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Contributions of intestinal P-glycoprotein and CYP3A to oral bioavailability of cyclosporin A in mice treated with or without dexamethasone

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Abstract

The contributions of P-glycoprotein (P-gp) and CYP3A to the oral bioavailability (BA) of cyclosporin A (CyA) were separately evaluated by using wild-type and *mdr1a/1b* knockout mice treated with dexamethasone (DEX). Mice were treated with DEX (1 or 75 mg/kg/day, i.p.) daily for 7 days, and the blood concentrations of CyA were measured after an i.v. or p.o. dose of CyA (10 mg/kg) at 1.5 h after the last DEX treatment. The BA values of CyA in wild-type and *mdr1a/1b* knockout mice were similar, 0.25 and 0.287, respectively. As regards expression of *mdr1a* and *CYP3A* mRNAs, expression of mdr1a mRNA was weakest in the duodenum, the main absorption site of CyA, along the whole intestine of wild-type mice, while expression of CYP3A was strongest in the duodenum of both types of mice. After treatment with 1 and 75 mg/kg DEX, the BA values decreased to 43 and 25% of the control in wild-type mice, respectively, and to 89 and 73% of the control in *mdr1a/1b* knockout mice, respectively. Expression of *mdr1a* mRNA in duodenum of wild-type mice was potently induced by DEX treatment. The expression of *CYP3A* mRNA in liver and duodenum of both strains was enhanced only by high-DEX treatment. These results suggest that P-glycoprotein plays only a small role in the absorption of CyA under physiological conditions, but the protein is readily induced by DEX and then functions as a more substantial absorption barrier to CyA than does CYP3A in the intestine.

Keywords: Cyclosporin A; Bioavailability; P-glycoprotein; Dexamethasone; CYP3A; *mdr1a/1b* knockout mice

1. Introduction

Cyclosporin A (CyA) is widely used for immunosuppression in patients after organ transplantation, but the level of blood concentration after oral administration is frequently unstable. Therefore, it is important to clarify the factors influencing the oral bioavailability (BA) of CyA. It is well known that CyA is a substrate of both the efflux transporter P-glycoprotein (P-gp) and the metabolizing enzyme cytochrome P450 (CYP3A) and these proteins in the intestinal epithelial cells both act to restrict drug absorption (Lown et al., 1997; Konishi et al., 2004; Lee et al., 2005). However, the effects of P-gp and CYP3A, present in the small intestinal epithelium, on the BA of CyA need to be separately evaluated. Since the development of *mdr1a/1b* knockout mice (Schinkel et al., 1997), the role of P-gp in drug pharmacokinetics has been intensively studied (Smit et al., 1998; Lan et al., 2000). On the other hand, because many subspecies of CYP3A exist, animals in which all CYP3A genes have been knocked out are not yet available.

It has been shown that dexamethasone (DEX) induces increased protein levels of P-gp and CYP (Zhao et al., 1993; Lake et al., 1998; Laurent and Leslie, 1998; Demeule et al., 1999). The nature of the drug interaction in patients treated with

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CyA and steroid in combination is, however, controversial; the blood concentration of CyA may be increased (Klintamalm and Sawe, 1984), or decreased (Hricik et al., 1990), or unaffected (Rocci et al., 1988). We have experienced a decrease of the blood concentration of tacrolimus after steroid pulse therapy in a living-donor liver transplant patient. Therefore, we examined the interaction between immunosuppressants and steroids, and we found that the BA values of CyA and tacrolimus were significantly decreased by DEX, which increased the expression of *mdr1a* and *CYP3A2* mRNAs and the levels of the corresponding proteins in the liver and small intestine of rats (Yokogawa et al., 2002; Shimada et al., 2002).

In this study, to evaluate separately the contributions of P-gp and CYP3A to the absorption of CyA, we examined the pharmacokinetics of CyA and the expression of P-gp and CYP3A, mainly in the small intestine, in wild-type mice and *mdr1a/1b* knockout mice treated with DEX at low and high doses.

2. Methods

2.1. Materials

Sandimmun® injection (cyclosporin A, CyA) and DEX were purchased from Novartis Pharma Co. Ltd. (Tokyo, Japan) and Wako Pure Chemical Industries (Osaka, Japan), respectively. Oligonucleotide primers were custom-synthesized by Amersham Pharmacia Biotech (UK). Other reagents were purchased from Sigma Co. (St. Louis, MO).

2.2. Animal experiments

Experiments were performed on male *mdr1a/1b* knockout mice (body weight 22–27 g, Taconic Farms Inc., NY, USA). We used male FVB/NJcl mice (body weight 23–26 g, SLC, Hamamatsu, Japan) as wild-type mice.

Mice were treated daily for 7 days with a corn oil solution of DEX (1 or 75 mg/kg/day, i.p.). The control mice were injected daily with corn oil alone for 7 days. Mice were fasted for 12 h prior to the CyA administration, but water were given freely.

CyA (10 mg/kg) was injected via the jugular vein in a volume of 50 μl or was orally administered in a volume of 200 μl at 1.5 h after the last DEX treatment. Blood samples were collected from the intraorbital venous plexus using a heparinized capillary tube under light ether anesthesia, at designated time intervals.

2.3. Measurement of blood concentration of CyA

Blood concentration of CyA was measured with a TDx analyzer using a commercial kit according to the manufacturer's instructions (Dainabot Co. Ltd., Tokyo, Japan). The TDx assay is a fluorescence polarization immunoassay (FPIA) reagent system for the measurement of CyA in whole blood (David-Neto et al., 2000). The measurement range of blood concentration was 25–1500 ng/ml. The cross-reactivities with metabolites of CyA were 19.4% for M1 and less than 5% for other metabolites.

2.4. Reverse transcriptase-polymerase chain reaction (RT-PCR) assay

Total RNA was isolated from the liver and small intestine (duodenum, jejunum and ileum) by using an Isogen Kit (Wako, Osaka). Synthesis of cDNA from the isolated total RNA was carried out using RNase H- reverse transcriptase (GIBCO BRL, Rockville, MD). Reverse transcription (RT) reactions were carried out in 40 mM KCl, 50 mM Tris–HCl (pH 8.3), 6 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM each of dATP, dCTP, dGTP and dTTP, 10 units of RNase inhibitor (Promega, Madison, WI), 100 pmol of random hexamer, total RNA and 200 units of the Moloney murine leukemia virus reverse transcriptase (Gibco-BRL, Berlin, Germany) in a final volume of 50 μl at 37 °C for 60 min. Polymerase chain reaction (PCR) was carried out in a final volume of 20 μl, containing 1 μl of RT reaction mixture, 50 mM KCl, 20 mM Tris–HCl (pH 8.3), 2.5 mM $MgCl_2$, 0.2 mM each of dATP, dCTP, dGTP and dTTP, 10 μM each of the mixed oligonucleotide primers and 1 unit of Taq DNA polymerase (Gibco-BRL). Primers used for mouse *mdr1a* were 5′-GAATTGGTGACAAAATCGGA-3′ and 5′-TGTCTATACTGGGCTTATTA-3′ (576 bp) (Croop et al., 1989), those for mouse *mdr1b* were 5′-GGA-ACTCTCGCTGCTATTAT-3′ and 5′-GGT TAG CTT CCA ACC ACT TA-3′ (486 bp) (Seree et al., 1998), those for mouse *CYP3A* were 5′-GAA GCA TTG AGG AGG ATC AC-3′ and 5′-GGG TTG TTG AGG GAA TCC AC-3′ (670 bp) (Seree et al., 1998), and those for mouse *β-actin* were 5′-TTC TAC AAT GAG CTG CGT GTG GC-3′ and 5′-CTC (A/G)TA GCT CTT CTC CAG GGA GGA-3′ (456 bp), as previously reported by Waki et al. (1995). Each cycle consisted of 30 s at 94 °C, 60 s at 60 °C and 75 s at 72 °C for *mdr1a* and *mdr1b*, 30 s at 94 °C, 60 s at 56 °C and 75 s at 72 °C for *CYP3A*, and 30 s at 94 °C, 60 s at 58 °C and 75 s at 72 °C for *β-actin*. PCR reaction was run for 30 cycles for *mdr1a*, *mdr1b* and *CYP3A*, and for 22 cycles for *β-actin*.

2.5. Data analysis

The pharmacokinetic parameters were estimated according to model-independent moment analysis as described by Yamaoka et al. (1978). The data were analyzed using Student's *t*-test to compare the unpaired mean values of two sets of data. The number of determinations is noted in each table and figure. A value of $P < 0.05$ or 0.01 was taken to indicate a significant difference between sets of data. Electrophoresis results were analyzed by using NIH Image software.

3. Results

3.1. Blood concentration–time courses of CyA after i.v. administration

The blood concentration–time courses of CyA following i.v. administration of CyA (10 mg/kg) at 1.5 h after the last administration of DEX (1 or 75 mg/kg/day, i.p., 7 days) or the vehicle in wild-type and *mdr1a/1b* knockout mice are shown in Fig. 1. The blood concentrations of CyA were sig-

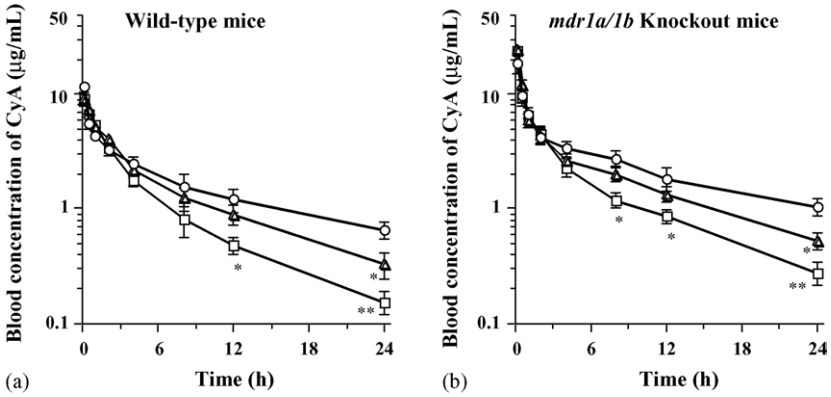

Fig. 1. The blood concentration–time courses of CyA after i.v. administration of CyA (10 mg/kg) in untreated and DEX-treated mice (1 or 75 mg/kg/day, i.p.). Wild-type (a) and *mdr1a/1b* knockout (b) mice were treated daily for 7 days with DEX prior to the CyA administration. CyA was administered at 1.5 h after the last DEX treatment. Each point and bar represent the mean ± S.E. of five mice. (◯) No treatment, (△) DEX treatment with 1 mg/kg/day, (□) DEX treatment with 75 mg/kg/day. *Significantly different from non-treated mice at $P < 0.05$, **significantly different from non-treated mice at $P < 0.01$.

nificantly decreased in wild-type mice, depending on the dose of DEX. The blood concentrations of CyA were also significantly decreased in *mdr1a/1b* knockout mice by DEX in a dose-dependent manner. But, the blood concentrations of CyA in *mdr1a/1b* knockout mice became higher than those in wild-type mice.

The pharmacokinetic parameters of CyA in wild-type mice and *mdr1a/1b* knockout mice are listed in Table 1. The value of the area under the blood concentration–time curve from 0 to 24 h ($AUC_{0–24\,h}$) of CyA after i.v. administration in *mdr1a/1b* knockout mice was significantly higher than that of wild-type mice, and the value of total clearance (CL_{tot}) of *mdr1a/1b* knockout mice was only about two-thirds of that of wild-type mice. The $AUC_{0–24\,h}$ values in both types of mice were lowered by DEX in a dose-dependent manner, while the values of total clearance (CL_{tot}) were significantly increased. Although the values of distribution volume at the steady-state (Vd_{ss}) of *mdr1a/1b* knockout mice tended to be smaller than those of wild-type mice, there was no significant difference.

3.2. Blood concentration–time courses of CyA after p.o. administration

The blood concentration–time courses of CyA following p.o. administration of CyA (10 mg/kg) at 1.5 h after the last administration of DEX (1 or 75 mg/kg/day, i.p., 7 days) or the vehicle in wild-type mice and *mdr1a/1b* knockout mice are shown in Fig. 2. The blood concentrations of CyA after p.o. administration were significantly decreased in wild-type mice, depending on the dose of DEX. The blood concentrations of CyA in *mdr1a/1b* knockout mice were little changed by low-DEX treatment, whereas they were significantly decreased by high-

Table 1
Pharmacokinetic parameters of CyA with or without DEX in wild-type and *mdr1a/1b* knockout mice

Parameters	Wild-type mice			*mdr1a/1b* Knockout mice		
	No treatment	DEX 1 mg/kg	DEX 75 mg/kg	No treatment	DEX 1 mg/kg	DEX 75 mg/kg
i.v. Administration						
$AUC_{0–24\,h}$ (μg h/ml)[a]	40.4 ± 5.6	36.2 ± 5.4 (90)	27.5 ± 2.4 (68)**	62.0 ± 7.8##	53.6 ± 4.5 (86)##	43.3 ± 4.0 (70)**,#
MRT (h)[b]	7.53 ± 1.81	6.01 ± 2.06	4.78 ± 0.89*	7.69 ± 1.79	5.99 ± 1.05	4.84 ± 0.89*
CL_{tot} (ml/h/kg)[c]	248 ± 34	276 ± 40	364 ± 31**	161 ± 20##	187 ± 17##	231 ± 25**,##
Vd_{ss} (l/kg)[d]	1.87 ± 0.64	1.66 ± 0.71	1.74 ± 0.42	1.24 ± 0.41	1.12 ± 0.27	1.22 ± 0.31
p.o. Administration						
$AUC_{0–24\,h}$ (μg h/ml)[a]	10.1 ± 1.7	3.89 ± 0.64 (39)**	1.72 ± 0.17 (7)**	17.8 ± 2.9##	13.7 ± 1.2 (77)##	9.05 ± 1.43 (51)**,##
BA[e]	0.250	0.107 (43)	0.0625 (25)	0.287	0.256 (89)	0.209 (73)

Mice were treated daily for 7 days with DEX (1 or 75 mg/kg) prior to the CyA administration. CyA (10 mg/kg) was administered at 1.5 h after the last DEX treatment. Pharmacokinetic parameters were estimated according to model-independent moment analysis. Each value in parenthesis represents the percent of each control. Each value represents the mean ± S.D. of five mice.

[a] Area under blood concentration–time curve from 0 to 24 h.
[b] Mean residence time from 0 to 24 h.
[c] Blood total clearance.
[d] Distribution volume at the steady-state.
[e] Bioavailability.
* Significantly different from each control in both groups at $P < 0.05$.
** Significantly different from each control in both groups at $P < 0.01$.
Significantly different from the value of each group in wild-type mice at $P < 0.01$.

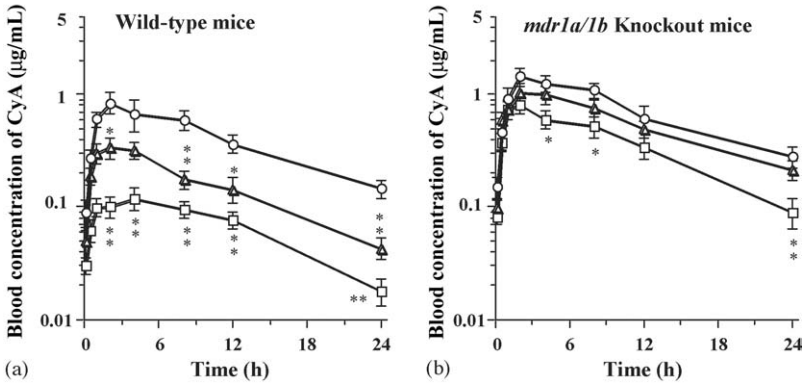

Fig. 2. The blood concentration–time courses of CyA after p.o. administration of CyA (10 mg/kg) in untreated and DEX-treated mice (1 or 75 mg/kg/day, i.p.). Wild-type (a) and *mdr1a/1b* knockout (b) mice were treated daily for 7 days with DEX prior to the CyA administration. CyA was administered at 1.5 h after the last DEX treatment. Each point and bar represent the mean ± S.E. of five mice. (◯) no treatment, (△) DEX treatment (1 mg/kg/day), (□) DEX treatment (75 mg/kg/day). *Significantly different from non-treated mice at $P < 0.05$, **significantly different from non-treated mice at $P < 0.01$.

DEX treatment. However, the tendency of decrease in blood concentration of CyA induced by DEX treatment in *mdr1a/1b* knockout mice was clearly smaller than that in wild-type mice.

As shown in Table 1, the $AUC_{0–24\,h}$ values of CyA after p.o. administration in wild-type mice treated with low and high doses of DEX were decreased to about 38 and 17% of the control values, respectively, whereas those in *mdr1a/1b* knockout mice were decreased to about 77 and 51% of the control values, respectively.

The oral bioavailability (BA) of CyA in wild-type mice was markedly decreased by DEX, but that in *mdr1a/1b* knockout mice was little changed by DEX.

3.3. RT-PCR analysis of mdr1a, mdr1b and CYP3A mRNAs in tissues

Fig. 3 shows the effects of DEX treatment on the expression levels of *mdr1a*, *mdr1b* and *CYP3A* mRNAs in three segments of small intestine (duodenum, jejunum and ileum) and liver in

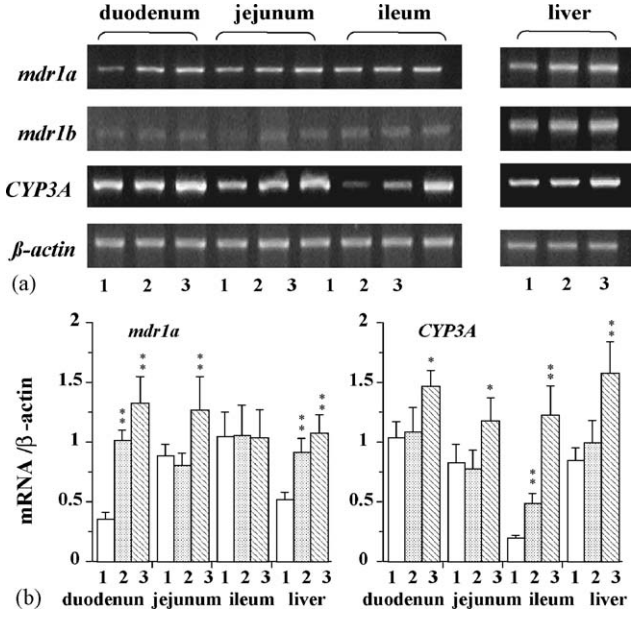

Fig. 3. (a and b) Effects of DEX on the expression of *mdr1a*, *mdr1b* and *CYP3A* mRNAs in liver and three segments of small intestine (duodenum, jejunum and ileum) of wild-type mice with or without DEX treatment at 1.5 h after the last DEX treatment. (a) The wild-type mice were given successive i.p. administrations of DEX (1 or 75 mg/kg/day) for 7 days. The sizes of the reverse transcriptase-polymerase chain reaction (*RT-PCR*) products are 576 bp (*mdr1a*), 486 bp (*mdr1b*) and 670 bp (*CYP3A*). (b) Relative expression of *mdr1a* and *CYP3A* mRNAs. Each column and bar represent the mean ± S.E. of five mice. *Significantly different from the DEX-untreated mice at $P < 0.05$, **significantly different from the DEX-untreated mice at $P < 0.01$. Lane 1, no treatment; lane 2, DEX treatment (1 mg/kg/day); lane 3, DEX treatment (75 mg/kg/day).

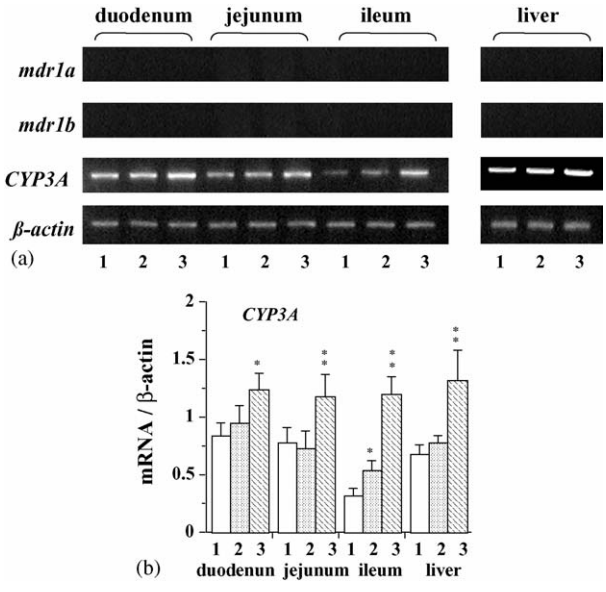

Fig. 4. (a and b) Effects of DEX on the expression of *mdr1a*, *mdr1b* and *CYP3A* mRNAs in liver and three segments of small intestine (duodenum, jejunum and ileum) of *mdr1a/1b* knockout mice with or without DEX treatment at 1.5 h after the last DEX treatment. (a) The *mdr1a/1b* knockout mice were given successive i.p. administrations of DEX (1 or 75 mg/kg/day) for 7 days. The sizes of the reverse transcriptase-polymerase chain reaction (*RT-PCR*) products are 576 bp (*mdr1a*), 486 bp (*mdr1b*), and 670 bp (*CYP3A*). (b) Relative expression of *CYP3A* mRNA. Each column and bar represent the mean ± S.E. of five mice. *Significantly different from the DEX-untreated mice at $P < 0.05$, **significantly different from the DEX-untreated mice at $P < 0.01$. Lane 1, no treatment; lane 2, DEX treatment (1 mg/kg/day); lane 3, DEX treatment (75 mg/kg/day).

wild-type mice. The expression of *mdr1a* mRNA in small intestine of DEX-untreated wild-type mice was highest in the ileum, followed by jejunum and duodenum, but that of *CYP3A* mRNA was in the opposite order. DEX dose-dependently induced the expression of these mRNAs in tissues having intrinsically low mRNA expression levels, but in tissues expressing high mRNA levels it was either ineffective or effective only at the high-dose. The expression of *mdr1a* mRNA in the liver of wild-type mice was significantly induced to the same extent by both low- and high-DEX treatment, and that of *CYP3A* mRNA was strongly induced by the high-DEX, but not the low-DEX treatment.

The expression levels of *mdr1b* mRNA in all three segments of small intestine of wild-type mice were very much lower than those of *mdr1a* mRNA, and the influence of DEX treatment on the expression of *mdr1b* mRNA could not be observed clearly. The expression of *mdr1b* mRNA in the liver was on a par with that of *mdr1a* mRNA, but DEX did not show a clear effect.

Fig. 4 shows the effects of low- and high-DEX treatments on *CYP3A* mRNA expression in duodenum, jejunum, ileum and liver at 1.5 h after the last DEX treatment in *mdr1a/1b* knockout mice. The expression levels of *CYP3A* mRNA and the effect of DEX in *mdr1a/1b* knockout mice were essentially similar to those in wild-type mice. It was confirmed that *mdr1a* and *mdr1b* mRNAs were not detectable in these tissues.

4. Discussion

In this study, we used wild-type and *mdr1a/1b* knockout mice to examine the contributions of P-gp and CYP3A to the intestinal absorption of CyA. The $AUC_{0–24\,h}$ values of CyA in *mdr1a/1b* knockout mice after i.v. administration and after p.o. administration were about 1.5 times and 1.8 times those in wild-type mice, respectively, and the CL_{tot} value in *mdr1a/1b* knockout mice was about two-thirds of that in wild-type mice (Table 1). These differences between the pharmacokinetic parameters of CyA in the two types of mice presumably reflect the lack of P-gp in *mdr1a/1b* knockout mice. However, the small difference in the BA values between these types of mice suggests that P-gp makes little contribution to intestinal absorption. Indeed, the expression level of *mdr1a/1b* mRNA was weakest in duodenum, which is the absorption site of CyA (Cakaloglu et al., 1993), along the small intestine of wild-type mice. The key factor in the clearance of CyA under normal conditions may be CYP3A activity in intestinal endothelium and liver, and P-gp may have only a limited effect on intestinal absorption of CyA.

It has been reported that DEX increased the protein levels of both P-gp and CYP in vitro (Zhao et al., 1993; Lake et al., 1998; Laurent and Leslie, 1998; Demeule et al., 1999). We previously reported that DEX increased the expression of *mdr1a* and *CYP3A2* mRNAs and the proteins in liver and intestine, and lowered the absorption of CyA and tacrolimus (Yokogawa et al., 2002; Shimada et al., 2002). In this study, DEX treatment decreased the $AUC_{0–24\,h}$ value and increased the CL_{tot} value of CyA to similar extents in wild-type mice and *mdr1a/1b* knockout mice after i.v. administration, while the $AUC_{0–24\,h}$ value of CyA in wild-type mice was greatly decreased compared with that in *mdr1a/1b* knockout mice by DEX treatment, and consequently the BA value in wild-type mice was markedly lower than that in *mdr1a/1b* knockout mice.

Generally, BA is given by the following equation:

$$BA = F_a \cdot F_g \cdot F_{liv}$$

where F_a, F_g and F_{liv} represent the availability for the absorption into the small intestinal membrane, the permeability of the small intestinal membrane and the permeability of the liver, respectively.

We found that the $AUC_{0–24\,h}$ values of CyA after p.o. administration in *mdr1a/1b* knockout mice given low- and high-dose DEX treatments were decreased significantly and dose-dependently, and the BA values were also decreased to 89 and 73% of that of untreated mice, respectively. On the other hand, we found that intestinal *CYP3A* mRNA expression is strongest in duodenum in untreated *mdr1a/1b* knockout mice, and the induction by high-dose DEX, though statistically significant, is weak in duodenum, despite the strong induction in other sites of the intestine and liver. Thus, in the case of *mdr1a/1b* knockout mice, it was considered that changes of F_g and F_{liv} induced by DEX treatment influence the BA value, but change of F_a does not, owing to the absence of P-gp. If only F_{liv} need be considered, the CL_{tot} values after i.v. and p.o. administrations should be decreased equally by treatment with DEX, so the BA should not be changed. However, the BA in *mdr1a/1b* knockout mice was decreased by high-dose DEX treatment, suggesting that a change in F_g occurs and causes the 27% decrease of BA. Thus, we conclude that CyA in small intestinal epithelium is metabolized by CYP3A.

We found that the $AUC_{0–24\,h}$ value of CyA after p.o. administration in wild-type mice was decreased significantly by DEX in a dose-dependent manner, and the extent of the decrease was much larger than that in *mdr1a/1b* knockout mice. The BA values of wild-type mice after treatments with low- and high-dose DEX were decreased to 43 and 25% of that in the untreated control, respectively. On the other hand, expression of *mdr1a* mRNA in duodenum of wild-type mice was induced equally strongly by low- and high-dose DEX treatments. Therefore, in the case of low-dose DEX treatment, it appears that F_g and F_{liv} do not change, whereas F_a is markedly decreased owing to induction of P-gp. We suggest that efflux via P-gp in the small intestine accounts for the decrease of BA in wild-type mice given low-dose DEX treatment.

Further, we found that the BA of wild-type mice given high-dose DEX treatment is greatly decreased. Strong induction of P-gp in duodenum and moderate induction of CYP3A in the liver were caused by high-dose DEX treatment. These changes in F_a and F_g synergistically result in a decrease of BA by 75%.

The dose of CyA used in this study is within the upper limit of the therapeutic dose range for infusion administration, while in the case of p.o. administration, the initial CyA concentration in the intestinal tract may be simply regarded as about 100 μM, as calculated from the volumes of the intestinal tract (1.5 ml/0.02 kg) (Davies and Morris, 1993) and the dosing solution (0.2 ml). It is reported that the efflux of CyA by P-gp may be saturated at an apical CyA concentration of 0.28 or 1 μM (in rodent or human, respectively) (Lee et al., 2005), and the

expression of P-gp in the duodenum is weak under normal conditions. Therefore, it is reasonable to consider that in the case of p.o. administration, the efflux via P-gp would not substantially influence the oral bioavailability of CyA. This is consistent with the observation that the $AUC_{0-24\,h}$ value of untreated *mdr1a/1b* knockout mice is increased only 1.8 times compared with that of untreated wild-type mice.

However, when the expression of P-gp is increased by DEX treatment, the value of $AUC_{0-24\,h}$ is markedly decreased to 1/3–1/6 of that in untreated wild-type mice. We speculate that the initial CyA concentration in the intestinal tract may be nearer the saturation level for P-gp efflux function, rather than the calculated figure of 100 μM mentioned above, because of the poor aqueous solubility of CyA and the adsorption of CyA on the gastrointestinal membrane (Jin et al., 2005). This would explain why enhancement of the P-gp efflux function by DEX treatment markedly decreased the $AUC_{0-24\,h}$ value. The blood concentrations of CyA in wild-type mice after p.o. administration were within the range of 0.01–2 μg/ml (0.08–1.7 μM), so transport of CyA across the blood–brain barrier may be restricted by P-gp efflux (Tsuji et al., 1993; Sakata et al., 1994). It is known that the regional distribution patterns of P-gp and CYP3A expression in small intestine of mice are similar to those in humans (Thorn et al., 2005), so the roles of P-gp and CYP3A in mice clarified in this study could be relevant to the clinical situation.

In conclusion, under normal conditions, CYP3A influences the BA value of CyA through metabolism in the small intestine in mice, but P-gp has little influence on the absorption of CyA. However, when P-gp is induced, e.g., by steroid, the intestinal absorption of CyA may be inhibited, resulting in a marked decrease of the BA value.

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