

Intestinal Ischemia-Reperfusion Suppresses Biliary Excretion of Hepatic Organic Anion Transporting Polypeptides Substrate

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Abstract - Purpose. Intestinal ischemia-reperfusion (I/R) causes gut dysfunction and promotes multi-organ failure. The liver and kidney can be affected by multi-organ failure after intestinal I/R. Organic anion transporting polypeptides (OATPs) and organic anion transporters (OATs) are recognized in a broad spectrum from endogenous compounds to xenobiotics, including clinically important drugs. Therefore, it is important for understanding the pharmacokinetics to obtain evidence of alterations in OATPs and OATs expression and transport activities. In the present study, we investigated the expression of rat Oatps and Oats after intestinal I/R. **Methods.** We used intestinal ischemia-reperfusion (I/R) model rats. Real-time PCR and Western blotting were used to assess mRNA and protein expression levels. Plasma concentration and biliary excretion of sulfobromophthalein (BSP), which is used as a model compound of organic anion drugs, were measured after intravenous administration in intestinal I/R rats. **Results.** Although Oat1 and Oat3 mRNA levels were not altered in the kidney, Oatp1a1, Oatp1b2 and Oatp2b1 mRNA levels in the liver were significantly decreased at 1-6 h after intestinal I/R. Moreover, Oatp1a1 and Oatp2b1 protein expression levels were decreased at 1 h after intestinal I/R. Plasma concentration of BSP, which is a typical substrate of Oatps, in intestinal I/R rats reperused 1 h was increased than that in sham-operated rats. Moreover, the area under the concentration-time curve (AUC_{0-90}) in intestinal I/R rats reperused 1 h was significantly increased than that in sham-operated rats. The total clearance (CL_{tot}) and the biliary clearance (CL_{bile}) in intestinal I/R rats reperused 1 h were significantly decreased than those in sham-operated rats. **Conclusions.** Oatp1a1 and Oatp2b1 expression levels are decreased by intestinal I/R. The decreases in these transporters cause alteration of pharmacokinetics of organic anion compound. The newly found influence of intestinal I/R on the expression and function of Oatps may be a key to perform appropriate drug therapy.

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INTRODUCTION

Intestinal ischemia-reperfusion (I/R) induces severe intestinal mucosa injury (1). Moreover, intestinal I/R induces remote organ failure, including the lung (2), liver (3) and kidney (4), resulting in multi-organ failure (5). Intestinal ischemia accounts for only about 1-2% of gastrointestinal illnesses but the incidence has been increasing considerably (6). The liver and kidney are major organs for detoxification of xenobiotics, including drugs. Hepatic or renal transporters contribute to the elimination of various compounds and prevent the accumulation of potentially harmful compounds in the bile or urine. Therefore, hepatic or renal transporters play a critical role in the host defense mechanism. The excretion of compounds mediated by transporters is vectorial transport from blood circulation to bile or urine. For the uptake step,

organic anion transporting polypeptides (OATPs), organic anion transporters (OATs) and organic cation transporters (OCTs) have been shown to be involved in the transport of organic anions and organic cations. For the efflux step, ATP-binding cassette (ABC) transporters, including P-glycoprotein (P-gp), multidrug resistance-associated proteins (MRPs) and breast cancer resistance protein (BCRP) have been shown to be involved in the transport of various compounds.

OATPs or OATs express predominantly in the liver or kidney, respectively, and are recognized in a broad spectrum from endogenous compounds to

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xenobiotics, including clinically important drugs. Therefore, it is important for understanding the pharmacokinetics to obtain evidence of alterations in OATPs and OATs expressions and transport activities. Indeed, functional changes in OATPs and OATs sometimes influence the pharmacological and toxicological effects of drugs as well as their pharmacokinetics (7, 8).

It is known that expression levels of ABC transporters are altered by intestinal I/R (9-14). These alterations occur in the intestine, liver and kidney. Moreover, pharmacokinetics of several ABC transporter substrates are altered by intestinal I/R (9, 12, 13, 15). However, there has been no report on alterations in expressions and transport activities of uptake transporters, including Oatps and Oats after intestinal I/R. In this study, we investigated the alterations in expression levels of Oatps and Oats after intestinal I/R. We also assessed transport activities of Oatps by examining excretion into bile of sulfobromophthalein (BSP), a typical substrate of Oatps.

MATERIALS AND METHODS

Chemicals

BSP was purchased from Sigma-Aldrich Japan (Tokyo, Japan). All other reagents were of the highest grade available and used without further purification.

Animals

Male Wistar rats, aged 6 weeks, were obtained from Jla (Tokyo, Japan). The rats were housed for at least 1 week (until reaching 250-350 g in weight). The housing conditions were the same as those described previously (16). 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 previous reports (10, 11, 16). The animals were anesthetized with sodium pentobarbital (30 mg/kg body weight, *i.p.* injection). Through a midline laparotomy, each rat was subjected to 30 min of ischemia by ligating small anastomosing vessels and occluding the superior mesenteric artery (SMA). Reperfusion was induced by removing the clamp. The abdomen was sutured during

reperfusion.

Assessments of biochemical parameters

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN) or creatinine were measured by a kit (Transaminase CII-Test-Wako, L type Wako UN or L type Wako creatinine F) purchased from Wako Pure Chemical Industries (Osaka, Japan).

Semi-quantitative real-time PCR

Total RNA was prepared from organs of rats using an ISOGEN (Nippon Gene, Tokyo, Japan) and an RNase-Free DNase Set (QIAGEN, Tokyo, Japan). Single-strand cDNA was made from 2 µg total RNA by reverse transcription (RT) using a ReverTra Ace (TOYOBO, Osaka, Japan). Semi-quantitative real-time PCR was performed using an ABI PRISM 7700 Sequence Detector (Applied Biosystems, Foster City, CA) with Platinum[®] SYBR[®] Green qPCR SuperMix-UDG (Invitrogen, Carlsbad, CA) as per the manufacturer's protocol. PCR was performed using rat Oatp1a1-specific primers, rat Oatp1a4-specific primers, rat Oatp2b1-specific primers or rat Oat3-specific primers through 40 cycles of 95 °C for 15 s, 50 °C for 30 s and 72 °C for 30 s; using rat Oat1-specific primers through 40 cycles of 95 °C for 15 s, 52 °C for 30 s and 72 °C for 30 s; using rat Oatp1b2-specific primers through 40 cycles of 95 °C for 15 s, 56 °C for 30 s and 72 °C for 30 s; or using rat GAPDH-specific primers all after pre-incubation at 50°C for 2 min and 95°C for 15 min. The primers specific to rOatp1a1, rOatp1a4, rOatp1b2, rOatp2b1, rOat1, rOat3 and rGAPDH were designed on the basis of sequences in the GenBank[™] database (accession no.: NM_017111, U95011, NM_031650, NM_080786, NM_017224, NM_031332 and AF106860, respectively). The sequences of the specific primers are shown in Table 1 (17-18). The PCR products were normalized to amplified GAPDH, which was the internal reference gene (housekeeping gene). Standard curves were prepared for each target and housekeeping gene. The standard curve was established between the threshold cycles (Ct) and the log₁₀(copy numbers) by using the Applied Biosystems sequence detection system software, version 1.9.1. The software calculates the relative amount of the target gene and the housekeeping gene based on the Ct.

Table 1 Primer sequences for real-time PCR

Gene	Gene bank accession	Sequences	Product size (bp)	References
rGAPDH	AF106860	Forward 5'-ATG GGAAGC TGG TCATCAAC-3' Reverse 5'-GTG GTT CAC ACC CAT CAC AA-3'	221	
rOatp1a1	NM_017111	Forward 5'-ACC TGG AAC AGC AGT ATG GAAAA-3' Reverse 5'-ACC GAT AGG CAAAT GCT AGG TAT-3'	163	St-Pierre et al., 2004
rOatp1a4	U95011	Forward 5'-TGT GAT GAC CGT TGATAA TTT TCC A-3' Reverse 5'-TTC TCC ACA TAT AGT TGG TGC TGAA-3'	81	St-Pierre et al., 2004
rOatp1b2	NM_031650	Forward 5'-CCT GTT CAA GTT CAT AGAGCA GCA-3' Reverse 5'-TGC CAT AGT AGG TAT GGT TAT ATT TC-3'	88	St-Pierre et al., 2004
rOatp2b1	NM_080786	Forward 5'-ACG ACT TTG CCC ACC ATAGC-3' Reverse 5'-CCA CGT AAA GGC GTA GCA TGA-3'	117	St-Pierre et al., 2004
rOat1	NM_017224	Forward 5'-GTG GTT GCT CCC CTA CTG CT-3' Reverse 5'-ATT CGG GTT GTC CTT GCT TG-3'	160	Komazawa et al., 2013
rOat3	NM_031332	Forward 5'-CAACAG CAC CAG AGA CAC CA-3' Reverse 5'-GAC TGAAGG CAG CAC CAG AG-3'	209	Komazawa et al., 2013

Western blot analysis

Lysates of liver homogenates were prepared from the livers of rats. The protein concentrations of these samples in clear supernatant were determined by the method of Lowry et al. with bovine serum albumin as a standard (19). Each sample was denatured at 100°C for 3 min in a loading buffer containing 50 mM Tris-HCl, 2% SDS, 5% 2-mercaptoethanol, 10% glycerol, 0.002% BPB and 3.6 M urea and separated on 4.5% stacking and 10% SDS polyacrylamide gels. Proteins were transferred electrophoretically onto nitrocellulose membranes or polyvinylidene difluoride (PVDF) membranes at 15 V for 90 min. The membranes were blocked with PBS containing 0.05% Tween 20 (PBS/T) and 10% or 1% non-fat dry milk for 1 h at room temperature. After being washed with PBS/T, the membranes were incubated overnight at room temperature with a rabbit monoclonal antibody to Oatp1a1 (Millipore, Bedford, MA) (diluted 1:500), a rabbit monoclonal antibody to Oatp1a4 (Santa Cruz Biotechnology, Santa Cruz, CA) (diluted 1:500), a goat monoclonal antibody to Oatp1b2 (Santa Cruz Biotechnology) (diluted 1:250), a rabbit monoclonal antibody to Oatp2b1 (Santa Cruz Biotechnology) (diluted 1:250) or a mouse monoclonal antibody to actin (Millipore) (diluted 1:500) and washed three times with PBS/T for 10 min each time. The membranes were subsequently incubated for 1 h at room temperature with horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (Santa Cruz Biotechnology), horseradish peroxidase-conjugated donkey anti-goat secondary antibody (Santa Cruz Biotechnology) or horseradish peroxidase-conjugated goat anti-mouse secondary antibody (Santa Cruz Biotechnology) at a dilution

of 1:2,000 and washed three times with PBS/T for 10 min each time. The bands were visualized by enhanced chemiluminescence according to the instructions of the manufacturer (Amersham Biosciences Corp., Piscataway, NJ). The densities of bands in each lane were determined densitometrically using Scion Image (Informer technologies, Inc, Abingdon, UK). The densities of Oatps were normalized to the band density of actin, which was the internal reference protein (housekeeping protein).

Intravenous administration study

The common bile duct of each rat was cannulated with polyethylene tubing (PE-10) to collect bile specimens. After intestinal I/R, a BSP solution (19.2 mg/mL) was injected at 19.2 mg/kg through the femoral vein. Blood samples were collected from a cervical vein at 1, 3, 15, 30, 45, 60, 75 and 90 min after injection. Plasma was obtained by centrifugation (850×g for 15 min at 4°C) of blood samples. Bile specimens were collected at 0-15, 15-30, 30-45, 45-60, 60-75 and 75-90 min after injection. The area under the concentration-time curve (AUC₀₋₉₀) was calculated using the trapezoidal rule from the concentration-time curve. The total clearance (CL_{tot}) and the biliary clearance (CL_{bile}) of BSP were calculated as 2-compartment model equation by Origin J: $C = A * \exp(-\alpha * t) + B * \exp(-\beta * t)$ (C; plasma concentration, t; time after administration), $CL_{tot} = D * \alpha * \beta / (A * \beta + B * \alpha)$ (D; dosage amount), $CL_{bile} = CL_{tot} * M_{bile} / D$ (M_{bile}; bile excretion amount).

Analytical procedure of BSP

One hundred µL of plasma samples and bile specimens were alkalinized by 100 µL of 1 N NaOH,

and were diluted in concentration ranging from 0 to 38.4 µg/mL by 1 N NaOH. The concentration of BSP was measured by absorbance at 580 nm using an absorption spectrometer (infinite® M200, TECAN, Yokohama, Japan).

Data analysis

Statistical significance was evaluated using unpaired Student's t-test. A value of p<0.05 was considered significant.

RESULTS

Effect of intestinal I/R on the liver and kidney

In an initial experiment, we investigated liver and kidney injuries after intestinal I/R. Plasma AST was significantly increased at 1-6 h and plasma ALT level was significantly increased at 1 h after intestinal I/R (Table 2). Although plasma creatinine level was not altered at any time after intestinal I/R, plasma BUN level was significantly increased at 1-6 h (Table 2). These results indicated that intestinal I/R induced liver and kidney dysfunction.

Alterations in mRNA levels of Oatps and Oats

Next, we assessed the hepatic Oatps and the renal Oats mRNA levels in intestinal I/R rats. Oatp1a1, Oatp1a4, Oatp1b2 and Oatp2b1 abundantly express in the rat liver. Oatp1a1, Oatp1b2 and Oatp2b1 mRNA levels in the liver were decreased at 1-6 h after intestinal I/R, but Oatp1a4 was not altered at any time (Fig. 1). On the other hand, Oat1 and Oat3 abundantly express in the kidney. Oat1 and Oat3 mRNA levels in the kidney were not altered at any time after intestinal I/R (Fig. 2).

Alterations in protein expression levels of Oatps

We investigated whether protein expression levels of Oatps correlated with mRNA levels. Oatp1a1 and Oatp2b1 protein expression levels were decreased at 1 h (Fig. 3A and 3D). These results were associated with mRNA levels. Although Oatp1b2 mRNA level was decreased at 1 h, Oatp1b2 protein expression level was not altered at any time (Fig. 3C). Oatp1a4 protein expression level, as well as mRNA level, was not altered at any time (Fig. 3B).

Pharmacokinetics of BSP after intravenous administration

BSP is a typical substrate of Oatps and about 70-80% of BSP is excreted to bile. Moreover, BSP is not metabolized by CYP enzymes and its quantity is easily determined. Based on these BSP characteristics, we used BSP for assessment of the transport activities of Oatps. Plasma concentration of BSP after intravenous administration was increased in intestinal I/R rats reperused 1 and 6 h compared with that in sham-operated rats (Fig. 4A and 4B). Moreover, BSP excretions to bile in intestinal I/R rats reperused 1 and 6 h were 0.85- and 0.89-fold decreased, respectively, though there was not statistical different (Fig. 4A and 4B). The AUC_{0-90s} in intestinal I/R rats reperused 1 and 6 h were significantly increased after intestinal I/R (Table 3). The CL_{tot}s and the CL_{bile}s in intestinal I/R rats reperused 1 and 6 h were significantly decreased after intestinal I/R (Table 3). In intestinal I/R rats reperused 24 h, the pharmacokinetics of BSP was recovered (Fig. 4C and Table 3).

Table 2 Time courses of plasma hepatic injury markers and renal injury markers

			1 h	6 h	24 h
Liver	AST	Sham	0.69±0.11	0.99±0.17	0.47±0.12
	(units/µL)	I/R	1.6 ±0.19 **	1.6 ±0.32 *	0.63±0.16
	ALT	Sham	2.8±0.15	7.7±1.8	3.9±0.83
	(units/µL)	I/R	4.3±0.30 **	8.6±0.37	4.5±1.1

Kidney	BUN	Sham	9.7± 3.0	10 ± 5.1	18 ± 7.3
	(mg/dL)	I/R	18 ± 7.3 *	23 ±11 *	15 ± 7.7
	Creatinine	Sham	3.5±0.80	2.5±0.18	2.9±0.24
	(mg/dL)	I/R	3.8±1.0	2.7±0.69	2.8±0.36

Each value represents the mean±S.D. of 3-7 rats (AST: Sham 3 rats at 1, 6 and 24 h, I/R 3 rats at 1, 6 and 24 h; ALT; Sham 3 rats at 1, 6 and 24 h, I/R 3 rats at 1, 6 and 24 h; BUN: Sham 6 rats at 1 h and 6 h and 5 rats at 24 h, I/R 7 rats at 1 h, 5 rats at 6 h and 6 rats at 24 h; Creatinine: Sham 4 rats at 1, 6 and 24 h, I/R 4 rats at 1 h and 3 rats at 6 and 24 h).

*P<0.05 significantly different from sham, **P<0.01.

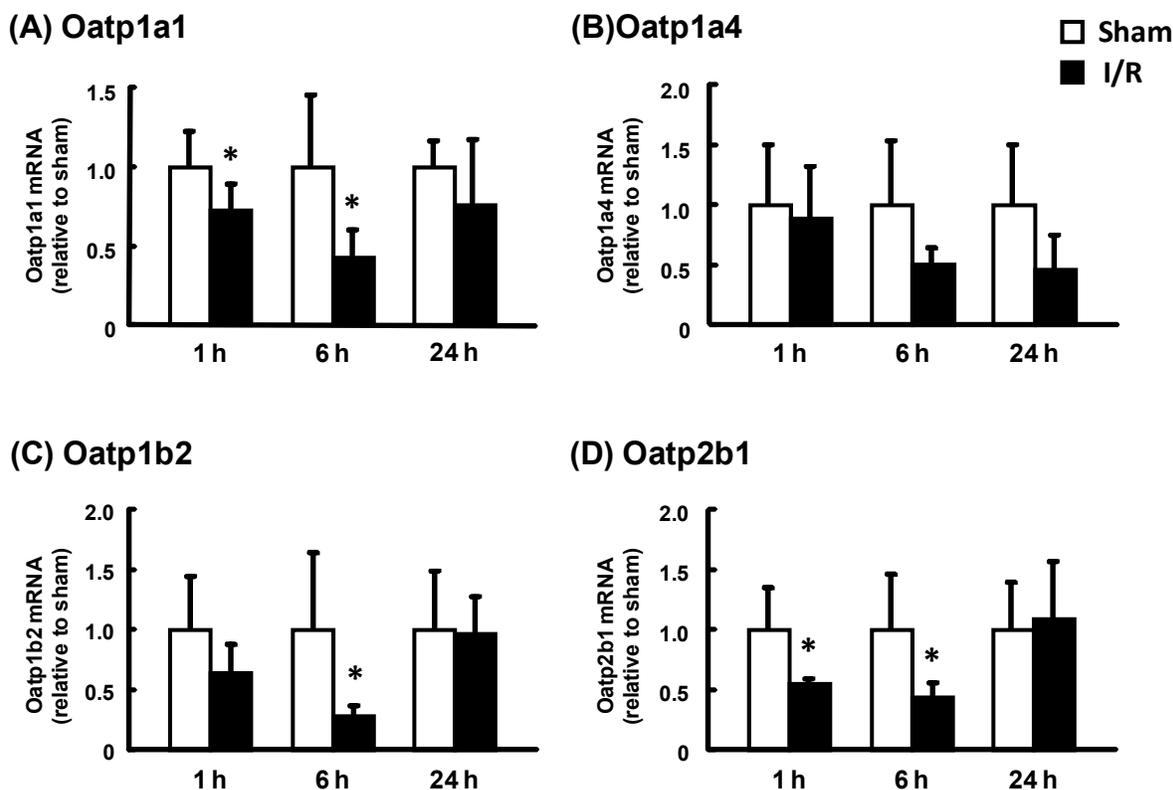


Figure 1. Time courses of the mRNA levels of Oatps in the liver after intestinal I/R. The PCR products were normalized to amplified GAPDH, which was the internal reference gene (housekeeping gene). White columns represent sham and black columns represent intestinal I/R. Each column represents the mean with S.D. of 3-6 rats (Oatp1a1: Sham 5 rats at 1, 6 and 24 h, I/R 5 rats at 1 and 6 h and 4 rats at 24 h; Oatp1a4: Sham 5 rats at 1, 6 and 24 h, I/R 4 rats at 1 h, 6 rats at 6 h and 3 rats at 24 h; Oatp1b2: Sham 5 rats at 1, 6 and 24 h, I/R 5 rats at 1, 6 and 24 h; Oatp2b1: Sham 5 rats at 1, 6 and 24 h, I/R 5 rats at 1, 6 and 24 h). * $P < 0.05$ significantly different from sham.

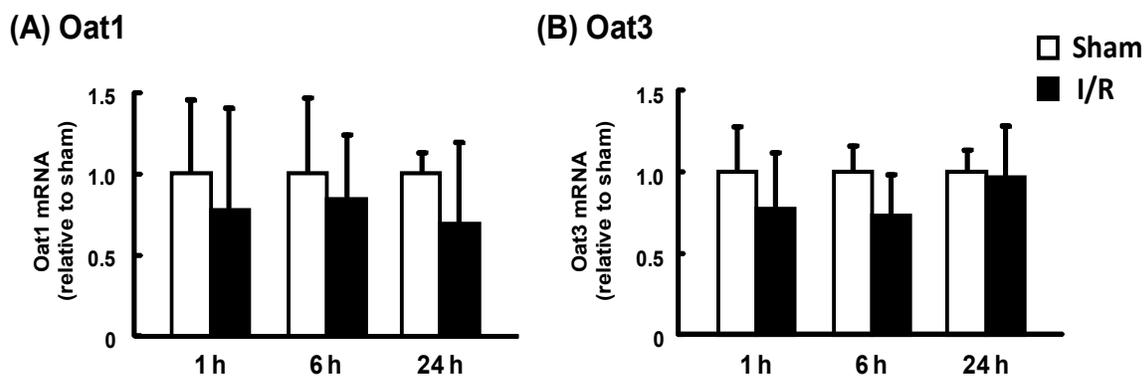


Figure 2. Time courses of the mRNA levels of Oats in the kidney after intestinal I/R. The PCR products were normalized to amplified GAPDH, which was the internal reference gene (housekeeping gene). White columns represent sham and black columns represent intestinal I/R. Each column represents the mean with S.D. of 3-5 rats (Oat1: Sham 4 rats at 1 h, 5 rats at 6 h and 4 rats at 24 h, I/R 5 rats at 1 and 6 h and 4 rats at 24 h; Oat3 Sham 3 rats at 1 h, 4 rats at 6 h and 5 rats at 24 h, I/R 3 rats at 1 h, 5 rats at 6 h and 4 rats at 24 h).

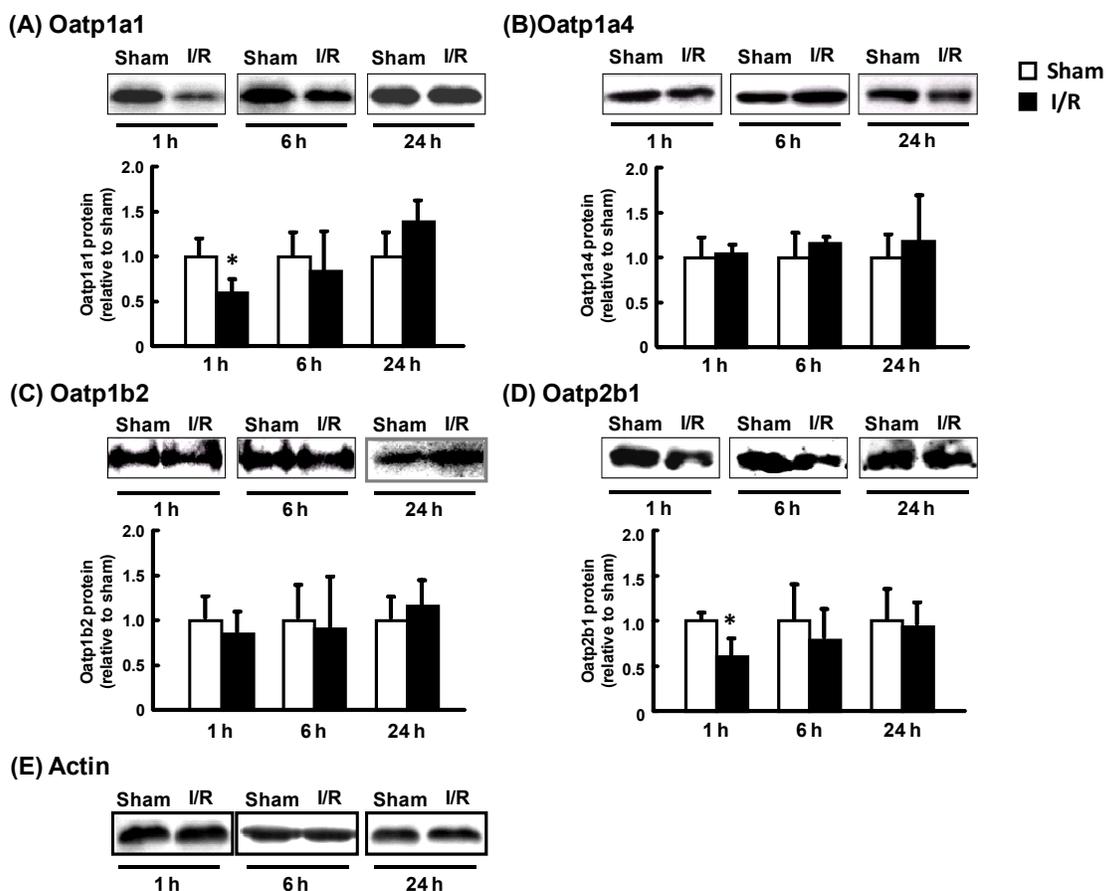


Figure 3. Time courses of the protein expression of Oatps in the liver after intestinal I/R. Whole cell lysates of rat liver at 1, 6 and 24 h after intestinal I/R were used for Western blot analysis. Western blot band intensity was determined by densitometry using Scion image program and was normalized to band intensity of β -actin, which was the internal reference gene (housekeeping gene). White columns represent sham and black columns represent intestinal I/R. Each column represents the mean with S.D. of 3-4 rats (Oatp1a1: Sham 3 rats at 1, 6 and 24 h, I/R 3 rats at 1, 6 and 24 h; Oatp1a4: Sham 3 rats at 1, 6 and 24 h, I/R 3 rats at 1, 6 and 24 h; Oatp1b2: Sham 4 rats at 1, 6 and 24 h, I/R 4 rats at 1, 6 and 24 h; Oatp2b1: Sham 3 rats at 1, 6 and 24 h, I/R 3 rats at 1, 6 and 24 h). * $P < 0.05$ significantly different from sham.

Table 3 Pharmacokinetic parameters of BSP after intestinal I/R

		AUC ₀₋₉₀ ($\mu\text{g/mL} \cdot \text{min}$)	CL _{tot} (mL/min/kg weight)	CL _{bile} (mL/min/kg weight)
1 h	Sham	740.3 \pm 278.5	14.8 \pm 3.83	10.7 \pm 3.23
	I/R	1261 \pm 580.9*	9.88 \pm 3.04*	6.21 \pm 2.47*
6 h	Sham	1024 \pm 539.6	11.5 \pm 4.53	8.71 \pm 4.76
	I/R	2104 \pm 427.0**	5.27 \pm 1.64*	3.44 \pm 0.815*
24 h	Sham	948.7 \pm 312.7	11.5 \pm 3.71	8.36 \pm 3.33
	I/R	1152 \pm 605.4	10.6 \pm 5.81	7.37 \pm 2.97

Each value represents the mean \pm S.D. of 4-8 measurements.
* $P < 0.05$ significantly different from sham.

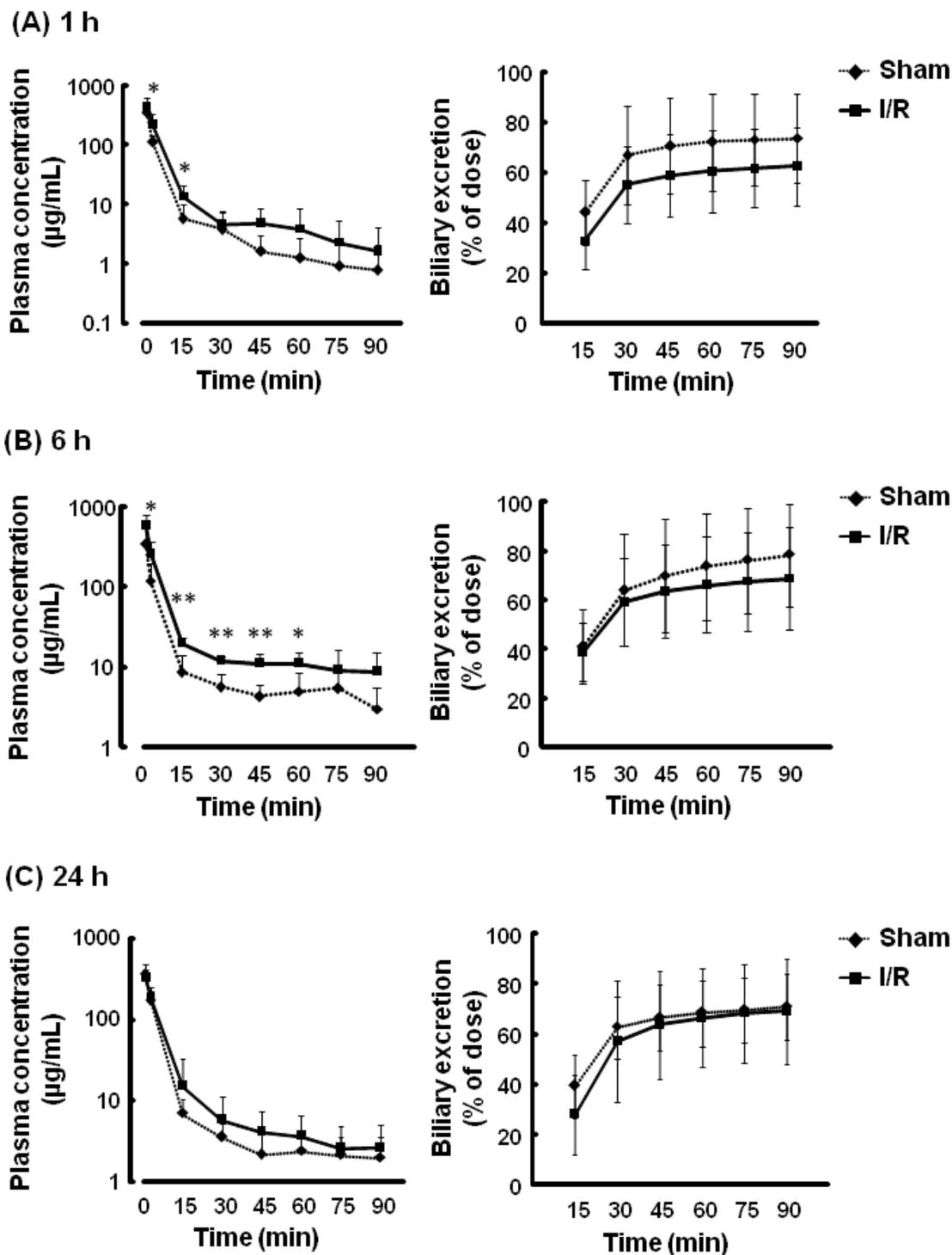


Figure 4. Time profiles of the plasma concentration and cumulative biliary excretion of intravenously administered BSP after intestinal I/R. Reperfusion times were 1 h (A), 6 h (B) and 24 h (C). BSP was injected at a single dose of 19.2 mg/kg after intestinal I/R. Blood and bile samples were collected at the specified times after BSP injection. Closed diamonds represent sham and closed squares represent intestinal I/R. Each point represents the mean with S.D. of 4-8 rats (Sham 8 rats at 1 h and 5 rats at 6 and 24 h, I/R 7 rats at 1 h, 5 rats at 6 h and 4 rats at 24 h). * $P < 0.05$ significantly different from sham, ** $P < 0.01$.

DISCUSSION

The liver and kidney are major organs for detoxification of xenobiotics, including drugs. Intestinal I/R induces severe intestinal mucosa injury. Remote organs, including the liver and kidney, are also damaged by intestinal I/R. Plasma hepatic and renal injury markers significantly increased at 1-6 h in our intestinal I/R model rats (Table 2). These results indicated that our intestinal I/R model rats could be used as a model for multi-organ failure after intestinal I/R.

In clinical practice, patients usually take many kinds of drugs at the same time. Thus, changes in pharmacokinetics of drugs that have strong pharmacological effects can lead to serious adverse consequences. Pharmacokinetics of drugs may be influenced by pathophysiological conditions (20-22). Transporters can be major determinants of the pharmacokinetic, safety, and efficacy profiles of drugs (23). Oatp1a1 and Oatp2b1 protein expression levels were decreased at 1 h after intestinal I/R (Fig. 3A and 3D). It has been known that intestinal I/R induces alteration of protein expression at early stage of reperfusion, even at 1 h after I/R (10, 12), though changes in protein expressions need a longer time than those in mRNA levels. Oxidative stress derived from reactive oxygen species (ROS) production occurs during ischemia and early stage of reperfusion (24). Thus, the production of ROS during ischemia and early stage of reperfusion may cause the alteration of Oatp1a1 and Oatp2b1 protein expression, as well as those of mRNA. Indeed, ROS are known to decrease Oatps expression (25). Although mRNA levels of Oatp1a1, Oatp1b2 and Oatp2b1 were decreased at 6 h after intestinal I/R, these protein levels in intestinal I/R rats were same as those in sham-operated rats. The decreases in Oatps expression levels by ROS were recovered with time, though mRNA levels were still decreased (25). These findings also suggested that ROS mainly caused the decreases in Oatp1a1 and Oatp2b1 expression levels after intestinal I/R. It has been also known that intestinal I/R induces the release of inflammatory cytokines, and these are known to decrease Oatps expression. We previously reported that serum concentration of IL-6 significantly increased at 3 to 6 h in our intestinal I/R model rats (11, 26). Because Oatp1a1 and Oatp2b1 expressions were decreased at 1 h after intestinal I/R, IL-6 might not cause the decrease in Oatps expressions after intestinal I/R. It has been known that Oatp2b1 expresses not only in the liver but also in the intestine (27). Although Oatp2b1 mRNA

level was not altered in the jejunum after intestinal I/R, Oatp2b1 mRNA level in the ileum was significantly decreased at 6 h after intestinal I/R (data not shown). These results suggest that the alteration of Oatp2b1 expression in the intestine might affect the absorption of Oatp2b1 substrate drugs.

BSP is well known as anionic prototype substance and commonly used as a hepatobiliary function indicator. It has been known that Oatp1a1, Oatp1a4, Oatp1b2 and Oatp2b1 transport BSP into cells. Thus, these Oatps have influences on the uptake of BSP from blood circulation to hepatocytes. At 1 h after intestinal I/R, plasma concentration of BSP was higher and biliary excretion of BSP was lower than those in sham-operated rats (Fig. 4A). These results suggested that the decreases in Oatp1a1 and Oatp2b1 expression levels at 1 h after intestinal I/R might cause the suppression of liver uptake of BSP, resulting in the high AUC₀₋₉₀, the low CL_{tot} and CL_{bile} (Table 3). The CL_{tot} and CL_{bile} values in this study are almost consistent with the previous report in other group (28). Moreover, the difference of CL_{tot} between intestinal I/R rats reperfused for 1 h and sham-operated rats was almost same as the difference of CL_{bile} (Table 3), suggesting that the low CL_{tot} value of BSP in intestinal I/R rats reperfused for 1 h was caused by the suppression of biliary excretion. Hepatic blood flow also affects pharmacokinetics of drugs, as well as uptake to hepatocytes by transporters. Hepatic bile flow is reduced at early stage of reperfusion (29). This finding suggested that decrease in hepatic blood flow might be another factor of the decrease in CL_{tot} and CL_{bile} of BSP. Although Oatps protein expression was recovered at 6 h (Fig. 3), pharmacokinetics of BSP was affected by intestinal I/R (Fig. 4B and Table 3). It has been known that MRP2/Mrp2, which expresses on the canalicular membrane, has an influence on the efflux BSP from hepatocytes to bile. In our previous study, Mrp2 expression in the liver was decreased at 6 h after intestinal I/R (11). Thus, the low biliary excretion of BSP in intestinal I/R rats reperfused for 6 h (Fig. 4B and Table 3) might be caused by the decrease in Mrp2 expression (11). When Mrp2 function is suppressed, Mrp3 excretes Mrp2 substrates from hepatocytes to blood circulation. Thus, BSP accumulated in the hepatocytes might be efflux to blood by Mrp3, resulting in the high plasma concentration of BSP in intestinal I/R rats reperfused for 6 h (Fig. 4B and Table 3). Oatp1a1 expresses in the kidney (27), as well as in the liver, and has an influence on a renal reabsorption of

organic anion compound (30). However, the urinary excretion of BSP was much lower (0.3% of dose) (31) than the biliary excretion (70-80% of dose). This finding suggests that renal Oatp1a1 has little influence on a BSP pharmacokinetics.

AST and ALT levels in sham-operated rats at 6 h were highest than those in sham-operated rats at the other times (Table 2). AST level in sham-operated rats at 6 h was significantly increased than that at 1 h or 24 h (p values of both 1 h vs 6 h and 6 h vs 24 h were less lower 0.01 using one-way ANOVA followed by Tukey's test). We did not investigate the factors inducing this alteration by sham-operation, but it is possible that laparotomy may have contributed to this increment. Indeed, several workers reported that laparotomy induced the stress, and produced several stress hormones, particularly catecholamine and adrenocorticotropin (ACTH) (32, 33).

In conclusion, Oatp1a1 and Oatp2b1 protein expression levels are decreased by intestinal I/R. The decrease in these transporters causes high plasma concentration and low biliary excretion of organic anion compound. The newly found influence of intestinal I/R on the expression and function of Oatps may be a key to perform appropriate drug therapy.

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