Pharmacokinetics of Cyclosporin A after Intravenous Administration to Rats in Various Disease States

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We examined the pharmacokinetic profile of cyclosporin A (CyA) after intravenous administration to rats prepared as models of various disease states found in patients who receive organ transplantations. After intravenous bolus administration to normal rats, the total blood clearance (CL) of CyA showed a dose-dependent increase. The CL was reduced in anemic (ANE) rats prepared by venesection, in carbon tetrachloride-induced acute hepatic failure (AHF) rats, and in glycerol-induced acute renal failure (ARF) rats. On the other hand, the volume of distribution at a steady state (V.) of CyA increased significantly in ANE and aged (AGE) rats. CyA distribution was tissue-specific, and the tissue CyA concentration was disease state-dependent. Linear relationships between the CyA concentration in whole blood and various tissues (liver, kidney, and heart) were found in AGE, ANE, and AHF animals. However, in ARF rats, tissue concentration was not increased to a great extent in comparison with the other disease models, even though the whole blood CyA concentration was increased. The tissue per blood concentration ratio (K.), which represented the CyA tissue transfer from systemic circulation, was influenced by the disease state. In the liver, in particular, the K. increased in the AHF and AGE groups, whereas it decreased in the ANE and ARF rats. The CL of CyA was negatively related to the erythrocyte per plasma concentration ratio (E/P), and the E/P exhibited disease state-dependent changes, suggesting that this ratio is a valuable indicator for predicting variations in CyA total blood clearance in organ transplant patients during episodes of anemia, nephrotoxicity and hepatotoxicity.

Keywords cyclosporin A; pharmacokinetics; rat disease model; erythrocyte-to-plasma distribution; tissue transfer

Cyclosporin A (CyA), a potent immunosuppressive agent, is widely used for the inhibition of graft rejection in renal, hepatic, cardiac, lung, pancreatic, and bone marrow transplantation. The immunosuppressive activity of this drug is related to its selective action against T lymphocytes, which play a central role in the induction of immune responsiveness. In clinical practice, this drug has had a great influence on successful results in transplantation. CyA blood concentration monitoring is advocated, however, to avoid its serious side effects, i.e., nephrotoxicity and hepatotoxicity. Reasonable correlations have been noted between the concentration of CyA and immunosuppressive response in various in vitro tests, but this correlation is much less obvious in vivo. Indeed, via blood monitoring only, it is often difficult to distinguish between the clinical signs of its major adverse effects and the lack of therapeutic efficacy. Further, the pharmacokinetics of CyA is affected by several physiological factors in relation to a patient's disease state after transplantation. To date, no optimal drug regimen suitable for use during the disease states found in transplant patients has been established.

Administered CyA is distributed widely to blood components, and it is therefore considered that the pharmacokinetic profile of CyA is affected by changes in these components. However, no clear relationship between these factors has yet been established. In a previous series of studies, we have demonstrated the characteristics of CyA distribution in human blood. The erythrocyte uptake of CyA is influenced by plasma lipids, albumin, and hematocrit. Because the distribution of CyA in human blood is regulated by two different binding properties, those in the plasma and the erythrocyte fraction, the quantity of unbound CyA, which is the form in which this drug exerts its immunosuppressive response, may depend on these distribution properties in blood. Therefore, considering all possible factors that may be related to the erythrocyte-to-plasma distribution of CyA, we believe that disease state-dependent changes in blood constituents may affect the disposition and efficacy of CyA.

The purposes of this investigation have been: (1) to elucidate changes in CyA transition in blood and in its distribution to tissues in various disease states which are observed in patients receiving organ transplantation, and (2) to show how the erythrocyte-to-plasma distribution is related to CyA pharmacokinetics. We examined various disease states in rats, namely anemia, hepatotoxicity, and nephrotoxicity, as well as advanced age, and then we investigated CyA pharmacokinetics following intravenous administration.

Materials and Methods
Chemicals CyA was kindly supplied by Sandoz, Ltd., Basle, Switzerland. Cremophor® EL, for the preparation of CyA injection, was purchased from Nacalai Tesque, Ltd., Kyoto, Japan. All other chemicals used were of analytical grade.

Preparation of Test Solution
The standard formulation was prepared in a mixture of absolute ethanol and Cremophor® EL (7:13) at CyA concentrations ranging from 25 to 100μg/ml. CyA test solutions for intravenous administration were then prepared by diluting these standard solutions 10-fold with saline to give final CyA concentrations of 2.5 to 10μg/ml. At this time, the final concentration of ethanol and Cremophor® EL in each test solution was 3.5% (v/w) and 6.5% (w/w), respectively.

Animal Preparation
In all experiments, male Sprague-Dawley rats were used. In this study, we induced three major disease states found in patients receiving organ transplantations. We also used a group of aged rats. Acute renal failure (ARF) was induced in rats by a subcutaneous injection of glycerol (5 ml/kg) in divided doses. The CyA experiments were performed at 48 h after the injection of glycerol. Acute hepatic failure (AHF) was induced in rats by the oral administration of 50% carbon tetrachloride in corn oil (2 ml/kg). The subsequent CyA experiments were also performed at 48 h after the oral administration of carbon tetrachloride. Anemia (ANE) was induced by carrying out a venesection (12 ml/kg) from the left carotid artery. The CyA experiments were performed at 3 h after this treatment.

Aged (AGE) rats were animals 35 to 50 weeks old; they had received normal food during rearing. The mean body weights of the normal (control; CNT) animals and those of the ARF, AHF and ANE rats were 255±18 g.
i.e., there were no differences in body weight between these disease models. The mean body weight of the AGE group was 486 ± 41 g. After the completion of these pretreatments to induce disease states, a polyethylene cannula (d.i.d. 0.5 mm; o.d. 0.8 mm; Duran Pykals, Australia) was surgically introduced into the left carotid artery to obtain blood samples at various times. This procedure was carried out while the animals were anesthetized. An intraperitoneal injection of sodium pentobarbital was used for this purpose; 40 mg/kg for CNT and AGE, and 20 mg/kg for ARF, AHF and ANE. Before the intravenous administration of CyA to these disease models, a portion of blood was withdrawn and subjected to biochemical measurements. Albumin, blood urea nitrogen (BUN), creatinine concentration and transaminase activity (GOT, GPT) in plasma were determined colorimetrically using assay kits purchased from Iyotoro Co., Ltd., Tokyo, Japan. The hematocrit (HCT) was measured by a centrifugation method.

**Results**

### Biochemical Tests in Rat Disease Models

The results of biochemical tests in rat disease models are shown in Table I. Plasma GPT and GPT increased 10-fold above normal levels in carbon tetrachloride-induced AHF rats, but there was no significant increase in renal function markers in this disease model. In glyceral-induced ARF rats, plasma creatinine and BUN increased significantly above the normal range, but no significant differences were found in hepatic function markers between the ARF and CNT groups. Albumin was reduced below the normal range in the ARF, AHF and ANE groups. Further, the mean hematocrit values were significantly reduced in the ARF and ANE animals. In ANE rats, in particular, both albumin and hematocrit decreased by one-half in comparison with the CNT group. There were no significant differences in the results of these biochemical tests between the AGE animals and the CNT group.

### I. V. Bolus Administration to Normal Rats

The mean whole blood concentration–time profiles after i.v. bolus administration of CyA to the CNT group at three different doses are shown in Fig. 1. There were no differences in body weight between the three dosage groups. CyA disappeared from the systemic circulation with three-exponential decay (Fig. 1a). The total blood clearance (CL) at doses of 2.5, 5.0, and 10 mg/kg were 3.107 ± 0.188, 3.406 ± 0.654 and 3.954 ± 1.105 mL/min/kg, respectively, and the CL showed a dose-dependent increase (Fig. 1b).

### I. V. Bolus Administration to AHF, ARF, ANE and AGE

The mean whole blood concentration–time profiles after i.v. bolus administration of CyA, 5.0 mg/kg, to AHF, ARF, ANE and AGE rats are shown in Fig. 2. The pharmacokinetic parameters after i.v. bolus administration

### Table I. Biochemical Data in Normal and Disease Model Rats

<table>
<thead>
<tr>
<th>Items</th>
<th>CNT</th>
<th>ANE</th>
<th>AGE</th>
<th>AHF</th>
<th>ARF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (mg/dl)</td>
<td>3.86 ± 0.18</td>
<td>1.88 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.80 ± 0.10</td>
<td>3.31 ± 0.14</td>
<td>3.65 ± 0.12</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>44.5 ± 2.3</td>
<td>24.5 ± 3.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.5 ± 1.7</td>
<td>46.3 ± 4.0</td>
<td>41.0 ± 2.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.15 ± 0.05</td>
<td>0.67 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.19 ± 0.08</td>
<td>1.25 ± 0.15</td>
<td>5.27 ± 0.42&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>22.3 ± 2.5</td>
<td>11.5 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.7 ± 2.0</td>
<td>26.4 ± 3.2</td>
<td>47.5 ± 6.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GPT (IU/I)</td>
<td>68.9 ± 10.2</td>
<td>38.6 ± 11.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>78.7 ± 12.3</td>
<td>829.2 ± 79.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.3 ± 13.9</td>
</tr>
<tr>
<td>GPT (IU/I)</td>
<td>25.4 ± 0.8</td>
<td>19.2 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.1 ± 1.0</td>
<td>458.5 ± 88.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.3 ± 0.92</td>
</tr>
</tbody>
</table>

Results are given as the mean ± S.D. of 4 to 5 determinations. <sup>a</sup> Plasma samples were obtained from rats after the treatments to induce disease states. <sup>b</sup> Significantly different from the appropriate control (p < 0.01), <sup>c</sup> Significantly different from the appropriate control (p < 0.05).

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TABLE II. Pharmacokinetic Parameters of CyA Following i.v. Bolus Administration to Disease Model Rats

<table>
<thead>
<tr>
<th>Items</th>
<th>CNT</th>
<th>ANE</th>
<th>AGE</th>
<th>AHF</th>
<th>ARF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>258</td>
<td>252</td>
<td>486</td>
<td>253</td>
<td>249</td>
</tr>
<tr>
<td>$t_{1/2,β}$ (h)</td>
<td>4.38 ± 1.12</td>
<td>16.06 ± 3.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.58 ± 0.69</td>
<td>8.55 ± 3.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.33 ± 4.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>$AUC_{0-∞}$ (μg h/ml)</td>
<td>25.36 ± 4.51</td>
<td>49.75 ± 10.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.05 ± 0.53</td>
<td>35.46 ± 4.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.43 ± 12.47&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>$CL_{α}$ (ml/min/kg)</td>
<td>3.406 ± 0.654</td>
<td>1.760 ± 0.408&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.962 ± 0.498</td>
<td>2.373 ± 0.233&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.821 ± 0.309&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>$MRT$ (h)</td>
<td>8.60 ± 2.10</td>
<td>25.39 ± 5.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.00 ± 1.24</td>
<td>13.30 ± 3.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.60 ± 3.26&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>$V_{α}$ (l/kg)</td>
<td>1.687 ± 0.178</td>
<td>2.582 ± 0.323&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.135 ± 0.271&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.879 ± 0.430</td>
<td>1.986 ± 0.580</td>
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</tbody>
</table>

CyA was administered to rats intravenously (5.0 mg/kg body wt). Results are given as the mean ± S.D. of 4 to 5 determinations. <sup>a</sup>Significantly different from the appropriate control ($p<0.01$). <sup>b</sup>Significantly different from the appropriate control ($p<0.05$).

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Fig. 1. Mean Whole Blood Concentration versus Time Curves of CyA after i.v. Bolus Administration to Normal Rats (a), and Relationship between $CL_{α}$ of CyA and Dose Administered (b)

Each point represents 3 to 4 individual determinations, and is expressed as mean ± S.D.

Key: ■—■, 2.5 mg/kg; O—O, 5 mg/kg; ▲—▲, 10 mg/kg.

for AHF, ARF, ANE and AGE rats are shown in Table II. In the CNT group, the mean values for $t_{1/2,β}$, $AUC_{0-∞}$, $CL_{α}$, $V_{α}$, and $MRT$ were 4.38 ± 1.12 h, 25.36 ± 4.51 μg h/ml, 3.406 ± 0.654 ml/min/kg, 8.60 ± 2.10 h, and 1.687 ± 0.178 l/kg, respectively. In the AGE, AHF and ARF animals, the whole blood concentration of CyA at the distribution phase after i.v. bolus administration decreased significantly in comparison with the CNT. In the ANE, AGE and AHF animals, CyA disappeared from the systemic circulation with three exponential decay, as occurred with the CNT; however, the concentration of CyA in these groups at 480 min after i.v. bolus administration was increased significantly ($p<0.01$) in comparison with CNT. In the ARF group, however, the secondary peak concentration was found between 1.5 and 4 h after the first distribution phase, yet the concentration at 480 min after administration was also significantly increased ($p<0.01$) in comparison with CNT. The $t_{1/2,β}$ was significantly prolonged in ANE, AHF and ARF, whereas in AGE, the $t_{1/2,β}$ was only slightly increased in comparison with CNT. The $CL_{α}$ was significantly reduced ($p<0.05$) in ANE, AHF and ARF, and the $MRT$ was significantly prolonged in these three groups

($p<0.05$). The $V_{α}$ was significantly increased in ANE and AGE, while in AHF and ARF, it was only slightly increased in comparison with CNT.

Relationships between Blood CyA Concentration and Tissue CyA Concentration in Disease Model Rats

Figure 3 shows the relationships between tissue CyA concentration and whole blood CyA concentration in the liver, kidney, and heart. The concentration of CyA in tissues varied in a tissue- and disease-dependent manner. In each of the tissues, the plots were divided roughly into two classes based on disease states. One category included AGE, ANE and AHF, and the other included only ARF. Based on the CNT standard, the tissue CyA concentration in ANE and AHF increased in each tissue as the respective blood CyA concentrations increased. On the other hand, although the blood CyA concentration in ARF was increased in comparison with the CNT level, the tissue CyA concentration was unchanged in the kidney, and was actually reduced in the liver and heart. To further elucidate the tissue transfer
of CyA in the various disease states, the tissue per whole blood concentration ratio ($K_b$) was calculated for each animal (Fig. 4). In the ARF group, the $K_b$ was significantly reduced ($p < 0.01$) in the liver, kidney, and heart compared with the CNT. In the AHF, ANE and AGE groups, in comparison with CNT, there were no significant changes in the $K_b$ values in the kidney and heart. In the liver, however, the $K_b$ was affected by disease states; namely, the $K_b$ was significantly increased in AHF ($p < 0.01$) and AGE ($p < 0.05$), whereas it was reduced in ANE and ARF.

Relationship between Total Blood Clearance ($CL_t$) and CyA Erythrocyte-to-Plasma Distribution Ratio ($E/P$) Figure 5 shows the relationship between the $CL_t$ and the $E/P$. Compared to the CNT values, the $E/P$ were significantly reduced in ANE, ARF and AHF. We found that the $CL_t$ was negatively related to the $E/P$.

Discussion

Generally, it has been determined that the concentration of a drug in plasma is more closely related to clinical responses or tissue toxicity because the plasma fraction, including any unbound drug, is adjacent to the tissues. In this study, however, we used whole blood samples to measure CyA concentration in blood. The reason we used whole blood samples is, firstly, to minimize changes in the reduction of blood constituents with the passage of time during i.v. study by withdrawing minimal volumes of collected blood, and secondly, to examine the blood-to-tissue transfer of CyA in various disease states because protein bound CyA in blood plays an important role in CyA tissue transfer. Although the CyA blood clearance values calculated from the HPLC measurements in rats were about 2- to 4-fold lower than those of the plasma clearance reported elsewhere, the blood clearance provided a reasonable picture of the physiological pharmacokinetics involved, since CyA binds to erythrocytes and has a higher partition ratio between the plasma and erythrocyte fraction (around 3) at body temperature (37 °C). The in vitro free fraction of CyA in human blood is less than 5% at 37 °C, and the drug is distributed widely to blood components because of its extremely lipophilic nature.

Generally, the plasma or serum unbound drug concentration is a good index for estimating the pharmacological effects of a drug, and the protein binding of drugs in systemic circulation affects their pharmacokinetic profiles and their pharmacological effects. However, in the case of CyA, although a reasonable correlation has been noted between its concentration and immunosuppressive response in various in vitro tests, this correlation is much less obvious in vivo. In our previous in vitro investigations, we found that the erythrocyte uptake of CyA was retarded by increased albumin or lipid concentrations in the plasma fraction, whereas the erythrocyte concentration was reduced by increases in the hematocrit or by decreased CyA blood concentration. Although unbound
CyA cannot be measured directly, these phenomena suggest that the concentration of unbound CyA in blood is regulated by two blood fractions for which CyA has different affinities, namely, erythrocytes and plasma. Therefore, we believe that disease state-dependent changes in blood constituents could affect CyA pharmacokinetics involving tissue transfer and, thus, its pharmacological efficacy.

The total blood clearance of CyA, CLv, after i.v. bolus administration to CNT animals was found to increase dose-dependently (Fig. 1b). Since CyA is extensively metabolized by microsomal cells, CLv is defined as the product of the extraction ratio in the liver and the total blood flow through the liver. In the experiment shown in Fig. 1, normal rats of the same age were used for each dosage group, and it was therefore considered that there were no differences in the blood flow through the liver in any rat group. Hence, it is likely that the extraction of CyA by the liver may be concentration-dependent. In relation to this speculation, Lemaire et al. proposed that CyA bound to erythrocytes or plasma proteins was more easily able to dissociate and equilibrate into tissues than unbound CyA, and this bound CyA might be readily available for transport into the liver. In other words, if protein-bound CyA in the systemic circulation increases dose-dependently, the uptake of CyA by microsomal cells will increase. This is an interesting view, which confirms the relationship between CyA dose and the CLv. Gupta et al. found that the intake of high fat meals enhanced CyA clearance in normal human subjects; this observation supports the speculation of Lemaire et al. However, the precise mechanisms underlying the relationship between bound CyA and its uptake by microsomal cells still remain unclear. In contrast to the CyA concentration in the CNT rats, the concentration of CyA at the distribution phase decreased significantly in the AGE, AHF, ARF groups (Fig. 2). These phenomena suggest the existence of disease state-dependent deep compartments or the participation of another tissue compartment. An increase in adipose tissue is expected to be a deep compartment in AGE. An increase in CyA tissue permeability or transfer at the distribution phase from blood circulation to tissues is expected in AHF and ARF.

In addition, Lemaire et al. reported that the uptake of CyA into tissues is markedly limited by binding to plasma proteins and erythrocytes. In this study of CyA tissue transfer, therefore, we evaluated the tissue transfer by calculating the blood-to-tissue ratio (Ki). The blood-to-liver transfer of CyA exhibited disease state-dependent changes (Figs. 3, 4). The Ki in the AHF rats increased significantly in the liver, probably due to the reduction of cytochrome P-450 oxidase produced by carbon tetrachloride. The Ki in AGE rats also increased in the liver, probably due to a reduction in metabolic clearance or an increase in adipose constituents in microsomal cells. In ANE rats, the Ki in liver tended to decrease. This phenomenon can also be explained in terms of the speculation of Lemaire et al. However, in both the AHF and ANE animals (Fig. 3), the concentration of CyA in whole blood at 480 min after i.v. bolus administration increased in comparison with the CNT. One of the major causes of the elevation of CyA concentration in blood under anemic conditions may be the suppression of hepatic uptake due to a reduction in bound CyA related to the reduction of the erythrocyte fraction in blood. Accordingly, anemia during immunosuppressive therapy with CyA is considered to represent an emergent state, as does hepatotoxicity, and an optimal dose adjustment is thus required.

It has been reported that renal dysfunction did not affect CyA pharmacokinetics. Therefore, it has generally been accepted that a dose adjustment is not always necessary when a nephrotoxic episode occurs. However, we found that the mean of t1/2 in the ARF rats was prolonged about 2.5-fold, and the CLv was significantly reduced in comparison with that in the CNT (Table II). Also, a secondary concentration peak was found after i.v. bolus administration (Fig. 2). In addition, in these animals, the Ki was significantly reduced in the liver, kidney and heart in comparison with the other disease model rats (Fig. 4). These observations in ARF animals are contrary to the concept that renal dysfunction does not affect CyA pharmacokinetics. The reduction of tissue transfer in ARF rats may be due to the reduction of CyA solubility, and thus, tissue permeability, as a result of edema induced by renal dysfunction; the appearance of the secondary peak concentration suggests the participation of one more type of tissue compartment, i.e., lymphatic circulation. In attempting to find evidence to support the concept that renal dysfunction has no effect on CyA pharmacokinetics, Follath et al. compared the pharmacokinetic results of renal and bone marrow transplants. They found that the systemic clearance of CyA in patients with advanced renal failure (creatinine clearance $<5\text{ml/min}$) was $0.369 \pm 0.08 \text{l/h/kg}$ (by HPLC, whole blood measurements), whereas that in patients with bone marrow transplantation without renal failure was $0.56 \pm 0.06 \text{l/h/kg}$. Hence, they concluded that there was no difference in the systemic clearance of CyA, irrespective of the presence or absence of renal failure. However, they showed no detailed data on changes in blood constituents due to renal failure, patients' age, the hemotogenous conditions of either disease group, or the CyA dose administered. Therefore, it is hard to say that these clearances had not been compared on the same conditions. Although we can not always consider the disease state of glycerol-induced ARF is identical to the actual renal dysfunction related to transplants, it is of some interest to determine the regulatory mechanisms underlying CyA tissue distribution in glycerol-induced ARF. Nevertheless, for clinical practice, further investigations are required to elucidate the relationship between renal dysfunction and CyA pharmacokinetics.

As shown in Fig. 5, the E/P increased significantly in the AHF, ARF and ANE groups. In addition, the CLv of CyA changed in a manner corresponding with alterations in the E/P. In one of our previous reports, we showed that the E/P increased in a renal transplant patient when hepatotoxicity or nephrotoxicity was exhibited. When the renal and hepatic functions were stable, the CyA E/P was relatively constant, around 4, whereas when renal or hepatic function was unstable, the E/P increased up to around 12. Since the erythrocyte uptake of CyA is enhanced by a decrease in plasma proteins (and/or lipoproteins), or by a decrease in the hematocrit, we concluded that an elevation of the CyA E/P during an unstable phase in renal or hepatic function after transplantation is dependent upon a decrease in these physiological factors due to renal or
hepatic dysfunction. In the present in vivo studies, although we separately investigated the effects of various disease states on the pharmacokinetics of CyA, it is considered that the disease states which appear in transplant populations are not always independent. It is generally accepted that hematogenous functions and the synthesis of albumin and lipoproteins are suppressed during episodes of hepatotoxicity and nephrotoxicity. Indeed, transplant patients often exhibit anemia due to intraoperative blood loss, or to renal or hepatic dysfunction. In bone marrow transplant patients in particular, the hematocrit value exhibits hematogenous function-dependent changes. Therefore, an alteration in the $E/P$, which is closely related to alterations in blood constituents, will be a valuable indicator for predicting the $CL_s$ of CyA at various disease states after transplantation.

The present in vivo investigations using rat disease models provided valuable information for the therapeutic drug monitoring of CyA in transplant patients. The $E/P$, corresponding to the changes in blood constituents due to various disease states, is a valuable indicator for predicting alterations in CyA $CL_s$ when anemia, hepatotoxicity or nephrotoxicity is exhibited in transplant patients. To establish an optimal drug regimen method for CyA during episodes of toxicity or anemia, we are now examining the relationship between CyA monitoring data and the properties of CyA distribution in blood.

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