both drugs are effective primary immunosuppressants in liver transplantation.32 In these studies, the dose of cyclosporine was adjusted to achieve a C2 level within the target range of 1,300–1,700 ng/mL and 800–1,200 ng/mL in the first 3 months for renal and liver transplant patients, respectively. Different C2 targets are appropriate depending on adjunctive immune suppression, level of immunologic risk, cyclosporine tolerability, risk of renal toxicity and postoperative periods.33 Since a patient with a low C2 may be either a low or a delayed absorber of cyclosporine, or be a normal absorber who is receiving a low dose, C2 monitoring alone might be insufficient to differentiate between these types of patients, and measurements at additional time-points are needed.33 Nonetheless, it is essentially difficult to determine the optimal therapeutic range of cyclosporine as well as tacrolimus in an individual patient.

### Inhibition of Calcineurin Phosphatase Activity by Cyclosporine and Tacrolimus

The strategy to evaluate the pharmacological effects of cyclosporine and tacrolimus includes the measurement of calcineurin phosphatase activity in circulating blood.14-31 Table 1 shows a summary of reports assessing calcineurin...
There are many reports on calcineurin activity in healthy controls at usual trough blood cyclosporine concentrations. Patients treated with cyclosporine have about 50% less calcineurin activity than controls. Renal transplant patients treated with cyclosporine have lower calcineurin activity than peripheral blood leukocytes, and studies with tacrolimus are limited. The pharmacodynamics of cyclosporine in peripheral blood leukocytes, and studies with tacrolimus are limited.

There are many reports on calcineurin activity in transplant patients. Halloran's group has extensively examined the pharmacodynamics of cyclosporine and tacrolimus in peripheral blood leukocytes, and suggested that calcineurin activity is closely related with blood cyclosporine concentrations in kidney transplant patients. Renal transplant patients treated with cyclosporine have about 50% less calcineurin activity than healthy controls at usual trough blood cyclosporine concentrations.

There are many reports on calcineurin activity in transplant patients treated with cyclosporine, but studies with tacrolimus are limited (Table 1). Koeefo-Nielsen et al. reported that calcineurin activity was maximally suppressed 2 hr post-administration of tacrolimus, and returned to baseline levels by 6 hr, which was inconsistent with the concentration-time profile of tacrolimus. Blanchet et al. and Millán et al. showed a good correlation between calcineurin activity in lymphocytes and blood tacrolimus concentrations measured at 2 hr after dosing in liver transplant patients.

We compared the relationship between calcineurin phosphatase activity in PBMCs and blood drug concentrations of tacrolimus and cyclosporine in 40 de novo LDLT patients (Fig. 2). The calcineurin activity in patients receiving cyclosporine showed a steep decline according to the increase in blood drug concentrations, and reached a plateau above a specific blood concentration, approximately 700 ng/mL, which was similar to a study measuring the inhibition of stimulated interleukin-2 production in whole blood by cyclosporine. A population pharmacodynamic analysis using the maximum effect (Emax) model demonstrated the mean estimate of the blood concentration that gives a half-maximal effect (EC50) for cyclosporine to be 200 ng/mL. Moreover, tacrolimus showed less dynamic change in its effect on calcineurin activity with a population mean estimate for the EC50 of 26.4 ng/mL, higher than the upper limit of the therapeutic range (20 ng/mL). Koeefo-Nielsen et al. also reported that stable renal patients treated with tacrolimus and cyclosporine displayed different calcineurin activity profiles. The pharmacological effect of tacrolimus in vitro was reported to be 30–90 times greater than that of cyclosporine, and no pharmacological effect of tacrolimus was observed in FKBP12-deficient mice. Taking these findings into consideration, tacrolimus may have unknown molecular mechanism(s) of action for immunosuppression, in addition to its inhibitory effect on calcineurin. Additionally, the discrepancy in the calcineurin activity profile after treatment with cyclosporine and tacrolimus might partly explain the difference in the monitoring strategy for each of these drugs: namely, C0 monitoring is enough for adjusting the dosage of tacrolimus, but C2 monitoring is needed for cyclosporine.

Based on the population analysis, large interindividual variability in the EC50 value was demonstrated in patients receiving both cyclosporine and tacrolimus (mean %CV, 84.0% and 81.4%, respectively). The variability may be explained by the difference in drug concentrations in

### Table 1. Measurement of calcineurin phosphatase activity in transplant patients

<table>
<thead>
<tr>
<th>Calcineurine inhibitor</th>
<th>Author(s) (Reference)</th>
<th>Transplant type</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclosporine (CsA)</td>
<td>Pai et al. (36)</td>
<td>Stem-cell (n = 33)</td>
<td>CaN with acute GVHD was lower than that without GVHD.</td>
</tr>
<tr>
<td></td>
<td>Battik et al. (37)</td>
<td>Renal (n = 4)</td>
<td>CaN rapidly changed with blood levels.</td>
</tr>
<tr>
<td></td>
<td>Battik et al. (38)</td>
<td>Renal (n = 62)</td>
<td>CaN was 50% reduced compared with controls at trough time points.</td>
</tr>
<tr>
<td></td>
<td>Piccinini et al. (39)</td>
<td>Renal (n = 30)</td>
<td>Time course of CaN was independent from CsA pharmacokinetics.</td>
</tr>
<tr>
<td></td>
<td>Halloran et al. (40)</td>
<td>Renal (n = 4)</td>
<td>CaN closely related with CsA blood concentration.</td>
</tr>
<tr>
<td></td>
<td>Caruso et al. (41)</td>
<td>Renal (n = 15)</td>
<td>No relationship was found between CsA pharmacokinetics and CaN. Baseline CaN correlated with AUA0–12.</td>
</tr>
<tr>
<td></td>
<td>Millán et al. (42)</td>
<td>Renal (n = 65)</td>
<td>CaN correlated with C2, even in combined therapy with mycophenolate mofetil.</td>
</tr>
<tr>
<td></td>
<td>Brunet et al. (43)</td>
<td>Renal (n = 15)</td>
<td>CaN correlated with C2.</td>
</tr>
<tr>
<td></td>
<td>Sanquer et al. (44)</td>
<td>Stem-cell (n = 31)</td>
<td>CaN predicted acute GVHD.</td>
</tr>
<tr>
<td></td>
<td>Fukudo et al. (45)</td>
<td>Liver (n = 40)</td>
<td>CsA displayed greater CaN inhibition than tacrolimus. EC50 of CsA = 200 ng/mL.</td>
</tr>
<tr>
<td></td>
<td>Koefo-Nielsen et al. (46)</td>
<td>Renal (n = 40)</td>
<td>CaN displayed greater CaN inhibition than tacrolimus.</td>
</tr>
<tr>
<td></td>
<td>Fukudo et al. (47)</td>
<td>Liver (n = 14)</td>
<td>Baseline CaN correlated with AUA0–24.</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>Koefo-Nielsen et al. (48)</td>
<td>Renal (n = 21)</td>
<td>No single tacrolimus concentration did not correlate with AUA0–6. CaN at each time well correlates with AUA0–6.</td>
</tr>
<tr>
<td></td>
<td>Blanchet et al. (49)</td>
<td>Liver (n = 20)</td>
<td>CaN correlated with C2, but not C0.</td>
</tr>
<tr>
<td></td>
<td>Millán et al. (42)</td>
<td>Renal (n = 65)</td>
<td>CaN correlated with C2, even in combined therapy with mycophenolate mofetil.</td>
</tr>
<tr>
<td></td>
<td>Fukudo et al. (45)</td>
<td>Liver (n = 40)</td>
<td>CsA displayed greater CaN inhibition than tacrolimus. EC50 of tacrolimus = 26.4 ng/mL. QD improved pharmacokinetics of CsA and provided an effective CaN inhibition.</td>
</tr>
</tbody>
</table>

CsA: cyclosporine, CaN: calcineurin activity, GVHD: graft-versus-host disease, AUA: area under the CaN-time curve, C0: trough concentration, C2: blood concentration 2 hr post dose, EC50: the blood concentration that gives a half-maximal effect, QD: once daily.
PBMCs and/or the difference in function or content of proteins relating to the pharmacodynamics such as immunophilins and calcineurin. Since P-glycoprotein is also expressed in PBMCs, it might contribute to the difference in drug distribution into the cells.54,55) In addition, since extensive intraindividual variability compared to the assay variability was also indicated in calcineurin activity on treatment with both drugs (8.5 and 8.6 pmol/min/mg protein for cyclosporine and tacrolimus, respectively).45) Pharmacodynamic monitoring of calcineurin phosphatase activity might be useful to evaluate the immunosuppressive degree on each occasion in each patient.

Relationship between Pharmacokinetics and Pharmacodynamics of Cyclosporine

We recently demonstrated that a once daily (QD) administration improved the oral absorption of a microemulsion of cyclosporine compared with ordinary twice daily (BID) administrations, and helped to achieve effective inhibition of calcineurin early after LDLT.47) According to the blood concentration data for cyclosporine in the QD and BID regimens, the $C_2$ level significantly correlated with the AUC0–4 (Fig. 3A), as previously reported.27,28) However, a negative, but not significant, relationship was observed between the pharmacokinetics (AUC0–4) and pharmacodynamics (area under the calcineurin activity-time curve for 12 hr, AUA0–12) (Fig. 3B),47) because of the large interindividual variability in the pharmacodynamics. Caruso et al.41) reported no significant correlation of the $C_0$ or AUC0–12 of cyclosporine with calcineurin activity at baseline or AUA0–12 in renal transplant patients. Similarly, no significant correlation between the AUC0–24 and AUA0–24 was observed in our LDLT patients (Fig. 3C). However, the AUA0–24 significantly correlated with the calcineurin activity at trough time points for both once daily or twice daily regimens of cyclosporine (Fig. 3D). Caruso et al.41) also reported that the baseline calcineurin activity correlated with AUA0–12 in renal transplant patients treated with cyclosporine. Koefoed-Nielsen et al.48) reported that AUA0–6 was significantly correlated with calcineurin activity at baseline and AUA0–12 in renal transplant patients treated with cyclosporine. Based on these findings, monitoring of calcineurin activity at trough time would be useful to predict the overall calcineurin activity during the dosing period in transplant patients given cyclosporine as well as tacrolimus.

Relationship between Calcineurin Phosphatase Activity and Clinical Outcome

The relationship between calcineurin phosphatase activity and clinical outcome has been little investigated. Pai et al.36) reported that the calcineurin activity of patients with acute GVHD who were taking cyclosporine was lower than that of patients without GVHD, and suggested that cyclosporine-resistant GVHD is not the result of inadequate suppression of calcineurin activity. We reported the calcineurin activity in LDLT patients undergoing nephrotoxicity or acute rejection.45) Patients with nephrotoxicity treated with tacrolimus had significantly higher trough concentrations and lower levels of calcineurin activity than those without nephrotoxicity (Figs. 4A, B).45) In addition, patients with acute rejection had significantly lower tacrolimus trough concentrations and higher levels of calcineurin activity than those without a rejection episode (Figs. 4C, D).45) In the same report, we also showed the relationship between calcineurin activity and the occurrence of acute rejection and nephrotoxicity in LDLT patients treated with cy-
Fig. 3. Correlation between pharmacokinetic parameters (A) or between exposure and response (B and C) and between pharmacodynamic parameters (D) of cyclosporine in LDLT patients.

A: area under the concentration-time curve from 0 to 4 hr (AUC_{0–4}) versus blood concentration at 2 hr post-dose (C_2) for all points in the twice daily (BID, open circles) and once daily (QD, closed circles) groups. B: Mean area under the calcineurin activity-time curve for 12 hr (AUA_{0–12}) on postoperative day 6 (open circle) and 27 (open square) in the BID group, and on postoperative day 6 (closed circle) and 13 (closed square) in the QD group was compared with mean AUC_{0–4}, respectively. Error bar indicates SD. C: AUA_{0–24} versus AUC_{0–24} for all points in the BID (open circles) and QD (closed circles) groups. D: AUA_{0–24} versus calcineurin phosphatase activity at trough time point (CaN_0) for all points in the BID (open circles) and QD (closed circles) groups.

closporine. Namely, patients without acute rejection had higher C_2 levels of cyclosporine than those with an episode and C_0 levels within the therapeutic range (< 300 ng/mL). The mean calcineurin activity in patients receiving cyclosporine without a rejection episode was suppressed more, while that in the 2 patients experiencing acute rejection was higher than the above level. The mean C_0 level, but not C_2 level, of cyclosporine was significantly higher in patients with than those without nephrotoxicity. The mean calcineurin activity at the trough time point and 2 hr post-dose were comparable between the patients with and without nephrotoxicity. The C_2 monitoring of cyclosporine therefore may be effective for the prevention of acute rejection, and the risk of nephrotoxicity will be reduced by C_0 monitoring in transplant patients treated with cyclosporine.

Sanquer et al. have recently reported that calcineurin phosphatase activity in PBMCs may be a functional index with which to predict acute GVHD after allogenic stem-cell transplantation. Their hypothesis is that calcineurin activity reflects two distinct biological pathways: the degree of activation of T lymphocytes and the inhibitory effect of cyclosporine, and the final activity of calcineurin results from the balance between these two pathways. The proportion of interleukin-2-producing CD8^+ T cells has been shown to be predictive of acute rejection after liver transplantation. Taking these findings into consideration, a pharmacodynamic assessment of calcineurin activity as well as interleukin-2 production, in combination with classical therapeutic drug monitoring, may be useful for determining the individual therapeutic range of tacrolimus and cyclosporine in patients following transplantation.

Conclusion

The characteristics of the inhibitory effect on calcineurin phosphatase activity in PBMCs differ between tacrolimus and cyclosporine, which might explain partly the difference in the therapeutic monitoring of these two drugs, namely C_0 monitoring for tacrolimus and C_2 monitoring for cyclosporine. Extensive inter- and intraindividual variability in calcineurin activity for both drugs and an association between acute rejection and increased calcineurin activity are indicated. Additionally, calcineurin phosphatase activity at
Pharmacodynamic Monitoring of Calcineurin Inhibitors

trough time points would be a single surrogate predictor for the overall calcineurin activity throughout the dosing period. Monitoring of calcineurin phosphatase activity might be useful to determine the therapeutic range of tacrolimus and cyclosporine concentrations for an individual patient treated with a calcineurin inhibitor.

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