Relationship between Apparent Total Body Clearance of Cyclosporin A and Its Erythrocyte-to-Plasma Distribution Ratio in Renal Transplant Patients

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To establish an optimal method for determining a cyclosporin A (CyA) regimen based on physiological changes that occur during immunosuppressive therapy, the relationship between apparent CyA body clearance (CL/f) and the CyA erythrocyte-to-plasma distribution ratio (CyA-EP) was examined using clinical time courses obtained during routine monitoring. The CyA-EP, which was calculated by a multiple regression formula using routine data, was increased during renal dysfunction involving the normal recovery phase after transplantation, during nephrotoxicity, during acute tubular necrosis, and during acute renal rejection. CyA total body clearance (CL), calculated by multiplying CL/f and converted bioavailability, f, (which is equal to 0.009 × LD, where LD represents the CyA level in blood per dose ratio), showed hyperbolic decay with increasing CyA-EP (the mean CL was defined as follows: CL = 0.937/CyA-EP), whereas f showed exponential decay with increasing CyA-EP (the mean f was defined as follows: f = 0.593 × exp(−0.155 × CyA-EP)). These findings suggest that total CyA body clearance and its bioavailability were suppressed during the renal dysfunction phase. Hence, the mean CL/f as a function of the CyA-EP was given by the following equation: CL/f = 1.390 × exp(0.204 × CyA-EP)/CyA-EP. Since the CyA-EP reflects a patient's disease state and alterations in the CyA pharmacokinetic profile, these model formulae should provide an adequate method for determining a CyA dosage regimen for several disease states after renal transplantation.

Keywords cyclosporin A; physiological pharmacokinetics; erythrocyte-to-plasma distribution; population pharmacokinetics; renal transplantation

Cyclosporin A (CyA), a potent immunsuppressive agent, is widely used for the inhibition of graft rejection in renal, hepatic, cardiac, lung, pancreatic, and bone marrow transplantation.1–3 In clinical practice, this drug has had a great influence on successful results in transplantation. As a drug for therapeutic drug monitoring, CyA was selected to avoid serious side effects, i.e., nephrotoxicity and hepatotoxicity.4, 5 Reasonable correlations have been noted between CyA concentration and the immunosuppressive response in various in vitro tests, although this correlation is much less obvious in vivo.6 Further, the pharmacokinetics of CyA are affected by changes in several physiological factors in relation to a patient's disease state after transplantation. To date, no optimal drug dosage regimen suitable for use during the disease states found in transplant patients has yet been established.7

In a series of previous reports in relation to CyA distribution in human blood,8, 9 we found that this distribution was regulated by two different properties, those in plasma and those in erythrocyte fraction. Alterations in the CyA erythrocyte-to-plasma distribution ratio (CyA-EP) thus reflected physiological changes in blood constituents due to disease states, and prompted a shift of CyA into tissues. In addition, we found that the immunosuppressive response, determined by lymphocyte proliferation, increased during renal dysfunction or hepatotoxicity with an increase in the CyA-EP.10 Thus, we concluded that monitoring the CyA-EP would be more useful for predicting clinical efficacy and the risk of developing renal and hepatic dysfunction than monitoring CyA plasma or whole blood levels alone. Furthermore, we believe that the use of the CyA-EP concept for CyA therapeutic monitoring will allow for the adequate administration of CyA.

In this study, to establish an optimal method for determining a CyA dosage regimen during immunosuppressive therapy after renal transplantation, we examined the relationship between the apparent total body clearance of CyA and CyA-EP.

MATERIALS AND METHODS

Patients and Sample Treatment Twenty-seven clinical time courses of CyA, obtained from 7 renal transplant patients (6 males and 1 female) during therapeutic monitoring, were used for analysis. Immunosuppression after renal transplantation was achieved with a triple therapy consisting of CyA, prednisolone, and azathioprine. Informed consent was obtained from all the patients. Before renal transplantation, physicians described to their patients outline of renal transplantation, immunosuppressive therapy with CyA, the smaller effects and adverse effects of CyA and several clinical tests used to evaluate clinical efficacy, including blood-collecting for the CyA pharmacokinetic study. Physicians also obtained each patient's agreement, which became part of his or her medical records, accompanied by patient's
signature. The patients received oral CyA (3.380–8.152 mg/kg/d), given in two divided doses at 12-h intervals during the study periods. Blood samples were collected into heparinized tubes on or after the third day after a change in oral dose (steady state). Collections were carried out before administration (6:00 a.m.) and at 1, 2, 3, 4, 6, 8, 10 and 12 h after the administration of CyA. Whole blood samples were kept at −80°C until the day of assay. Rejection episodes were diagnosed by biopsy, by a rapid increase in serum creatinine (S-CRE) and body weight due to systemic edema, and by a rise in the number of leukocytes and the presence of fever. Nephrotoxicity episodes were diagnosed by the presence of a persistent increase in S-CRE without fever.

**Biochemical Tests**  As factors that regulate CyA-EP, we selected the hematocrit (HCT, %) and triglyceride (TG, mm) and cholesterol (CHO, mm) levels. Hepatic and renal functions were assessed by glutamate pyruvate transaminase activity (GPT, IU/l), and by S-CRE (mg/dl), respectively. The results for these biochemical tests were obtained by employing the SHINE computer on-line system of Shiga University of Medical Science.

**Data Analysis**  The area under the curve from time zero to 12 h (AUC₀₋₁₂, μg·h/ml) after oral administration of the drug was calculated using a linear trapezoidal approximation. Then the apparent body clearance of CyA (CLᵢf, 1/h/kg) was calculated using the following equation:

\[
CLᵢf = D/AUC₀₋₁₂
\]

where \(D\) represents one oral dose (mg/kg), \(f\) represents the bioavailability of CyA and CLᵢf is given as a hybrid form. The CyA-EP was then given by the following equation:

\[
\text{CyA-EP} = 6.0831 - 0.2944 \times (\text{TG} + \text{CHO}) - 0.0037
\times (C_p) - 0.0553 \times (\text{HCT}) + 0.0463 \times (\text{BW}) + 0.4447
\times (\text{S-CRE}) - 0.0366 \times (\text{AGE})^{11}
\]

where \(C_p\) represents the CyA concentration (ng/ml) in whole blood at trough, TG + CHO, BW, CRE, and AGE represent the sum of plasma lipids (mm) correlated to the quantity of lipoproteins in plasma, to the patient’s body weight (kg), to serum creatinine (mg/dl), and to the patient’s age (in years), respectively. To determine the relationships between variables obtained from routine clinical data, we used an extended non-linear least squares method, MULTIELS,\(^{12}\) implemented on a PC-9821 Cs2 microcomputer. On the MULTIELS, variations due to different blood-collating times were treated as intra-individual variations within a patient because time-dependent change (which is intrinsic for individuals) in the CyA pharmacokinetics are observed during immunosuppressive therapy with CyA after transplantation.\(^{13}\) Linear regression analysis was used in bivariate analysis. For the test of differences of means, we used the Welch t-test, since the variance between data for the classified groups was not equal.

**Drug Assay**  Concentrations of CyA in whole blood were measured by our high-performance liquid chromatographic (HPLC) method reported previously,\(^{14}\) or by a fluorescence polarization immunoassay method with monoclonal fluorescence antibody tracer (m-FPIA). Briefly, a liquid–liquid extraction procedure involving a delipolyzing process with dextran sulfate sodium salt and a silica gel column were used for the separation of the drug. The mobile phase consisted of 90% hexane and 10% ethanol, both by volume. The column temperature was maintained at 60°C to avoid broadening the peak, and the flow rate was set at 1.0 ml/min. The wavelength of the ultraviolet detector was set at 214 nm. Cyclosporin D was used as an internal standard. The m-FPIA was carried out automatically, on the TDx® assay system, according to the Abbott assay manual (Abbott Laboratory).\(^{15}\) Measurements by HPLC have a one-to-one correspondence to those of the m-FPIA because both these assay methods detect the unchanged form of CyA in blood.\(^{16}\)

**RESULTS**

Figure 1 shows all time courses from 7 patients obtained during routine therapeutic monitoring. There were great inter- and intra-individual variations in the transit of blood CyA after administration. The correlation coefficient, determined by bivariate linear regression analysis between CyA dose and trough level was 0.064 (\(p > 0.5\)), suggesting that it would be difficult to adjust the CyA dose using the CyA trough level only. Patient B had an episode of acute tubular necrosis (ATN) following renal transplantation and received hemodialysis on and after day 22. Thus, this patient’s AUC₀₋₁₂ was markedly reduced in comparison with the other patients’ AUC₀₋₁₂ within the same treatment periods. Patient D had an episode of acute renal rejection on day 183 after renal transplantation, with markedly increased S-CRE and body weight. The time course was obtained two weeks later (day 196). The AUC₀₋₁₂ on day 196 was markedly reduced compared to that on day 48, despite there being no change in CyA dose. Nephrotoxicity was observed in patients E, F, and G on days 72, 60, and 60 after renal transplantation, respectively. During the nephrotoxic phase in patients E and F, their AUC₀₋₁₂ were markedly reduced, and the time required to reach peak concentration was markedly delayed in patient F on day 60.

To clarify changes in the apparent total body clearance, CLᵢf, and the CyA-EP, we classified these time courses in relation to the time after transplantation and to the renal function marker (S-CRE) (Table I). There was no episode of hepatotoxicity assessed by GPT (<40 IU/l). For expedience, the early phase of immunosuppressive therapy, within 30 d after renal transplantation, was considered a recovery phase from the operation, and persistent elevation of S-CRE without fever for more than 30 d was considered to be due to nephrotoxicity. The CLᵢf during the normal recovery phase was significantly higher (\(p < 0.01\)) than that in the no-episode group more than 30 d after renal transplantation. Moreover, the CLᵢf in the nephrotoxic phase more than 30 d after renal transplantation was also significantly increased in comparison with that in the no-episode group (\(p < 0.01\)). Although the number of time courses during ATN or acute renal rejection were small, the CLᵢf was markedly higher than that in the no-episode
Fig. 1. Time Courses of Blood CyA after Oral Doses at Different Phases of Immunosuppressive Therapy in Seven Renal Transplant Patients (A—G)
Numbers on the panels represent the time after transplantation (days, upper) and one oral dose of CyA (mg/kg, lower).

| Table 1. CL/f and CyA-EP When Data Were Classified According to Renal Function and Time (Days) after Renal Transplantation |
|---|---|---|---|---|
| Period (Days) | n | S-CRE (mg/dl) | CL/f (l/h/kg) | CyA-EP |
| Within 30 d | | | | |
| ATN<sup>a</sup> | 7—14 | 2 | 11.6 | 1.302 | 9.989 |
| Normal recovery<sup>b</sup> | 7—30 | 14 | 4.50 ± 4.21 | 0.759 ± 0.290 | 5.653 ± 1.770 |
| More than 30 d | | | | |
| No episode<sup>c</sup> | 40—50 | 7 | 1.78 ± 0.49 | 0.536 ± 0.220 | 3.916 ± 0.513 |
| Nephrotoxicity<sup>d</sup> | 40—76 | 3 | 1.99 ± 0.12 | 0.871 ± 0.691 | 4.009 ± 0.670 |
| Rejection<sup>e</sup> | 196 | 1 | 7.7 | 1.028 | 8.772 |

<sup>a</sup> Data obtained from patient B.  
<sup>b</sup> No episodes of hepatotoxicity. S-CRE level was rapidly reduced to around normal range within 30 d after the operation.  
<sup>c</sup> S-CRE and GPT, <1.5 mg/dl and <40 IU/l, respectively.  
<sup>d</sup> Persistent increase in S-CRE without fever and increase in body weight.  
<sup>e</sup> Data obtained from patient D.
group. The values for CyA-EP in the normal recovery phase, ATN, and/or rejection were also markedly higher than those in the no-episode group. Figure 2 shows the relationship between CyA-EP and renal function in a frequency histogram. The values of CyA-EP for the no-episode group were found to be around 3.0 to 4.0, whereas those for renal dysfunction groups due to ATN, acute renal rejection, and/or nephrotoxicity were 3 to 11. In particular, episodes of ATN and rejection provided higher increases in the CyA-EP. These findings, shown in Table I and Fig. 2, suggest that states of renal dysfunction increase the CyA-EP.

To elucidate the relationship between the $CL/f$ and the CyA-EP from routine clinical data after oral administration, we selected an index to reflect CyA bioavailability ($f$). In this study, we estimated $f$ using a conversion formula defined by the following equation:

$$f_c = 0.009 \times LD$$

where $f_c$ and $LD$ represent converted bioavailability and CyA trough level in whole blood (ng/ml) per dose ratio, respectively. The constant in Eq. 3 represents exchange rates calculated by comparing ranges of $f$ reported elsewhere\(^{12,17}\) and our $LD$ data. As shown in Fig. 3, since the $LD$ has a significant correlation with the $AUC_{0-12}$ per dose ratio $(AUC_{0-12}/D)$, this ratio being related to the absorption rate of CyA from the intestinal tract after oral administration,\(^{18}\) the $f_c$ calculated by the conversion formula reflects the bioavailability of CyA after oral administration. We then estimated the total body clearance of CyA($CL_t$) by the following equation:

$$CL_t = (CL/f) \times f_c$$

Figure 4 shows the relationships between the $CL_t$ and the CyA-EP, and between the $f_c$ and the CyA-EP. The

Fig. 2. Frequency of CyA-EP Classified by Renal Dysfunction

Key: ◆, normal recovery within 30d; □, no-episode for more than 30d; ◐, nephrotoxicity of more than 30days' duration; ■, rejection at day 196 in patient D; □, ATN in patient B.

Fig. 3. Linear Correlation between $AUC_{0-12}$ and $LD$

$a)$ $AUC_{0-12}$ per CyA oral dose ratio. $b)$ CyA trough level per CyA oral dose ratio. The regression equation of the $AUC_{0-12}/D$ on the $LD$ was $Y = 0.0274X + 0.640 (r = 0.654, p < 0.001)$.

Fig. 4. Relationships between $CL_t$ and CyA-EP, and between $f_c$ and CyA-EP

$a)$ A hyperbolic relationship was found for $CL_t$ versus CyA-EP. The mean population trace was given by the equation: $CL_t = 0.937$/CyA-EP. $b)$ An exponential relationship was found for $f_c$ versus CyA-EP. The mean population trace was given by the following equation: $f_c = 0.993 \times \exp(-0.155 \times \text{CyA-EP})$. Constants in these equations were estimated by MULTIELS.
**Fig. 5. Model to Account for the Relationship between CL/f and CyA-EP**

This correlation is given as a hybrid form of the equations in Fig. 4. The mean population trace is given by the equation: \( CL/f = 1.390 \times \exp(0.204 \times \text{CyA-EP}) / \text{CyA-EP}. \) Constants in this equation were estimated by MULTIELS.

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**TABLE II. List of Converged Parameters by MULTIELS**

<table>
<thead>
<tr>
<th>Model</th>
<th>Mean parameters</th>
<th>95% C.I.</th>
<th>Variances</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eq. 5</td>
<td>( P_1 = 0.937 )</td>
<td>0.832—1.046</td>
<td>( \sigma^2 = 6.979 \times 10^{-2} )</td>
</tr>
<tr>
<td>Eq. 6</td>
<td>( P_2 = 0.593 )</td>
<td>0.540—0.646</td>
<td>( \sigma^2 = 8.728 \times 10^{-3} )</td>
</tr>
<tr>
<td></td>
<td>( P_3 = 0.155 )</td>
<td>0.127—0.182</td>
<td>( \sigma^2 = 1.769 \times 10^{-2} )</td>
</tr>
<tr>
<td>Eq. 7</td>
<td>( P_4 = 1.390 )</td>
<td>1.245—1.535</td>
<td>( \sigma^2 = 8.518 \times 10^{-3} )</td>
</tr>
<tr>
<td></td>
<td>( P_5 = 0.204 )</td>
<td>0.186—0.221</td>
<td>( \sigma^2 = 1.341 \times 10^{-1} )</td>
</tr>
</tbody>
</table>

a) Model equations defined on MULTIELS. b) 95% confidence interval. c) Variances of inter-individual variations (omega) and intra-individual variation (sigma).

CL₄ and \( f_c \) decreased with increasing CyA-EP. Therefore, we fitted decay curves as a function of the CyA-EP to \( CL_f \) versus CyA-EP and \( f_c \) versus CyA-EP data. From the Akaike’s Information Criteria (AIC)\(^{11}\) computed by MULTIELS,\(^{12}\) hyperbolic and exponential relationships, respectively, were found in \( CL_f \) versus CyA-EP and \( f_c \) versus CyA-EP. In the MULTIELS, the model equations for the \( CL_f \) and \( f_c \) as a function of the CyA-EP were defined as follows:

\[
CL_f = \frac{(P_1 + \eta_1) \times \text{CyA-EP} + \epsilon_1}{\eta_2 \times \exp((P_3 + \eta_3) \times \text{CyA-EP}) + \epsilon_2}
\]

\[
f_c = \frac{(P_3 + \eta_3) \times \exp((P_3 + \eta_3) \times \text{CyA-EP}) + \epsilon_2}{\eta_4 \times \exp((P_3 + \eta_3) \times \text{CyA-EP}) + \epsilon_2}
\]

where \( P_1, P_2, \) and \( P_3 \) represent mean parameters. \( \eta_1, \eta_2, \) and \( \eta_3 \) represent inter-individual variations, and \( \epsilon_1 \) and \( \epsilon_2 \) represent intra-individual variations and measurement error.

Figure 5 shows a model that accounts for the relationship between \( CL/f \) and CyA-EP. In Fig. 4, we found that \( CL_f \) and \( f_c \) decreased with the hyperbolic or exponential relationships; therefore, the relationship between the \( CL/f \) and the CyA-EP in the MULTIELS was given by a hybrid form defined by:

\[
CL/f = CL_f' = (P_4 + \eta_4) \times \exp((P_4 + \eta_4) \times \text{CyA-EP}) / \text{CyA-EP} + \epsilon_3
\]

where \( P_4 \) and \( P_5 \) represent mean parameters, and \( \eta_4 \) and \( \eta_5 \) represent inter-individual variations. \( \epsilon_3 \) represents intra-individual variation and measurement error. The solid line in Fig. 5 represents the mean population of \( CL/f \) as a function of CyA-EP computed by MULTIELS.\(^{12}\)

Table II represents converged parameters by MULTIELS for 3 model equations (Eqs. 5—7). All population means (\( P_1 - P_5 \)) are within a 95% confidence interval, suggesting that these population means are estimated at significance levels of less than 0.05.

**DISCUSSION**

Despite the requirement for an optimal CyA regimen that reflects physiological changes in CyA pharmacokinetics when CyA monitoring is performed, no method suitable for use in the disease states found in transplant patients has yet been established.\(^{7}\) In this report, to establish a CyA oral regimen using the CyA-EP, we examined the underlying relationship between the \( CL/f \) and the CyA-EP using routine clinical data. The pharmacokinetics of CyA is very complex, since CyA is affected by physiological changes due to several disease states.\(^{13}\) Various pharmacokinetic studies have been performed using the concept of a compartment model\(^{17,20}\), however, the results of these investigations have not commonly been translated into clinical practice. Indeed, as shown in Fig. 1, we could not fit the compartment model to the data in patient F because of the appearance of a secondary peak concentration and the time-lag, and because of the marked delay in time required to reach the peak concentration. Therefore, we used a non-parametric method to obtain the \( CL/f \) in this study.

CyA is widely distributed into such blood constituents as erythrocytes and plasma lipoproteins.\(^{21–23}\) During disease states after transplantation, i.e., nephrotoxicity, hepatotoxicity, and anemia, the amounts of blood constituents to which CyA binds and/or is distributed are physiologically altered.\(^{9}\) Therefore, changes in the CyA-EP reflect patient disease states after transplantation. In addition, we have found that the total body clearance...
of CyA after intravenous administration had a negative correlation with the CyA-EP in rat disease models.\textsuperscript{240} These findings suggest that the CyA-EP is a useful monitoring device for predicting alterations in CyA pharmacokinetics and changes in disease states after renal transplantation. To obtain direct measurements of the CyA-EP, however, the CyA concentration in both whole blood and plasma (separated at 37 °C) must be measured, yet it is very tedious to perform these assays during routine CyA monitoring because the equilibrating process at 37 °C to separate plasma, and a specific method such as HPLC or radioimmunoassay with a monoclonal antibody to measure CyA in plasma, are required. From this perspective, in a previous study\textsuperscript{213} we created a model formula (Eq. 2) to predict the CyA-EP from blood CyA measurements of routine trough level monitoring by m-FPIA on the automatic analysis system (TDx)\textsuperscript{15} and various types of simple biochemical tests. This model formula provides estimates of the CyA-EP from these routine data, and allows us to predict alterations in patient's disease states and CyA total body clearance.\textsuperscript{240} In this study, for the practical use of this formula (Eq. 2) as a monitoring indicator for CyA monitoring, we examined the relationships between the calculated CyA-EP by Eq. 2 and the $CL/f$ from CyA concentration versus time curves.

It has been generally accepted that renal dysfunction did not influence CyA pharmacokinetics, since the urinary excretion of unchanged CyA is less than 6% of the dose administered. However, physiological changes in CyA binding and/or distributing constituents due to renal dysfunction could affect unbound CyA in the blood circulation and alter CyA pharmacokinetics. In our previous study,\textsuperscript{186} using a one compartment open model with a zero-order absorption process, we found that CyA absorption from the intestinal tract was retarded during renal dysfunction. In addition, the zero-order absorption rate constant, standardized by the CyA distribution volume, correlated with changes in such physiological parameters as the hematocrit and creatinine clearance due to renal dysfunction. On the other hand, the total body clearance of CyA after intravenous administration to rat disease models with glycerol-induced renal dysfunction was reduced in comparison with that in normal rats.\textsuperscript{240} These observations suggest that the total body clearance of CyA and its bioavailability may be independent of each other, but that both parameters depend on renal function. The analytical results shown in Figs. 2 and 4 certainly support these speculations; that is to say, CyA total body clearance and CyA absorption from the intestinal tract during the renal dysfunction phase, involving the early phase of recovery from the operation, ATN, acute rejection, and nephrotoxicity, were reduced with an increase in the CyA-EP. Regarding the CyA-EP at which a patient's disease state was stable, the range of $C_{\text{total}}-\text{EP}$ was 3.0 to 4.0.

Our final purpose was to establish an optimal CyA regimen involving the CyA-EP. Since the value for CyA-EP is obtained by simple calculation and since it reflects physiological changes due to several disease states,\textsuperscript{111} the formula showing the relation between the $CL/f$ and the CyA-EP allows us to estimate $CL/f$ from routine monitoring results (Fig. 5). The biphasic pattern of the relationship between the $CL/f$ and the CyA-EP is essentially dependent on relative changes in CyA total body clearance and its bioavailability (Fig. 4). From Fig. 5, it can be seen that an increase in the $CL/f$ means that with increasing CyA-EP, the patient is in a disease state of renal dysfunction, and with decreasing CyA-EP, this means enhanced CyA total body clearance. Gupta et al.\textsuperscript{173} reported that an increase in plasma lipids enhanced CyA total body clearance in healthy subjects. Since the CyA-EP is reduced with a rise in plasma lipids,\textsuperscript{111} the formula in Fig. 5 clearly explains the relationship between total body clearance and the increased plasma lipids reported by Gupta et al.\textsuperscript{173} As there were no time courses available during hepatotoxicity, we could not clearly determine the relationships between the $CL/f$ and the CyA-EP during this disease state. Since the CyA-EP increases due to a reduction of blood constituents with which CyA binds and/or is distributed,\textsuperscript{111} and CyA total body clearance decreases with increasing CyA-EP\textsuperscript{240} during hepatotoxicity, the relationship between the $CL/f$ and the CyA-EP during hepatotoxicity agrees with the relationship shown in Fig. 4a. On the other hand, theoretically, the oral bioavailability, $f$, is defined as follows: $f=Q/(Q+D/AUC_{0-12})$,\textsuperscript{259} where $Q$ and $D$ represent the hepatic blood flow and CyA oral dose, respectively. Since CyA is mainly metabolized by the cytochrome P-450 mixed oxidase system in liver,\textsuperscript{259} the $AUC_{0-12}$ increases with a reduction in CyA total body clearance during hepatotoxicity. Therefore, according to the above equation, it is considered that $f$ during hepatotoxicity may increase with a rise in the CyA-EP if the hepatic blood flow is unchanged. Nevertheless, no rapid increase in the $CL/f$ with increasing CyA-EP (Fig. 5) is expected during hepatotoxicity.

In conclusion, we succeeded in creating a model formula to explain the relationship between $CL/f$ and CyA-EP. We found that the total body clearance and bioavailability of CyA decreased with increases in the CyA-EP. Since the CyA-EP reflects physiological changes due to several disease states which appear during immunosuppressive therapy after renal transplantation, these model formulae (Eqs. 5—7) as a function of the CyA-EP should be useful devices for adequate adjustments of the oral CyA dose during several disease states. Although we could not demonstrate a concrete method for a CyA dosage regimen using these model formulae under existing conditions, for example, by employing the Bayesian approach,\textsuperscript{271} using these model formulae for estimating individual parameters will be one possible method. To adopt the concept of the CyA-EP for the routine monitoring of CyA, we are now undertaking a pharmacokinetic or multicollinear analysis using these formulae in mass populations. The results of this work will be presented in a subsequent report.

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