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Decrease in oral bioavailability of ciclosporin by intravenous pulse of methylprednisolone succinate in rats

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Abstract

We examined the effects of high-dose methylprednisolone on the bioavailability of orally administered ciclosporin in rats. To emulate the clinical protocol of methylprednisolone pulse therapy, methylprednisolone sodium succinate (MPS), a prodrug of methylprednisolone, was intravenously administered as repeated doses (66.3 mg kg^{-1}) for 3 days. The area under the blood ciclosporin concentration versus time curve after oral administration was significantly reduced by 60% by pulse treatment with MPS. Based on our previous finding that the total body clearance of ciclosporin was reduced by about 20% by the same methylprednisolone pulse protocol, the extent of reduction in the oral bioavailability of ciclosporin was estimated to be approximately 50%, indicating a drug interaction between high-dose methylprednisolone and orally administered ciclosporin, which affected the absorption process. In rats treated with MPS, an in-situ efflux experiment using rhodamine-123 demonstrated that the reverse transport function of P-glycoprotein (P-gp) in the small intestine was significantly enhanced, although there was no significant increase in the intestinal microsomal activity of triazolam α- and 4-hydroxylation, metabolic probes for CYP3A. In addition, a significant decrease was observed in the amount of secreted bile acids serving as an enhancer of gastrointestinal absorption of ciclosporin in MPS treatment. To directly estimate the absorptive capacity, an in-situ absorption test was conducted using a closed-loop of small intestine in control and MPS-treated rats. Intestinal absorption of ciclosporin was significantly decreased, not only in the absence of bile flow but also by treatment with MPS, which well reflected the change in the in-vivo pharmacokinetic behaviour of ciclosporin after methylprednisolone pulsing. These results demonstrate that bioavailability of ciclosporin is markedly reduced by MPS pulse treatment, and the mechanism of this interaction was confirmed to involve enhancement of small-intestinal P-gp function and decrease in bile secretion.

Introduction

Methylprednisolone, a synthetic steroid hormone, is widely used in the treatment of allergic, immunologic and inflammatory disorders. When needed for urgent management of flares in the disease status, a short-term high dose of methylprednisolone sodium succinate (MPS), a hydrophilic esterified prodrug of methylprednisolone, is intravenously administered as pulse therapy. Ciclosporin, a strong immunosuppressant with a narrow therapeutic window, is generally applied to control graft rejection in solid organ transplant recipients and graft-versus-host disease after allogenic bone marrow transplantation, and has been increasingly used to suppress a variety of hypersensitive autoimmune responses (Kahan 1989; Faulds et al 1993; Perico & Remuzzi 1997). In clinical practice, methylprednisolone is frequently administered in combination with ciclosporin to promote their synergic effects, irrespective of dosage and administration forms of ciclosporin. However, pharmacokinetic behaviour of ciclosporin is easily affected by co-administration of other drugs, thus impeding successful therapeutic control without close monitoring to ensure an adequate blood ciclosporin concentration (Campana et al 1996; Dresser et al 2000). We have recently demonstrated in experiments using rats that

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pulse treatment with MPS significantly increases the total body clearance of intravenously administered ciclosporin by induction of hepatic CYP3A (Konishi et al 2004). However, ciclosporin is also administered via the oral route (e.g. for maintenance therapy in the remission period and for the replacement of intravenous administration after subsidence of acute episodes). However, ciclosporin is susceptible to drug interactions during the intestinal absorption process when administered orally, because a number of physiological factors contribute to its first-pass elimination and absorptive capacity (Akhlaghi & Trull 2002). Yokogawa et al (2002) reported that the bioavailability of ciclosporin was markedly decreased by intraperitoneal administration of dexamethasone to rats, and that this phenomenon was a consequence of modulation in the expression of CYP3A2 and P-glycoprotein (P-gp) in the gastrointestinal tract. However, there is no information regarding the change in the disposition of orally administered ciclosporin in MPS pulse treatment, even though these agents are frequently administered in combination.

This study investigated whether intravenous high-dose MPS pulse treatment can alter the bioavailability of ciclosporin in rats, and examined likely mechanisms that modulate the pharmacokinetic disposition of orally administered ciclosporin.

Materials and Methods

Reagents and experimental animals

Ciclosporin was generously donated by Novartis Pharma KK (Tokyo, Japan). MPS, triazolam and rhodamine-123 (Rho123) were purchased from Sigma Chemical Co. (St Louis, MO). Other chemicals and solvents were of analytical grade.

Male Sprague-Dawley rats (Japan SLC Inc., Hamamatsu, Japan) were acclimatized for at least 2 days before assignment to experimental groups at 7–9 weeks of age (200–300 g), and were housed in wire-bottom cages to avoid contact with faecal excrement, in a clean room maintained at 23 ± 2°C with a relative humidity 55 ± 10% and 12-h light–dark cycle. Rats were allowed free access to regular animal diet and drinking water, with the exception of food deprivation overnight before sacrifice. The rats used in this study were handled in accordance with the Guidelines for Animal Experimentation of Shiga University of Medical Science, and the experimental protocol was approved by the Animal Care and Use Committee of Research Center for Animal Life Science.

Treatment with MPS

MPS was dissolved in physiological saline at a final concentration of 66.3 mg mL^{-1} and intravenously administered to rats as a substitute for methylprednisolone. In the examination of the effect of pre-treatment, MPS was injected into the tail vein at a dose of 66.3 mg kg^{-1} (equivalent to 50 mg kg^{-1} methylprednisolone) for 3 consecutive days (designated as MPS-treated rats). In the experiment involving co-administration of MPS, a solution of MPS was injected into the jugular vein of untreated rats as a single dose of 66.3 mg kg^{-1}. Control rats received an equivalent volume of saline alone instead of MPS solution at the same time.

Oral administration of ciclosporin and measurement of systemic blood concentration of ciclosporin

Rats were fasted overnight with free access to drinking fluid. Ciclosporin was suspended in 0.3% sodium carboxymethylcellulose solution to make a concentration of 2.5 mg mL^{-1}. MPS-treated and control rats were orally administered ciclosporin suspension at a dose of 5 mg kg^{-1} using a feeding tube, 24 h after the last injection. In experiments of co-administration of MPS, ciclosporin suspension (5 mg kg^{-1}) was orally administered to untreated rats, immediately after the single injections of MPS solution (66.3 mg kg^{-1}) or an equivalent volume of saline. Blood samples were withdrawn using heparinised sampling tubes without constraint from the contralateral jugular vein under light ether anaesthesia at 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 h after oral ciclosporin administration. Whole blood samples were stored at −20°C until assayed.

Preparation of rat small intestinal microsomes

The rats were killed by bolus injection of KCl solution under pentobarbital anaesthesia, and small intestinal microsomes were prepared according to the methods described by Bonkovsky et al (1985) and Mohri & Uesawa (2001) with modifications. A 30-cm portion of upper jejunum was excised, and the lumen was washed with Solution A (sodium-potassium phosphate buffer containing (in mM): 1.5 KCl, 96 NaCl, 27 sodium citrate and 0.23 phenylmethylsulfonyl fluoride (PMSF), pH 7.3). After incubating for 15 min at 37°C in Solution A, the content of the intestinal lumen was replaced and filled with ice-cold Solution B (sodium-potassium phosphate buffer containing (in mM): 2.7 KCl, 137 NaCl, 1.5 EDTA, 0.5 dithiothreitol and 0.23 PMSF, pH 7.2). The sample segment was placed on a glass plate on ice and tapped gently with fingers to harvest epithelial cells into Solution B. The luminal fluid was collected, with the procedure being repeated three times. All samples of luminal fluid were combined and centrifuged at 800 *g* for 10 min. Precipitated cells were re-suspended in Solution C (5 mM histidine, 0.25 M sucrose, 0.5 mM EDTA and 0.23 mM PMSF, pH 7.0) after rinsing by centrifugation at 800 *g*, then homogenized in the same solution using a Potter-type homogeniser. The homogenate was centrifuged at 10 000 *g* for 15 min. The supernatant fractions were centrifuged at 105 000 *g* for 60 min to obtain microsomes. The pellet was suspended in 100 mM sodium-potassium phosphate buffer (pH 7.4) to make a protein concentration of 5 mg mL^{-1}. These procedures were carried out below 4°C. The protein concentration was determined by the method of Lowry et al (1951) using bovine serum albumin as a standard.

Enzyme assay of triazolam hydroxylation in rat small intestinal microsomes

The enzyme reaction mixture (1 mL) consisted of an NADPH-generating system (0.5 mM $NADP^+$, 5 mM glucose-6-phosphate, 5 mM $MgCl_2$, 2 IU mL^{-1} glucose-6-phosphate dehydrogenase), 70 mM sodium-potassium phosphate buffer (pH7.4), 0.5 mg mL^{-1} microsomes and 200 μM triazolam. Triazolam was dissolved in methanol in advance, and the methanol concentration in the reaction mixture was set at 2.5%. The reaction was started by adding the microsomal suspension, then incubated at 37°C for 20 min. The reaction was stopped by addition of 2 mL of acetonitrile. Production of α- and 4-hydroxytriazolam were measured by the HPLC method (Kanamitsu et al 2000).

In-situ exsorption of Rho123 across rat jejunum

Rats fasted overnight were anaesthetized by sodium pentobarbital (50 mg kg^{-1}, i.p.) and fixed in a supine position. The abdominal cavity was opened by a midline incision. The jejunum (15-cm length from the Traiz's ligament) was used as an in-situ loop, which was emptied of its contents with physiological saline pre-warmed at 37°C using a syringe. An inlet silicone cannula (o.d. 5 mm, i.d. 3 mm) was inserted into the proximal end of the lumen, and was connected to the perfusion system using a peristaltic pump (SJ-1220; Atto, Tokyo). The distal end of the jejunum was also catheterized with an outlet silicone cannula to serially collect perfusate. The abdominal cavity was closed, and then the loop was perfused with Dulbecco's phosphate-buffered saline containing 25 mM glucose (pH 7.4, 37°C) in an uncirculated mode at a rate of 1 mL min^{-1}. Rho123 was dissolved in 5% mannitol at a concentration of 38 μg mL^{-1}, and injected into the jugular vein (0.19 mg kg^{-1}) after the loop had been equilibrated with the perfusate. Effluents of jejunal lumen were collected in light-shielded test tubes at designated time points up to 2 h after Rho123 administration. Blood samples were withdrawn from the contralateral jugular vein at the intermediate time point of effluent collection, and the plasma fraction was separated by centrifugation. The samples were subjected to analysis within a sampling day.

The concentration of Rho123 in the plasma and effluent was determined by fluorescent measurement at wavelengths of 485 nm for excitation and 546 nm for emission, using an RF-510 fluorescence spectrophotometer (Shimadzu, Kyoto).

In-situ intestinal absorption of ciclosporin in the presence or absence of bile flow

Rats were fasted overnight and fixed in a supine position under anaesthesia with sodium pentobarbital (50 mg kg^{-1}, i.p.). After laparotomy by a midline incision, a 15-cm length segment from the duodenum to upper jejunum was isolated. The lumen of the segment was washed with pre-warmed physiological saline using a syringe, and the remaining saline solution was expelled with air. Both ends were carefully ligated with silk sutures so as to preserve the vascular supply, and the top ligature was placed just above the common bile duct allowing bile to flow freely into the looped-sac for the experiments in the presence of bile flow. In the experiments without bile flow, the bile duct was severed before other procedures. After placement of a polyethylene cannula (o.d. 0.8 mm, i.d. 0.5 mm) into the portal vein for blood sampling to measure ciclosporin concentration in whole blood, ciclosporin suspension (0.125%) was transmucosally instilled into the looped sac at a dose of 5 mg kg^{-1} using a 27-gauge hypodermic needle, followed by closure of the abdominal cavity. Blood was collected every 15 min up to 2 h after the intra-loop instillation of ciclosporin, and the portal cannula was filled with heparin solution (100 U mL^{-1} in physiological saline) during sampling intervals. Whole blood samples were stored at −20°C until assayed.

Measurement of bile flow and amount of bile acid

Rats fasted overnight were anaesthetized by sodium pentobarbital (50 mg kg^{-1}, i.p.) and fixed in a supine position. A polyethylene cannula (o.d. 0.8 mm, i.d. 0.5 mm) was inserted into the bile duct, and bile was serially collected up to 7 h. The concentration of bile acids was measured by colorimetric assay using a commercial reagent kit coupling with 3α-hydroxysteroid dehydrogenase and diaphorase (Kyokuto Pharmaceutical Industrial Co. Ltd, Tokyo, Japan).

Calculation of pharmacokinetic parameters of orally administered ciclosporin and intravenously administered Rho123

The ciclosporin concentration in whole blood was determined by a fluorescence polarization immunoassay technique using the TDxFLx system (Abbot Laboratories, Tokyo, Japan) according to the directions provided by the manufacturer. Cross-reactivity with ciclosporin metabolites was 19.4% for AM9, 6.7% for AM1 and less than 5% for others.

Standard pharmacokinetic parameters of orally administered ciclosporin were obtained by model-independent analysis. The elimination rate constant at the terminal phase (ke) was determined by linear regression of the log-linear portion of plots of blood ciclosporin concentration against time. The area under the blood ciclosporin concentration versus time curve after oral administration (AUC_{po}) was calculated by a linear trapezoidal approximation from time zero to the last sampling point, with the addition of a correction term by extrapolation to infinity using the ratio of the last measured concentration to ke. Apparent body clearance (CL_{tot}/F) was calculated by dividing the oral ciclosporin dose (5 mg kg^{-1}) by AUC_{po}, where CL_{tot} and F represent the total clearance and bioavailability of ciclosporin, respectively, and CL_{tot}/F represents a hybrid parameter.

The area under the plasma Rho123 concentration versus time curve after intravenous administration (AUC_{Rho}) was calculated by a linear trapezoidal integration from

time zero to the last sampling point, and total clearance (CL_t) was determined by dividing the intravenous Rho123 dose by the AUC_{Rho}. Exsorption clearance (CL_{exp}, mL min^{-1} per 15-cm jejunum) was calculated by dividing the amount of Rho123 excluded from the systemic circulation to the jejunal effluent by the AUC_{Rho}.

Statistical analysis

The results are expressed as the means ± s.d. One-factorial data were compared between the control and MPS-treated rats using unpaired Student's *t*-test. Two-factorial data from two groups were compared by two-way analysis of variance, followed by Tukey's post-hoc test. A *P*-value less than 0.05 was considered significant.

Results

In-vivo pharmacokinetic profile of ciclosporin after MPS treatment

Time courses of the blood ciclosporin concentration in control and MPS-treated rats after oral administration of ciclosporin are shown in Figure 1. Blood ciclosporin levels of MPS-treated rats were markedly lower than those of the control rats at each sampling point. Table 1 shows the pharmacokinetic parameters of ciclosporin in the two groups. In rats treated with MPS, there was about a 60% decrease in the AUC_{po} and concurrent increases in the CL_{tot}/F compared with those in control rats. However, there were no significant differences in the pharmacokinetic parameters of orally administered ciclosporin between control and treated rat groups, when MPS was concomitantly given as a single dose (data not shown).

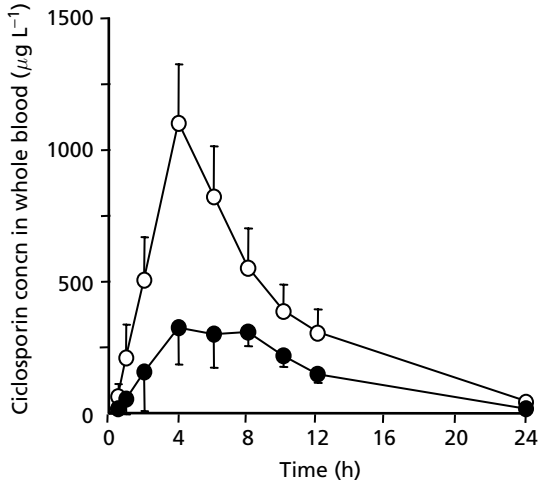

Figure 1 Whole blood concentration–time course of ciclosporin after oral administration to control (○) and MPS-treated rats (●). Rats intravenously received MPS (66.3 mg kg^{-1}) for 3 consecutive days before oral administration of ciclosporin (5 mg kg^{-1}). Control rats were administered vehicle alone instead of MPS. Each point with a bar represents the mean ± s.d. of 5 experiments.

Table 1 Pharmacokinetic parameters of ciclosporin orally administered to control and MPS-treated rats

Parameters	Control rats	MPS-treated rats
ke (h^{-1})	0.16 ± 0.02	0.18 ± 0.03
AUC (h mg L^{-1})	8.73 ± 1.82	3.56 ± 0.94**
CL_{tot}/F (L h^{-1} kg^{-1})	0.57 ± 0.11	1.41 ± 0.38**
F	0.316	0.154

Pharmacokinetic parameters were calculated according to model-independent analysis. Each value represents the mean ± s.d. of 5 determinations. F was estimated by dividing the CL_{tot} value reported in our previous paper (Konishi et al 2004) by the calculated CL_{tot}/F. **$P < 0.01$ vs control.

Effects of MPS treatment on induction of small intestinal CYP3A and P-gp

The effect of MPS pulsing on induction of CYP3A expressed in the rat small intestine was evaluated by measuring the activity of triazolam α-hydroxylase and 4-hydroxylase, metabolic probes for CYP3A (Kanamitsu et al 2000), in microsomes of intestinal epithelial cells obtained from control and MPS-treated rats. In control rats, triazolam α-hydroxylation and 4-hydroxylation activity was 0.315 ± 0.130 nmol min^{-1} mg^{-1} and 0.571 ± 0.225 nmol min^{-1} mg^{-1}, respectively (n = 4). In MPS-treated rats, the activity of triazolam α- and 4-hydroxylation was 0.395 ± 0.181 nmol min^{-1} mg^{-1} and 0.661 ± 0.307 nmol min^{-1} mg^{-1}, respectively (n = 4). There were no significant differences in either enzyme's activity between the two rat groups.

Exsorption rate of Rho123, an excellent substrate of P-gp, from blood into the intestinal lumen was measured by the in-situ single perfusion method using jejunal segments to assess P-gp function in the small intestine in control and MPS-treated rats. The CL_{exp} of intravenous administered Rho123 was significantly increased by treatment with MPS (Figure 2A). In addition, a slight

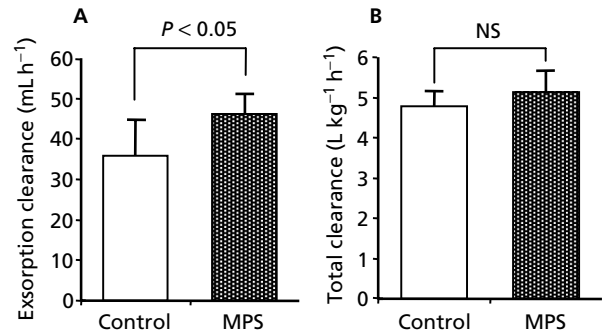

Figure 2 Changes in exsorption clearance (CL_{exp}) (A) and total clearance (CL_t) (B) of Rho123 by MPS pulse treatment. Rats were intravenously administered MPS (66.3 mg kg^{-1}) for 3 consecutive days before intravenous administration of Rho123 (0.19 mg kg^{-1}). Control rats received vehicle alone instead of MPS. Each column with a bar represents the mean ± s.d. of 5 or 6 experiments. NS, not significant.

tendency toward increase in the CL_t of Rho123 was observed in MPS-treated rats, although the difference was not significant (Figure 2B).

Change in bile secretion by MPS treatment

Figure 3 shows the profile of the bile flow rate and the amount of bile acids secreted into the bile in control and MPS-treated rats. Compared with controls, MPS treatment produced no significant change in the total bile flow over the 7-h collection period (8.01 ± 0.76 mL in MPS-treated rats vs 7.82 ± 0.87 mL in control rats). However, the total amount of bile acids was significantly ($P < 0.01$) reduced by 32% in MPS-treated rats (43.3 ± 6.7 μmol in MPS-treated rats vs 64.4 ± 7.3 μmol in control rats).

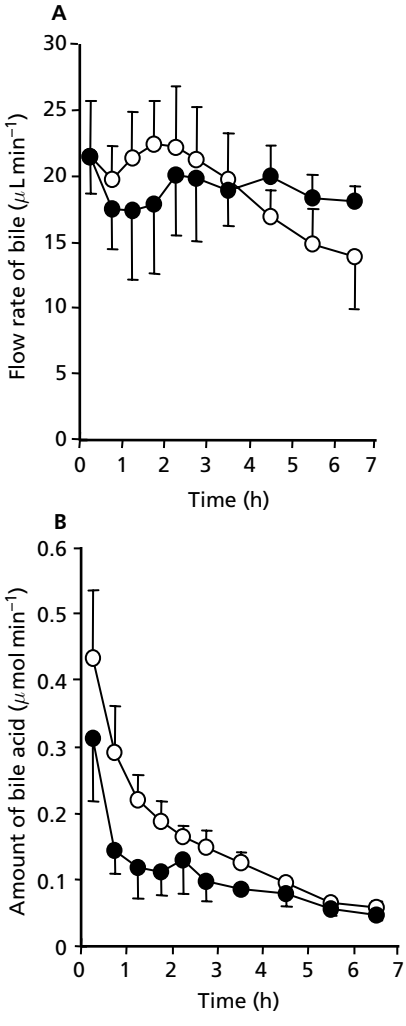

Figure 3 Effect of MPS pulse treatment on the biliary flow rate (A) and the amount of secreted bile acids (B) in rats. Each point with a bar represents the mean ± s.d. of 5 or 6 experiments. ○, control rats; ●, MPS-treated rats.

Effect of MPS treatment on in-situ intestinal absorption of ciclosporin

Differences in intestinal absorption of ciclosporin were directly examined using the closed-loop method in control and MPS-treated rats, in the presence or absence of bile flow. Figure 4 shows the time course of blood ciclosporin concentration in the portal vein after instillation of ciclosporin suspension into the respective loops. The order of mean blood ciclosporin level at each sampling point was as follows: control rats with bile flow > MPS-treated rats with bile flow > control rats without bile flow > MPS-treated rats without bile flow. The AUC values of ciclosporin from time zero to 120 min (last sampling point) in the individual groups are shown in Table 2. There were significant differences ($P < 0.01$) between control and MPS-treated rats and between the groups with and without bile flow.

Discussion

Ciclosporin undergoes extensive biotransformation by hepatic CYP3A after entry to the systemic circulation. In contrast, orally administered ciclosporin shows a low bioavailability because of poor absorption and small intestinal first-pass elimination via CYP3A and P-gp. Therefore, physiological and biological changes in absorption sites in the small intestine can affect absorption of ciclosporin, and this issue is recognized as a major cause of pharmacokinetic interactions of ciclosporin with therapeutic agents, food

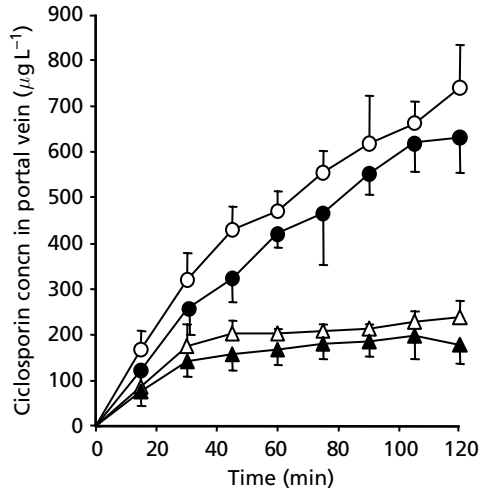

Figure 4 Differences in intestinal absorption profile of ciclosporin in control and MPS-treated rats in the presence or absence of bile flow. MPS treatment was performed as described in the text. Ciclosporin suspension (0.125%) was injected into the looped sac at a dose of 5 mg kg^{-1}. Ciclosporin concentration in whole blood in portal vein was successively measured. The AUC was calculated to estimate intestinal absorptive capacity of ciclosporin. Each point with a bar represents the mean ± s.d. of 5–7 experiments. ○, control with bile flow; ●, MPS-treatment with bile flow; △, control without bile flow; ▲, MPS-treatment without bile flow.

Table 2 Effect of MPS pulse on intestinal absorption of ciclosporin in the presence or absence of biliary supply

	AUC ($h\,mg\,L^{-1}$)	
	With bile	Without bile
Control rats	0.897 ± 0.071	0.360 ± 0.015
MPS-treated rats	0.768 ± 0.045	0.296 ± 0.045

Whole blood concentration of ciclosporin in the portal vein was serially measured after instillation of ciclosporin into the looped intestine and the AUC was calculated to estimate intestinal absorption of ciclosporin. Each value represents the mean ± s.d of 5–7 determinations. $P < 0.01$, control vs MPS-treated rats; $P < 0.01$, group with bile flow vs group without bile flow (two-way analysis of variance followed by Tukey's test).

and beverages (Campana et al 1996). In this study, it was demonstrated that the CL_{tot}/F of ciclosporin was increased by about 2.5 fold following repeated pulse treatment with MPS, although a single co-administration of MPS did not induce any significant change in ciclosporin disposition. Using the same MPS treatment protocol as in this study, we recently reported that pulsed methylprednisolone increases the CL_{tot} of intravenously administered ciclosporin by about 20% due to the enhancement of hepatic metabolism in rats (Konishi et al 2004). By comparing the pharmacokinetic parameters of ciclosporin after intravenous and oral administration in rats treated with MPS, the extent of the reduction in the bioavailability (F) of orally administered ciclosporin was estimated to be approximately 50%. This finding indicates that pulsed methylprednisolone has a much greater impact on the disposition of oral ciclosporin than intravenous ciclosporin.

As ciclosporin is not a drug with a high hepatic extraction ratio (Akhlaghi & Trull 2002), the first-pass hepatic removal probably makes only a minor contribution to pre-systemic elimination of orally administered ciclosporin. Thus, a marked decrease in the bioavailability of oral ciclosporin is ascribable mainly to intestinal events before its influx into the portal vein. Yokogawa et al (2002) demonstrated that repeated intraperitoneal injections of dexamethasone to rats led to marked increases in CYP3A2 and P-gp in the small intestine at mRNA and protein levels, and consequently decreased the blood levels of orally administered ciclosporin. Based on these findings, we first examined the inducibility of intestinal CYP3A by pulsed methylprednisolone by measuring the enzyme activity of triazolam α-hydroxylation and 4-hydroxylation in intestinal microsomes as metabolic probes for CYP3A. However, there was no significant increase in either enzyme's activity in MPS-treated rats, suggesting that MPS pulse treatment has little impact on the activity of intestinal microsomal CYP3A responsible for the first-pass elimination of ciclosporin. These observations concur with the previous findings that the inducing potency of methylprednisolone for CYP3A is not as strong as that of dexamethasone (Schuetz & Guzelian 1984; Pichard et al 1992). On the other hand, P-gp, an ATP-dependent efflux pump, is expressed in abundance in the apical brush-border membrane of the epithelium of small intestine. We measured the amount of Rho123 exsorbed from the bloodstream into the intestinal lumen using a single perfusion method after its intravenous administration, as P-gp extensively mediates the intestinal secretory transport of Rho123 (Hsing et al 1992; Lee et al 1994; Yumoto et al 1999). There was about a 20% increase in CL_{exp} of Rho123 in MPS-treated rats relative to the controls. This indicates that the efflux function of intestinal P-gp is clearly enhanced by methylprednisolone pulsing. CYP3A and P-gp work cooperatively as enteric defences against excessive uptake of xenobiotics, whereas their expression in tissues is likely to be regulated at least partially by independent signal transduction systems, as shown by some types of corticosteroids (Salphati & Benet 1998; Sérée et al 1998). It has been demonstrated that dexamethasone tended to potently up-regulate P-gp compared with CYP3A in the rat intestine, and that its induction potency is more prominent in enteric mucosal P-gp than hepatic P-gp and microvessel endothelial P-gp expressed in the blood–brain barrier (Yokogawa et al 2002; Perloff et al 2004). These findings are indicative of relatively specific inducibility of intestinal P-gp by steroids. Although methylprednisolone has a high affinity to P-gp due to the presence of a methyl moiety at the 6α-position and a hydrogen-bond donor at the 11-position in its chemical structure (Oka et al 2002; Yates et al 2003), there has not been any available information regarding the effect of methylprednisolone on induction of intestinal P-gp. The daily dose of methylprednisolone frequently exceeds 500 mg in pulse therapy, and is much higher than that of dexamethasone used in clinical trials and in previous fundamental studies with animal models. Thus, a significant increase in P-gp function by high-dose methylprednisolone appears to be a reasonable consequence, considering that dose dependency exists in the induction behaviour of corticosteroids (Pichard et al 1992). However, the magnitude of change in the bioavailability of oral ciclosporin was much greater than that of the efflux capacity of P-gp in MPS-treated rats. To explain this discordance, we assumed that other important factors should be involved in the reduced ciclosporin bioavailability in MPS-treatment.

Because of its hydrophobic property, it is considered that solubilization of ciclosporin into mixed micelles is required for enteric passive transport. Cakaloglu et al (1993) showed that intestinal absorption of ciclosporin was markedly decreased in the absence of continuous bile flow. It has also been reported that reduction in oral ciclosporin absorption occurs in rats with renal dysfunction (Shibata et al 1999, 2000), and that the impaired absorption is attributed to the decrease in secretion of bile acids into the small intestinal lumen (Igarashi et al 2003; Shibata et al 2004). According to previous reports using rodents, methylprednisolone had no effect on bile secretion at a single dose of $10\,mg\,kg^{-1}$ (Chanussot et al 1992), although bile lipid composition was altered after long-term intramuscular administration of MPS (Yarimagan & Bor 1986). In contrast, we

clarified that MPS pulse treatment significantly decreased the excreted amount of bile acids while bile flow was unchanged. The observed difference in the responsiveness of bile production to MPS treatment is probably due to experimental disparities in the dose of methylprednisolone and its administration period; our results strongly suggest that the suppression of biliary secretion can be regarded as one of the underlying mechanism of drug interaction between high-dose intravenous MPS and orally administered ciclosporin.

To directly estimate the change in absorptive capacity of ciclosporin in the small intestine to validate the proposed mechanism regarding the pharmacokinetic interference with oral ciclosporin by methylprednisolone pulsing, an in-situ absorption test was conducted using a closed-loop of small intestine obtained from control and MPS-treated rats in the presence or absence of bile flow. Intestinal absorption of ciclosporin was evaluated by measuring its blood concentration in portal vein to avoid involvement of first-pass metabolism in the liver. In agreement with the previous findings (Cakaloglu et al 1993), the amount of absorbed ciclosporin was markedly decreased in the absence of bile flow. In addition, a significant reduction in ciclosporin absorption was ascertained in MPS-treated rats regardless of whether bile was supplied into lumen. The obtained results were compatible with the in-vivo disposition behaviour of ciclosporin, providing conclusive evidence that the decreased bioavailability of ciclosporin observed after the high-dose MPS was caused by the combined effects of enhancement in intestinal P-gp function and suppression of biliary supply.

MPS pulse treatment greatly differs from its maintenance therapy with respect to the daily dose. In practice, considerable decreases in blood levels of intravenously administered ciclosporin and paclitaxel were observed in transplant patients receiving methylprednisolone pulse (Ptachcinski et al 1987; Monsarrat et al 1998). Considering the converging systemic exposure of methylprednisolone, a likely explanation for the occurrence of these events is the induction of hepatic CYP3A, because hepatic CYP3A is a major catalyst involved in the biotransformation of these agents. Recently, it was found in clinical practice that the blood concentration of orally administered tacrolimus, an immunosuppressant, was drastically reduced by pulse methylprednisolone therapy (625 mg daily) for 3 days (Shimada et al 2002). This clinical finding strongly suggests the reduced bioavailability of tacrolimus after methylprednisolone pulsing, since tacrolimus is subject to intestinal exclusion by P-gp (van Gelder 2002), similarly to ciclosporin.

Conclusion

We demonstrated that the systemic bioavailability of ciclosporin is markedly reduced by MPS pulse treatment, and the extent of change in the pharmacokinetic disposition of orally administered ciclosporin is much greater than that of intravenous ciclosporin after methylprednisolone pulsing. The mechanism of this interaction was confirmed to involve not only enhancement of small intestinal P-gp function but also decrease in the bile secretion. Therefore, careful monitoring of blood ciclosporin concentration should be mandatory when MPS pulse therapy is concomitantly conducted with oral administration of ciclosporin, taking its narrow therapeutic range into account.

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