Pharmacokinetics of FK-506, a Novel Immunosuppressant, after Intravenous and Oral Administrations to Rats

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A pharmacokinetic study of FK-506 (FK), a novel immunosuppressant being about hundred times more potent than cyclosporin A (CyA) in the in vitro experiment, has been performed in rats after intravenous and oral administrations at two doses, 1.0 and 5.0 mg/kg. As compared with CyA, plasma, bile, urine and lymph FK levels were determined by a fluorescence high-performance liquid chromatographic method with chemiluminescence detection. Each compartmental pharmacokinetic parameters were calculated by area/moments analysis. After the i.v. injection of FK at 1.0 and 5.0 mg/kg, the total plasma clearance, $CL_{tot}$, was 7.028 ± 0.076 (mean ± S.E.) and 7.651 ± 0.755 ml/min, steady-state distribution volume, $V_{ss}$, was 4623 ± 28 and 4201 ± 529 ml/kg, elimination half-life at the terminal elimination phase, $t_{1/2}$, was 2.36 ± 0.25 and 2.54 ± 0.29 h, and the volume of the initial distribution space, $V_{i}$, was 1336 ± 150 and 1065 ± 115 ml/kg, respectively. By comparing the pharmacokinetic parameter with CyA, the $CL_{tot}$ of FK is about three times greater than that of CyA. There is not a significant difference on $t_{1/2}$ between CyA and FK. The $V_{i}$ and $V_{ss}$ of FK are 4—5 times greater than that of CyA. Therefore, higher clearance of FK is ascribed not only to the faster elimination from the rat body but also to the greater distribution space in the body. The mean percentage of FK transferred into the thoracic lymphatics over 6 h were 0.09 ± 0.02% (1.0 mg/kg) and 0.16 ± 0.02% (5.0 mg/kg), respectively. As the mean percentages of FK excreted into both the bile and urine for 6 h after i.v. injection were 0.0744 ± 0.0177% and 0.0073 ± 0.0018%, respectively, the main elimination pathway of this drug is thought to be the metabolism in the body. To the other groups of rats, FK was administered intraduodenally, 5.0 mg/kg, in two kinds of liquid preparations, and both the systemic and lymphatic availabilities were studied. The mean systemic availabilities of FK were 11.3% and 23.5% from the two preparations. The lymphatic availability of this drug over the experimental period, 6 h, was less than 0.2%. These results suggest that FK distributes more extensively in the rat body than CyA.

Keywords — FK-506; immunosuppressant; cyclosporin A; pharmacokinetics; intravenous injection; oral administration; rat

Introduction

FK-506 (FK), a macrolide antibiotic extracted from the fermentation broth of Streptomyces tsukubaensis,1) was shown to have a strong immunosuppressive activity against a mixed lymphocyte reaction (MLR).2) Although FK is structurally quite different from cyclosporin A (CyA), FK shares many of the pharmacological properties of CyA such as inhibitory effects on the production of various interleukins (IL-2, IL-3 and gamma-interferon) and on the expression of alloantigen-induced human lymphocyte IL-2 receptors (IL-2R) and transferrin receptors.2,3) FK inhibits the expression of IL-2R on both CD4+ and, more markedly, on human CD8+ T cells.4) This impairment of T-cell responses was achieved at concentrations of FK that, like CyA, had no effect on mouse bone marrow colony formation in vitro or on the proliferation of certain leukaemic cells. The in vitro pharmacological activity of FK is reported to be about 100 times more potent than that of CyA.5) Furthermore, the in vivo and in vitro minimum effective doses of this novel immunosuppressant are about one tenth those of CyA.5,6) The doses of 4.4 and 1 mg/kg FK have been reported to impair splenic immunoglobulin M (IgM) plaque-forming cell

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responses to sheep erythrocytes in mice and rats, respectively. FK also prolonged the graft survival by intramuscular injection of dose at 1 mg/kg/d in heterotropic cardiac allotransplantation in the rats.\(^7\) In these models, CyA had a similar effect at approximately 10-fold higher doses.\(^8,9\) The difference, in the extent to which FK is more potent than CyA in vitro compared with in-vivo systems, may simply reflect the difference of the rates of kinetic process in the body, namely the rate of absorption and/or the rate of elimination from the body. In our pharmacokinetic studies on CyA in the rat, the plasma concentration-time profile of CyA, after both intravenous (i.v.) and oral administrations, was examined and was analyzed with a non-compartment analysis approach.\(^10,11\)

In a previous paper, we reported a new specific assay method for FK in the rat plasma, lymph, urine, and bile by high-performance liquid chromatography (HPLC) using chemiluminescence detection after pre-labelling with dansyl hydrazine as a fluorescent reagent.\(^12\) This assay procedure is specific for unmetabolized FK in the rat body fluids and has a high sensitivity for measuring small amounts of this drug in the biological fluids obtained at the doses corresponding to the clinical use of this strong immunosuppressant.

The present study was performed to determine the basic pharmacokinetic characteristics of this new potent immunosuppressant after both i.v. and oral dosings to rats. It was also of interest to compare the kinetic profile of this drug with that of CyA.

**Experimental**

**Chemicals and Reagents** — FK was a gift from Fujisawa Pharmaceutical Co., Ltd. (Osaka, Japan). Pharmaceutical surfactant, polyoxyethylated, 60 \(\mu\)mol, castor oil (HCO60\(^\circ\) ), was obtained from Nikko Chemicals Ltd. (Tokyo, Japan). Propylene glycol (PG) was obtained from Nacalai Tesque Ltd. (Kyoto, Japan), Dansyl hydrazine (DH) and bis (2,4,6-trichlorophenyl) oxalate (TCPO) were of reagent grade from Tokyo Kaseikogyo Ltd. (Tokyo, Japan). HPLC-grade methanol and \(H_2O_2\) (30\%) were obtained from Wako Pure Chemicals Ltd. (Osaka, Japan). All other chemicals used were of analytical grade. Sep-pak\(^{\circ}\) C\(_{18}\) cartridges were 1.0 ml size and were obtained from Millipore (Massachusetts, USA).

**Animals** — Male Wistar rats (SLC, Shizuoka, Japan), weighing 400—450 g, were used throughout the study. The rats were housed in pairs under controlled environmental conditions and fed on commercial food pellets.

**Preparation of Test Solution** — The i.v. solution of FK was prepared in a 5\% (w/v) HCO-60 solution. The concentrations of FK in the i.v. solution for bolus injection were 0.8 mg/ml for low dose study (1.0 mg/kg) and 4.0 mg/ml for high dose study (5.0 mg/kg).

Two oral test solutions, PG solution and PG solution containing HCO60 (5\%w/v), were used in this study. The concentration of FK in the oral test solutions were 4.0 mg/ml.

**Animal Preparation** — i.v. Bolus Study: Twelve rats were divided into three groups, A, B and C. All the rats were fasted overnight but had free access to water. Under anesthesia by an intraperitoneal injection of sodium pentobarbital, 45 mg/kg, surgery was performed in the three groups of the rats. In groups A and B rats, the effect of the administered dose, 1.0 and 5.0 mg/kg, on the plasma disposition kinetics and lymphatic transfer of FK were studied. In group C rats, the excretory properties of FK into both the bile and urine were studied at a 5.0 mg/kg dose level.

Group A and B Rats: A polyethylene cannula (i.d., 0.5 mm; o.d., 0.8 mm, Dural Plastics, Australia) was introduced into the left carotid arteries of these groups of rats to obtain blood samples at various times. A modification of the method of Bollman et al.\(^13\) was used for the collection of lymph from the thoracic lymph duct. A heparin-filled polyvinyl cannula (i.d., 0.5 mm; o.d., 1.2 mm; Dural Plastics) was threaded about 3 mm into the thoracic lymph duct. A drop of tissue cement (Aron Alpha\(^{\circ}\), Sankyo Co., Ltd., Tokyo) was applied to the hole in the lymphatic to seal it and to fix the cannula in place. After collecting blank blood and lymph samples, 1.25 ml of the FK test solution per kg of rat body weight was injected into the femoral
vein of the rats, corresponding to a FK dose of 1.0 and/or 5.0 mg/kg. Group A rats received i.v. dose of FK at 1.0 mg/kg, and group B rats received that at the dose level of 5.0 mg/kg. The continuous output of the lymph from the thoracic lymph duct was collected in hourly fractions in tared culture tubes for 6 h, and their volumes were determined by weighing, assuming a density of 1.0 mg/µl. Blood samples (100—200 µl) were drawn into a heparinized microcentrifuge tubing at 0, 2, 5, 10, 20, 30, 90, 150, 210, 270 and 330 min after drug administration. Between samplings, the cannula was filled with heparinized saline to maintain its potency.

Group C Rats: In this group, the cannulation into the carotid artery and thoracic lymph duct were not performed. Instead, both the common bile duct and the urinary bladder were cannulated according to the standard technique reviewed by Cocchetto et al. to collect the bile and urine samples. After the i.v. injection of the FK test solution at the dose level of 5.0 mg/kg, both the bile and the urine samples were collected in hourly fractions in the tared culture tubes for 6 h. The bile samples were protected from light during the collection period. The bile and urine volumes were determined by weighing, assuming a density of 1.0 mg/µl. The samples were then stored at -20 °C until analyzed.

Oral Study: Eight rats, group D and E, were used in this study. The rats were fasted overnight but had free access to water. Under anesthesia cannulas were surgically introduced into both the left carotid artery and the thoracic lymph duct. After collecting blank blood and lymph samples, 1.25 ml of the oral test solution per kg of rat body weight was injected into the duodenum of the rats, corresponding to a FK dose of 5.0 mg/kg. Group D rats received a PG solution and group E rats received a PG solution containing HCO60. After administration, the pore made in the duodenum was closed with a drop of tissue cement and the abdominal incision was sutured surgically. The continuous output of the lymph from the thoracic lymph duct was collected in hourly fractions in tared culture tubes for 6 h, and their volumes were determined gravimetrically. Blood samples (100—200 µl) were drawn into heparinized microcentrifuge tubes at 0, 15, 30, 60, 120, 180, 240, 360 and 480 min after administration. Between samplings, the cannula was filled with heparinized saline to maintain its potency.

Sample Treatment and HPLC Assay in Biological Fluids — All the blood samples were centrifuged at 37 °C to obtain the plasma fraction. The plasma, lymph, bile and urine samples were frozen immediately after collection and stored in a freezer at -20 °C until analyzed. The concentration of FK was measured using an HPLC method with chemiluminescence detection, as described previously. After defrosting of the plasma, lymph, bile and urine samples, 100 to 200 µl aliquots of the rat plasma, lymph and bile samples were used for the FK assay after extraction into ethyl acetate. In the case of urine samples, the specimen was diluted ten times with saline. Thereafter, 50 µl of the diluted sample was used for the extraction. The extraction procedure was as we reported previously. Briefly, after the addition of 4 ml of water, 100 µl of isooamylalcohol and 5 ml of ethyl acetate, FK was extracted into the ethyl acetate phase. Four milliliters of the ethyl acetate phase was taken into a clean tube and evaporated to dryness at 40 °C under a stream of nitrogen gas. To the evaporated residue in the tube were added 1 ml of HCl: acetonitrile (80:µl: 100 ml) mixture and 100 µl of 0.05% (w/v) DH solution. After incubation for 5 min at 60 °C, 100 µl of sodium pyruvate solution was added and warmed for 10 min at 25 °C to accelerate the degradation of the excess DH reagent. To the resulting mixture were added 3 ml of water and 4 ml of n-hexane:ethyl acetate (9:1) mixture. The dansyl hydrazone of FK was extracted with this organic solvent and the extract was evaporated to dryness under a stream of nitrogen gas. The residue was dissolved with 150 µl of 50% methanol solution (methanol:water = 1:1) and was then added to Sep-Pak cartridge. After washing with 5 ml of 70% methanol solution, dansyl hydrazone of FK was eluted with five ml of 80% methanol solution. The eluate was collected and was evaporated to dryness at 40 °C under a stream of nitrogen. The resulting residue was redissolved by the addition of 200 µl of mobile phase (70% methanol solution) of which 50 µl aliquot was
injected into the HPLC system.

The HPLC system used in this report is the same as we reported as a column-switching system for the analysis of dansyl hydrazone of FK. The pretreated sample was injected on the pre-column and dansyl hydrazone of FK was first adsorbed to the pre-column (20 × 4.6 mm i.d., packed with Wakogel RP-18, 10 μm) with the first mobile phase, methanol:water (7:3), during 5 min. Thereafter, the line was switched to the second mobile phase, methanol:water (9:1), which is more hydrophobic than the previous mobile phase. Dansyl hydrazone of FK was transferred on an analytical column (Chemcosorb RP-18, 5 μm, 4.6×250 mm) with this mobile phase. The flow rates of the two mobile phases were always 1.0 ml/min. Twenty minutes after injection, the HPLC system was ready for a new cycle. Levels were estimated by the chromatographic technique of comparing peaks obtained from rat body fluid sample with curves obtained from the body fluid to which were added known amounts of FK. The standard curves of FK added to the rat body fluids were linear over the range of 0—20 μg/ml for plasma, lymph, bile and lymph sample respectively and passed through the origin.

Pharmacokinetic Analysis — The terminal elimination rate constant, β, for the FK concentration–time curves after i.v. and oral dosings was determined by linear regression at least three data points from the terminal portion of the plasma concentration–time plots. The area under the plasma concentration–time curve (AUC) after i.v. administration, AUC_{i.v.}, was calculated using the logarithmic trapezoidal rule up to the last measured plasma concentration C_{p(last)}, and extrapolated to infinity by addition of the correction term C_{p(last)}/β. The AUC obtained after oral administration, AUC_{oral}, was calculated to maximum concentration with the linear trapezoidal rule and after that to the last measured plasma concentration with the logarithmic trapezoidal rule and the addition of the correction term after last measured point to the infinity, namely C_{p(last)}/β. The area under the first moment curve to the last measured plasma concentration after i.v. injection, AUMC_{i.v.}, was calculated with the linear trapezoidal rule and with the addition of the correction term after last measured point to the infinity, namely t_{(last)}C_{p(last)}/β + C_{p(last)}/β^{1,15} The terminal elimination half-life, t_{1/2β}, was determined by dividing ln2 by β. The total plasma clearance, CL_{tot}, was determined by dividing the i.v. dose by the AUC_{i.v.}. The volume of the initial distribution space, V_{1}, was calculated by dividing the injected dose with the plasma concentration at the first collection time, 2 min, after i.v. injection of FK to rats. The volume of distribution at steady-state, V_{d,ss}, was calculated from the AUC_{i.v.} and AUMC_{i.v.} by the following equation, V_{d,ss} = dose/AUMC_{i,v} /AUC_{i,v}.^{2,15}

The parameters were determined for each individual. The values are expressed as their mean ± S.E. Statistical differences were assumed to be reproducible when p<0.05 (two-sided t-test).

Results

Mean plasma concentration–time profiles after the i.v. administration of FK to group A (1 mg/kg) and group B rats (5 mg/kg), are represented in Fig. 1. FK disappeared from the systemic circulation with two-exponential decay as shown in this figure. The pharmacokinetic parameters for these groups of rats are shown

![Fig. 1. Plasma Concentration–Time Profiles of FK after i.v. Bolus Injection to Two Groups (A and B) of Rats at Two Dose Levels (●, 1.0 mg/kg; ○, 5.0 mg/kg) to Rats in Comparison with That Obtained with CyA (△, 3.5 mg/kg)](image-url)
### Table I. Pharmacokinetic Parameters of FK and CyA Obtained after i.v. Injection to Rats

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Rat group</th>
<th>CyA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A</td>
<td>Group B</td>
</tr>
<tr>
<td>$\text{CL}_{\text{tot}}$ (ml/min)</td>
<td>7.028 ± 0.076</td>
<td>7.651 ± 0.755</td>
</tr>
<tr>
<td>$V_t$ (ml/kg)</td>
<td>1336 ± 150</td>
<td>1065 ± 115</td>
</tr>
<tr>
<td>$V_{d,ss}$ (ml/kg)</td>
<td>4623 ± 28</td>
<td>4201 ± 529</td>
</tr>
<tr>
<td>$t_{1/2\beta}$ (h)</td>
<td>2.36 ± 0.25(^a)</td>
<td>2.54 ± 0.29(^a)</td>
</tr>
<tr>
<td>$AUC_{i.v.}$ (µg·h/ml)</td>
<td>0.711 ± 0.063</td>
<td>3.802 ± 0.450</td>
</tr>
</tbody>
</table>

Group A and B rats received i.v. doses of FK at 1.0 and 5.0 mg/kg, respectively. The pharmacokinetic parameter for CyA were determined from the data published by us, where the i.v. injected dose was 3.5 mg/kg.\(^{11}\) Each value represents the mean ± S.E. \(^{a}\) Not significantly different from CyA group.

In Table I. In group A rats, the mean terminal elimination half-life, $t_{1/2\beta}$, was 2.36 ± 0.25 h, the mean total plasma clearance, $\text{CL}_{\text{tot}}$, was 7.028 ± 0.076 ml/min and $V_{d,ss}$ was 4.623 ± 0.028 l/kg. Furthermore, $V_t$ was calculated and the result is also shown in the table. The mean value is 1.336 ± 0.150 l/kg. When the administered dose was increased to 5.0 mg/kg in group B rats, the plasma disappearance pattern was similar to that obtained at low dose level, 1.0 mg/kg (Fig. 1). Significant differences were not detected with the three parameters, $t_{1/2\beta}$, $\text{CL}_{\text{tot}}$ and $V_{d,ss}$ between the two groups. In addition, the $AUC_{i.v.}$ value increased in accordance with the injected dose from 0.711 ± 0.063 µg·h/ml (1.0 mg/kg) to 3.802 ± 0.450 µg·h/ml (5.0 mg/kg). Therefore, a linear pharmacokinetics is applicable to the plasma disappearance profile of FK in this dose range. Figure 1 also shows the plasma disappearance curve of CyA injected to rats at the dose level of 3.5 mg/kg, although this data is cited from our previous report.\(^{11}\) The pharmacokinetic parameters for CyA were calculated and the result is also shown in Table I. As a significant difference is not detected with the elimination half-lives, $t_{1/2\beta}$, of these two immunosuppressants, the difference observed in $\text{CL}_{\text{tot}}$ of FK and CyA is ascribed to the difference of $V_t$ of these two drugs.

With respect to the lymphatic transfer of FK after i.v. administration to these two groups of rats, the results are shown in Table II. The mean peak lymph FK levels were 0.35 ± 0.05 µg/ml (1.0 mg/kg) and 2.86 ± 0.32 µg/ml (5.0 mg/kg), respectively. However, the percentage amounts of FK transferred into the lymphatics over the experimental period, 6 h, were 0.09 ± 0.02% and 0.16 ± 0.02% of the injected dose at 1.0 and 5.0 mg/kg, respectively. Therefore, the distribution of FK to the lymphatic circulation is extremely low.

Table III shows the excretory properties of FK both into the bile and the urine in group C rats over 6 h after i.v. injection, although the results

### Table II. Lymphatic Delivery of FK in Rats after i.v. and Oral Administrations

<table>
<thead>
<tr>
<th>Administration route and preparation</th>
<th>Rat group</th>
<th>Dose (mg/kg)</th>
<th>Peak lymph level (µg/ml)</th>
<th>Percentage amount of FK transferred over 6 h (% of dose)</th>
<th>Lymph flow (ml/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.v.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solution A</td>
<td>A</td>
<td>1.0</td>
<td>0.35 ± 0.05</td>
<td>0.09 ± 0.02</td>
<td>0.42 ± 0.03</td>
</tr>
<tr>
<td>Solution B</td>
<td>B</td>
<td>5.0</td>
<td>2.86 ± 0.32</td>
<td>0.16 ± 0.02</td>
<td>0.41 ± 0.05</td>
</tr>
<tr>
<td>Oral</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PG(^a)</td>
<td>D</td>
<td>5.0</td>
<td>1.82 ± 0.80</td>
<td>0.12 ± 0.07</td>
<td>0.34 ± 0.06</td>
</tr>
<tr>
<td>PG-HCO60(^b)</td>
<td>E</td>
<td>5.0</td>
<td>3.30 ± 0.51</td>
<td>0.18 ± 0.02</td>
<td>0.21 ± 0.02</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E. \(^{a}\) The solvent was PG. \(^{b}\) The solvent was PG containing HCO60 (5 w/v).
TABLE III. Excretion of FK into Rat Bile and Urine after i.v. Injection

<table>
<thead>
<tr>
<th>Excretory route</th>
<th>Peak FK level (µg/ml)</th>
<th>Percentage amount of FK excreted for 6 h (% of dose)</th>
<th>Flow rate (ml/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bile</td>
<td>0.774 ± 0.216</td>
<td>0.0744 ± 0.0177</td>
<td>1.42 ± 0.01</td>
</tr>
<tr>
<td>Urine</td>
<td>0.203 ± 0.009</td>
<td>0.0073 ± 0.0018</td>
<td>0.16 ± 0.02</td>
</tr>
</tbody>
</table>

Four rats (group C) received FK at 5.0 mg/kg in this experiment. Each value represents the mean ± S.E.

were obtained with one dose level, 5.0 mg/kg. The excreted amounts of unchanged FK in both the bile and urine were very low and averaged 0.0744 ± 0.0177% (bile) and 0.0073 ± 0.0018% (urine) of the i.v. injected dose, respectively.

The availabilities of FK to both the systemic circulation and the thoracic lymphatics were studied by administering this drug into the duodenum of another two groups of rats, groups D and E, at 5.0 mg/kg, in two kinds of liquid preparations. Figure 2 shows the mean plasma FK concentration–time curves after intraduodenal administration of this drug. As shown in Fig. 2, the mean peak plasma FK levels were 259 ± 88 ng/ml (mean peak time was 30 min) for group D rats (PG preparation) and 416 ± 169 ng/ml (mean peak time was 30 min) for group E (PG containing HCO60 preparation), respectively. The AUCoral values were calculated with these two groups of rats, 0.428 ± 0.038 µg-h/ml for group D rats and 0.893 ± 0.258 µg-h/ml for group E rats, respectively. By comparing the mean values of the AUCoral obtained after oral dosing of this drug to the mean AUCi.v. obtained after i.v. injection to group B rats (5.0 mg/kg), the systemic availabilities of FK from the two preparations were determined. The mean systemic availabilities were 11.3% for PG preparation (group D) and 23.5% for PG containing HCO60 preparation (group E), respectively. Table II shows the lymphatic availability of FK after oral dosing to these groups of rats. Mean peak lymph levels were 1.82 ± 0.80 µg/ml for PG preparation and 3.30 ± 0.51 µg/ml for PG containing HCO60 preparation, respectively. The percentage amounts of FK transferred into the lymphatics for 6 h were less than 0.2% in both preparations. Therefore, the lymphatic transfer of this novel immunosuppressant after intraduodenal administration is low.

Discussion

FK is a novel potent immunosuppressant being much more potent than CyA. The pharmacological action of FK was demonstrated at a cellular level and the mechanism by which FK shows an immunosuppressive activity is almost the same as proposed with CyA as precisely described in the section of introduction.3,16) FK shows such an in vitro action on T-cell responses with approximately one-hundredth the concentration as compared to CyA. Moreover, in the in vivo experimental transplantation models such as dog cardiac and kidney transplantations, the effective dose of FK was reported to be 1 mg/kg, where that of CyA was 20—30 mg/kg.5-9)

According to our previous reports concerning CyA, the formulation from which high lymph CyA concentration was obtained showed strong immunosuppressive activity.17-19) Therefore,
lymphatic deliveries of FK and CyA were studied in this report. However, the lymphatic transport characteristics of the two immunosuppressants cannot explain the difference in pharmacological activities of FK and CyA. Hess et al. showed that CyA acts directly on helper T (Th) cells to produce its immunosuppressive effect.\textsuperscript{20} CyA appears to selectively inhibit Th cell performance in MLR. Both CyA and FK inhibit the generation of lymphokines including IL-2, macrophage migration inhibition factor, macrophage-activating factor, and \( \tau \)-interferon.\textsuperscript{21,22} Th cells circulate in the body on the flow of blood stream. In addition, FK shares the same pharmacological mechanism with CyA. However, the biophase for these two immunosuppressants has not yet been made clear.\textsuperscript{23} One possibility to explain the difference in the pharmacological activity of the two immunosuppressants, in the extent to which FK is more potent than CyA \textit{in vitro} compared with \textit{in vivo} system, is the authentic pharmacological activity of this novel immunosuppressant. Another possibility is the difference in the disposition characteristics, rate of metabolism in the body and rate of excretion from the body, of these two immunosuppressants. In this report, both the availability and disposition kinetics of FK were studied in comparison with CyA to ascertain the above possibilities.

Here, we found the result that the total plasma clearance of FK was about three times greater than that of CyA. However, there is no significant difference in \( t_{1/2b} \) between the two drugs. Furthermore, \( V_{d,ss} \) and \( V_1 \) of FK are approximately four or five times greater than that obtained with CyA. Therefore, we may state that the distribution characteristic of FK differs from that of CyA. Generally, the concentration of the unbound drug molecule in the plasma fraction of the central circulation is the driving force for the disposition of drugs in the body.\textsuperscript{24} Pellegrin et al. studied the hepatic clearance of gitoxin using an isolated perfused liver preparation.\textsuperscript{25} The gitoxin concentrations in the perfusate were compared with and without albumin. By the addition of albumin into the perfusate, the distribution volume of gitoxin decreased approximately one-third. Therefore, a plasma protein binding study could give a new insight to this point. For CyA, an average free fraction of 1.3\% was reported by Henricsson,\textsuperscript{26} though tritiated CyA was used in an equilibrium dialysis method. In the case of extremely hydrophobic drugs like FK, it is not easy to obtain a reliable amount of the free fraction experimentally without using a labeled compound with radioisotopes.

The percentage amounts of FK excreted into the bile and urine are as small as CyA. According to our previous study on CyA, the mean percentage recoveries of CyA into both the bile and urine for 24 h were 1.2\% and 0.8\%, respectively.\textsuperscript{11} Therefore, the main disposition route of FK is thought to be from metabolism in the body, probably in the liver. With respect to the metabolism of FK in the liver, we are now performing a kinetic study by infusing this drug from two administration routes, an intraportal route and i.v. one, and the results will be published in the following paper.

According to the study of Venkataramanan et al. using transplant patients, the systemic availability of FK was reported to be 27\%.\textsuperscript{27} Though an enzyme immunoassay method was used to measure plasma FK levels, the systemic availability reported in the patients is almost the same obtained in this report. With respect to the lymphatic availability of FK, less than 0.2\% of the administered dose was transferred into the thoracic lymph duct both after i.v. and oral administrations. With CyA, the lymphatic availability was reported to be about 0.24\% of the i.v. injected dose (3 mg/kg)\textsuperscript{28} and 0.2\% of the oral dose (5 mg/kg).\textsuperscript{11} Therefore, it appears that the lymphatic transfer of the two immunosuppressants is almost the same order as in rats.

A basic pharmacokinetic study on FK has been performed in comparison with CyA. The absorption and elimination characteristics of FK and CyA were almost of the same order. However, FK distributes more rapidly and extensively than CyA. Especially, \( V_{d,ss} \) and \( V_1 \) of FK are about four or five times greater than that of CyA. As CyA and FK act on the lymphatic cells, especially T-cells, we are now studying the distribution characteristics of the two drugs in the blood compartment.
References


