Evidence of Different Pharmacokinetics Including Relationship among AUC, Peak, and Trough Levels between Cyclosporine and Tacrolimus in Renal Transplant Recipients Using New Pharmacokinetic Parameter
—Why Cyclosporine Is Monitored by C_2 Level and Tacrolimus by Trough Level—

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MATERIALS AND METHODS

Patients Twelve hours of monitoring of CYA and TAC blood concentrations was performed on 20 patients who were administered a CYA microemulsion formulation at 41.8±20.5 (mean±S.D.) d after renal transplantation and on 24 patients who were administered TAC at 41.4±21.4 d after transplantation. This monitoring was routinely carried out once before hospital discharge. The CYA-administered patients consisted of 11 males and 9 females aged 42.7±1.0 (mean±S.D.) years, and the mean (S.D.) body weight of these patients was 53.5±10.0 kg. The TAC-administered patients consisted of 15 males and 9 females aged 46.5±11.3 years, and their mean body weight was 52.3±9.9 kg (Table 1). None of the patients had digestive or liver dysfunction, which may affect the absorption or metabolism of CYA or TAC. All patients received a triple immunosuppressive-drug regimen, which consisted of calcineurin inhibitors (CYA or TAC), methylprednisolone, and either mycophenolate mofetil or azathioprine. Other drugs known to affect the pharmacokinetics of CYA or TAC were not administered to these patients. Informed consent was obtained from all patients included in this study.

The clinical efficacy of calcineurin inhibitors administered to renal transplant patients is considered to be a strong function of the area under the concentration time curve (AUC). Interestingly, monitoring timings of blood concentrations for two similar calcineurin inhibitors, cyclosporine (CYA; Neoral®) and tacrolimus (TAC; Prograf®) are different. Namely, CYA blood concentration is usually monitored at 2 h after administration (C_2) for peak concentration (C_p) and trough concentration (C_t). In the literature, data describing such characteristics of CYA and TAC have been presented in the past. However, each of these patient groups had different backgrounds. We have attempted to examine the behavior of blood concentration curves simultaneously for both CYA and TAC by establishing controlled groups of renal transplant patients with similar clinical backgrounds. Furthermore, we have analyzed the correlation with C_p and C_t versus AUC implementing area under the trough level (AUTL), or area above the trough level (AATL) as new pharmacokinetic parameters, such that C_t for CYA and C_p for TAC have been verified using controlled clinical data. We have also found distinct differences in the pharmacokinetics between CYA and TAC with the relationships between AUC, C_p, and C_t.

Key words area under the trough level; cyclosporine; tacrolimus; trough concentration; blood concentration at 2 h after administration; area under the concentration time curve

Cyclosporine (CYA) and tacrolimus (TAC) are calcineurin inhibitors with similar action mechanisms. However, there have not been any detailed reports on the pharmacokinetic differences of relationship among are under the concentration time curve (AUC), peak concentration (C_p), and trough concentration (C_t), regarding CYA and TAC after oral administration in a controlled group. AUC of CYA has been reported to be associated with clinical efficacy. On the other hand, the relationship between AUC of TAC and the clinical efficacy of TAC has not been evaluated precisely. However, it is generally thought that AUC is the most reliable pharmacokinetic parameter to predict the clinical efficacy of calcineurin inhibitors including TAC. CYA blood concentration is often monitored by blood concentration at 2 h after administration (C_t) to be more significantly associated with AUC, and TAC blood concentration is monitored by C_p. It has yet to be explained why only AUC of CYA correlates more significantly with C_t, and we could not clearly account for why the monitoring timings differ between CYA and TAC. Therefore, we investigated this issue to compare the blood concentration curve patterns and pharmacokinetic parameters of CYA and TAC in renal transplant recipients by using new pharmacokinetic parameters such as area under the trough level (AUTL) and area above the trough level (AATL). These parameters are parts of AUC and are dependent on C_t and C_p, respectively as described later.
**Evaluation of Pharmacokinetic Parameters for CYA and TAC** All patients were administered equally divided doses of CYA or TAC twice daily within 30 min after meals. Blood samples were then taken both before, and at intervals of: 1, 2, 3, 4, 6, and 12 h after CYA or TAC was administered in the morning, respectively. CYA concentrations were measured by fluorescence polarization immunoassay (FPIA), and TAC concentrations were measured by enzyme immunoassay (EIA).

The pharmacokinetic parameters for CYA and TAC were evaluated. We devised AUTL and AATL as new pharmacokinetic parameters (Fig. 1). AUTL was calculated from the area under the line between the 

\[ C_t \] just before administration (hour 0) and \[ C_i \] just before the next administration (hour 12). AATL was calculated to subtract AUTL from \( AUC \). The correlation coefficients between AUTL and AATL versus \( AUC \) were analyzed to verify whether AUTL, namely \( C_i \), is more conduciile to the area in \( AUC \), or AATL, namely \( C_p \), in CYA and TAC, respectively. Then the AUTL/AUC\%, a ratio accounting for AUTL in \( AUC \), was estimated. AUTL/AUC\% would be a new parameter that can represent the relationship between \( AUC \) and \( C_i \) or \( C_p \). The ratio of \( C_p \) and \( C_i \) (\( C_p/C_i \)), \( AUC \) per dose (D) per body weight (BW) (\( AUC/D/BW \)), \( C_p/D/BW \) and \( C_i/D/BW \) were also compared between the CYA- and TAC-treated recipients, alike.

**Statistics** We used two-tailed unpaired \( t \)-tests and variance analysis for comparisons of mean ages, body weights, mean days after transplantation, and the mean pharmacokinetic parameters between the CYA- and TAC-treated recipients. Fisher’s exact probability tests were used to compare the proportion of males and females between the two recipient groups. Pearson’s correlation coefficients were used to show correlation coefficients between AUTL and AATL versus \( AUC \) in CYA- and TAC-treated recipients. These analyses were performed with Statview. In each case, two-sided \( p \) values less than 0.05 were considered to be significant.

**RESULTS**

The pharmacokinetic parameters of calcineurin inhibitors were compared between the 20 CYA-treated recipients and 24 TAC-treated recipients. There was no significant difference in the basic profiles including age, gender, period after transplantation, and BW between CYA- and TAC-treated recipients. D/BW were 2.27±0.82 mg/kg in the CYA group, and 0.99±0.05 mg/kg in the TAC group (Table 1). AUTL/\( AUC \)% of CYA and TAC, respectively. The AUTL/AUC\% of CYA was 41.9±6.9\% (\( p<0.0001 \)) (Fig. 2). The ratio of the value of \( C_p/D/BW \) between CYA and TAC were almost similar, the ratio of \( AUC/D/BW \) between CYA and TAC was 1.48. However, \( C_i/D/BW \) of CYA was 77.1±23.6 (ng/ml)/(mg/kg), which was significantly lower than that of TAC (160.0±91.8, \( p<0.0005 \)), and the ratio of \( C_i/D/BW \) between CYA and TAC was 0.48.

**Table 1. Profiles of Renal Transplant Recipients Administered CYA and TAC**

<table>
<thead>
<tr>
<th>Value</th>
<th>CYA (n=20)</th>
<th>TAC (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>42.7±1.0</td>
<td>46.5±11.3</td>
</tr>
<tr>
<td>Gender (m/f)</td>
<td>11/9</td>
<td>15/9</td>
</tr>
<tr>
<td>Days after transplantation</td>
<td>41.8±20.5</td>
<td>41.4±21.4</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>53.5±10.0</td>
<td>52.3±9.9</td>
</tr>
<tr>
<td>Dose (mg)</td>
<td>119.5±45.07</td>
<td>4.7±2.59</td>
</tr>
<tr>
<td>Dose/body weight (mg/kg)</td>
<td>2.27±0.82</td>
<td>0.09±0.05</td>
</tr>
</tbody>
</table>

**Table 2. Comparison of Pharmacokinetic Parameters between CYA and TAC**

<table>
<thead>
<tr>
<th>Value</th>
<th>CYA (n=20)</th>
<th>TAC (n=24)</th>
<th>( p ) value</th>
<th>CYA/TAC ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUTL/AUC%</td>
<td>41.9±6.9</td>
<td>73.4±8.1</td>
<td>&lt;0.0001</td>
<td>0.57</td>
</tr>
<tr>
<td>( C_p/C_i )</td>
<td>6.00±1.78</td>
<td>1.93±0.43</td>
<td>&lt;0.0001</td>
<td>3.11</td>
</tr>
<tr>
<td>( AUC/dose/kg (ng/ml·h)/(mg/kg) )</td>
<td>2323±447</td>
<td>2507±1255</td>
<td>N.S.</td>
<td>0.92</td>
</tr>
<tr>
<td>( C_p/dose/kg (ng/ml)/(mg/kg) )</td>
<td>433.1±90.3</td>
<td>292.6±135.7</td>
<td>&lt;0.0005</td>
<td>1.48</td>
</tr>
<tr>
<td>( C_i/dose/kg (ng/ml)/(mg/kg) )</td>
<td>77.1±23.6</td>
<td>160.0±91.8</td>
<td>&lt;0.0005</td>
<td>0.48</td>
</tr>
</tbody>
</table>
TAC was higher with AUTL ($r^2=0.9029$; Fig. 5c) than AATL ($r^2=0.7123$; Fig. 5d).

**DISCUSSION**

The immunosuppressive action mechanisms of CYA and TAC are almost the same except for a difference in their binding proteins; *i.e.*, cyclophilin for CYA and FK-binding protein (FKBP) for TAC. AUC of CYA is generally thought the most reliable pharmacokinetic parameter for the evaluation of its clinical efficacy. Therefore, CYA blood concentration is monitored by AUC for 0 to 4 h after administration ($AUC_{0-4}$) in many institutions after the use of CYA microemulsion formulation. Alternatively, monitoring was also carried out by $C_2$ level, which is reported to correlate most closely with AUC as a simple monitoring method. On
the other hand, $AUC$ of TAC is generally thought the most reliable pharmacokinetic parameter to predict the clinical efficacy as well as CYA, though the relationship between $AUC$ of TAC and the clinical efficacy of CYA has not been evaluated precisely. Blood concentration of CYA is usually suggested to monitor by $C_{yt}$ substituted for $C_{t}$ because $AUC$ is more significantly correlated with $C_{yt}$ than $C_{t}$, though $AUC$ blood concentration is monitored by $C_{t}$. However it has yet to be explained as to why only $AUC$ of CYA correlates more significantly with $C_{yt}$ than $C_{t}$ and we had been clearly unable to explain as to why the monitoring points differ between CYA and TAC, despite of the same calcineurin inhibitors. Therefore, we examined these issues by comparing the new pharmacokinetic parameters. We proved that the pharmacokinetic parameters were clearly different between CYA and TAC. Though CYA and TAC relatively gave almost the same bioavailability ($AUC/D/BW$), CYA had higher $C_{yt}$ and a sharper blood concentration curve than TAC did. Though it was better known that CYA had a sharper blood concentration curve and TAC had a gentler curve, there have been no reports that $AUC$ corrected by dose per body weight ($AUC/D/BW$), namely relative bioavailability was similar between CYA and TAC, and that $C_{yt}$ of CYA was relatively lower than TAC, and that $C_{yt}$ of CYA was higher. It is thought that there is a difference of pharmacokinetics between CYA and TAC, because the absorption rate constant of CYA is probably higher than that of TAC as CYA and TAC have almost the same half life.

It suggests an important problem that there is a distinct difference of blood concentration curve to have higher peak ($C_{yt}$ is higher and $C_{t}$ is lower, relatively) like CYA, or the concentration curve not to make peak to keep minimum effective concentration ($C_{p}$ is lower and $C_{t}$ is higher, relatively) like TAC in effective and adverse reaction shown on Fig. 4, even if same drugs have same values of $AUC$. The mono-pharmacokinetic parameters of only $AUC$ are insoluble fundamentally. Pharmacokinetic analysis, including $C_{t}$ and $C_{yt}$, and not only $AUC$, will be required.

Furthermore, we used AUTL/$AUC$% as a new parameter being able to present the relationship among $AUC$, $C_{yt}$, and $C_{t}$. It is important that these problems be solved to use AUTL/$AUC$%. Therefore, it was presumed that the $AUC$ of CYA was largely dependent on $C_{yt}$, as the AUTL/$AUC$% was less than 50%. On the contrary, TAC gave lower $C_{yt}$ and gentler blood concentration curves. As AUTL/$AUC$% of TAC was more than 70%, it was presumed that the $AUC$ was largely dependent on $C_{p}$ and the blood concentration curve was approximate to one of continuous intravenous infusion.

As described above, to confirm that $AUC$ of CYA and TAC were dependent on $C_{yt}$ and $C_{t}$ respectively, we compared the correlation coefficients ofAUTL and AATL versus $AUC$ in both drugs. $AUC$ of CYA was more significantly associated with AATL than AUTL (Figs. 5a, b). Conversely, $AUC$ of TAC was more significantly associated with AUTL (Figs. 5c, d). We demonstrated that the degree of involvement of $C_{p}$ and $C_{t}$ in the area of $AUC$ was different between CYA and TAC. We have proved that $C_{yt}$ monitoring points for CYA, and $C_{t}$ monitoring points for TAC have been appropriate in this study, because the areas in $AUC$ of CYA and TAC were significantly dependent on $C_{yt}$ and $C_{t}$ respectively. This result verified the difference of pharmacokinetics between CYA and TAC including the relationship among $AUC$, $C_{yt}$, and $C_{t}$ using similar clinical backgrounds in renal transplant patients.

Aside from this, the new parameter, AUTL has been also useful as follows; switching to continuous intravenous infusion from oral administration, and vice versa. Continuous infusion of CYA has been carried out more frequently in bone-marrow transplant recipients than renal transplants. Miller et al. reported that the adjustment of CYA level to be 450—500 ng/ml by continuous infusion is well tolerated and effective in the prevention of acute graft-versus-host disease (GVHD). On the other hand, Ogawa et al. reported that the incidence of acute GVHD in bone-marrow transplant recipients treated with continuous infusion of CYA with a target level to be 250—400 ng/ml was significantly higher than that in recipients treated with twice-daily infusions. We previously calculated a conversion value utilizing the ratio of AUTL (=AUC/AATL) and $AUC$ for predicting a target blood concentration when switched to continuous infusion from oral administration, so that the target blood concentration of CYA administered by continuous infusion necessary was 2.55 times of $C_{t}$ by oral administration. The calculated CYA target level in continuous infusion corroborates the results of the CYA target level reported by Miller et al. As described above, it is thought that the AUTL was useful when switched to continuous intravenous infusion from oral administration, and vice versa.

CONCLUSION

In conclusion, although there has never been a report given to account for the distinct differences in the pharmacokinetics between CYA and TAC including the association among $AUC$, $C_{yt}$, and $C_{t}$, we clearly proved the differences in the pharmacokinetics between CYA and TAC in renal transplant recipients by using AUTL/$AUC$%, and we demonstrated with controlled clinical data why $AUC$ of CYA correlated more significantly with $C_{yt}$, and why CYA blood concentration must be monitored by $C_{yt}$ and why TAC blood concentration must be monitored by $C_{t}$. We will further investigate the optimal pharmacokinetics of calcineurin inhibitors including the relationship among $C_{t}$, $C_{yt}$ and $AUC$ to investigate relationship between AUTL/$AUC$% and clinical efficacy.

A new pharmacokinetic parameter AUTL/$AUC$% should also be examined to investigate the difference of relationship between $AUC$, $C_{yt}$, and $C_{t}$ in many drugs. Pharmacokinetic analysis in the future will need to consider not only $AUC$ separately, but also $AUC$ including its relationships with $C_{yt}$ and $C_{t}$, when evaluating clinical efficacy and adverse reactions.

REFERENCES