Intestinal P-glycoprotein Expression is Multimodally Regulated by Intestinal Ischemia-Reperfusion

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ABSTRACT - Purpose. Reactive oxygen species (ROS) have multiple physiological effects that are amount-dependent. ROS are one of the causes of intestinal ischemia-reperfusion (I/R) injury. In this study, we investigated whether the amount of ROS and the degree of intestinal I/R injury affect the expression level of P-glycoprotein (P-gp). **Methods.** We used hydrogen peroxide (H_2O_2) as ROS in *in vitro* experiments. Intestinal I/R model rats, which were subjected 15-min ischemia (I/R-15), were used in *in vivo* experiments. **Results.** P-gp expression in Caco-2 cells was increased in response to 1 μM of H_2O_2 but decreased upon exposure to 10 mM of H_2O_2. We previously reported that P-gp expression is decreased after intestinal I/R with 30-min ischemia (I/R-30), which time a large amount of ROS is generated. I/R-15 induced slightly less mucosal and oxidative injury than did I/R-30. P-gp expression in the jejunum was increased at 1 h after I/R-15, and ileal paracellular permeability was increased. The blood concentration of tacrolimus, a P-gp substrate, was lower during 0-20 min but was higher during 40-90 min post-administration compared with that in the sham-operated rats. P-gp expression in the ileum was decreased at 6 h after I/R-15, due to abnormal localization of P-gp, resulting in a high blood tacrolimus concentration in rats reperfused for 6 h. **Conclusions.** ROS multimodally regulate P-gp expression depending on its amount. This is important for understanding the pattern of P-gp expression after intestinal I/R.

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INTRODUCTION

It is well known that reactive oxygen species (ROS) have multiple physiological effects that are amount-dependent. When the amount of ROS is moderate, ROS act as second intracellular messengers that play roles in the regulation of immunostimulation, cell growth and apoptosis (1, 2). On the other hand, When the amount of ROS is excessive, ROS cause cell death by induction of lipid peroxidation and alteration of protein conformation after oxidation of cysteine and methionine residues (3). Intestinal ischemia-reperfusion (I/R) occurs in various clinical settings such as small intestinal transplantation, intestinal surgery, circulatory shock, and strangulation ileus. It has been shown that the small intestine is highly sensitive to I/R (4). ROS, which are derived from xanthine oxidase activation, play an important role in acute intestinal I/R injury (5,

6). ROS induce membrane lipid peroxidation followed by loss of intestinal barrier function. Therefore, the degree of intestinal I/R injury can be characterized by the amount of generated ROS.

The small intestine is important for absorption of nutrients and drugs. P-glycoprotein (P-gp), which belongs to the ATP-binding cassette (ABC) superfamily, is expressed on the brush border membrane (BBM) of the small intestine and mediates the efflux of a broad range of endogenous and xenobiotic compounds, including clinical drugs. Several studies have shown that P-gp expression level is altered by intestinal I/R (7-9) and that the bioavailability of P-gp substrates is changed after

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intestinal I/R (7, 10, 11). However, results regarding alteration of P-gp expression are not consistent among those studies. We previously reported that the expression level of P-gp was significantly decreased in intestinal I/R rats subjected to 30-min ischemia (I/R-30) and 6-h reperfusion (8). Moreover, Tomita et al. (9) reported that P-gp expression was decreased in intestinal I/R model rats subjected to 60-min ischemia and 1-h reperfusion. On the other hand, Omae et al. (7) reported that the expression level of P-gp was increased in intestinal I/R model rats subjected to I/R-30 and 24-h reperfusion.

Some procedural differences exist among these intestinal I/R model rats. We subjected the rats to ischemia by ligating small anastomosing vessels and occluding the superior mesenteric artery (SMA) (8). Omae et al. (7) subjected the rats to ischemia by occluding the SMA without ligating small anastomosing vessels. Tomita et al. (9) subjected the rats to a longer duration of ischemia than the durations in the other studies. Thus, there are differences in the magnitude of I/R injury depending on amount of generated ROS among those studies.

In this study, we examined whether the amount of ROS affects the expression of P-gp in Caco-2 cells, which are a model of the human intestinal epithelium. Then we carried out an additional study to investigate P-gp expression in intestinal I/R model rats subjected to a short duration of ischemia. We previously reported a decrease in P-gp expression in I/R-30 rats. Since the degree of mucosal injury after intestinal I/R is in proportion to the duration of ischemia (12), we subjected the rats to 15-min ischemia (I/R-15) in this study.

MATERIALS AND METHODS

Chemicals
Hydrogen peroxide (H_2O_2) and tacrolimus were purchased from Wako (Osaka, Japan). Fluorescein isothiocyanate–dextran (FD-4) was purchased from Sigma (Tokyo, Japan). All other reagents were of the highest grade available and used without further purification.

Cell culture
Caco-2 cells obtained from Riken Cell Bank (Tsukuba, Japan) were maintained in plastic culture flasks (Corning Costar Corp., Cambridge, MA). The cell culture conditions were the same as those

described previously (13). In the present study, Caco-2 cells were used between passages 45 and 60. For H_2O_2 addition studies, H_2O_2 dissolved in the medium (pH 7.4) was added to cells at various concentrations for a period of 6 h.

Animals
Male Wistar rats, aged 6 weeks, were obtained from Jla (Tokyo, Japan). The rats were housed for at least 1 week (until reaching 190-280 g in weight). The housing conditions were the same as those described previously (13). The experimental protocols were reviewed and approved by the Hokkaido University Animal Care Committee in accordance with the "Guide for the Care and Use of Laboratory Animals".

Intestinal I/R model
Surgical procedures were carried out as described in a previous report (8). The animals were anesthetized with sodium pentobarbital (30-40 mg/kg body weight, *i.p.* injection). Through a midline laparotomy, each rat was subjected to 15 min of ischemia by ligating small anastomosing vessels and occluding the SMA. Reperfusion was induced by removing the clamp. The abdomen was sutured during reperfusion.

Assessment of function of the tight junction
Function of the tight junction was assessed by mucosal to serosal transport of FD-4. The experiment was performed as described previously (11). Infinite® M200 (Tecan, Kawasaki, Japan) was used to measure FD-4. The permeation rate of FD-4 was expressed as an apparent permeability coefficient (P_{app}) according to the following equation: $P_{app}=dQ/dt /SC_0$, where dQ/dt is the linear appearance rate of mass in the receiver solution, S is the exposed area (0.64 cm^2), and C_0 is the initial concentration of FD-4 (100 μg/mL).

Real-time PCR
Real-time PCR was performed as described previously (8). PCR was performed using rat mdr1a-specific primers through 40 cycles of 94°C for 15 s, 50°C for 30 s and 72°C for 30 s, using rat mdr1b-specific primers through 40 cycles of 95°C for 15 s, 50°C for 30 s and 72°C for 30 s or using human MDR1-specific primers through 40 cycles of 94°C for 15 s, 58°C for 30 s and 72°C for 30 s, in all cases after pre-incubation at 50°C for 2 min and at 95°C for 15 min, or using human or rat GAPDH-specific

primers. The primers specific to hMDR1, hGAPDH, rmdr1a, rmdr1b, and rGAPDH were designed on the basis of sequences in GenBank™ database (accession no.: NM_000927, NM002046, NM_133401, NM_012623, and AF106860, respectively). The sequences of the specific primers were as follows: the sense sequence was 5'-TGC TCA GAC AGG ATG TGA GTT G-3' and the antisense sequence was 5'-AAT TAC AGC AAG CCT GGA ACC-3' for hMDR1, the sense sequence was 5'-AAG GTC ATC CCT GAG CTG AA-3' and the antisense sequence was 5'-TTC TAG ACG GCA GGT CAG GT-3' for hGAPDH, the sense sequence was 5'-GCA GGT TGG CTG GAC AGA TT-3' and the antisense sequence was 5'-GGA GCG CAA TTC CAT GGA TA-3' for rmdr1a, the sense sequence was 5'-CTG CTA TCA TCC ACG GAA CC-3' and the antisense sequence was 5'-GCT GAC GGT CTG TGT ACT GTT G-3' for rmdr1b, and the sense sequence was 5'-ATG GGA AGC TGG TCA TCA AC-3' and the antisense sequence was 5'-GTG GTT CAC ACC CAT CAC AA-3' for rGAPDH. The PCR products were normalized to amplified GAPDH, which was the internal reference gene (housekeeping gene).

Western blot analysis
Protein extracts from Caco-2 cells and intestinal brush border membrane vesicles (BBMVs) were used for Western blot analysis. The protein extracts from Caco-2 cells and intestinal BBMVs were prepared by the same methods as those described previously (8, 13). Protein concentrations of these samples in clear supernatants were determined by the method of Lowry et al. (14) with bovine serum albumin as a standard. Western blot analysis was performed as described previously (8). A mouse monoclonal antibody to MDR1 (Santa Cruz Biotechnology, Santa cruz, CA) or mouse anti-actin monoclonal antibody (Chemicon, Temecula, CA) (diluted 1:500) was used. Horseradish peroxidase-conjugated goat anti-mouse secondary antibody (Santa Cruz Biotechnology) was used as a secondary antibody. The bands were visualized by enhanced chemiluminescence according to the instructions of the manufacturer (Amersham Biosciences Corp., Piscataway, NJ).

Oxidative injury assessment
The extent of ROS-induced oxidative damage was assessed indirectly by measuring the level of malondialdehyde (MDA), an intermediate product of lipid peroxidation, as described previously (15). For this study, a PBS suspension of Caco-2 cells and tissue homogenate were used. Thiobarbituric acid (TBA) solution consisted of 2.6 mM TBA, 918 mM trichloroacetic acid, 0.3 mM HCl, and 1.8 mM 2,6-di-tert-butyl-4-metylphenol in 22% ethanol. The reaction mixture contained 0.2 mL of the test sample, 0.2 mL of 8.1% SDS, 1.5 mL of 20% acetic acid solution (pH 3.5), and 1.5 mL of TBA solution. The mixture was heated at 95°C for 60 min. After cooling with tap water, 2.5 mL of n-butanol or 1.0 mL of distilled water and 5.0 mL of n-butanol were added (Caco-2 cells or tissue homogenate, respectively), and the mixture was shaken vigorously. After centrifugation at 720×g for 5 min, the absorbance of the organic layer (upper layer) was measured at 535 nm with 1,1,3,3-tetraethoxypropane as a standard. The amount of MDA was corrected by protein content.

Pharmacokinetic studies in rats
Tacrolimus administration was performed by modifications of the method of Yamaguchi et al. (16). Rats were anesthetized with sodium pentobarbital (30-40 mg/kg body weight, *i.p.* injection). The abdominal cavity was opened via a middle incision, and tacrolimus solution (0.5 mg/mL) was administered via the upper duodenum at 1.0 mg/kg body weight. The administration volume of tacrolimus solution was 2.0 mL/kg body weight. Blood samples (each 0.3 mL) were collected into tubes from a jugular vein at 0, 5, 10, 20, 40, 60 and 90 min. Tacrolimus concentrations were assayed in whole blood by PRO-Trac™ II ELISA. PRO-Trac™ II ELISA was performed according to the manufacturer's protocol. The absorbance was read at 450 nm by an absorption spectrometer (Infinite® M200, TECAN JAPAN). The area under the concentration-time curve (AUC) of tacrolimus was calculated using the trapezoidal rule from the concentration–time curve. The maximum drug concentration (C_{max}) and the maximum drug concentration time (T_{max}) were calculated by Origin J. Total clearance (CL) was calculated by the following equation: CL=D/AUC, where D is the dosage amount.

STATISTICAL ANALYSIS

Statistical significance was evaluated using Dunnett's

test (*in vitro* experiments) or Student's *t*-test (*in vivo* experiments). A value of p<0.05 was considered significant.

RESULTS

MDA level in H_2O_2-treated Caco-2 cells

To investigate the effect of H_2O_2, one of the ROS, on the extent of oxidative injury, Caco-2 cells were exposed to H_2O_2 for 6 h. The level of MDA, a reliable marker for lipid peroxidation, was increased in a concentration-dependent manner (Fig. 1).
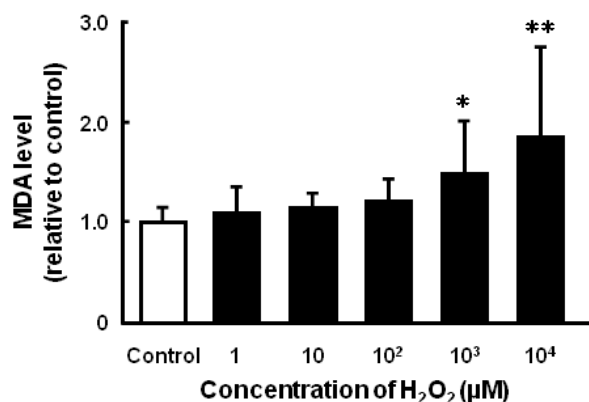

Figure 1. Effect of hydrogen peroxide on lipid peroxide in Caco-2 cells. Caco-2 cells were exposed to hydrogen peroxide for 6 h. Control was incubated in the medium for 6 h. Each column represents the mean with S.D. of 9-18 measurements. *P<0.05 significantly different from the control. **P<0.01.

P-gp expression in H_2O_2-treated Caco-2 cells

Next, to clarify the effect of H_2O_2 concentration on P-gp expression level, we examined the change in mRNA level of MDR1, which encodes P-gp, in Caco-2 cells. The mRNA level of MDR1 was increased in response to 1 μM H_2O_2 but was decreased in response to more than 1 μM H_2O_2 in a concentration-dependent manner (Fig. 2A). The mRNA level of MDR1 was significantly decreased upon exposure to 10 mM H_2O_2 compared with that in control cells (Fig. 2A). Moreover, P-gp protein expression was increased in response to 1 μM H_2O_2 but decreased in response to more than 1 μM H_2O_2 in a concentration-dependent manner (Fig. 2B). This result was associated with the level of MDR1 mRNA.

MDA levels in intestinal I/R-15 rats

Since the degree of I/R injury is increased with prolongation of the duration of ischemia to more than 15 min in rats (12), we used intestinal I/R-15 rats to induce slight intestinal I/R injury compared to intestinal I/R-30 rats in this study. Firstly, to confirm that intestinal I/R injury is induced even by intestinal I/R-15, we evaluated the occurrence of oxidative injury after intestinal I/R-15 by measuring the levels of MDA. The MDA levels in the jejunum and ileum were increased at 1 h after intestinal I/R-15 compared to those in sham-operated rats (Fig. 3A and 3B). These results indicated that oxidative injury in the intestine was induced by intestinal I/R-15.

Intestinal paracellular permeability in intestinal I/R-15 rats

Intestinal I/R is well known to affect intestinal paracellular permeability (17). Although P_{app}s of FD-4 through jejunal membranes of intestinal I/R-15 rats reperfused for 1 h and 6 h were not altered, P_{app} of FD-4 through jejunal membranes of intestinal I/R-15 rats reperfused for 24 h was significantly decreased (Fig. 4A). On the other hand, P_{app}s of FD-4 through ileal membranes of intestinal I/R-15 rats reperfused for 1 h and 6 h were significantly higher than those in sham-operated rats (Fig. 4B).
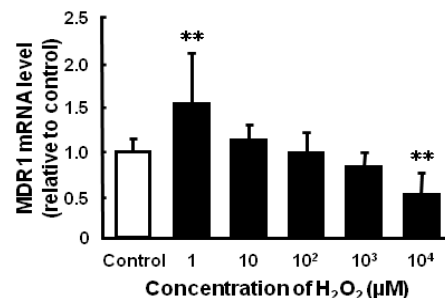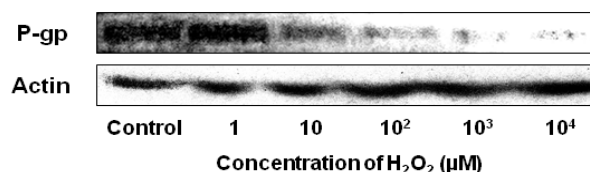

Figure 2. Effects of hydrogen peroxide on MDR1 level (A) and P-gp expression level (B) in Caco-2 cells. Caco-2 cells were exposed to hydrogen peroxide for 6 h. Control was incubated in the medium for 6 h. Each column represents the mean with S.D. of 5-8 measurements. **P<0.01 significantly different from the control. Western blot data shown are typical results from three independent experiments.

P_{app} of FD-4 through ileal membranes of intestinal I/R-15 rats reperfused for 24 h was almost the same as that in sham-operated rats (Fig. 4B).

P-gp expression in intestinal I/R-15 rats

We examined mRNA levels of mdr1a and mdr1b, which encode P-gp, and P-gp expression levels in the jejunum and ileum after intestinal I/R-15. In the jejunum, as shown in Fig. 5, the mRNA levels of mdr1a and mdr1b were increased at 1 h after intestinal I/R-15. The mdr1b mRNA level was also increased at 6 h after intestinal I/R-15 (Fig. 5B). On the other hand, the mdr1a mRNA level was decreased at 24 h after intestinal I/R-15 (Fig. 5A). The expression level of P-gp in the jejunal BBM was increased at 1 h but was not significantly altered at 6 or 24 h after intestinal I/R-15 (1.8-fold at 1 h, 0.81-fold at 6 h and 1.0-fold at 24 h) (Fig. 5C). In the ileum, mdr1a and mdr1b mRNA levels were not altered at any time after intestinal I/R-15 (Fig. 6A and 6B). On the other hand, the expression level of P-gp in the ileal BBM was 0.62-fold decreased at 6 h after intestinal I/R (Fig. 6C) but was not altered in the ileal homogenate at any time (0.96-fold at 1 h, 1.1-fold at 6 h and 1.0-fold at 24 h) (data not shown).

Pharmacokinetics of tacrolimus in intestinal I/R-15 rats

Finally, we investigated whether the pharmacokinetics of tacrolimus is affected by intestinal I/R. Fig. 7 shows the time courses of blood tacrolimus concentration after intraintestinal administration at a dose of 10 mg/kg. The blood tacrolimus concentration in intestinal I/R-15 rats reperfused for 1 h was lower during 0-20 min but higher during 40-90 min compared with that in sham-operated rats (Fig. 7A). T_{max} in intestinal I/R-15 rats reperfused for 1 h was higher than that in sham-operated rats, but AUC_{0-90} in intestinal I/R rats reperfused for 1 h was almost the same as that in sham-operated rats (Table 1). The blood tacrolimus concentration in intestinal I/R-15 rats reperfused for 6 h was higher than that in sham-operated rats (Fig. 7B). AUC_{0-90}, T_{max} and C_{max} in intestinal I/R-15 rats reperfused for 6 h were 1.6-fold, 1.4-fold and 1.5-fold higher than those in sham-operated rats, respectively, but AUC_{0-90} and C_{max} were not significantly different (Table 2). The blood tacrolimus concentration in intestinal I/R-15 rats reperfused for 24 h was almost the same as that in sham-operated rats (Fig. 7C). Pharmacokinetic parameters in intestinal I/R-15 rats reperfused for 24 h were also the same as those in sham-operated rats (Table 2).

DISCUSSION

One of the mechanisms of intestinal I/R injury is thought to be excessive production of ROS. ROS have multiple physiological effects that are amount-dependent (18). Moderate amount of ROS acts as an intracellular signaling molecules that are indispensable to the living body. In contrast, excessive amount of ROS induce severe oxidative organ injury. We focused on this bilateral character of ROS and investigated the effect of the amount of ROS on P-gp expression.
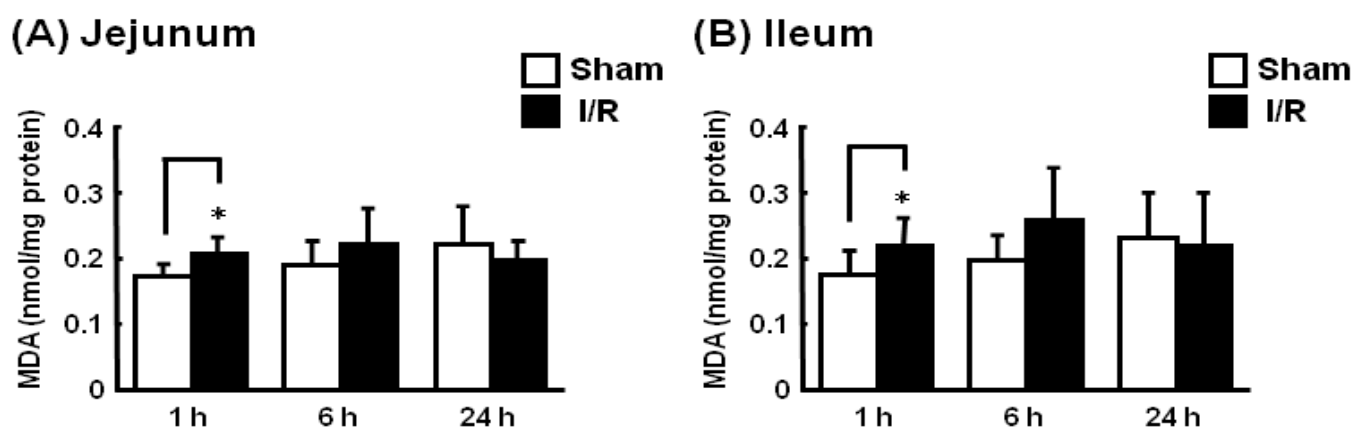

Figure 3. Time courses of MDA levels in the jejunum (A) and ileum (B) after intestinal I/R-15. Each column represents the mean with S.D. of 3-7 measurements. *; P<0.05 significantly different from sham.
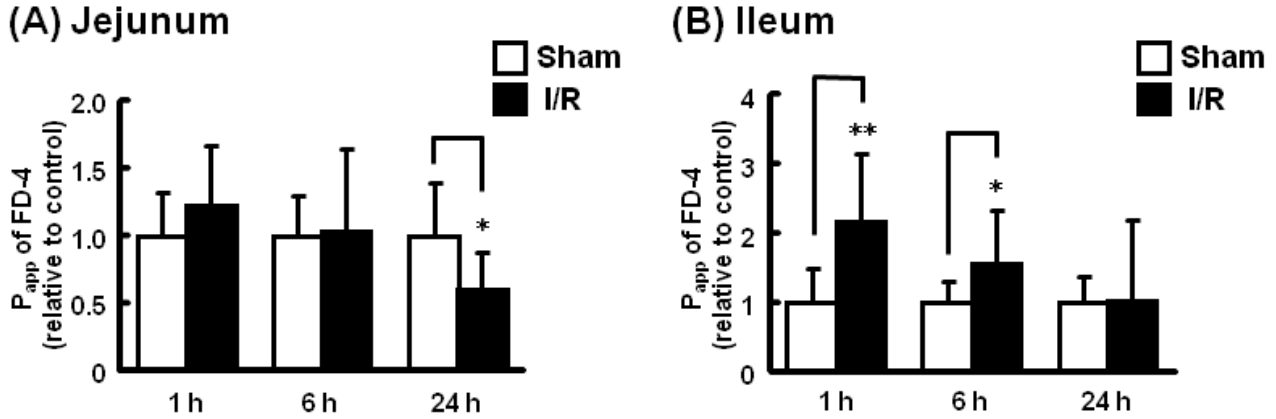
Figure 4. Time courses of P_{app} of FD-4 through jejunal (A) and ileal (B) membranes of intestinal I/R-15 rats. Each column represents the mean with S.D. of 11-15 measurements (n=4-5). *; P<0.05 significantly different from sham. **P<0.01.
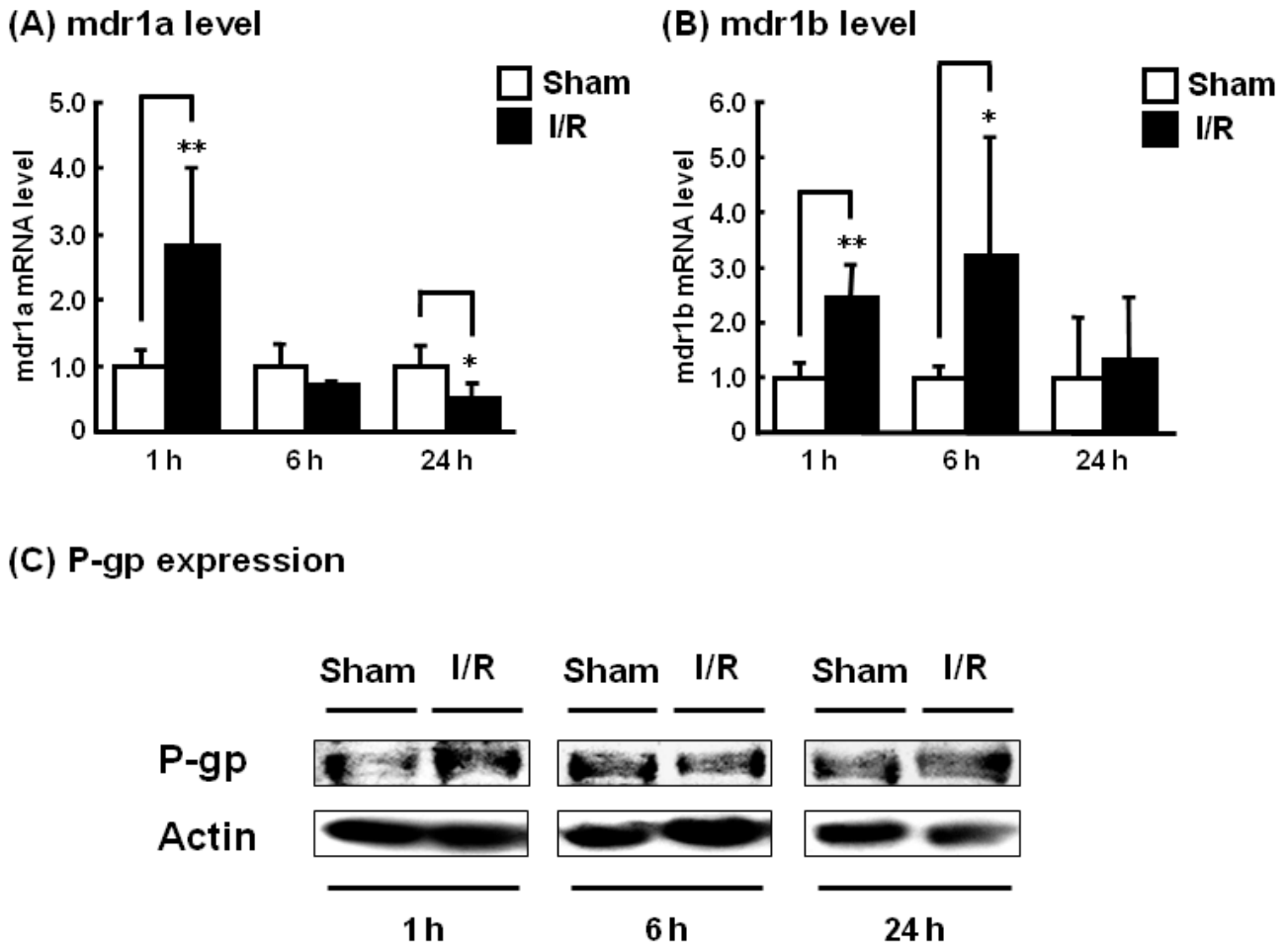

Figure 5. Time courses of mdr1a (A), mdr1b (B) and P-gp (C) levels in the jejunum after intestinal I/R-15. Each column represents the mean with S.D. of 4-6 measurements. *P<0.05 significantly different from sham. **P<0.01. Western blotting using jejunal BBMVs at 1, 6 and 24 h after I/R-15. Western blot data shown are typical results from three independent experiments.
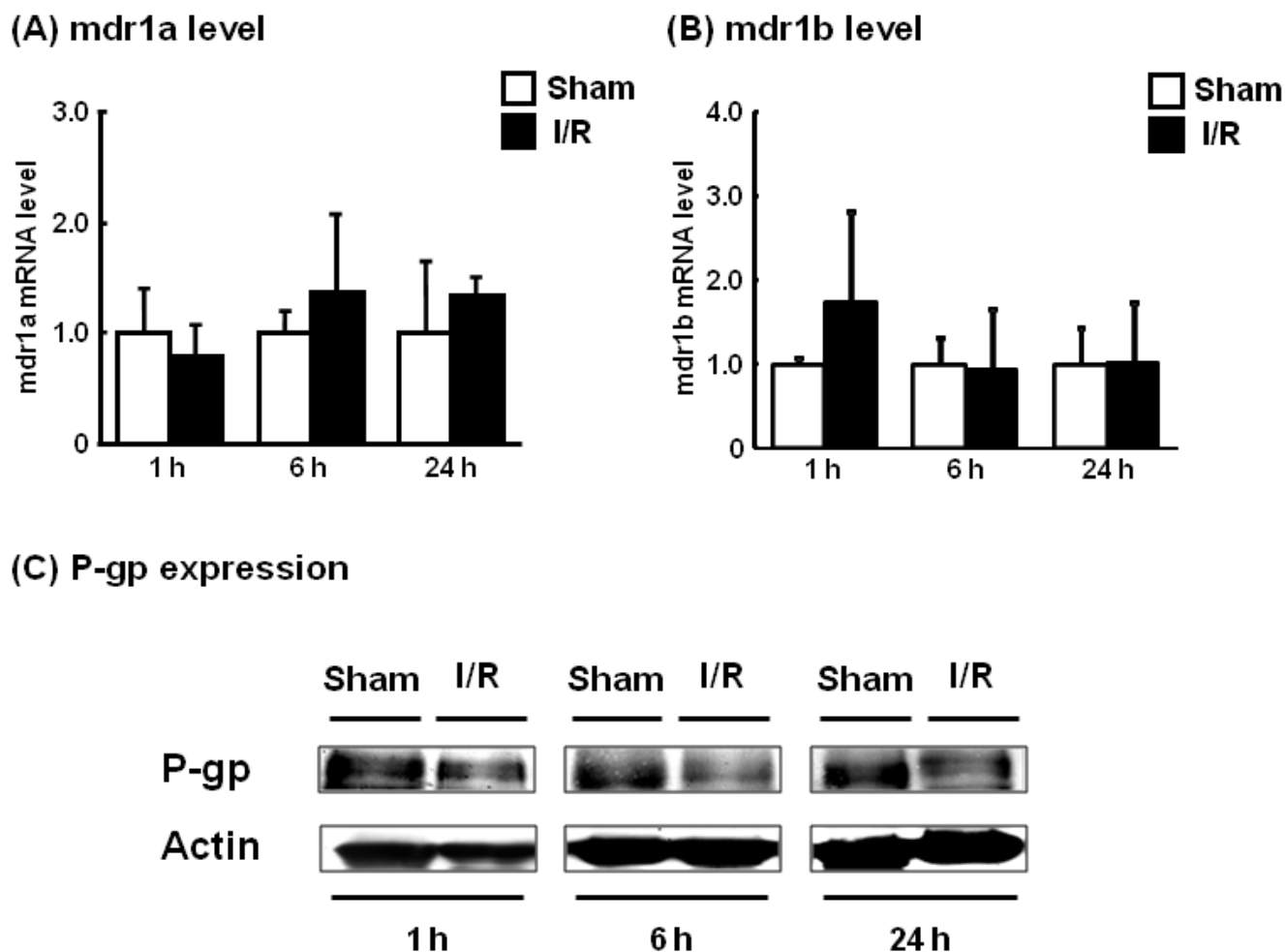
Figure 6. Time courses of mdr1a (A), mdr1b (B) and P-gp (C) levels in the ileum after intestinal I/R-15. Each column represents the mean with S.D. of 4-6 measurements. Western blotting using ileal BBMVs at 1, 6 and 24 h after I/R-15. Western blot data shown are typical results from three independent experiments.

Firstly, we assessed the effects of various amounts of ROS on P-gp expression in Caco-2 cells, which are widely used as an intestinal model. P-gp expression in Caco-2 cells was increased in response to 1 μM of H_2O_2 but was decreased upon exposure to 10 mM H_2O_2. Moreover, the MDR1 mRNA level with 1 μM to 10 mM H_2O_2 showed a negative correlation with the extent of oxidative stress evaluated by measuring MDA level (r: -0.900, $p < 0.05$). We therefore concluded that P-gp expression is increased by a small amount of ROS but is decreased by a large amount of ROS, which is accordance with the results of a previous study of Nagira et al. using *tertiary*-butylhydroperoxide (19). In our previous study, we revealed that I/R-30 caused severe damage of the intestinal mucosa and that mdr1a mRNA level

was decreased at 6 h after I/R-30 in the ileum, resulting in a decrease in P-gp expression level (8). MDA level was increased by 2.1 fold in intestinal I/R-30 rats (15) and was increased by 1.9 fold in Caco-2 cells exposed to 10 mM H_2O_2 (Fig. 1). These findings indicated that the small intestine after I/R-30 was affected by severe oxidative stress that was similar to or greater than that induced in Caco-2 cells exposed to 10 mM H_2O_2. Boros et al. (12) reported that mucosal injury level after intestinal I/R is significantly increased with prolongation of the duration of ischemia in rats to more than 15 min. I/R-15 rats have been used to assess diverse phenomena caused by slight I/R injury (20), and I/R-30, but not I/R-15, has been shown to induce epithelial destruction and DNA fragmentation (21).
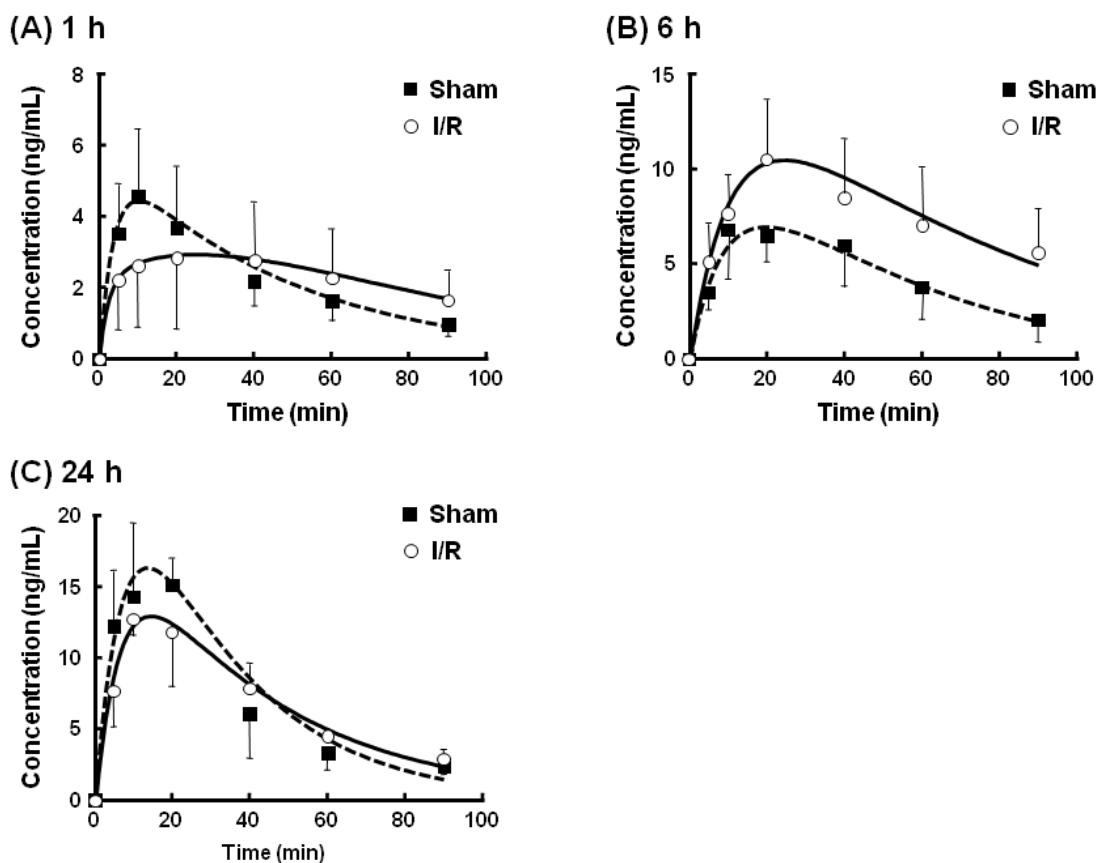
Figure 7. Blood concentration of tacrolimus after intraintestinal administration in intestinal I/R-15 rats.   The reperfusion times were 1 h (A), 6 h (B) and 24 h (C). Each point represents the mean ±S.D. of 3-6 measurements.

Table 1. Pharmacokinetic parameters of tacrolimus after intestinal I/R

		AUC_{0-90} ((ng/mL)·min)	T_{max} (min)	C_{max} (ng/mL)
1 h	Sham	206±71	11.1±4.0	4.6±1.8
	I/R	213±128	26.8±10.1[*]	3.2±1.8
6 h	Sham	413±145	19.1±1.6	7.1±2.2
	I/R	676±237	25.8±1.4[**]	10.9±3.0
24 h	Sham	639±162	13.9±4.3	17.0±1.9
	I/R	632±71	13.6±3.2	13.7±2.7

Each value represents the mean± S.D. of 3-5 measurements.
*; $P < 0.05$ significantly different from sham. **; $P < 0.01$.

Based on those reports, we decided to make the duration of ischemia shorter than 30 min and we used I/R-15 rats in this study to investigate the effects of slight I/R injury on expression of P-gp in the small intestine. Maximum MDA levels in the jejunum and ileum in intestinal I/R-15 rats (Fig. 4) were lower

than those in intestinal I/R-30 rats (15). These findings suggest that shortening the duration of ischemia attenuates the oxidative injury in the small intestine after I/R, which is accordance with several reports (12, 20, 21). In intestinal I/R-15 rats, mdr1a (Fig. 5A) and mdr1b (Fig. 5B) mRNA levels in the jejunum were increased at 1 h after intestinal I/R, resulting in an increase in P-gp expression level (Fig. 5C). This significant up-regulation of P-gp expression was not observed in intestinal I/R-30 rats (8). These results suggest that ROS have a bilateral effect on P-gp expression in a living body, being consistent with the results of *in vitro* experiments (Fig. 2). Several studies have shown that P-gp expression level is altered by intestinal I/R, but results regarding alteration of P-gp expression are not consistent among those studies (7-9). Some procedural differences exist among these intestinal I/R model rats, such as ischemia duration, grade of ischemia and reperfusion time, which have been shown to affect the extent of intestinal I/R injury (9, 12, 22). Thus, there were differences in the amount of generated ROS after intestinal I/R among those studies, resulting in the difference in the alteration of P-gp expression. In the ileum, P-gp expression in the ileal BBM was decreased at 6 h after intestinal I/R-15 (Fig. 6C), though mdr1a and mdr1b mRNA levels (Figs. 6A and 6B) and P-gp protein expression in the ileal homogenate (data not shown) were not decreased. Takizawa et al. (23) reported abnormal localization of P-gp in the ileal membrane during intestinal I/R. This finding suggests that abnormal localization of P-gp also occurs in our intestinal I/R-15 model rats.

P-gp expression level in the intestine is an important pharmacokinetic factor for the immunosuppressant tacrolimus in small intestinal transplantation (24). Blood concentration of tacrolimus in intestinal I/R-15 rats reperfused for 1 h was lower during 0-20 min post-administration but higher during 40-60 min post-administration compared with that in sham-operated rats. Simomura et al. (25) reported that the site of absorption of tacrolimus was the whole ileum in jejunum-resected rats, resulting in T_{max} of tacrolimus being significantly higher than that in sham-operated rats. Considering this finding, the increase in jejunal P-gp expression level in intestinal I/R-15 rats causes the lower blood tacrolimus concentration in the period from 0 to 20 min post-administration, resulting in T_{max} of tacrolimus being significantly higher than that in sham-operated rats. The paracellular pathway to the lateral intercellular space *via* tight junctions between epithelial cells is another transport pathway that can function as a drug absorption route (26, 27).

Ileal paracellular permeability was 2.2-fold increased at 1 h after intestinal I/R-15 (Fig. 4B), resulting in an increase in absorption of tacrolimus from the ileum. The increase in ileal paracellular permeability in intestinal I/R-15 rats reperfused for 1 h might cause the higher blood tacrolimus concentration in the period from 40 to 90 min post-administration. In I/R-15 rats reperfused for 6 h, blood tacrolimus concentration was higher than that in sham-operated rats (Fig. 7). The high blood tacrolimus concentration in intestinal I/R-15 rats could be explained by the induction of absorption of tacrolimus resulting from the decrease in P-gp expression (Fig. 6) and the increase in paracellular permeability (Fig. 4B) in the ileum. In intestinal I/R-30 rats, P-gp expression in the ileum was also decreased at 6 h (8). The tacrolimus concentration in intestinal I/R-30 rats reperfused for 6 h was higher than that in sham-operated rats (data not shown). AUC_{0-90}, T_{max} and C_{max} in intestinal I/R-30 rats reperfused for 6 h were 2.2-fold, 1.7-fold and 1.7-fold higher than those in sham-operated rats, respectively. However, the mechanisms of the decrease in P-gp expression were different in intestinal I/R-15 rats and intestinal I/R-30 rats. The decrease in P-gp expression in intestinal I/R-15 rats might be caused by abnormal localization of P-gp, but that in intestinal I/R-30 rats was caused by the decrease in mdr1a mRNA level. The mechanism of this abnormal localization of P-gp in intestinal I/R-15 rats is not clear. Further detailed investigation is needed to elucidate this regulation of P-gp in intestinal I/R-15 rats. Jenunal paracellular permeability was significantly decreased at 24 h after intestinal I/R-15. We currently have little information to explain the phenomenon. It is known that the protein expression levels of claudin-1 and -4, which play a crucial role in the barrier function of tight junctions, is significantly increased at 24 h after intestinal I/R (28). This finding suggests that the increase in claudin-1 and -4 expression might cause the decrease in jejunal paracellular permeability at 24 after intestinal I/R. In sham-operated rats, AUC_{0-90} and C_{max} were increased with experimental time. We did not investigate the factors leading to the alterations of AUC_{0-90} and C_{max} by the sham operation, but it is possible that laparotomy may have contributed to these alterations. Indeed, several workers reported that laparotomy induced the production of stress hormones (29, 30).

Intestinal ischemia remains a serious complication of intestinal surgery including small intestinal transplantation. Tacrolimus is a key drug used in organ transplantation. Tacrolimus shows large intra- and inter-individual pharmacokinetic variability,

and that increases the risk of rejection and graft loss (31). Thus, modifying the dosage of tacrolimus is necessary for transplant recipients to secure better postoperative control. The present findings provide useful information for immunosuppressive therapy with tacrolimus.

In conclusion, we clarified that P-gp expression in Caco-2 cells was increased in response to 1 μM of H_2O_2 but decreased upon exposure to 10 mM of H_2O_2. Moreover, P-gp expression in the jejunum was increased at 1 h after intestinal I/R-15, when slight oxidative injury is occurring, resulting in reduction of the absorption rate of tacrolimus. In the ileum, P-gp expression in the BBM was decreased at 6 h after intestinal I/R-15, due to abnormal localization of P-gp, resulting in a high blood tacrolimus concentration. In an intestinal I/R condition, the duration of ischemia is variable. The results of this study suggest that ROS multimodally regulate P-gp expression depending on its amount. It is important to concentrate on the amount of generated ROS after intestinal I/R for understanding the pattern of P-gp expression.

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