Enhanced Absorption of Cyclosporin A by Complexation with Dimethyl-β-cyclodextrin in Bile Duct-Cannulated and -Noncannulated Rats

KOIZUMI MIYAKE, Hidetoshi ARIMA, Tetsumi IRIE, Fumitoshi HIRAYAMA, and Kenato UEKAMA*

Faculty of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862-0973, Japan.
Received August 21, 1998, revised October 12, 1998

The enhancing effects of dimethyl-β-cyclodextrin (DM-β-CyD) on the absorption of cyclosporin A (CsA) after oral administration to rats under bile duct-cannulated and -noncannulated conditions were investigated. The dissolution rate of CsA was markedly augmented by complexation with DM-β-CyD. In a closed loop in situ study, DM-β-CyD considerably increased the cumulative amounts of CsA in the mesenteric venous blood after injection of the aqueous CsA suspension into the small intestinal sac of rats. In addition, the cumulative amount ratio of M1, the dominant metabolite of CsA in rats, to CsA in the mesenteric venous blood for up to 40 min after the injection of the CsA-DM-β-CyD suspension into the sac was lower than that of the CsA suspension alone. DM-β-CyD inhibited the bioconversion of CsA in the small intestinal microsomes of rats. These results indicate that the bioconversion of CsA was abated by complexation with DM-β-CyD. An in vivo study revealed that DM-β-CyD increased the transfer of CsA to blood, not lymph, with low variability in the absorption after oral administration of the CsA suspension to rats. The variability of bioavailability of DM-β-CyD complex was lower than that of Sandimmune, although the extent of bioavailability of DM-β-CyD was only a little higher than that of Sandimmune. The bioavailability of CsA or its DM-β-CyD complex was appreciably decreased by the cannulation of the bile duct of rats, and the extent of the lowering in the bioavailability in the presence of DM-β-CyD was much less serious than that of CsA alone. The present results suggest that DM-β-CyD is particularly useful in designing oral preparations of CsA with an enhanced bioavailability and a reduced variability in absorption.

Key words  dimethyl-β-cyclodextrin; cyclosporin A; oral absorption; complexation; bile acids; bioavailability

Cyclosporin A (CsA, Fig. 1) is an immunosuppressive drug and is used primarily in the treatment of autoimmune diseases and prevention of allograft rejection after organ transplantation. However, CsA is known to exhibit low oral bioavailability and a wide range of variability in absorption. One of the reasons for such drawbacks is the very low aqueous solubility of CsA which has a high molecular weight of 1202 daltons. The poor dissolution property of CsA has limited the design of dosage form in various administration routes and has presented a substantial challenge to pharmaceutical scientists. For example, Sandimmune, a currently manufactured formulation of CsA has employed oil- and alcohol vehicles (oral solution and soft gelatin capsules). In Neoral, a new oral formulation of CsA, the drug is incorporated in a microemulsion consisting of a surfactant, a lipophilic solvent and a hydrophilic solvent and ethanol.

Cyclodextrins (CyDs) form inclusion complexes with lipophilic drugs as guests and thus improve their water solubility. There are a few reports on the use of CyDs to improve the pharmaceutical characteristics of CsA: one is that α-CyD improved the solubility of CsA in ophthalmic solutions, and another is that dimethyl-α-cyclodextrin (DM-α-CyD) formed a higher order complex with CsA and significantly enhanced the oral bioavailability of CsA in rats. In a previous paper, we demonstrated by means of the solubility method and mass and nuclear magnetic resonance spectroscopies that the low aqueous solubility of CsA is significantly improved by inclusion complexation with dimethyl-β-cyclodextrin (DM-β-CyD) and the hydrophobic side chains of CsA amino acids such as butenylthreonine and leucine are involved in the complex formation (Fig. 1). In this continuing study, we mainly focused on the in vivo absorption of CsA after oral administration of CsA-DM-β-CyD complex to rats under bile duct-cannulated and -noncannulated conditions.

Fig. 1. The Chemical Structures of CsA and M1 and M17 Metabolites

* To whom correspondence should be addressed. © 1999 Pharmaceutical Society of Japan
because bile acids are known to affect significantly the absorption of lipophilic drugs such as CsA.

MATERIALS AND METHODS

Materials CsA and its dominant and subdominant metabolites in rats (M1 and M17, respectively, Fig. 1) were donated from Shiseido Co. (Yokohama, Japan). Sandimmune and cyclosporin D (CsD) were obtained from Sandoz Co. (Tokyo, Japan). DM-β-CyD was obtained from Nihon Shokuhin Kako, Co. (Tokyo, Japan). Other chemicals and solvents were of analytical reagent grade and double-distilled water was used throughout the study.

Preparation of Complex of CsA with DM-β-CyD The solid CsA-DM-β-CyD complex in a molar ratio of 1:10 was prepared by the kneading method as reported previously, using ethanol–water as a solvent. The calculated amounts of CsA and DM-β-CyD were weighed and triturated with a small amount of ethanol–water and the slurry was kneaded thoroughly for about 40 min. After evaporation of the solvent, the solid complexes were dried under reduced pressure at room temperature for 3 d and stored in a desiccator.

Dissolution Studies The dissolution rates of CsA and its DM-β-CyD complex were measured by the dispersed amount method. The powder sample (<100 mesh, equivalent to 1 mg CsA) was added to 100 ml second fluid (pH 6.8) of Japanese Pharmacopeia XIII (JPXIII) and stirred at 100 rpm at 37 °C. At appropriate intervals, an aliquot (1.0 ml) of the dissolution medium was withdrawn using a pipette with a cotton plug. The volume in the vessel was replaced with the second fluid (pH 6.8) of JPXIII disintegration test after each sampling. n-Hexane (4 ml) was added to the aliquot (1.0 ml), and the mixed solution was shaken and centrifuged. The organic phase (3.0 ml) was transferred to a glass tube and was evaporated to dryness. The residue was reconstituted with 100 μl of mobile phase and 70 μl of sample was injected onto HPLC column. The HPLC was performed under the following conditions by Shibata et al.15: apparatus, a Hitachi L-6000 machine (Tokyo, Japan) equipped with Lichrosorb Si-60 column (4.6 mm × 250 mm; GL-Science, Tokyo, Japan); column temperature, 50 °C; mobile phase, n-hexane–ethanol (17:3, v/v); UV detection, 214 nm; flow rate, 1.0 ml/min.

In Situ Closed Loop Studies Male Wistar rats, 250—300 g, were fasted overnight, and anesthetized with 25% ethyl carbamate in saline (6 ml/kg). Following laparotomy, about a 10 cm segment of jejunum was cannulated at both ends to form the in situ closed loop of the small intestine (sac). All the mesenteric veins supplying the sac were identified, and were cannulated with polyethylene tubing (Intramedic PE-50, Japan Becton Dickinson, Tokyo, Japan) for venous blood collection. One milliliter of CsA suspension (200 μg/ml) in the absence and presence of DM-β-CyD (20 mg/ml) in phosphate buffered saline (pH 7.4) was injected into the sac. All the mesenteric venous blood draining from the sac was collected. During in situ closed loop study, rats were kept on a hot-plate at 37 °C. After 40 min, the amounts of CsA in the whole blood were measured by using HPLC. The HPLC assay of CsA and its metabolites (M1 and M17) was performed according to Khoschour et al. In brief, 2 ml of HCl solution (0.18 M), 50 μl of CsD solution (as an internal standard) and 4 ml of diethyl ether were added to the samples. After shaking (15 min) and centrifugation (10000 g, 5 min), the ether phase was transferred to another glass tube containing 2 ml of NaOH solution (0.1 M). After shaking (10 min) and centrifugation (10000 g, 5 min), 3 ml of the ether phase was transferred to a glass tube and evaporated to dryness. The residue was reconstituted with 100 μl of mobile phase and then 70 μl of the sample was injected onto the HPLC column. HPLC assay was carried out under the following conditions: apparatus, a Hitachi L-6000 machine (Tokyo, Japan) equipped with YMC Pack CN column (5 μm, 6.0 mm × 150 mm, Kyoto, Japan); mobile phase, n-hexane–isopropanol (9:1, v/v); UV detection, 214 mm; flow rate, 1.3 ml/min.

In Vitro Metabolism Studies of CsA in Rat Small Intestinal Microsomes Male Wistar rats, 250—300 g, were fasted overnight prior to the studies. The induction of cytochrome P4503A (CYP3A) in rat enteroctyes was performed according to the methods of Kolars et al.17 One ml of olive oil suspension containing dexamethasone, a CYP3A inducer, was injected intraperitoneally at a dose of 80 mg/kg/d for 2 d. After the second injection of the oily suspension, the rats were fasted for 16—18 h prior to phlebotomy lethality and laparotomy. Then microsomes were prepared from rat epithelial cells according to the method of Bonkovsky et al.,18 and all the steps were carried out on ice. Briefly, the enteroctyes from a single rat small intestine were isolated in 5 ml of “solution C” (5 mm histidine, 0.25 mm sucrose, 0.5 mm Na2EDTA, 40 mg/ml phenylmethylsulfonyl fluoride, pH 7.0), and were washed twice with 20 ml of “solution C” and centrifuged at 800 g for 10 min. The cells were resuspended in 5 ml of “solution C” and homogenized with 20 up-and-down strokes using a Potter-Elvejhem homogenizer. The homogenate was centrifuged at 15000 g for 10 min, the supernatant was carefully removed with a Pasteur pipette into a new tube. To aggregate microsomes, 1.25 ml of ice-cold CaCl2 (52 mm) was added in supernatant. The tube was gently inverted five times and allowed to stand in ice for 15 min, and was centrifuged at 20000 g for 10 min. The supernatant was decanted, and the microsomal pellet was resuspended in 1 ml of 0.1 M phosphate buffered saline (pH 7.4) to obtain a final protein content of 11 μg/ml. In the CsA metabolism studies, 100 μl of microsomal solution (protein concentration, 11 μg/ml) and 200 μl of CsA solution (5.75 μm) with or without DM-β-CyD (23.0 μm) in the glass tube were incubated for 4 min at 37 °C. The reaction was started with the addition of 30 μl of NADPH solution (11.5 μM) and stopped by the addition of 1 ml of HCl solution (0.18 M) at an appropriate time. The concentrations of CsA and its metabolites were assayed by using the HPLC method, as described in the in situ closed loop studies section.

In Vivo Absorption Studies Male Wistar rats, 250—270 g, were fasted overnight, and were orally administered the aqueous suspension containing CsA, its DM-β-CyD complex or Sandimmune (equivalent to 10 mg/kg). At appropriate intervals, rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (30 mg/kg) and a heparinized polyethylene cannula was implanted into the thoracic lymph duct. Lymph sample (ca. 350 μl) was collected after cannulation into the thoracic lymph duct, and at the same time, a blood sample (1 ml) was collected from the
heart. The plasma and lymph levels of CsA were assayed by using the HPLC method described below. Plasma and lymph samples (300 µl) were added to 200 µl of 0.1 molar dextran sulfate sodium salt solution containing 50 mM MgCl₂, 4 ml of n-hexane and 40 µl of CsD solution (10 µM) as the internal standard. This mixture was agitated on a reciprocating shaker for 30 min, and then centrifuged at 1000 g for 5 min at room temperature. The organic phase was transferred to another glass tube and evaporated under reduced pressure at 40°C. The resultant residue was dissolved in 100 µl of mobile phase, and then 70 µl of the solution was injected onto the HPLC column. The HPLC conditions were the same as those described in the dissolution study section. The area under the plasma CsA concentration–time curves (AUC) and the mean residence times of the drug in the plasma (MRT) were calculated by moment analysis. The bias for the plasma CsA concentration was calculated with the following equation: (each datum—the mean of data)/the mean of data×100. The plasma sample after the intravenous administration of CsA oily solution (5 mg/kg) was collected and then the plasma CsA level was assayed by using the HPLC to calculate the absolute bioavailability.

Statistical Analysis  Data are given as means±S.E.M. Statistical significance of mean coefficients was determined by Student’s t-test, and statistical analysis of the data shown in Fig. 8 was performed by analysis of variance followed by Scheffe’s test. p values for significance were set at 0.05.

RESULTS

Dissolution Study  It is well known that dissolution of the drug in aqueous solution is the rate-limiting step for the absorption of poorly water-soluble drugs. We examined the dissolution rate of CsA in JPHXIII second fluid (pH 6.8) by the dispersed amount method. As shown in Fig. 2, the dissolution rate of CsA was markedly augmented by complexion with DM-β-CyD.

In Situ Closed Loop Study  Figure 3 shows the cumulative amounts of the intact CsA in the mesenteric venous blood after the injection of the CsA suspension with or without DM-β-CyD into the small intestinal sac of rats in the in situ closed loop system. When CsA alone was applied into the sac, the cumulative amounts of CsA in the blood gradually increased as a function of time. The amounts of CsA in the blood was markedly augmented by the addition of DM-β-CyD.

The effect of DM-β-CyD on the metabolism of CsA into the intestinal mucosa was examined in the in situ closed loop system. As shown in Fig. 4, of the metabolites of CsA in rats, only the M1 metabolite was detected under this experimental condition. The cumulative concentration ratio of the metabolite M1 to CsA in the mesenteric venous blood up to 40 min after the injection of CsA-DM-β-CyD complex into the sac was lower than that of CsA alone. These results indicated that the bioconversion of CsA to M1 was abated by the addition of DM-β-CyD. In addition, the results suggest the possibility that DM-β-CyD inhibited CYP3A activity in the small intestinal microsome of rats. Thus, we examined the effect of DM-β-CyD on the bioconversion of CsA in the small intestinal microsome of rats. In the absence of DM-β-CyD, the amounts of the intact CsA in the microsome decreased in a time-de-
pended manner and concomitantly the amounts of metabolites M1 and M17 gradually increased (Fig. 5). In the presence of DM-β-CyD, the level of intact CsA was slightly decreased, and that of M1 was only very slightly increased, and M17 was below the detection limit (Fig. 5). These in situ and in vitro results suggest that DM-β-CyD inhibits the biocconversion of CsA during the pass through the small intestinal mucosa of rats.

**In Vivo Oral Absorption Behavior of CsA** Bile duct-cannulated and -noncannulated rats were used to study the effects of bile on the oral absorption of CsA in vivo. Figure 6(A) shows the plasma concentration–time profiles of CsA after oral administration of the aqueous suspension containing CsA or its DM-β-CyD complex, or Sandimmune in bile duct-noncannulated rats. The pharmacokinetic parameters of CsA are listed in Table 1. Under the present experimental design, plasma sample was collected once from a rat, not seven points, because the multiple and intermittent blood samplings were difficult due to a concomitant sampling of lymph. Thus, the mean values of pharmacokinetic parameters, not including standard deviation values, are listed in Table 1. When the aqueous suspensions containing CsA alone or its DM-β-CyD complex were administered, the complexation with DM-β-CyD increased the AUC value and the maximum levels in plasma (C_{max}) of CsA about 4.7 and 2.8 fold, respectively, and also increased the times required to reach the peak plasma concentration (T_{max}) value and the MRT value. DM-β-CyD enhanced the absolute bioavailability (F value) of CsA from 5.3% to 24.8%. For comparison, the AUC, C_{max}, and T_{max} values of DM-β-CyD complex were only slight greater than those of Sandimmune. Figure 7 shows the variation in plasma levels of CsA after oral administration of the suspensions to bile duct-noncannulated rats. It is clear that the bias of plasma CsA level after the oral administration of DM-β-CyD complex was lower than that of CsA alone suspension and Sandimmune at all the sampling points.

Figure 6(B) shows that the lymph concentration–time pro-

---

**Table 1. Pharmacokinetic Parameters of CsA after Oral Administration of the Aqueous Suspension Containing CsA or Its DM-β-CyD or Sandimmune (Equivalent to 10 μg/kg CsA) to Bile Duct-Noncannulated or -Cannulated Rats**

<table>
<thead>
<tr>
<th>System</th>
<th>AUC (μg·h/ml)</th>
<th>C_{max} (μg/ml)</th>
<th>T_{max} (h)</th>
<th>MRT (h)</th>
<th>F (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-cannulated rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug alone</td>
<td>1.62</td>
<td>0.33</td>
<td>3.00</td>
<td>4.62</td>
<td>5.43</td>
</tr>
<tr>
<td>With DM-β-CyD</td>
<td>7.58</td>
<td>0.92</td>
<td>5.00</td>
<td>5.41</td>
<td>24.8</td>
</tr>
<tr>
<td>Sandimmune</td>
<td>6.90</td>
<td>0.82</td>
<td>4.00</td>
<td>6.60</td>
<td>22.6</td>
</tr>
<tr>
<td>Cannulated rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug alone</td>
<td>0.49</td>
<td>0.10</td>
<td>2.80</td>
<td>4.43</td>
<td>1.6</td>
</tr>
<tr>
<td>With DM-β-CyD</td>
<td>2.88</td>
<td>0.57</td>
<td>2.60</td>
<td>4.15</td>
<td>9.4</td>
</tr>
</tbody>
</table>

Each value represents the mean of 4—5 rats.
files of CsA after oral administration of these suspensions to bile duct-noncannulated rats. When Sandimmune was administered orally, the CsA levels in the lymph markedly increased compared with those of CsA alone and DM-β-CyD complex. On the other hand, the levels were changed only very slightly by complexation with DM-β-CyD in the suspension system. These results indicate that DM-β-CyD following the oral administration to the bile duct-noncannulated rats considerably enhances the transfer of CsA into the blood, not into the lymph.

We examined the effects of DM-β-CyD on the bioavailability of CsA after oral administration of the aqueous suspension containing CsA or its DM-β-CyD complex to bile duct-cannulated rats. Compared with the noncannulated rats, the plasma CsA levels after oral administration of the suspension containing CsA or its DM-β-CyD complex significantly decreased in the cannulated rats (Fig. 8), suggesting that the oral absorption of CsA is markedly abated by the absence of bile irrespective of DM-β-CyD. On the other hand, the F value in the presence of DM-β-CyD was 5.9-fold higher than that of CsA alone in the cannulated rats, and the F value in the presence of DM-β-CyD in the cannulated rats was still 1.8-fold higher than that of CsA alone in the non-cannulated rats. These results suggest that DM-β-CyD acts as a substitute for bile in solubilizing CsA, thus making it available for absorption in the small intestine of rats.

DISCUSSION

We previously reported that the solubility of CsA markedly increased due to complexation with DM-β-CyD, e.g. the solubility of CsA increased 87-fold in the presence of $5.0 \times 10^{-2}$ M DM-β-CyD. Thus, these findings suggested that the complexation of CsA with DM-β-CyD increases the dissolution rate in the gastrointestinal tracts and oral bioavailability. In fact, the dissolution rate of CsA was markedly augmented by the complexation with DM-β-CyD (Fig. 2). The enhancing effect of DM-β-Cyd on the dissolution rate of CsA was superior to that of α-Cyd and was comparable to that of DM-α-Cyd (data not shown). In addition, the enhancing effects of these CyDs were consistent with the order of magnitudes of the stability constants of the complexes, i.e. the stability constants were determined to be 150 m$^{-1}$, 1060 m$^{-1}$ and 1050 m$^{-1}$ for the α-Cyd, DM-α-Cyd and DM-β-Cyd complex, respectively. Thus, the enhanced dissolution rate of CsA in complexation with CyDs may be due to the increase in the solubility of CsA.

Recently, it has become widely known that the extent of the oral absorption of CsA is influenced not only by the physicochemical characteristics of CsA but also by the physiological conditions in the intestine, such as the activity of CYP3A4, which is associated with the metabolism of CsA in the small intestinal wall, P-glycoprotein, which is associated with the efflux of CsA from the small intestinal wall, bile acids and foods. Thus, the in situ closed loop studies demonstrated that DM-β-Cyd enhanced the transfer of CsA from the small intestinal lumen into the mesenteric venous blood (Fig. 3). It was conceivable that this enhancing effect of DM-β-Cyd could be attributed to the faster dissolution rate of the drug. In addition, the cumulative amount ratio of M1 to CsA in the blood up to 40 min after the injection of the CsA suspension into the intestinal sac was markedly decreased by the addition of DM-β-Cyd. These results suggest that DM-β-Cyd inhibits the bioconversion of CsA in the
small intestinal mucosa. There are two possible mechanisms by which DM-β-CyD inhibits the bioconversion of CsA: 1) DM-β-CyD decreases the CYP3A activity in the small intestine, and/or 2) DM-β-CyD increases the amount of CsA uptake in the small intestinal mucosa, leading to enzymatic saturation. In fact, the former possibility may be supported by the findings that DM-β-CyD stabilized CsA in the small intestinal microsomes in vitro (Fig. 5). However, we should pay attention to the idea that the uptake of DM-β-CyD into the mucosa might be prerequisite for DM-β-CyD to exert some effects on the activity of CYP3A. It is generally recognized that the gastrointestinal absorption of CyDs, including DM-β-CyD, in an intact form is limited due to their bulky and hydrophilic nature. Therefore, the former mechanism might be ruled out. In a recent study, a saturable metabolism of CsA was observed as its dose increased. These results suggest that DM-β-CyD inhibits the bioconversion of CsA into M1, probably through the enzyme saturation rather than its direct effect on enzyme activity.

It is also well known that P-glycoprotein affects the oral absorption of CsA. Recently, Demeule et al. reported that the portion of CsA that binds to calcineurin, which corresponds to amino acid residues 4—9 of CsA (Fig. 1), plays a role in the interaction of CsA with P-glycoprotein. Our previous study revealed that DM-β-CyD preferentially included the hydrophilic side chains of CsA amino acids such as butinythreonyl and leucine in the cavity. These results suggested that the affinity of CsA to P-glycoprotein changes by complex formation with DM-β-CyD. However, we do not know the effects of DM-β-CyD on the efflux activity of P-glycoprotein under these experimental conditions. The effects of DM-β-CyD on the expression or activity of P-glycoprotein is currently under study in Caco-2 cells.

CsA exhibits poor bioavailability if administered using the currently preferred system of an oily solution and furthermore exhibits wide intra- and inter-individual variations as well. Thus, we evaluated the in vivo absorption of CsA after oral administration of the aqueous suspension containing CsA or its DM-β-CyD complex to rats, and this was compared with that of Sandimmune. In the present study, DM-β-CyD significantly increased the CsA bioavailability with low variability compared with CsA alone suspension. In addition, the variability in bioavailability of DM-β-CyD complex was lower than that of Sandimmune, although the extent of bioavailability of the DM-β-CyD complex was little or not different from that of Sandimmune (Figs. 6(A) and 7 and Table 1). In fact, the coefficients of variation in the blood levels of CsA after the oral administration of the suspension containing CsA, or its DM-β-CyD complex and Sandimmune were 62.5%, 22.3% and 40.5% in the bile duct-noncanulated rats, respectively. This improvement in oral bioavailability for CsA afforded by DM-β-CyD is attributed to the increase in the dissolution rate of the drug and the saturable first pass metabolism.

CsA is known to inhibit the protein phosphatase activity of calcineurin and to block the dephosphorylation and nuclear import of NF-AT transcription factors, especially NF-AT4, in CD4+ and CD8+ T cells. Thus, we determined the CsA level in lymph after oral administration of CsA suspension to rats; however unfortunately, DM-β-CyD did not increase the CsA level in lymph (Fig. 6(B)). The lack of the enhancing effect of DM-β-CyD could be attributed to the higher aqueous solubility of CsA-DM-β-CyD complex, because more lipophilic compounds tend to be transported into lymph via gastrointestinal tracts. Actually, this idea may be supported by the findings for the much higher CsA lymph levels after the oral administration of Sandimmune to rats. Nevertheless, DM-β-CyD may have an advantage as a candidate for a CsA carrier, because DM-β-CyD augmented the CsA level in the blood where T cells flow sufficiently.

It is well known that bile acids help the oral absorption of CsA when it was administered as the standard oily solution. Thus we studied the effects of DM-β-CyD on the oral absorption of CsA after the administration of the CsA suspension to bile duct-cannulated rats. When the CsA or its DM-β-CyD complex suspension was administered to the cannulated rats, the AUC value and Cmax value of CsA in the plasma significantly decreased as compared with those in noncannulated rats (Fig. 8 and Table 1). However, the extent of the lowering in the bioavailability in the presence of DM-β-CyD was much less serious than that of CsA alone (Table 1). The differences in the oral bioavailability between CsA alone and DM-β-CyD complex may be explained by the extent of the dissolved amount of CsA in the gut solution of rats. Thus, it is likely that DM-β-CyD constitutes at least some part of the solubilizing activity of bile acids in the small intestine of rats.

In conclusion, DM-β-CyD improved the oral bioavailability with the low variability in vivo as evidenced by the faster dissolution rate of CsA and the saturable first pass effect. The present results clearly suggest the potential use of DM-β-CyD in improving the oral absorption of CsA as a liquid formulation.

Acknowledgements We thank Mr. Kiyokazu Yunomae for technical assistance.

REFERENCES

4) Humbert H., Therapie, 52, 353—357 (1997).
(1997).
86 (1994).
6652 (1997).