Mechanism of decrease of oral bioavailability of cyclosporin a during immunotherapy upon coadministration of amphotericin B

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Running Title: Interaction of oral cyclosporin A/amphotericin B

Abbreviations: CyA, cyclosporin A; AMB; amphotericin B; BA, bioavailability; CYP, cytochrome P450; P-gp, P-glycoprotein; DEX, dexamethasone; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, γ-glutamyltranspeptidase; BUN, blood urea nitrogen; $AUC_{0\text{-}24h}$, area under the blood concentration-time curve from 0 to 24 h; MRT, mean residence time; CL_{tot}, total clearance; Vd_{ss}, distribution volume at the steady-state

ABSTRACT: The trough level of blood concentration of cyclosporin A (CyA) in a patients receiving immunotherapy was observed to decrease following coadministration of amphotericin B (AMB). We confirmed this clinical observation in experiments using Wistar rats intravenously given AMB (1.5 or 3.0 mg/kg) or saline (control) for 4 days, followed by CyA (10 mg/kg). The blood concentration of CyA after iv or po administration in both AMB groups was significantly decreased compared with the control. The oral bioavailability of CyA after 1.5 or 3.0 mg/kg AMB treatment was decreased to 67 or 46%, respectively, of that of the control group. AMB treatment increased the expression levels of mdr1a and mdr1b mRNAs in duodenum to about 3 times the control, and expression of CYP3A2 mRNA in liver was increased to about twice the control. The induction of their mRNAs by AMB treatment increased the level of P-gp and CYP3A2 protein to about twice the control. These findings suggest that the oral bioavailability of CyA is reduced as a result of both increased efflux transport via P-glycoprotein in the duodenum and an increased first-pass effect of CYP3A2-mediated hepatic metabolic activity, induced by AMB. We suggest that careful monitoring of CyA levels is necessary in the event of AMB administration to patients receiving immunotherapy with CyA.

Keywords: cyclosporin A; amphotericin B; P-glycoprotein; CYP3A; drug interaction; oral bioavailability

Introduction

There have been many reports of drug interactions involving cyclosporin A (CyA), and clinically, we have encountered a patient receiving immunotherapy with CyA in whom the blood concentration of CyA was decreased upon coadministration of amphotericin B (AMB) (Fig. 1).

It is well known that CyA is a substrate of both the efflux transporter P-glycoprotein (P-gp) and the metabolic enzyme cytochrome P450 (CYP3A) [1-3]. P-gp and/or CYP3A limit the oral bioavailability of digoxin [4], rifampin [4], vinblastine [5], dextromethorphan [6] and CyA [7, 8]. We have already shown that the blood concentration of CyA is reduced by pre-treatment with dexamethasone [9], cyclophosphamide [10] or levothyroxine [11] in rats, owing to induction of P-gp and CYP3A2 in liver and intestine. In those reports, we demonstrated that the oral bioavailability of CyA is primarily controlled by the level of CYP3A in the upper small intestine under physiological conditions, whereas after treatment with inducers, P-gp in the upper intestine also plays a significant role as an absorption barrier to CyA. Thus, we speculated that the reason for the decrease of blood concentration of CyA in the patient mentioned above might have been induction of P-gp and CYP. However, no report is currently available on drug interaction between CyA and AMB.

Therefore, in this study, we examined the mechanism of the decrease of blood concentration of CyA by using rats treated with AMB.

Materials and methods

Chemicals

Fungizone® injection (amphotericin B, AMB) and Sandimmun® injection (cyclosporin A, CyA) were purchased from Bristol-Myers Squibb Co. Ltd. (Tokyo, Japan) and Novartis Pharma Co. Ltd. (Tokyo, Japan), respectively. Oligonucleotide primers were custom-synthesized by Amersham Pharmacia Biotech (UK). Other reagents were purchased from Sigma Co. (MO, USA).

Animal Experiments

All animal experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of the University of Kanazawa.

A 150 μL aliquot of AMB (1.5 or 3.0 mg/kg/day) was intravenously administered to male 8-week-old Wistar rats (Japan SLC Co., Hamamatsu, Japan) *via* a tail vein daily for 4 days. The vehicle control rats received were distilled water alone for 4 days. A 100 μL aliquot of CyA (10 mg/kg) was injected *via* the femoral vein at 24 h after the last treatment with AMB. Alternatively, a 500 μL aliquot of CyA (10 mg/kg) was orally administered at 24 h after the last treatment with AMB. Blood samples (200 μL each) were collected at designated time intervals from the jugular vein of untreated rats and AMB-treated rats under light ether anaesthesia.

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 [12].

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.

Determination of laboratory data

Blood samples were collected from the jugular vein under light ether anesthesia at 24 h after the last treatment with AMB, and the plasma was separated by centrifugation and stored at –30°C. The measurements of laboratory data were entrusted to SRL Co. Ltd. (Tokyo, Japan).

Reverse Transcriptase-polymerase Chain Reaction (RT-PCR) Assay

Total RNA was isolated from the liver and intestine 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). Reported primers were used for rat mdr1a (511 bp) [13], for rat mdr1b (451 bp) [13], for rat CYP3A2 (252 bp) [14], and for rat ß-actin (456 bp) [15]. Each cycle consisted of 30 sec at

94°C, 60 sec at 62°C, and 75 sec at 72°C for mdr1a, mdr1b, and CYP3A2, and 30 sec at 94°C, 60 sec at 58°C, and 75 sec at 72°C for ß-actin. The PCR reaction was run for 26 cycles for liver, for 34 cycles for intestine and for 22 cycles for ß-actin.

Preparation of Microsomes and Plasma Membrane Fraction

For preparation of microsomes, the liver was homogenized in three volumes of 100 mM Tris-HCl buffer (100 mM KCl, 1 mM EDTA, pH 7.4). Microsomes were prepared as reported previously [16] and stored at -80°C until use. The intestine was quickly removed and washed with buffer containing 2 mM HEPES, 0.9% NaCl and 0.5 mM phenylmethylsulfonyl fluoride (PMSF). Mucosa was scraped off with a slide glass on ice and homogenized in a buffer containing 300 mM mannitol, 5 mM EDTA, 5 mM HEPES and 1 mM PMSF (pH 7.1). The homogenate was centrifuged at 10,000 *g* for 20 min, and the supernatant was centrifuged at 105,000 *g* for 60 min at 4°C. The pellet was added to the buffer containing 500 mM KCl, 1 mM EDTA, 2 mM DTT and 50 mM KPB (pH 7.4) and again centrifuged at 105,000 *g* for 60 min at 4°C. The pellet was added to the buffer containing 1 mM EDTA, 2 mM DTT and 50 mM KPB (pH 7.4), then stored at -80°C until use.

For the preparation of plasma membrane, the liver was homogenized in 10 mM Tris-HCl buffer (pH 7.5) containing 2 mM $CaCl_2$ at 4°C. The homogenate was centrifuged at 3,500 *g* for 10 min, and the supernatant was then centrifuged at 15,000 *g* for 30 min. The pellet was washed, resuspended in 50 mM Tris-HCl buffer (pH 7.2), and twice centrifuged at 10,000 *g* for 5 min, then stored at -80°C until use. The intestine was quickly removed and washed with ice-cold isotonic saline containing 1 mM phenylmethylsulfonyl fluoride (PMSF). Mucosa was scraped off

with a slide glass on ice and homogenized in a buffer containing 250 mM sucrose, 50 mM Tris-HCl (pH 7.4), and 1 mM PMSF. The homogenate was centrifuged at 3,000 *g* for 10 min, and the supernatant was again centrifuged at 15,000 *g* for 30 min. The pellet was resuspended in 0.5 mL of a buffer containing 50 mM mannitol, 50 mM Tris-HCl (pH 7.4), and 1 mM PMSF, and stored at -80°C until use. Protein contents were measured according to the method of Lowry et al. [17].

SDS-PAGE and Immunoblotting

SDS-PAGE and immunoblotting with peroxidase/antiperoxidase staining of the plasma membrane for P-gp and of the microsomes for CYP3A were carried out essentially as described by Laemmli [18] and Guengerich et al. [19]. The amounts of sample protein of liver and intestine were 4 and 200 μg for CYP3A or 30 and 300 μg for P-gp, respectively. The sample protein was electrophoresed on 10% sodium dodecyl sulfate-polyacrylamide gel and transferred onto PVDF membrane filters (Millipore Co., Billerica, MA). After having been blocked with 5% skim milk, the filters were incubated overnight at 4°C with primary antibody, mouse anti-P-gp C219 (Dako Co., Carpinteria, CA) and rabbit anti-rat CYP3A2 antibody (Daiichi Pure Chemicals Co. Ltd., Tokyo, Japan), and for 1 h with secondary antibody, anti-mouse IgG HRP-linked antibody (Cell Signaling, Beverly, MA) and mouse anti-rabbit IgG-HRP (Santa Cruz Bio., Santa Cruz, CA). Thereafter, the sample was extensively washed with phosphate-buffered saline. The immunopositive band was detected by means of a light-emitting nonradioactive detection system (Amersham International plc, Little Chalfont, Buckinghamshire, UK) with Kodak X-Omat R film (Eastman Kodak Co., Rochester, NY).

Data Analysis

The pharmacokinetic parameters were estimated according to model-independent moment analysis as described by Yamaoka et al. [20]. 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. The electrophoresis results were analyzed by using NIH Image software.

Case report

The patient was a 23-year-old man (56 kg) who had received bone marrow transplantation for acute myelogenous leukaemia 2 months before. He had been receiving immunotherapy with oral administration of Neoral® capsule (Novartis Pharma Co. Ltd.; CyA, 60 mg/day, 2 times a day). The trough blood concentrations of CyA were well maintained at about 100 ng/mL. However, he developed *Aspergillus* pneumonia, which was treated with intravenous infusion with Fungizone® injection (AMB, 1-30 mg/day, over 6 h). Figure 1 shows the relationship between the blood concentration of CyA and the dose of AMB or CyA. Main other combined drugs with Saxizon® injection (Kowa Pharmaceutical Co. Ltd.; hydrocortisone sodium succinate, 50-100 mg/day) and the preparations of mixed amino acid and multiple vitamins had been used after the start of the transplantation.

The blood concentration of CyA in the patient gradually decreased to about one-third over the 12 days following the start of coadministration

of AMB. Although the CyA dose was increased to 80 mg at day 17 after the start of AMB treatment, the blood concentration of CyA did not increase immediately. Subsequently the blood concentration of CyA slowly recovered, reaching about 210 ng/mL with 120 mg/day of CyA after 34 days.

The levels of γ-glutamyltranspeptidase (γ-GTP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST), parameters of hepatic function, were 52, 45 and 65 IU/L, respectively, in the initial period, and subsequently remained stable within normal ranges. The value of serum creatinine as a parameter of renal function was initially 0.8 mg/dL, but increased slightly to 1.35 mg/dL at day 9 after the start of AMB treatment. Therefore, the AMB administration, which was initially 30 mg/day daily for the first 9 days, was reduced to the same dose every second day until day 40. Following the decrease of AMB dosage frequency, the serum creatinine level rapidly decreased to 0.8 mg/dL. These results suggest that the initial AMB treatment caused slight renal impairment.

Experimental results

Pharmacokinetics of CyA in rats with AMB treatment

Figure 2 shows the blood concentration-time courses of CyA after i.v. administration of Cy A (10 mg/kg) in control rats and in rats pretreated with AMB (1.5 or 3.0 mg/kg/day, i.v.) for 4 days. The blood concentration of CyA in the low-AMB group showed a significant, time-dependent decrease compared with the control, and that in the high-AMB group showed a similar decrease.

Figure 3 show the blood concentration-time courses of CyA after p.o. administration of CyA (10 mg/kg) in control rats and in rats treated with AMB. After p.o. administration, the concentration of CyA reached a maximum within 2-4 h in all three groups. The maximum blood concentrations (C_{max}) of CyA in the control, low-AMB and high-AMB groups were 1.16, 0.37 and 0.26 μg/mL, respectively. The blood concentration of CyA was significantly and dose-dependently decreased by the AMB treatment.

The pharmacokinetic parameters of CyA in the three groups are listed in Table 1. After the i.v. administration, the values of the area under the blood concentration-time curve from 0 to 24 h ($AUC_{0\text{-}24h}$) in the two AMB groups were significantly decreased compared with the control, and the values of total clearance (CL_{tot}) were significantly increased. The elimination half-life ($t_{1/2}$) in the high-AMB group was significantly faster than that in the control group. After the p.o. administration, the $AUC_{0\text{-}24h}$ values of the low- and high-AMB groups were significantly decreased to about 42 and 31% of the control, respectively. But, the $t_{1/2}$ value was not a significant difference among all three groups. The values of oral bioavailability of CyA of the low- and high-AMB group were decreased to about 67 and 46% of the control, respectively.

Laboratory data in rats after AMB treatment

Table 2 shows the laboratory data for rats treated or not treated with AMB (1.5 or 3.0 mg/kg). There was no clear difference in the body weight among the three groups. The laboratory data reflecting hepatic function were unaffected by the AMB treatment, except for the A/G ratio. The values of BUN and serum creatinine were significantly increased by the high-AMB treatment.

RT-PCR Analysis of mdr1a, mdr1b and CYP3A2 mRNAs in intestine and liver

Figures 4 shows the effect of AMB treatment (1.5 or 3.0 mg/kg/day, i.v., for 4 days) on the expression of mdr1a, mdr1b and CYP3A2 mRNAs in the duodenum, ileum and liver at 24 h after the last treatment. The expression levels of mdr1a and mdr1b mRNAs were significantly increased in the duodenum, but little changed in the ileum and liver by the AMB treatment. On the other hand, the expression of CYP3A2 was significantly induced, in the liver only, by both AMB treatments.

Expression of P-gp and CYP3A in intestine and liver

Figure 5 shows the effect of the AMB treatment (3.0 mg/kg/day, i.v., for 4 days) on the expression levels of P-gp and CYP3A proteins which were confirmed by Western blot analysis. The protein levels of P-gp in the duodenum was elevated to about twice the untreated control level by AMB treatment, but the level in the ileum and liver were hardly changed by AMB. On the other hand, the protein levels of CYP3A in the liver was elevated to about twice the control level by AMB treatment, while the level in the duodenum and liver showed little change.

Discussion

In order to understand why the blood concentration of CyA in a patient receiving immunotherapy decreased following coadministration of AMB, we examined the effect of repeated i.v. administration of AMB (1.5 or 3.0 mg/kg) for 4 days on the disposition kinetics of CyA in rats after i.v. or p.o. administration (Figs. 2, 3). We designed that AMB is administered with 1.5 or 3.0 mg/kg to rats because total clearance of AMB of rats is about 5-times higher than that of human (0.5 mg/kg) [21]. As we previously clarified that dexamethasone [9] and cyclophosphamide [10] within 4 days after the co-administration induce P-gp and CYP3A2, in this study the AMB co-administerd intravenously to rats for 4 days. The blood concentration of CyA after i.v. administration was significantly, though not dose-dependently, decreased in both AMB groups compared with the control. After p.o. administration, there was a significant, dose-dependent decrease of the blood concentration of CyA in both AMB groups. The oral bioavailability of CyA was clearly decreased by AMB treatment (Table 1).

It is well known that renal failure is the main side effect of AMB in clinical practice. There are some reports indicating that the disposition kinetics of CyA is influenced by renal failure in rats. Shibata et al. [22] reported that CYP3A and P-gp in liver and intestine are not likely to be involved in lowering the oral bioavailability of CyA in gentamicin-induced acute renal failure, and that a change in bile function is responsible for the marked decrease. Huang et al. [23] reported that the expression level of P-gp in rats with glycerol-induced acute renal failure was increased 2.5-fold in the kidney, but not in the liver or brain. Leblond et al. [24] reported that chronic renal failure is associated with a decrease in total liver CYP450 (mainly CYP2C11, CYP3A1 and CYP3A2) activity in rats, and

this leads to a significant decrease in drug metabolism. In the rats, hepatic function was not impaired, as judged from the laboratory data, whereas increases in the values of serum creatinine and BUN indicated an appreciable impairment of renal function by the high-AMB treatment (Table 2). However, the increases of serum creatinine and BUN were not large compared with the increases of about 3- to 5-fold and 2- to 5-fold in model rats with gentamicin- or glycerol-induced renal failure, respectively [22-24]. Therefore, we considered that the AMB-induced renal impairment was not great even in the high-AMB group, and may have had only a slight influence on the excretion of CyA, which is metabolized mainly by CYP3A.

We found that in our model the expression of mdr1a and mdr1b mRNAs was characteristically induced about 3-fold in the duodenum compared with the control, but was only slightly increased in the ileum and liver by both AMB treatments (Fig. 4). Also, the expression of CYP3A2 mRNA was increased about 2-fold over the control in the liver. These results are broadly consistent with our previous findings in mice treated with dexamethasone (DEX) [25, 26]. Unlike DEX, AMB did not induce a substantial increase of CYP3A2 mRNA expression in intestine, possibly because the dose levels of AMB were relatively low. This would be consistent with the relatively minor renal impairment. The studies with DEX [9-11, 26] indicated that increased mRNA levels of mdr1a, mdr1b and CYP3A2 mRNAs are well reflected in increased levels of P-gp and CYP3A2 proteins. In this study, also the induction of their mRNAs by AMB treatment increased the level of P-gp and CYP3A2 proteins (Fig. 5). Therefore, we consider that the oral bioavailability of CyA was reduced in our present animal model as a result of increased efflux transport via P-glycoprotein in the duodenum and an increased first-pass effect of hepatic CYP3A2 induced by AMB. But, it was not observed that there is a great

difference in the $t_{1/2}$ values of CyA among three groups. Therefore, we thought that the induce of CYP3A2 in the liver influences slightly to the disposition kinetics of CyA. Previously we suggested that, under physiological conditions, the oral bioavailability of CyA is mainly controlled by CYP3A in the upper intestine, rather than liver, but when P-gp is induced by steroid, the intestinal absorption of CyA may be inhibited [26]. Therefore, we consider that the decrease of BA by AMB treatment is cause by the intestinal absorption decrease via induced P-gp.

In the case of our patient, the stable trough values of blood CyA concentration during repeated p.o. administration of CyA were decreased after repeated intravenous infusion administration of AMB was started, and the decrease continued for 3 weeks. During this period, the laboratory data indicated relatively minor impairment of hepatic and renal function by AMB. Further, the patient has been received in combination with hydrocortisone after the start of the transplantation, however, it was not observed that the hydrocortisone treatment influences slightly on the plasma level of CyA because the low dose of hydrocortisone (50-100 mg/day). Therefore, we think that the clinical observations can also be explained mainly in terms of the induction of P-gp in intestine by the AMB treatment.

We previously clarified that the induction of P-gp and CYP3A2 continues for 2 weeks after the final DEX treatment [27]. Here, the patient received repeated administration of AMB for 40 days, so it was difficult to predict when the levels of P-gp and CYP3A2 expression would recover to the control level; therefore, we chose to increase the dose of CyA gradually. The level of blood CyA concentration began to increase 7 days after increasing the dose of CyA from 120 mg/day to 160 mg/day, and after the dose of CyA was further increased to 240 mg/day, the blood CyA concentration reached 210 ng/mL. Therefore, the blood CyA

concentration might have recovered to about 100 ng/mL within 40 days after the start of AMB treatment, if the dose had been kept at 120 mg/day throughout. In other words, the P-gp and CYP3A activities might return to the control levels within several weeks after the start of the AMB treatment in spite of repeating the AMB treatment, leading to normalization of CyA bioavailability. Further, there are species differences in hepatic metabolism and susceptibility to metabolic changes, so that further evaluations need to be done *in vitro* study using human hepatic microsome and/or human hepatocytes.

In conclusion, our results indicate that the oral bioavailability of CyA is decreased by coadministration of AMB because of an increase of expression of P-gp and CYP3A induced by AMB treatment. The results in this animal model were consistent with our clinical observations. Therefore, blood concentration of drugs that are substrates of P-gp and CYP3A, such as CyA, should be carefully monitored in patients when AMB is coadministered in combination with such drugs.

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Legends

Figure 1. Change of blood concentration-time courses of CyA in a patient (23-year-old man, 56 kg) receiving immunotherapy with CyA, upon coadministration of AMB. He received daily oral administration of CyA, and then was intravenously infused over 6 h with AMB (daily for the first 10 days, every other day for the next 15 days).
Keys: ● , CyA blood concentration; ○ , CyA dose; ◊ , AMB dose

Figure 2. Blood concentration-time courses of CyA after an i.v. administration of CyA (10 mg/kg) in untreated rats (○) and rats treated with AMB at 1.5 mg/kg (△) or 3.0 mg/kg (▲) for 4 days.
Rats were given CyA at 24 h after the last AMB treatment. Each point and bar represents the mean ± SD of four rats.
**Significant difference between the control group and both AMB groups at P <0.01.

Figure 3. Blood concentration-time courses of CyA after an p.o. administration of CyA (10 mg/kg) in untreated rats (○) and rats treated with AMB at 1.5 mg/kg (△) or 3.0 mg/kg (▲) for 4 days.
Rats were given CyA at 24 h after the last AMB treatment. Each point and bar represents the mean ± SD of four rats.
**Significant difference between the control group and both AMB groups at P <0.01.

Figure 4. Effect of AMB on the expression of mdr1a, mdr1b and CYP3A2 mRNAs in duodenum, ileum and liver.

The data represent the relative expression of the mRNAs obtained as mRNA/β-actin mRNA ratios in the low-AMB (1.5 mg/day, □) and high-AMB (3.0 mg/day, ■) groups by that in the control group (⬚) at 24 h after the last AMB treatment for 4 days.
*, **Significant difference from the control group at $P < 0.05$ and $P < 0.01$, respectively.

Figure 5. Western blot analysis of P-gp (a) and CYP3A (b) proteins in the duodenum, ileum and liver of rats with (■) or without (□)AMB treatment, at 24 h after the last AMB treatment for 4 days. *, **Significant difference from the control group at $P<0.05$ and $P < 0.01$, respectively.

Table 1. Pharmacokinetic parameters of CyA with or without AMB (1.5 or 3.0 mg/kg) in rats

Parameters	Non treatment	AMB treatment	
		1.5 mg/kg	3.0 mg/kg
i.v. administration			
$AUC_{0\text{-}24h}$ (μg h/mL) [a)]	45.0 ± 2.7	28.4 ± 1.2 **	30.5 ± 1.8 **
MRT (h) [b)]	5.96 ± 0.82	5.01 ± 0.51	4.67 ± 0.53 *
CL_{tot} (mL/min) [c)]	3.70 ± 0.22	5.87 ± 0.19 **	5.47 ± 0.31 **
Vd_{ss} (L) [d)]	1.32 ± 0.31	1.76 ± 0.35	1.53 ± 0.23
$t_{1/2}$ (h) [e)]	5.87 ± 0.78	6.03 ± 0.68	4.18 ± 0.83*
p.o. administration			
$AUC_{0\text{-}24h}$ (μg h/mL)	8.53 ± 0.78	3.61 ± 0.41**	2.66 ± 0.23 **
$t_{1/2}$ (h)	6.32 ± 0.95	5.86 ± 0.82	5.97 ± 0.93
Bioavailability (%)	19.0	12.7	8.7

Rats were intravenously or orally administered with CyA (10 mg/kg) at 24 h after the last AMB treatment in untreated rats and rats treated with AMB at 1.5 or 3.0 mg/kg for 4 days. Pharmacokinetic parameters were estimated according to model-independent moment analysis. Each value represents the mean ± SD of four 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)] elimination half-life

*, **Significant difference between the control group and both AMB groups at $P < 0.05$ and 0.01, respectively.

Table 2. Physical and biochemical data in rats treated with AMB

	Non treatment	AMB treatment	
		1.5 mg/kg	3.0 mg/kg
Body weight (g)	220±7	227 ±12	216 ±11
AST (IU/L)	84 ±5	82 ±8	88 ±14
ALT (IU/L)	40 ±7	35 ±2	38 ±13
Albumin (g/dL)	4.07 ±0.11	3.93 ±0.15	3.67 ±0.31
A/G ratio	2.23±0.15	1.97 ±0.25	1.70 ±0.31*
Total bilirubin (mg/dL)	0.14 ±0.04	0.15 ±0.08	0.14 ±0.06
BUN (mg/dL)	15.1 ±1.8	26.1 ±5.9*	46.8 ±9.3**
serum creatinine (mg/dL)	0.26 ±0.03	0.28 ±0.03	0.37 ±0.05**

Data were measured at 24 h after the last administration of AMB (1.5 or 3.0 mg/kg i.v.) for 4 days in rats. Each value represents the mean ± SD of four rats.

*, **Significant difference between the control group and both AMB groups at $P < 0.05$ and 0.01, respectively.

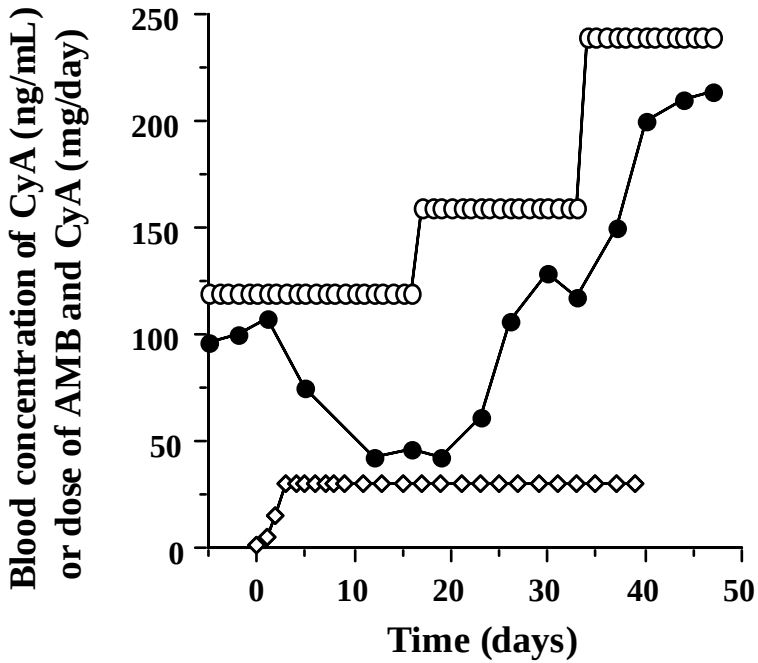
Blood concentration of CyA (ng/mL)
or dose of AMB and CyA (mg/day)
250
200
150
100
50
0
0
10
20
30
40
50
Time (days)

Fig.1

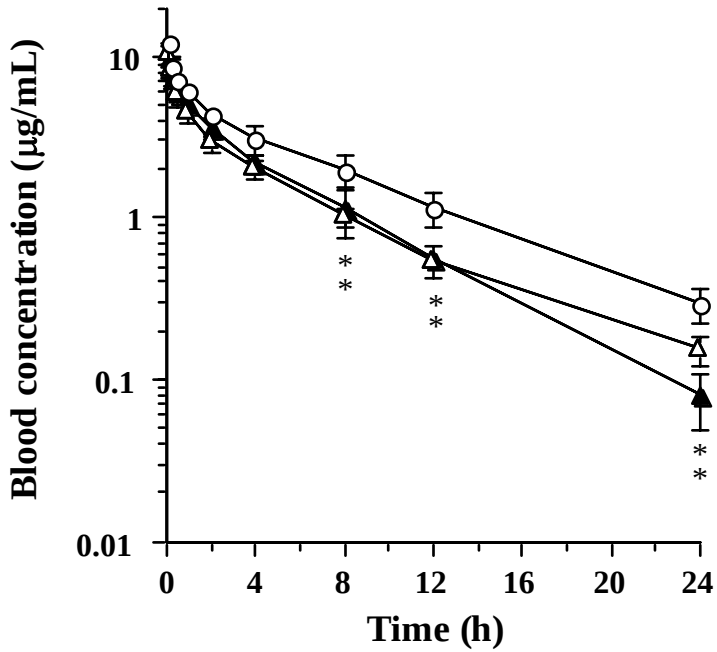

Fig. 2

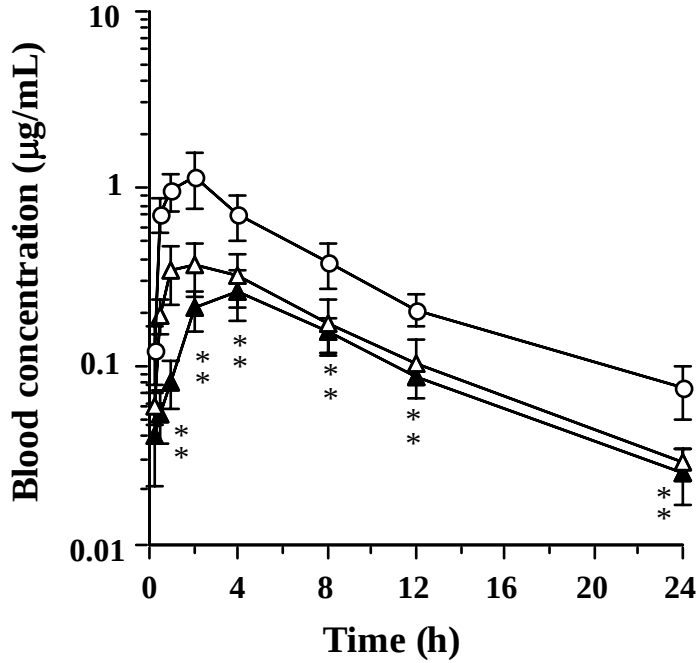

Fig. 3

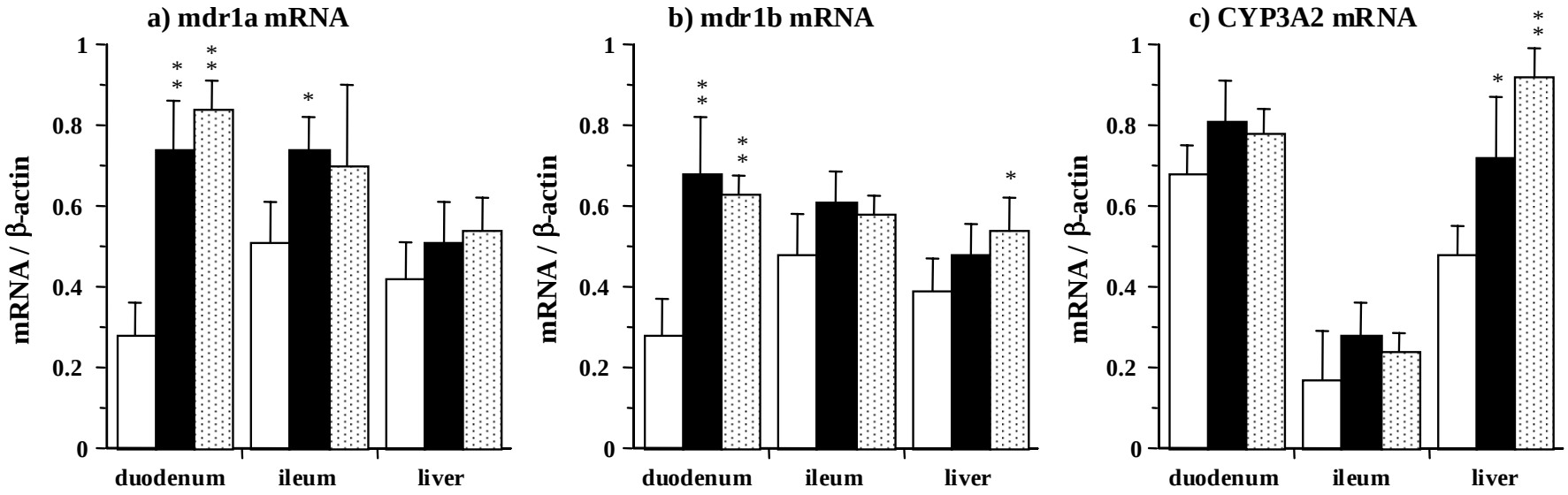
a) mdr1a mRNA
b) mdr1b mRNA
c) CYP3A2 mRNA
mRNA / β-actin
0
0.2
0.4
0.6
0.8
1
duodenum
ileum
liver
*
**

Fig. 4