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Increased systemic availability of cyclosporin A by formulation design: pharmacokinetic consideration on its transport

Kanji Takada [1], Yoshihiro Furuya [1], Hiroshi Yoshikawa [1], Shozo Muranishi [1], Tadaki Yasumura [2] and Takahiro Oka [2]

[1] *Department of Biopharmaceutics, Kyoto Pharmaceutical University, Kyoto (Japan)*
and [2] *The Second Department of Surgery, Kyoto Prefectural University of Medicine, Kyoto (Japan)*

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Summary

The systemic availability of cyclosporin A (CyA) from two different oral formulations was investigated in rats in relation to the lymphatic transport of CyA. The two test oral CyA formulations were a well-solubilized solution with a pharmaceutical additive, polyoxy 60 caster oil (HCO-60), and an olive oil formation. Each formulation was administered orally to two groups of 4 rats. After oral administration of the HCO-60 formulation, 7 mg/kg, the peak plasma CyA level and the area under the curve (AUC) were 1.5 times higher than obtained from the olive oil formulation. To clarify the contribution of the two absorption routes, portal and lymphatic, on the systemic availability of CyA, a pharmacokinetic study was performed by administering CyA to two more groups of 3 rats by two different administration routes, intraportal (i.p.) infusion and intravenous (i.v.) injection. The mean AUC after i.p. infusion, 7 mg/kg, was 11.32 ± 0.85(S.E.M.) $\mu g \cdot h/ml$ and after i.v. injection, 3.5 mg/kg, was 8.74 ± 0.85 (S.E.M.) $\mu g \cdot h/ml$. By analyzing these results with a physiologic pharmacokinetic model containing the liver and gastrointestinal (GI) tissue as metabolic eliminating organs for CyA, the increased systemic availability of CyA by formulation design was revealed to be due to increased absorption from the GI tract and the main transport route after being absorbed into the GI cells was revealed to be the portal venous system.

Introduction

Cyclosporin A (CyA), a cyclic endecapeptide having a molecular weight of 1201 (Wenger, 1982), is widely used in the inhibition of graft rejection in renal, hepatic, cardiac, lung, pancreatic and bone marrow transplantations (Cohen et al., 1984). The immunosuppressive activity of CyA is related to a selective action against T lymphocytes, which play a central role in the induction of immune responsiveness (Borel et al., 1977). Moreover, the lymphocytes circulate mainly in the lymphatic system in the body. By assuming that the immunosuppressive activity of CyA is related to the CyA concentrations in the lymphatic system, we developed a new CyA formulation having the potential to deliver more CyA into the lymphatics than the

Correspondence: K. Takada, Department of Biopharmaceutics, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607, Japan.

olive oil solution (Takada et al., 1985b; 1986a and b) Specifically, the lymphatic CyA concentration was about 20 times increased with our new well-solubilized CyA formulation by using one of the most popular pharmaceutical additives, polyoxy 60 caster oil (HCO-60). In addition, the immunosuppressive effect of CyA was significantly increased with our new formulation as compared to the olive oil solution in the rat heart transplantation model system (Yasumura et al., 1986; Takada et al., 1987). Briefly speaking, the mean survival of heart allograft in rats treated with a daily CyA dose in 8% HCO-60 solution, 2 mg/kg/day, for 1 week, was 21.8 ± 10.5 (S.D.) days, and two rats' hearts survived more than 40 days. In contrast, the mean survival time from the olive oil solution, 2 mg/kg/day for 1 week, was 12.8 ± 1.9 (S.D.) days. The control (non-treated) rats' mean survival time was 7.6 ± 0.6 (S.D.) days. However, the question remains as to whether the enhanced immunosuppressive activity of CyA is due to the increased lymphatic delivery of CyA or to its increased systemic availability. To solve this problem, a systemic availability study was performed with the two oral CyA formulations. Also a pharmacokinetic study was undertaken with parenteral CyA formulations to clarify the absorption route of CyA.

Materials and Methods

Drugs and reagents

CyA and CyD (cyclosporin D used as an internal standard) were kindly supplied by Sandoz Ltd., Basle, Switzerland, Polyoxy 60 caster oil (HCO-60) and polyoxyethylene glyceryl monooleate (TMGO-5) were obtained from Nikko Chemicals Co., Ltd. (Tokyo, Japan). All other reagents were of reagent grade commercially available.

Preparation of test solution

The olive oil formulation was prepared in a mixture of absolute ethanol, Nikkol TMGO-5, and olive oil (18 : 42 : 40). The HCO-60 formulation was prepared by dissolving CyA in 8.0%(w/v) HCO-60 solution followed by sonication at 25 °C for 5 min with an Ohtake 5202 sonicator (Tokyo, Japan). The final CyA concentration was 3.5 mg/ml in each test solution.

Animal preparation

Male Wistar rats weighing 400–500 g were used. Either 4 or 3 rats were used for each experimental group. The rats were fasted overnight but had free access to water. Under anesthesia by an intraperitoneal injection of sodium pentobarbital, 45 mg/kg, a polyethylene cannula (i.d., 0.5 mm; o.d., 0.8 mm, Dural Plastics, Australia) was surgically introduced into the left carotid artery to obtain blood samples at various times.

Oral study

Eight rats were divided into two groups. One group received a well-solubilized HCO-60 formulation and the other group received an olive oil formulation. One ml of each CyA test formulation per 500 g of rat body weight was injected into the duodenum of the rats, corresponding to a CyA dose of 7.0 mg/kg. After administration, the pore made in the duodenum was closed with a drop of tissue cement. Blood samples were drawn into heparinized microcentrifuge tubing at 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360 and 420 min. All the blood samples were immediately centrifuged at 37 °C to obtain the plasma fraction, because a marked temperature dependence of drug partitioning between red cells and plasma is reported (Follath et al., 1983). Just after collecting the final blood samples, at 7 h after oral administration, the whole gastrointestinal (GI) tract, namely from the stomach to the anus, was removed. Furthermore, the GI contents were washed out with 50 ml of isotonic phosphate buffer, pH 7.4, then mixed well. After the volume was measured with a volumetric flask, 1 ml of the solution was used for CyA assay. In contrast, the GI tissue was homogenized with 3 volumes of the isotonic phosphate buffer, pH 7.4. After measuring its whole volume, about 5 ml of the resulting homogenate was stored. The rat plasma sample, GI content sample and the GI homogenate sample thus obtained were stored in a freezer at -20 °C until analyzed.

Intravenous (i.v.) bolus study

Eight rats were used in this study. In the first group of 3 rats, both the common bile duct and the urinary bladder were cannulated according to the standard technique of Cocchetto et al. (1983) to collect the bile and the urine samples. The HCO-60 formulation containing CyA, 1.75 mg/ml, was administered from the femoral vein by a bolus injection at the dose level of 3.5 mg/kg. Blood samples were drawn into heparinized microcentrifuge tubes at 0, 2, 5, 10, 15, 20, 30, 40, 60, 90, 120, 180, 240, 300 and 360 min through a cannula introduced into the left carotid artery. In addition, the bile and the urine were collected over 24 h after administration. In the second group of 3 rats, the blood samples were collected from two sites, namely the carotid artery and the portal vein. To the portal vein, a disposal Vennula S-5 i.v. catheter (20G, Top Medical Corp., Tokyo, Japan) was inserted. After the i.v. administration of CyA, 3.5 mg/kg, blood samples were obtained from the carotid artery at 5, 10, 30, 60, 120, 240 and 360 min. Furthermore, blood samples were also obtained from the portal vein at 7, 12, 32, 62, 122, 242 and 362 min. All of the blood samples were immediately centrifuged at 37 °C to obtain the plasma fraction. Thus collected plasma, bile and urine samples of groups I and II were stored in a freezer at −20 °C until analyzed. In the third group of the remaining two rats, the reabsorption of CyA from the GI tract after excretion into the bile, the enterohepatic circulation, was studied. According to the method of Francis et al. (1982), into an anesthetized donor rat, CyA was injected, 3.5 mg/kg, from the femoral vein, and the bile was collected into a reservoir through a cannula. The fresh bile was immediately delivered into the duodenum of the recipient rat with a peristaltic pump. The blood samples were hourly collected for 12 h from the recipient rat through a cannula into the carotid artery. After centrifugation, the obtained plasma samples were also stored in a freezer at −20 °C until analyzed.

Intraportal (i.p.) infusion study

A disposable Vennula S-5 i.v. catheter was inserted into the portal veins of this group of rats. The HCO-60 formulation containing CyA, 3.5 mg/ml, was infused for 45 min using a variable-speed compact infusion pump, (model KN-201, Natsume Seisakusho Co., Tokyo, Japan) into the rat portal vein, 7.0 mg/kg. Through a cannula into the carotid artery, the blood samples were collected throughout the infusion period at 0, 5, 15, 30, 45 min, and up to 435 min postinfusion at 15, 45, 75, 105, 135, 195, 255, 315, and 435 min. The plasma was immediately obtained by centrifuging at 37 °C and was stored in a freezer at −20 °C until analyzed.

Drug assay

The CyA contents in plasma, bile, urine, in GI contents and in GI homogenate were determined with a high-performance liquid chromatographic (HPLC) procedure reported previously (Takada et al., 1985 a and b). 50–100 μl of the plasma, bile and urine samples were used for the CyA assay following extracted into diethyl ether. The extraction procedure was the same as we reported before (Takada et al., 1985a and b). Briefly speaking, after washing the residue of the ether extract with hexane, CyA was re-extracted into the mixture of carbon tetrachloride and 0.5 N NaOH (5 : 1). The separated carbon tetrachloride phase was transferred to a clean tube, and was evaporated to dryness with a stream of nitrogen at 50 °C. The resulting residue was dissolved in 200 μl of the mobile phase. An aliquot of 150 μl was then injected onto the column. With respect to the GI content and the GI homogenate, the same extraction procedure was used but with another initial volume of the samples, viz. 1 ml. A Hitachi 655 pump (Tokyo, Japan) including a Rheodyne 7125 sample injector was used for chromatographic analysis. The analytical column was a RP-18 Chemcosorb 5 μm (25 × 4.6 cm, Chemco Scientific Co., Ltd., Osaka, Japan). The UV detector was Hitachi 638-41. The column was maintained at 75 °C with a column heater. The mobile phase was composed of acetonitrile : water (70 : 3) and the flow rate was 1 ml/min (60 kg/cm^2). CyA and CyD eluted from the column was detected at 205 nm. Under these conditions, the retention times were 7 min for CyA and 9.5 min for CyD, respectively. No interfering peak was detected in all of these biological fluids used for blank stan-

dards or from rats who received CyA. Concentrations of CyA in the biological fluids were determined from calibration curves of peak area ratios of CyA to CyD. The standard curve of CyA added to the rat plasma was linear over the range of 0.2–20 μg/ml and passed through the origin. With the bile, the urine, the GI content and the GI homogenate, calibration curves were made over the range of 1–500 μg/ml and also passed through the origin.

Pharmacokinetic analysis

The terminal elimination rate constant, β, for the CyA concentration-time curves after i.v., oral or i.p. administration was determined by linear regression at least 4 data points from the terminal portion of the plasma concentration–time plots. The area under the plasma concentration–time curve after i.v. administration, AUC_{iv}, 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 term C_p(last)/β. The AUCs obtained after oral administration and i.p. infusion, AUC_{oral} and AUC_{ip}, were calculated to maximum concentration with the linear trapezoidal rule and after that to the last measured plasma concentration with the logarithmic trapezoidal rule with the addition of the correction term after last measured point to the infinity, namely C_p(last)/β. The half-life, $t_{1/2}$, was determined by dividing ln 2 by the terminal elimination rate constant, β. The total plasma clearance, CL, was determined by dividing the i.v. dose by the AUC_{iv}. The parameters were determined for each individual. The values are expressed as their mean ± S.D. Statistical differences were assumed to be reproducible when $P < 0.05$ (two-sides t-test).

A minimal model which describes the experimental data is shown in Fig. 1. It is assumed that CyA is delivered by the blood flow from the heart–lung subsystem to the GI tissue and the liver. We also assume that the metabolic disposition of CyA in the eliminating organs follows linear kinetics. In the term used in this model, Q is the cardiac output, Q_G and Q_{HA} mean the mesenteric arterial blood flow and the hepatic arterial blood flow, respectively. The F_G, F_H and

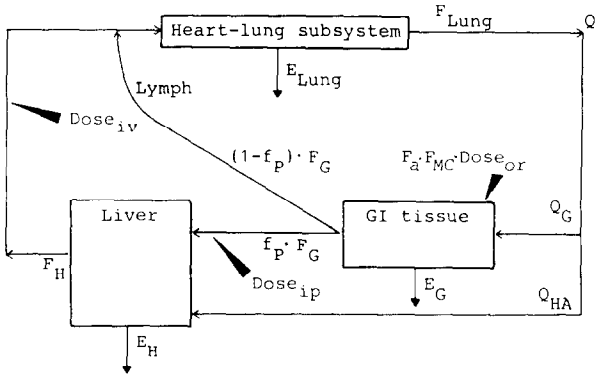

Fig. 1. A physiologic pharmacokinetic model for CyA administered i.v., orally (or) and i.p. Q, cardiac output; Q_G, blood flow to the GI system; Q_{HA}, blood flow to the liver; E_{Lung}, extraction ratio of CyA in the heart-lung subsystem; E_G, extraction ratio of CyA in the GI system; E_H, extraction ratio of CyA in the liver; F_{Lung}, availability in the heart-lung subsystem; F_G, availability in the GI system; F_H, availability in the liver; F_a, fraction of CyA absorbed from the GI tract into the mucosal cells; F_{MC}, fraction of CyA unmetabolized in the mucosal cells after being absorbed from the GI tract; f_p, fraction transported into the portal venous system after escaping the metabolic disposition in the GI system; $(1 - f_p)$, fraction transported into the lymphatic system after escaping the metabolic disposition in the GI system.

F_{Lung} represent the availabilities in the GI tissue, in the liver and in the heart–lung subsystem, respectively. In contrast, E_G, E_H and E_{Lungs} mean the extraction ratio of CyA in these eliminating organs.

When CyA is administered intravenously, CyA enters into the heart-lung subsystem almost directly, it namely does not receive the hepatic first-pass effect. After that, CyA distributes to the organs with the flow of cardiac output. As the eliminating organs, the GI tissue and the liver were assumed. After passing through the GI tissue, the fraction of CyA, F_G, will be available to both the portal venous system and the lymphatic system. By representing the fraction being transported into the portal venous system as f_p, the total fraction of CyA available to the lymphatic system will be equal to $(1 - f_p) \cdot F_G$. Though the fraction of CyA transported into the lymphatic system will directly enter into the heart–lung subsystem, the fraction of CyA transported into the portal system enters into the liver together with the CyA in the hepatic artery and receive the

metabolic disposition there. By taking these points into consideration, the AUC_{iv} obtained after the i.v. injection of CyA is given according to the transport function analysis (Miyazaki et al., 1978; Van Rossum et al. 1983; Takada, 1987). Namely,

$$AUC_{iv} = \left[F_{Lung} \cdot Dose_{iv}\right] \times \left[Q - F_{Lung} \cdot \{Q_G \cdot f_P \cdot F_G \cdot F_H + Q_G \cdot (1 - f_P) \cdot F_G + Q_{HA} \cdot F_H\}\right]^{-1} \quad (1)$$

In the case of the i.p. infusion of CyA, the CyA infused into the portal vein receives the hepatic first-pass effect. Then, the AUC_{ip} is represented with Eqn. 2:

$$AUC_{ip} = \left[F_{Lung} \cdot F_H \cdot Dose_{ip}\right] \times \left[Q - F_{Lung} \cdot \{Q_G \cdot f_P \cdot F_G \cdot F_H + Q_G \cdot (1 - f_P) \cdot F_G + Q_{HA} \cdot F_H\}\right]^{-1} \quad (2)$$

On the other hand, after the oral administration of CyA, we must consider several availabilities (Gillette, 1982). At first, CyA must be absorbed from the GI tract and the fraction absorbed from the GI tract into the mucosal cells is represented as F_a. As the drug-metabolizing enzymes are abundant in the mucosal cells, we cannot deny the possibility of the metabolism of CyA in the mucosal cells. By representing the fraction of CyA unmetabolized in the mucosal cells as F_{MC}, the amount of CyA absorbed and unmetabolized in the mucosal cells as F_{MC}, the amount of CyA absorbed and unmetabolized in the mucosal cells will be equal to $(F_{MC} \cdot F_a \cdot Dose_{oral})$, and this amount of CyA will be transported both into the portal venous system and into the mesenteric lymphatic system. After being transported into the portal blood, CyA must receive the hepatic first-pass effect and the fraction unmetabolized in the liver will reach the heart–lung subsystem. In contrast, the fraction of CyA transported into the mesenteric lymphatics will be directly, namely without first-pass effect, transported into the heart–lung subsystem. Then, the AUC_{oral} is given with Eqn. 3.

$$AUC_{oral} = \left[F_{Lung} \cdot F_{MC} \cdot F_a \cdot Dose_{oral} \cdot \{f_P \cdot F_H + (1 - f_P)\}\right] \times \left[Q - F_{Lung} \cdot \{Q_G \cdot f_P \cdot F_G \cdot F_H + Q_G \cdot (1 - f_P) \cdot F_G + Q_{HA} \cdot F_H\}\right]^{-1} \quad (3)$$

Based on these equations, precise pharmacokinetic analysis was performed and the result is described in the Discussion.

Results

After the oral administration of CyA, 7 mg/kg, in the two different formulations, i.e., a well-solubilized solution with HCO-60 and an olive oil solution, the plasma CyA levels vs time curves showed significantly different patterns between the two formulations as shown in Fig. 2. The peak plasma CyA levels obtained after the oral administration HCO-60 formulation was about 1.5 times greater than that obtained with the olive oil formulation. Table 1 shows the main pharmacokinetic parameter values. In AUC value, the systemic availability of CyA from the HCO-60 formulation is about 1.5 times greater than that obtained from the olive oil formulation. However, there was no significant difference in the peak times between the two formulations. At the end of the experiments, the whole GI tract was removed and the recoveries of CyA from the GI contents and GI tissue were measured. In the case of HCO-60 formulation, the percentage recoveries of CyA from the GI contents and GI tissue were $20.92 \pm 2.94\%$ (S.D.) and $8.20 \pm 1.43\%$ (S.D.), respectively. Also, $24.18 \pm 2.22\%$ and $20.34 \pm 0.58\%$ were obtained for the olive oil formulation. Totally, the mean percentage recoveries of CyA from the GI system were 29.12% for HCO-60 formulation and 44.52% for olive oil formulation. This implies that the absorption of CyA from the GI

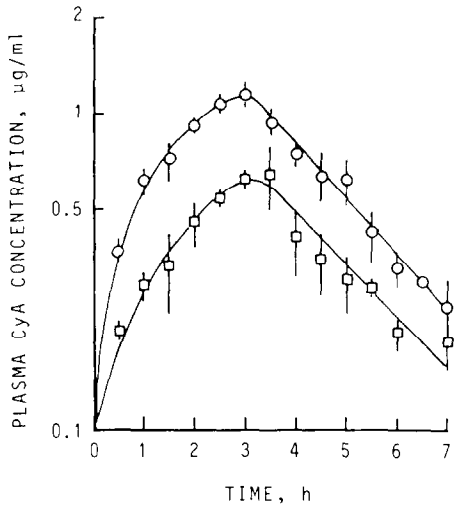

Fig. 2. Mean plasma concentration–time curves of CyA after oral administration of CyA, 7 mg/kg, to 4 rats in HCO-60 formulation (○) and in an olive oil formulation (□). Each point represents the 4 individual determinations, and is expressed as the mean ± S.E.M.

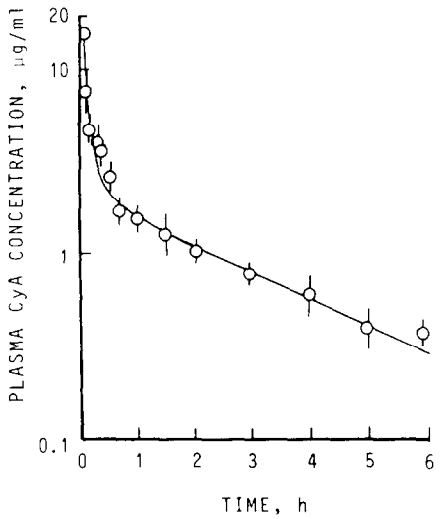

Fig. 3. Mean plasma concentration–time curve of CyA after i.v. bolus injection of CyA, 3.5 mg/kg, to 3 rats. Each point represents the 3 individual determinations, and is expressed as the mean ± S.E.M.

tract was improved by administering CyA in HCO-60 formulation.

When CyA was injected into the rat femoral vein, 3.5 mg/kg, plasma CyA levels declined bi-exponentially as shown in Fig. 3. The AUC, elimination rate constant and total plasma clearance values are also shown in Table I. The total plasma clearance was 166.3 ± 16.5 ml/h. The mean excreted amount of CyA into the rat bile for 24 h was 9.22 ± 5.45 μg, which corresponds to about 1.2% of the injected CyA dose. Furthermore, two more rats were used to study the reabsorption of CyA from the GI tract after being excreted into the bile. However, CyA was not detected, namely under the lower detection limit (0.2 μg/ml) of our HPLC assay method, in the plasma of the acceptor rat. Therefore, we may state that the amount of CyA excreted into the bile does not contribute the systemic availability of CyA. In the case of urinary excretion, the percentage recovery into the rat urine for 24 h was

TABLE 1

Pharmacokinetic parameters of CyA following administration to rats by 3 different administration routes

Formulation	Administration route	Dose (mg/kg)	$AUC_{0\rightarrow\infty}$ (μg · h/ml)	Total plasma clearance, CL (ml/h)	Elimination rate constant, β (h^{-1})	Half-life $t_{1/2}$ (h)
HCO-60 solution	oral	7.0	4.78 ± 0.23		0.453 ± 0.031 *	1.55 ± 0.12
Olive oil solution	oral	7.0	3.46 ± 0.17		0.431 ± 0.055 *	1.63 ± 0.12
HCO-60 solution	i.v.	3.5	8.74 ± 0.85	166.3 ± 16.5	0.390 ± 0.025	2.09 ± 0.27
HCO-60 solution	i.p.	7.0	11.32 ± 0.85	226.3 ± 17.3 **	0.346 ± 0.028 *	3.23 ± 0.47

Oral, intraduodenal administration of CyA, 7.0 mg/kg, to 4 rats. I.v., intravenous bolus injection of CyA, 3.5 mg/kg, to 3 rats. I.p., intraportal infusion of CyA, 7.0 mg/kg, for 45 min to 3 rats. Each value represents the mean ± S.D.

* not significantly different from the iv data ($p < 0.05$).

** As CyA receives hepatic first-pass effect, this value shows the apparent clearance, CL/F_H.

less than 1.0% of the injected dose, namely 0.8%. Therefore, the contribution of both the biliary and urinary excretory processes on the total elimination of CyA from the rat body is negligible. The main elimination pathway for CyA in the rat is thought to be via the systemic metabolism. Though the oral dose was 7 mg/kg, half of the dose, 3.5 mg/kg, was used in this i.v. study because of low availability of CyA (Kahan et al., 1983). By assuming that there is a linear relationship between the oral dose and the plasma CyA levels, the mean AUC_{iv} value at 7 mg/kg, the same dose level as the oral studies, was estimated to be 17.48 $\mu g \cdot h/ml$. By comparing the mean AUC_{oral} values obtained after the administration of the oral two CyA formulations with thus estimated mean AUC_{iv} value, the systemic availabilities of CyA from the two oral formulations were calculated. Namely, the systemic availability of CyA from HCO-60 formulation was 27.3% and that from the olive oil formulation ws 19.8%.

The carotid arterial CyA levels vs time curve and the portal venous CyA levels vs time curve obtained after the i.v. injection of CyA, 3.5 mg/kg, to the other group of 3 rats are shown in Fig. 4. As the blood samples were obtained from the two

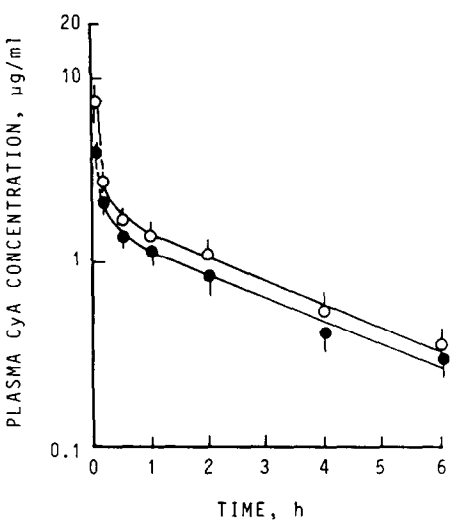

Fig. 4. Carotid arterial (open symbols) and portal venous (closed symbols) concentrations of CyA after i.v. bolus injection, 3.5 mg/kg, to 3 rats. Each point represents the mean ± S.E.M.

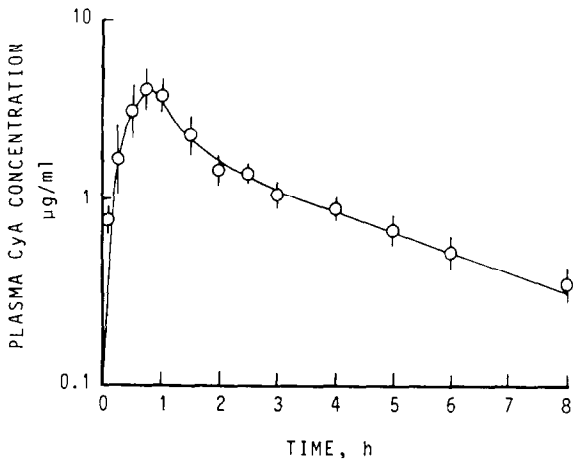

Fig. 5. Mean plasma concentration–time curve of CyA following 45-min intraportal infusion of CyA, 7 mg/kg, to 3 rats. Each point represents the mean ± S.E.M.

different sites, the sample points were decreased to half the amount of the above i.v. experiment. The calculated AUC values of the two curves are 7.12 ± 0.75 (S.D.) $\mu g \cdot h/ml$ for the carotid arterial CyA levels and 6.50 ± 0.84 (S.D.) $\mu g \cdot h/ml$ for the portal venous CyA levels, respectively. This means that about 10% of the amount of CyA entered into the GI system is extracted during the passage through the GI system and that about 90% of the amount of CyA is available to the portal venous system.

Fig. 5 shows the mean plasma concentration–time curve for CyA over the entire period of the study for the 3 rats in whom a constant rate infusion into the portal vein was performed for 45 min at a dose level of 7.0 mg/kg. The plasma CyA levels gradually increased until the end of the infusion, and after that declined biexponentially. The pharmacokinetic parameter values are also represented in the table. By comparing the mean AUC_{ip} value obtained after i.p. infusion of CyA with the estimated AUC_{iv} value (17.48 $\mu g \cdot h/ml$) at the dose level of 7 mg/kg, systemic availability of CyA after i.p. administration was estimated to be 64.7%. This means that about 35% of the amount of CyA infused into the portal vein was extracted by the liver before entering into the systemic circulation.

Discussion

Based on the assumption that the immunosuppressive activity of CyA is related to its concentration in the lymphatic system, we tried to develop a new formulation which would promote the lymphatic delivery of CyA (Takada et al., 1985; 1986a and b). Previous studies showed that the lymphatic CyA concentration was about 20 times greater by administering CyA in our new well-solubilized formulation with HCO-60 than administering in an olive oil solution. In addition, an intensified immunosuppressive effect of our HCO-60 formulation was confirmed with a rat heart transplantation model (Yasumura et al., 1986; Takada et al., 1987). However, we could not deny the possibility that the intensified immunosuppressive effect of the HCO-60 formulation was ascribed to the increased systemic availability of CyA. As we suspected, the systemic availability of CyA was also increased with the HCO-60 formulation as compared to the oily solution. To clarify whether the absorbed CyA mainly enters into the portal venous system or into the mesenteric lymphatic system, a pharmacokinetic study was performed by administering CyA i.v. and i.p. Our data in this paper have demonstrated that the main elimination process of CyA from the rat body is the metabolism in the body. As main metabolic organs, the liver, the lung and the GI tissue are generally considered (Collins et al., 1982). Therefore, a physiologic pharmacokinetic model shown in Fig. 1 is proposed, where these 3 essential organs are included.

As an administration route for CyA, we used 3 different routes, namely oral administration, i.v. injection and intraportal infusion. The relationship between the AUCs obtained after these 3 different administration routes and the physiologic pharmacokinetic parameters are represented from Eqns. 1–3. On the other hand, concerning the metabolic fate of CyA in the body, it was reported that the liver is the main metabolic organ for CyA (Buice et al., 1985). In addition, Gridelli et al. (1986) demonstrated that the GI tissue also has a high activity in the metabolism of CyA after being absorbed from the GI tract. However, there has been no report on the metabolic disposition of CyA in the lung up to now. Therefore, we may assume that the availability of CyA through the heart-lung subsystem is almost equal to unity. By introducing $F_{Lung} = 1.0$ into Eqn. 1–3, the fraction of CyA transported into the portal venous system after being absorbed from the GI tract, f_P, is determined by rearranging these equations under the condition that the doses administered from 3 different administration routes are equal. Namely,

$$f_P = \frac{AUC_{oral} - F_a \cdot F_{MC} \cdot AUC_{iv}}{F_a \cdot F_{MC} \cdot (AUC_{ip} - AUC_{iv})} \tag{4}$$

To determine the value of f_P, we must estimate the values of the two availabilities, F_a and F_{MC}. The value of F_a, the fraction of CyA absorbed from the GI tract, was estimated using the results concerning the recoveries of CyA from the rat GI contents and the GI tissue after oral administration. Namely, $F_a = 0.709$ for HCO-60 formulation and $F_a = 0.555$ for the olive oil formulation. In contrast, it is impossible to determine the value of F_{MC}, the fraction of CyA unmetabolized in the mucosal cells after being absorbed from the GI tract, from the oral experiment. Fig. 6 shows more precisely the fate of CyA in the GI tissue in relation to the two delivering routes, namely from the systemic circulation and from the GI tract after oral administration. When systemic blood enters into the GI tissue with the mesenteric blood flow, only some part of the mesenteric blood is distributed to the mucosal cells of the GI tissue. Then, the fraction of CyA delivered to the mucosal cells is defined as f_{MC}. By assuming that the drug-metabolizing enzymes are localized in the mucosal cells, the fraction unmetabolized in the mucosal cells, $F_{MC}f_{MC}$, will be available to both the portal venous system and the mesenteric lymphatic system. Therefore, the portal availability of CyA, $f_P \cdot F_G$, is represented as Eqn. 5.

$$f_P \cdot F_G = (1 - f_{MC}) + f_{MC} \cdot F_{MC} \cdot f_P' \tag{5}$$

where f_P' means the fraction of CyA entering into the portal venous system after escaping the metabolism in the mucosal cells. As Biber et al. (1973) reported that only 20% of the mesenteric blood supply is distributed to the mucosa of the

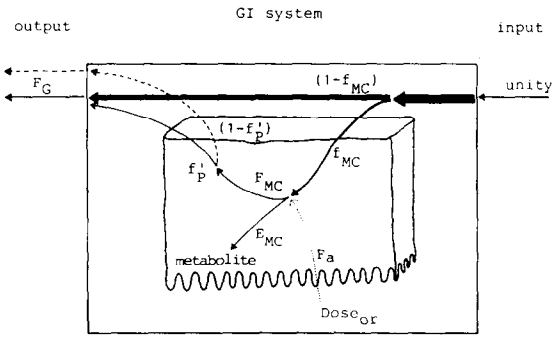

Fig. 6. Proposed model for the metabolic disposition of CyA in the GI system in relation to its administration routes. CyA is delivered to the GI system with the mesenteric blood flow, and a steady state was assumed, where the input to the system is unity and the output from the system is F_G, the fraction unmetabolized in the system. F_{MC}, fraction delivered to the mucosal cells. $(1 - F_{MC})$, fraction being not delivered to the mucosal cells; E_{MC}, fraction extracted in the mucosal cells by metabolism; F_{MC}, fraction unmetabolized in the mucosal cells; f'_P, fraction transported into the portal venous system; $(1 - f'_P)$, fraction transported into the mesenteric lymphatic system. In the case of the oral administration, the amount of CyA ($F_a \cdot Dose_{or}$), is absorbed into the mucosal cells where the drug-metabolizing enzymes are abundant. The amount of CyA unmetabolized there, ($F_a \cdot F_{MC} \cdot Dose_{oral}$), will be available to both the portal venous and mesenteric lymphatic systems.

GI tract, we may introduce their value as f_{MC}, namely $f_{MC} = 0.2$. In addition, according to the pharmacokinetic data of Ueda et al. (1983), less than 0.5% of the i.v. injected CyA dose, 3 mg/kg, was transported into the thoracic lymphatics of the rats over 10 h. This suggests that the lymphatic transport of CyA being delivered from the mesenteric blood supply is negligible. Then, we may assume that both f_P and f'_P are almost equal to unity. By introducing these values into Eqn. 5, the following equation is obtained.

$$F_G = 0.8 + 0.20 \cdot F_{MC} \quad (6)$$

The availability of CyA through the GI tissue, F_G, may be estimated by comparing the two AUC values both in the portal venous system and in the mesenteric arterial system which is almost equal to the carotid arterial system. In this study, one group of 3 rats were used to estimate the value of F_G, and the calculated value for F_G was 0.912 ± 0.028 (S.D.). Therefore, by introducing this value into Eqn. 6, we can estimate the value of F_{MC}, namely $F_{MC} = 0.560$ was obtained. Finally, by introducing the values of thus obtained F_a and F_{MC}, as well as the calculated AUC values of which the i.v. one was corrected to a dose of 7 mg/kg, into Eq. 4, the f_P value was determined with respect to the two formulations. The f_P value for the HCO-60 formulation was 0.88 and f_P value for olive oil formulation was 1.03. This result suggests that almost all of the absorbed CyA enters into the portal venous system after being administered as an olive oil formulation and that in the case of HCO-60 formulation the fraction of CyA transported into the portal venous system was decreased to be 88% and the remaining fraction (12%) entered into the mesenteric lymphatic system

In conclusion, the systemic availability of CyA was increased by the formulation change of CyA, namely about 1.5 times increased by administering CyA in a HCO-60 formulation than in the olive oil formulation. This enhanced systemic availability of CyA was ascribed to the increased absorption of CyA from the GI tract into the mucosal cells. However, the main transport route after being absorbed into the mucosal cells was suggested to be the portal route.

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