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Modulation of *mdr1a* and *CYP3A* gene expression in the intestine and liver as possible cause of changes in the cyclosporin A disposition kinetics by dexamethasone

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Abstract

We investigated the effect of dexamethasone (DEX) on the disposition kinetics of cyclosporin A (CyA) and the mechanism of this drug interaction. Rats were treated with DEX (1 or 75 mg/kg per day, i.p.) once a day for 1–7 days, and the blood concentration of CyA was measured after an i.v. or p.o. dose of CyA (10 mg/kg) at 1.5 hr after the last DEX treatment. In rats treated with a low dose of DEX (1 mg/kg), the blood concentration of CyA after i.v. administration was unchanged compared with that of untreated rats, whereas the blood concentration after oral administration was significantly decreased, and this decrease was dependent on the duration of DEX administration. The total clearance (CL_{tot}) of CyA was unchanged, but the bioavailability was significantly decreased to about one-third of that in DEX-untreated rats after 7 days of DEX treatment. At this time, the expression of *mdr1a* mRNA and P-gp in the liver and intestine was increased, whereas CYP3A2 was unaffected at both the mRNA and protein levels. In rats treated with a high dose of DEX (75 mg/kg), the blood concentration of CyA was significantly decreased after both i.v. and p.o. administrations compared with those of untreated rats. The bioavailability of CyA was decreased, and the CL_{tot} was significantly increased. The P-gp and CYP3A2 in the liver and intestine were increased at both the mRNA and protein levels. Our results indicate that the drug interaction between CyA and DEX is a consequence of modulation of P-gp and *CYP3A2* gene expression by DEX, with differential dose-dependence.

Keywords: Dexamethasone; Cyclosporin A; P-Glycoprotein; *mdr1a*; *CYP3A2*; Expression; Pharmacokinetics

1. Introduction

Concomitant administration of multiple drugs, as is often done in current medical practice, may result in changes of disposition kinetics, such as adsorption, clearance or tissue distribution. Various mechanisms of drug interactions have been reported. Clinically, potent immunosuppressive agents, CyA and glucocorticoids are widely used in organ transplantation therapy. However, the possible drug interaction in combination therapy with CyA and DEX remains controversial; the blood CyA concentration may be increased [1–3], decreased [4] or unchanged [5] by DEX.

It is well known that CyA and DEX are both substrates of P-glycoprotein (P-gp). P-gp is encoded by the *mdr* gene and is expressed not only in multidrug-resistant cancer cells, but also in various normal tissues such as the adrenal, kidney, liver, small intestine, colon, and capillary endothelium in the brain [6–8]. Schinkel *et al.* [9] reported that the distribution of several P-gp substrates, such as ivermectin, vinblastine, digoxin, CyA and DEX, to the brain is significantly increased in *mdr1a* knockout mice, compared with normal mice. We also reported that the brain distribution of tacrolimus is dominated by the P-gp-mediated drug efflux and the absorption of tacrolimus from the gastrointestinal tract is limited in part by P-gp [10].

On the other hand, cytochrome P450 is the major phase I drug-metabolizing enzyme, and its gene subfamily human CYP3A4 is a major form in the intestine as well as the liver [11]. It has been reported that CyA is mainly metabolized by CYP3A4, and also partly by CYP1A2 or CYP2C6, and that *CYP3A4* mRNA in man corresponds to *CYP3A2* mRNA in rat [12,13].

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In vitro studies have elucidated that DEX induces P-gp and/or cytochrome P450 family (CYP) members [14–17]. Recently, Greiner *et al.* [18] found that rifampin treatment increases intestinal P-gp content 3.5-fold in humans, therefore, the drug interaction between digoxin and rifampicin involves modulation of intestinal P-gp gene expression. Moreover, McCune *et al.* [19] demonstrated that DEX at doses used clinically increased CYP3A4 activity in both healthy volunteers and human hepatocyte cultures. These findings suggest that when DEX is used in combination therapy with substrates of P-gp and/or CYP, it may change the efficacy or side effects of the combined drugs in patients. But there is little available information on pharmacokinetically elucidating the interaction through modulating gene expression in *in vivo* study.

In this study, we show that DEX changes the disposition kinetics of CyA in rats and this drug interaction is a result of modulation of *mdr1a* and *CYP3A2* gene expression by DEX.

2. Materials and methods

2.1. Materials

Sandimmun® injection (CyA) and DEX were purchased from Novartis Pharma Co. Ltd. (Tokyo, Japan) and Wako Pure Chemical Industries (Osaka, Japan), respectively.

2.2. Animal experiments

Male Wistar rats weighing 280–300 g (Japan SLC Co., Hamamatsu, Japan) were treated daily for 1, 2, 4 or 7 days with a corn oil solution of DEX (1 or 75 mg/kg per day, i.p.) to examine the dependence on the duration and the dose of DEX treatment. The control rats were injected daily with corn oil alone for 1, 2, 4 or 7 days.

A 100 μL aliquot of a distilled water of CyA (10 mg/kg) was injected *via* the femoral vein or 500 μL (10 mg/kg) was orally administered at 1.5 hr after the last treatment with DEX. Rats were fasted for 12 hr prior to the CyA administration, but water were given freely. Blood samples (each 200 μL) were collected at designated time intervals from the jugular vein under light ether anesthesia and were stored at −30° until assay.

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 [20]. The measurement range of blood concentration was 25–1500 ng/mL. The cross-reactivities with the 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 all tissues 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° 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 rat *mdr1a* were 5′-AGA AAC AGA GGA GCG CCA TT-3′ and 5′-GAA TTC AAC TTC AGG ATC CG-3′ (511 bp) [21], those for rat *CYP3A2* were 5′-AGT AGT GAC GAT TCC AAC ATA T-3′ and 5′-TCA GAG GTA TCT GTG TTT CCT-3′ (252 bp) [22], and those for rat 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.* [23]. Each cycle consisted of 45 s at 94°, 60 s at 60°, and 75 s at 72° for *mdr1a* and β-actin, and 45 s at 94°, 60 s at 55°, and 75 s at 72° for *CYP3A2*. PCR reaction was run for 26 cycles for *mdr1a* and *CYP3A2* for 22 cycles for β-actin.

2.5. Preparation of plasma membrane fraction and microsomes

For the preparation of plasma membrane, the liver and intestine were homogenized in 100 mM Tris–HCl, pH 7.4. The homogenate was centrifuged at 1500 *g* for 15 min, and the supernatant was then centrifuged at 100,000 *g* for 60 min. The pellet was washed, resuspended in Tris buffer, and stored at −80° until analysis. The rat liver microsomes were prepared as described previously [24] and stored at −80° until analysis. Rat intestinal microsomes were prepared in the presence of phenylmethylsulfonyl fluoride (0.2 M) in 0.2 mM HEPES buffer pH 7.1 (washing buffer). Protein concentrations were measured according to the method of Lowry *et al.* [25].

2.6. SDS-PAGE and immunoblotting

Sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) and immunoblotting with peroxidase/antiperoxidase staining of the plasma membrane for P-gp and the microsomes for CYP3A2 were carried out essentially as described by Laemmli [26] and Guengerich *et al.* [27]. The

sample protein (100 μg) was electrophoresed on 10% and transferred onto nitrocellulose membrane filters (Schleicher & Schuell, Dassel, Germany). After having been blocked with 5% skim milk, the filters were incubated overnight with 1 μg/mL primary antibody, goat anti-rat CYP3A2 antibodies (Daiichi Pure Chemicals Co. Ltd., Tokyo, Japan) and C219 (Dako Co., CA, USA), and for 1 hr with secondary antibody, biotinylated anti-goat IgG (Vector Laboratories Inc., CA, USA). Thereafter, the sample was extensively washed with phosphate-buffered saline. The immunopositive band was detected by means of a light-emitting non-radioactive detection system (Amersham International plc, Little Chalfont, Buckinghamshire, UK) and exposure to a Kodak X-Omat R film (Eastman Kodak Co., Rochester, NY).

2.7. Data analysis

The pharmacokinetic parameters were estimated according to model-independent moment analysis as described by Yamaoka *et al.* [28]. 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 $P < 0.05$ was taken to indicate a significant difference between sets of data.

3. Results

3.1. Blood concentration–time courses of CyA after a low dose of DEX

The blood concentration–time courses of CyA after i.v. or p.o. administration of 10 mg/kg of CyA in rats treated with a low dose of DEX (1 mg/kg per day, i.p.) are shown in Fig. 1. The control rats were injected daily with corn oil alone for 1, 2, 4 or 7 days. However, the blood concentration–time courses of CyA among these rats were not clearly different from that in corn oil-untreated rats (data not shown), therefore, rats injected daily with corn oil alone for 7 days were used as the control.

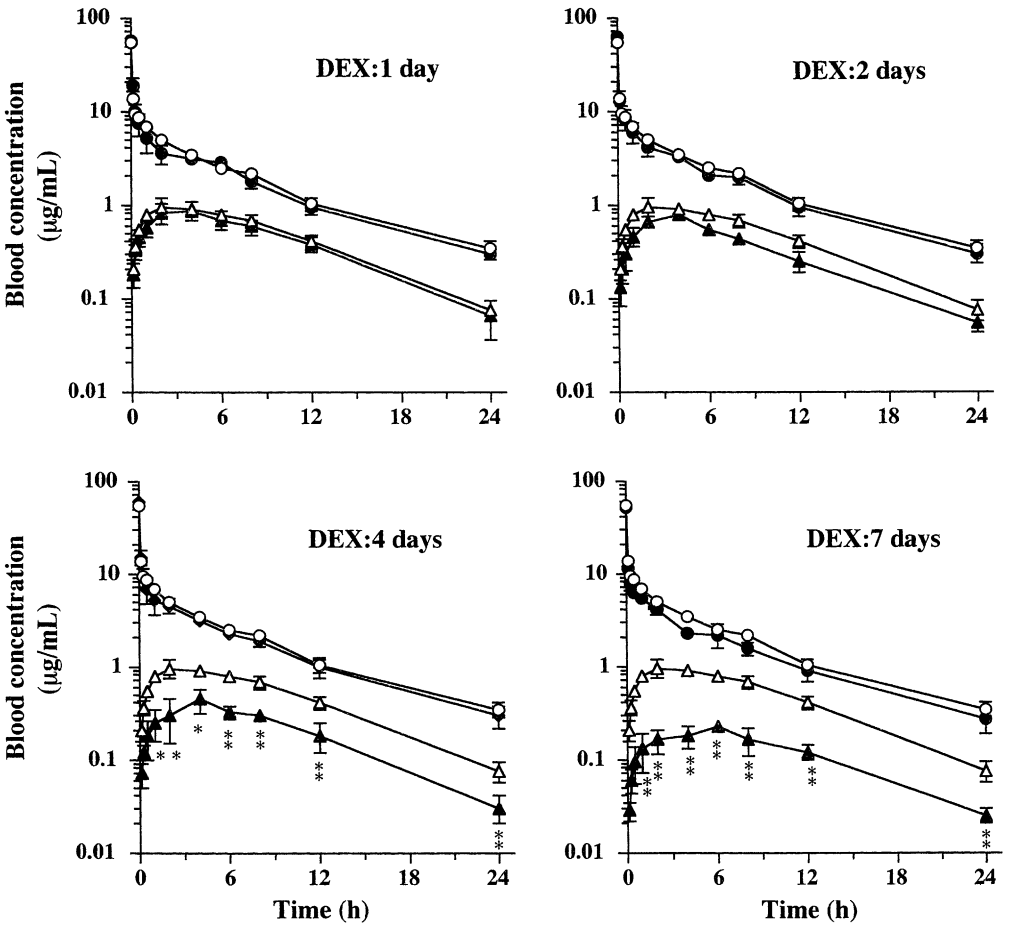

Fig. 1. The blood concentration–time courses of CyA after i.v. or p.o. administration of CyA (10 mg/kg) in untreated and DEX-treated rats (1 mg/kg per day, i.p.). Rats were treated daily for 1, 2, 4 or 7 days with DEX prior to the CyA administration. CyA was administered at 1.5 hr after the last DEX treatment. Each point and bar represent the mean ± SE of four rats; i.v. (○) and p.o. (△) administration in non-treated rats; i.v. (●) and p.o. (▲) administration in DEX-treated rats. *, **Significantly different from non-treated rats at $P < 0.05$ and 0.01, respectively.

Table 1
Pharmacokinetic parameters of CyA after a single i.v. or p.o. administration of CyA (10 mg/kg) in untreated and DEX-treated rats (1 mg/kg per day, i.p.)

Parameters	No treatment	DEX treatment (1 mg/kg per day)			
		1 day	2 days	4 days	7 days
i.v. administration					
$AUC_{0-24\ hr}$ (μg hr/mL)	51.2 ± 4.4	46.5 ± 6.6	48.0 ± 7.6	47.8 ± 8.4	41.1 ± 6.6
MRT (hr)	5.42 ± 0.57	5.44 ± 0.66	5.41 ± 0.81	5.50 ± 0.81	5.40 ± 0.93
Vd_{ss} (mL/kg)	1057 ± 137	1170 ± 204	1125 ± 227	1150 ± 265	1312 ± 294
CL_{tot} (mL/hr/kg)	195 ± 18	215 ± 32	208 ± 30	209 ± 34	243 ± 38
p.o. administration					
$AUC_{0-24\ hr}$ (μg hr/mL)	11.4 ± 1.1	10.2 ± 1.6	7.71 ± 0.82[a,b]	5.07 ± 0.64[a,b,c]	2.78 ± 0.36[a,b,c,d]
Bioavailability (%)	22.2	21.9	16.1	10.6	6.8

Rats were treated daily for 1, 2, 4 or 7 days with DEX prior to the CyA administration. CyA was administered at 1.5 hr after the last DEX treatment. Pharmacokinetic parameters were estimated according to model-independent moment analysis. Each value represents the mean ± SD of four rats.
[a] Significantly different from control rats at $P < 0.01$.
[b] Significantly different from DEX-treated rats for 1 day at $P < 0.05$.
[c] Significantly different from DEX-treated rats for 2 days at $P < 0.01$.
[d] Significantly different from DEX-treated rats for 4 days at $P < 0.01$.

The blood concentrations of CyA after i.v. administration were not significantly different from those of untreated rats. On the other hand, the blood concentrations of CyA after oral administration were significantly lower than those of untreated rats, to an extent that depended on the number of prior DEX treatments.

The pharmacokinetic parameters of CyA after administration of CyA to rats treated with the low dose of DEX are listed in Table 1. These parameters showed no change after the i.v. administration of CyA, but the values of the area under the blood concentration-time curve from 0 to 24 hr ($AUC_{0-24\ hr}$) after the p.o. administration were significantly lower than those of untreated rats. The bioavailability of CyA in the rats given the low concentration of DEX for 7 days was about one-third of that in untreated rats.

3.2. Blood concentration–time courses of CyA after a high dose of DEX

The blood concentration–time courses of CyA after i.v. or p.o. administration of 10 mg/kg of CyA in rats treated with high dose of DEX (75 mg/kg per day, i.p.) are shown in Fig. 2. The blood concentrations of CyA after the i.v. administration were significantly decreased compared with those of untreated rats, to an extent that depended on the number of prior DEX treatments. Similarly, after p.o. administration, the CyA concentrations were significantly decreased compared with those of untreated rats.

The pharmacokinetic parameters of CyA after i.v. administration of CyA in rats treated with a high dose of DEX are listed in Table 2. The values of $AUC_{0-24\ hr}$ after the i.v. administration of CyA were significantly decreased,

Table 2
Pharmacokinetic parameters of CyA after a single i.v. or p.o. administration of CyA (10 mg/kg) in untreated and DEX-treated rats (75 mg/kg per day, i.p.)

Parameters	No treatment	DEX treatment (75 mg/kg per day)			
		1 day	2 days	4 days	7 days
i.v. administration					
$AUC_{0-24\ hr}$ (μg hr/mL)	51.2 ± 4.4	41.5 ± 3.2[a]	32.4 ± 3.2[b,c]	28.8 ± 4.2[b,c]	23.4 ± 3.8[b,c]
MRT (hr)	5.42 ± 0.57	5.11 ± 0.61	3.38 ± 0.35[b,c]	3.33 ± 0.42[b,c]	3.34 ± 0.55[a,c]
Vd_{ss} (mL/kg)	1057 ± 137	1232 ± 303	1041 ± 137	1156 ± 208	1423 ± 310
CL_{tot} (mL/hr/kg)	195 ± 18	241 ± 30	308 ± 32[b,d]	347 ± 52[b,d]	426 ± 70[b,c,e]
p.o. administration					
$AUC_{0-24\ hr}$ (μg hr/mL)	11.4 ± 1.1	8.34 ± 1.43[a]	3.17 ± 0.43[b,c]	1.65 ± 0.47[b,c,e]	0.88 ± 0.16[b,c,f,g]
Bioavailability (%)	22.2	20.1	9.8	5.7	3.8

Rats were treated daily for 1, 2, 4 or 7 days with DEX prior to the CyA administration. CyA was administered at 1.5 hr after the last DEX treatment. Pharmacokinetic parameters were estimated according to model-independent moment analysis. Each value represents the mean ± SD of four rats.
[a] Significantly different from control rats at $P < 0.05$.
[b] Significantly different from control rats at $P < 0.01$.
[c] Significantly different from DEX-treated rats for 1 day at $P < 0.01$.
[d] Significantly different from DEX-treated rats for 1 day at $P < 0.05$.
[e] Significantly different from DEX-treated rats for 2 days at $P < 0.05$.
[f] Significantly different from DEX-treated rats for 2 days at $P < 0.01$.
[g] Significantly different from DEX-treated rats for 4 days at $P < 0.05$.

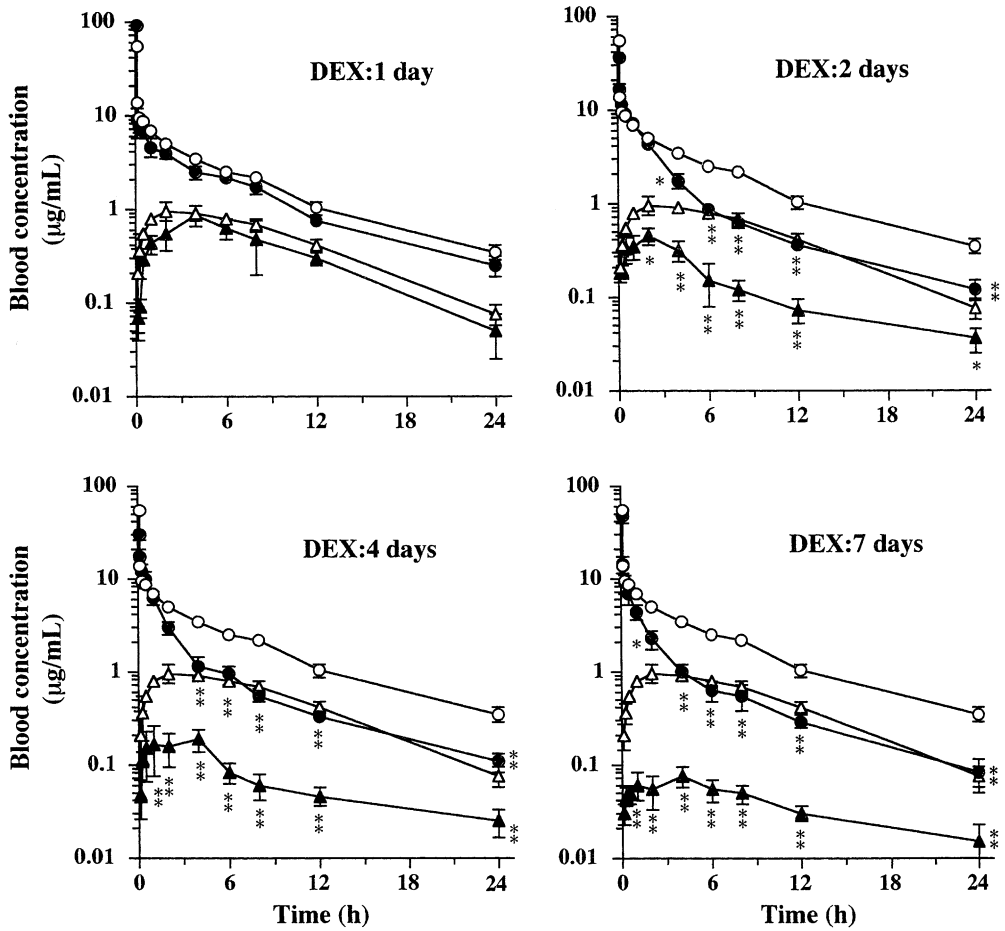

Fig. 2. The blood concentration–time courses of CyA after i.v. or p.o. administration of CyA (10 mg/kg) in untreated and DEX-treated rats (75 mg/kg per day, i.p.). Rats were treated daily for 1, 2, 4 or 7 days with DEX prior to the CyA administration. CyA was administered at 1.5 hr after the last DEX treatment. Each point and bar represent the mean ± SE of four rats; i.v. (○) and p.o. (△) administration in untreated rats; i.v. (●) and p.o. (▲) administration in DEX-treated rats. *, **Significantly different from untreated rats at $P < 0.05$ and 0.01, respectively.

depending on the number of prior DEX treatments, whereas the values of CL_{tot} were significantly increased. But the values of Vd_{ss} showed no significant difference between the untreated and DEX-treated groups. The bioavailability of CyA in the rats given the high dose of DEX for 7 days was about one-sixth of that in untreated rats.

3.3. RT-PCR analysis of mdr1a and CYP3A2 mRNAs in tissues

Fig. 3 shows the effect of low (1 mg/kg per day) and high (75 mg/kg per day) doses of DEX on the expressions of *mdr1a* and *CYP3A2* mRNAs in the brain, liver and intestine. In the rats treated with the low dose of DEX, the expression of *mdr1a* mRNA in all tissues increased, depending on the number of prior DEX treatments. But, *CYP3A2* mRNA expression in the liver in DEX-treated rats was unchanged compared with that of untreated rats. In rats given the high dose of DEX, the expression of *mdr1a* mRNA tended to increase in all tissues, and *CYP3A2* mRNA expression in the liver and intestine also increased.

3.4. Western blot analysis of P-gp and CYP3A2 in the liver and intestine

Fig. 4 shows the P-gp and CYP3A2 levels in the liver and intestine after 7-day treatment with the low or high dose of

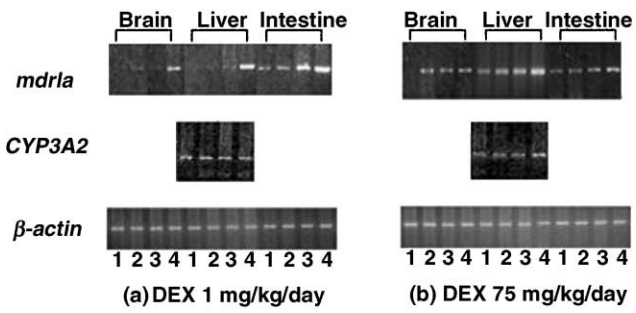

Fig. 3. Effects of DEX on the expression of *mdr1a* and *CYP3A2* mRNAs in isolated tissues of untreated and DEX-treated rats. Rats were given successive i.p. administrations of DEX (1 or 75 mg/kg per day) for 0, 2, 4, or 7 days. The sizes of RT-PCR products are 511 bp (*mdr1a*) and 252 bp (*CYP3A2*). Lane 1: non-treatment; lane 2: two treatments; lane 3: four treatments; lane 4: seven treatments.

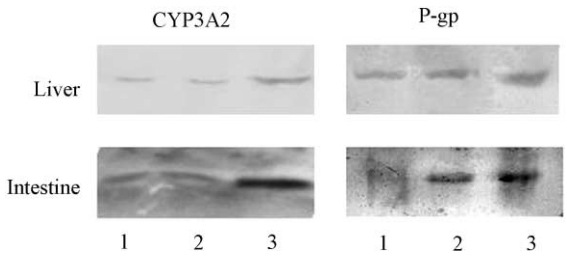

Fig. 4. Western blot analysis of P-gp and CYP3A2 proteins in liver and intestine of untreated and DEX-treated rats. Rats were given successive i.p. administrations of DEX (1 or 75 mg/kg per day for 7 days). Lane 1: non-treatment; lane 2: DEX treatment (1 mg/kg per day); lane 3: DEX treatment (75 mg/kg per day).

DEX. P-gp in the liver and intestine was increased by both low- and high-DEX treatments, compared with the untreated control. CYP3A2 was increased only by the high-DEX treatment.

4. Discussion

In this study, we demonstrated that DEX influences the disposition kinetics of CyA, and showed that this is a new type of drug interaction resulting from modulation of the gene expressions of the transporter P-gp and the metabolic enzyme CYP3A2 by DEX.

Laurent and Leslie [15] reported that expression of P-gp in the liver was decreased by DEX treatment in female rats, but was increased in male rats. Demeule *et al.* [16] clarified that the expression of P-gp was increased more than 4.5- and 2-fold in the liver and lung, respectively by DEX (1 mg/kg per day for 4 days), while it was decreased by 40% in the kidney. On the other hand, Lake *et al.* [17] demonstrated that CyA is mainly metabolized by CYP3A2 in rat liver and intestine, and DEX (50 mg/kg) induces CYP3A2 expression. However, little information has been available regarding the influence of the induction of gene expression by DEX on the disposition kinetics of coadministered drug(s) *in vivo*.

We found that in the case of the low-DEX treatment (1 mg/kg per day), the blood concentration–time course of CyA did not change after i.v. administration, but it was significantly decreased after p.o. administration, depending on the duration of DEX treatment (Fig. 1). The pharmacokinetic parameters of CyA such as the CL_{tot} value were unchanged, but the bioavailability decreased to about one-third of that in the untreated control (Table 1). Terao *et al.* [29] indicated that the blood concentration of CyA after p.o. administration is regulated by P-gp in the apical membrane of intestinal epithelial cells. It is well known that P-gp exists in various organs and tissues, such as the brush border of renal proximal tubules, the biliary membrane of hepatocytes, and the apical membrane of intestinal mucosal cells [30–32]. In this study, the expression of *mdr1a* mRNA in the brain, liver and intestine increased after a low dose of DEX (1 mg/kg), depending on the duration of DEX administration (Fig. 3); in particular, the expression of P-gp was markedly increased in the intestine (Fig. 4). The *CYP3A2* mRNA and CYP3A2 protein levels were unchanged after low-DEX treatment. We conclude that successive treatments with a low dose of DEX results in increased expression of P-gp in the intestine, and this is associated with a decrease in the bioavailability of CyA. In our study, the bioavailability of CyA (22.2%) in the control is in agreement with the reported value (12–23%) of Ueda *et al.* [33].

On the other hand, high-DEX treatment (75 mg/kg per day) decreased the blood concentration of CyA not only after p.o. administration, but also after i.v. administration (Fig. 2). The CL_{tot} of CyA in high-DEX-treated rats was significantly increased, depending on the duration of DEX treatment (Table 2). Expression of both P-gp and CYP3A2 in the liver and intestine was clearly increased at both the mRNA and protein levels after the high dose of DEX (75 mg/kg) (Figs. 3 and 4). These findings might explain why the high dose treatment with DEX caused both an increase in the CL_{tot} value and a decrease in the bioavailability of CyA.

Clinically, Hricik *et al.* [4] reported that steroid hormones decreased the blood level of CyA and increased the CL_{tot} of CyA. Our results support their observation. In summary, we found that the drug interaction between DEX and CyA appears to be a result of modulation of gene expression by DEX. Interestingly the effects of DEX on *mdr1a* and *CYP3A2* gene expressions were apparent at different dose levels of DEX. Further studies on the mechanisms of these effects are in progress.

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