Effects of Intestinal Ischemia/Reperfusion on P-Glycoprotein Mediated Biliary and Renal Excretion of Rhodamine123 in Rat

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Summary: To analyze the effects of I/R on P-gp function in liver and kidney, biliary and urinary excretions of rhodamine123 as a substrate of P-gp were examined in rats. The effects of reperfusion time on change and recovery of P-gp function were also examined. The biliary and renal clearance of rhodamine123 significantly decreased at 3 hr after reperfusion, but returned to control levels at 24 hr after reperfusion. These results suggest that intestinal I/R-induced decrease in P-gp-mediated biliary and renal excretion of rhodamine123 is likely due to impairment of P-gp-mediated transport ability. The level of P-gp protein in liver decreased and that of iNOS mRNA increased at 3 hr after reperfusion and both levels returned to control levels at 24 hr after reperfusion. No marked change in the levels of P-gp protein and iNOS mRNA was observed in kidney at 3 hr and 24 hr after reperfusion. Thus, decrease in biliary excretion of rhodamine123 would appear due in part to decrease in expression of P-gp, caused by increase in lipid peroxidation levels through iNOS mRNA.

Keywords: intestinal ischemia/reperfusion; liver; kidney; P-glycoprotein-mediated transport; inducible nitric oxide synthesis (iNOS)

Introduction

Intestinal ischemia injury is an important clinical problem in several disorders. Intestinal ischemia leads to depletion of cellular energy and accumulation of toxic metabolites, resulting in cell damage and death. Reperfusion exacerbates ischemia-induced mucosal injury via synthesis of reactive oxygen species (ROS), which is connected to neutrophil infiltration and release of inflammatory reaction mediators. Intestinal mucosal lesions after ischemia and reperfusion (I/R) injury include: loss of activity in brush border enzyme, cellular death (necrosis and apoptosis) and increase in intestinal permeability. I/R injury of the intestine and other organs have been found related to decrease in drug metabolism activity.

Important intestinal drug absorption, transport and metabolism after oral administration for drug bioavailability has been documented for many compounds. The liver and kidney have crucial functions for the elimination of endogenous and exogenous substances transported by ATP Binding Cassette (ABC) transporter such as P-gp and converted to more hydrophilic compounds by cytochrome P450 and/or conjugating enzyme to be excreted into the bile or urine.

Although the sequence of development of intestinal mucosal injury after I/R has been studied intensively, most studies have focused on the relatively late phase after reperfusion. We established an in vivo system for assessing early I/R injury using rat intestine, in which lipid peroxidation caused by hanging artery and vein acts as a trigger of the injury. Using this model, we showed that reperfusion for 60 min after ischemia for 60 min induces dysfunction of intestinal P-glycoprotein (P-gp) in a recent paper. In particular, we elucidated the quantitative evaluation of P-gp dysfunction using rhodamine123 as the substrate of P-gp and cyclosporine A, verapamil as an inhibitor of P-gp.

This study clarifies whether intestinal I/R modifies P-gp-mediated biliary and renal transport system in rats. Rhodamine123 was chosen as the model drug, since this compound is primarily excreted into the bile and urine in unchanged form. To evaluate the contribution of P-gp-mediated transport to the hepatobiliary and renal excretion of rhodamine123, we used western blotting to measure the expression of P-gp protein levels isolated from the liver and kidney at different times after reperfusion.
with ischemia for 60 min. We measured the expression of mRNA of inducible nitric oxide synthesis (iNOS) using RT-PCR, a trigger of lipid peroxidation based on oxidative stress.

Materials and Method

Materials: Rhodamine123, cyclosporine A, verapamil, tetraethylammonium (TEA) and cimetidine were purchased from Sigma Co. Ltd. (St. Louis, MO). All other reagents were of analytical grade or better.

Animals and Experimental Design: Male Wistar rats (eight weeks old) were purchased from Japan SLC Ltd. (Shizuoka, Japan). All animal experiments were performed according to the guidelines of Tokyo University of Pharmacy and Life Sciences. The animals were fasted for 18 hr before starting the experiment. Water was freely given while fasting. Rats were anesthetized by Nembutal (pentobarbital sodium, 50 mg/kg) and cannulated with polyethylene tubes in the right jugular vein for rhodamine123 administration and blood collection. All experiments were done under Nembutal anesthesia and body temperature was maintained at 37°C with a heat lamp. The superior mesenteric artery and vein in rat were occluded by hanging using surgical-sutures (Natsume No. 3) connected to a spring balance for 60 min as indicated in the previous paper10) (ischemia condition), followed by reperfusion based on cutting of sutures (reperfusion condition). Based on a previous paper,19 the hanging forces of blood vessel during ischemia used in this study were 50 g and 100 g. The fifty and 100 g loads of hanging force were designated as the 50 g load and 100 g load groups, respectively.10 For rhodamine123 clearance experiments, control rats (sham operation group) and rats treated by ischemia for 1 hr with 50 g load and 100 g load were used for reperfusion. Biliary and renal excretion of rhodamine123 was investigated in the control and treated rats at different times after reperfusion.

Experiments of Biliary and Renal Excretion: Cannulation (silicone tubing, Silascon® Kaneka Medix Co.) was conducted on the jugular vein for administration and sampling. The bile duct and urinary bladder were also cannulated for bile and urine collection, respectively. Bile and urine were collected in preweighted tubes at 20-min intervals for 60 min throughout the experiment. Rhodamine123 dissolved in saline was administrated by i.v. injection, as a bolus with a volume of 2.8 mL/kg, followed by an injection of saline at the same volume via the cannula inserted in the jugular vein. Blood samples were taken at the midpoints of the bile and urine collection periods. Plasma samples were obtained by centrifugation of the blood samples at 3,000 × g for 10 min. The volumes of bile and urine samples were measured gravimetrically. All plasma, bile and urine samples were stored at −40°C until analysis.

Assay of Rhodamine123 in Bile and Urine: Bile and urine samples were diluted with saline solution 1000 and 10 fold, respectively. The diluted samples were determined at 485 nm and 546 nm for excitation and emission, respectively, with a fluorescence spectrometer (HITACHI FP6500, Tokyo, JAPAN).

Pharmacokinetics analysis: Pharmacokinetics parameters such as total clearance (CLtot) were calculated dividing the dose by area under the plasma concentration curve obtained by the program МULTI. The biliary clearance (CLbile) of rhodamine123 during each bile collection period was calculated by dividing the biliary excretion rate by plasma concentration obtained at the mid time point for that collection period of bile excretion. The renal clearance (CLr) of rhodamine123 was calculated by dividing the renal excretion rate by plasma concentration obtained for that collection period. The effects of intestinal I/R on the biliary and renal excretion of rhodamine123 were investigated at 3 and 24 hrs after reperfusion. The pharmacokinetic parameters at 3 and 24 hrs in text represent the values calculated from the data at 3 to 4 hr and 24 to 25 hr after reperfusion, respectively. The pharmacokinetics parameters in both liver and kidney evaluated by the excretion data from 3 to 4 hr and those from 24 to 25 hr after reperfusion are discussed below.

Evaluation of P-gp Mediated Transport: To elucidate the effects of cyclosporine A (0.3 mg/kg), cimetidine (0.03 mg/kg), TEA (0.02 mg/kg) and verapamil (0.06 mg/kg) on the biliary and renal excretion of rhodamine123 (0.01 mg/kg), each drug and rhodamine123 were intravenously coadministered. Blood, bile and urine were collected as described above.

Estimation of relative P-gp activity: The relative activities of P-gp were obtained as follows:10

\[
P_{\text{pass}} = \frac{(P_{\text{app}} - P_{\text{pass}})P_{\text{pass}}}{P_{\text{app}}}
\]

Ppass is the excretion clearance of rhodamine123 with inhibitor and Papp is the excretion clearance of rhodamine123 without the inhibitor in liver and kidney. In the present study, the values calculated from \((P_{\text{app}} - P_{\text{pass}})P_{\text{pass}}\). Ppass and Papp represent the excretion clearance or rhodamine123 with or without cyclosporine A in liver or verapamil in kidney, respectively.

Western Blotting: The liver and kidney were isolated in each rat at different times after reperfusion. The fractions of renal brush border membranes and bile canaliclar membranes were prepared using magnesium chloride precipitation10,12,13,15,16) Briefly, isolated kidneys were homogenized in a buffer containing 0.05 mg/mL phenylmethylsulfonylfluoride, 300 mM mannitol, 12 mM Tris and 5 mM EGTA (pH 7.1) with a tissue homogenizer. 10 mM aqueous magnesium chloride solution was added to the homogenate. The homogenate was centrifuged at 3,000 g for 10 min. The supernatant was then centrifuged at 42,000 g for 30 min. The pellet was
resuspended in 300 mM mannitol, 20 mM HEPES, 10 mM Tris and 4 mM magnesium chloride (pH 7.4). For bile canalicular membranes, thin liver slices were added to chilled buffer (300 mM mannitol, 5 mM EGTA, 18 mM Tris-HCl, 0.1 mM PMSF, at pH 7.4). The slices were homogenized using a polytron in 15 mL buffer for 45 sec. The polytron power output was closely regulated at 4,800 rpm with a powerstat. The solution was centrifuged at 48,000 g for 30 min. The resulting pellet was resuspended in buffer and 15 mM Mg²⁺ precipitation was followed by centrifugation for 15 min at 2,445 g. The supernatant was centrifuged at 48,000 g for 30 min to obtain the canalicular fraction. The protein expression levels of P-gp in the bile canalicil and renal brush border membrane fractions were evaluated by Western blotting. Western blotting using C219 monoclonal antibody (Alexis) for P-gp was performed as reported previously.(13)

**Preparation of RNA and cDNA Synthesis:** Total RNA was isolated from liver and kidney specimens using TRIzol reagents (Invitrogen Co. LTD., Paisly, UK) according to the manufacturer's instructions. Complementary DNA (cDNA) was prepared from total RNA using ABI Prism 7000 for real-time PCR according to the manufacturer's instructions. The two-step reaction mixture contained 2 µg RNA, 100 ng random hexamers, 0.5 mM dNTP mix (dATP, dCTP, dGTP, dTTP), 10 mM Tris-HCl (pH 8.4), 25 mM KCl, 5 mM MgCl₂, 10 mM DTT and 10 units RNaseOUT recombinant ribonuclease inhibitor.

**Analysis of mdr1a nNOS and iNOS Gene Expression in Intestines by Quantitative Reverse Transcription-Polymerase Chain Reaction (RT-PCR):** To perform real-time PCR, 96 well reaction plates with optical adhesive covers and ABI PRISM 7000 Sequence Detection System were used. Assay on-Demand Gene Expression Products were purchased for GAPDH, mdr1a, nNOS and iNOS (Table 1). Reverse transcription was performed for 1 µg of RNA using a CDNA High Capacity Archive kit (Applied Biosystems, Foster City, CA, USA) and random hexamers as primers Quantitative PCR was performed on an SDS 7000 system from Applied Biosystems using a Universal MasterMix (Applied Biosystems). PCR-conditions were 10 min at 90°C, followed by 40 cycles of 15 sec at 95°C and 1 min at 60°C. All assays were RNA-specific (spanning exon-exon junctions) pre-designed TaqMan Gene Expression Assays from Applied Biosystems (Table 1).

**Statistical analysis:** All results were expressed by means ± standard error (Mean ± S.E.). Statistical significance between two groups was analyzed using the Dunnett test, Tukey test and Student t test. P less than 0.1, 0.05 and 0.01 was considered significantly different.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence (5’-3’)</th>
<th>Amplicon Size</th>
<th>GeneBank ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
<td>Forward TAGGTTGACCCATCTTCTTG</td>
<td>102 bp</td>
<td>NM-017008</td>
</tr>
<tr>
<td>Reverse</td>
<td>TGGTAAACCGGCTCCGATA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>iNOS</td>
<td>Forward CTTGGTGAACCGGTTGTCCT</td>
<td>101 bp</td>
<td>5-71597</td>
</tr>
<tr>
<td>Reverse</td>
<td>CGACTCTTCCTGTCATGAGCA</td>
<td></td>
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<td>nNOS</td>
<td>Forward GCCCACTGCTCAACCTCGAA</td>
<td>122 bp</td>
<td>AJ-305233</td>
</tr>
<tr>
<td>Reverse</td>
<td>CACATGACCCCGGAAAGG</td>
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</table>

**Results**

**Effects of cyclosporine A, verapamil, TEA and cimetidine on biliary and renal excretion of rhodamine 123:** To investigate whether the active biliary secretion of rhodamine123 is mediated mainly by P-gp, the effects of the P-gp substrates, cyclosporine and verapamil and a typical organic cation transporter substrate, cimetidine and TEA, on the biliary and renal secretion of rhodamine123 were investigated in normal rats. As shown in Figure 1, cyclosporine A and verapamil significantly inhibited Cl_bile and Cl_r of rhodamine123, respectively, but cimetidine and TEA did not have inhibitory effect on either organ (Fig. 1).

**Effects of intestinal I/R on biliary and renal excretion of rhodamine 123:** Time-dependent effects of intestinal I/R on the biliary and renal excretion of rhodamine123 in rats after reperfusion are presented in Table 2. Twenty-four hr after intestinal I/R of 50 g and 100 g load groups, Cl_bile of rhodamine123 decreased (0.035 to 0.021 ml/min) and increased (0.035 to 0.040 ml/min), although the differences failed to reach the 5% level of statistical significance (0.05 < P < 0.1). No change in Cl_r of rhodamine123 was observed 24 hr after reperfusion (Table 2). In contrast, significant reduction in Cl_bile and biliary excretion rate of rhodamine123 was observed in rats 3 hr after reperfusion (Table 2). Cl_r of rhodamine123 also significantly decreased in rats 3 hr after reperfusion (Table 2).

**Effects of intestinal I/R on P-gp protein in liver and kidney:** We examined the levels of P-gp protein expression in liver and kidney at different times after reperfusion. Decrease in P-gp expression level in bile canalicil membrane fraction at 3 hr after reperfusion was observed with the 50 g load during ischemia compared with control conditions (Fig. 2a). The 100 g load significantly induced decrease in P-gp expression at 3 hr after reperfusion (Fig. 2a). The differences failed to reach the 10% level of statistical significance between 50 g and 100 g load groups at 24 hr after reperfusion. The recovery of decreased P-gp expression was observed in the 50 g load group at 24 hr after reperfusion, but not in the 100 g load group (Fig. 2a). No change in P-gp ex-
Fig. 1. Inhibitory effects of cyclosporine A, verapamil, cimetidine and tetraethylammonium on biliary (a) and renal (b) excretion clearance (CL_{bile}, CL_{r}) of rhodamine123

Data represent means and S.E. (n = 6 - 13 for each condition). ## significantly different from control value (p < 0.05, t-test). * Significantly different from control value (P < 0.05, Dunnett). N.S.: not significantly different from control value.

Table 2. Effects of intestinal I/R on biliary and renal excretion of rhodamine 123 in rats

<table>
<thead>
<tr>
<th>Time after Reperfusion</th>
<th>CL_{bile} (mL/min)</th>
<th>Biliary excretion rate (ng/min)</th>
<th>Bile flow rate (mL/min)</th>
<th>CL_{r} (mL/min)</th>
<th>Urinary excretion rate (ng/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hr control</td>
<td>0.035 ± 0.004</td>
<td>0.334 ± 0.031</td>
<td>0.887 ± 0.042</td>
<td>0.040 ± 0.0067</td>
<td>0.659 ± 0.240</td>
</tr>
<tr>
<td>3 hr 50 g</td>
<td>0.004 ± 0.001**</td>
<td>0.165 ± 0.025**</td>
<td>0.387 ± 0.064**</td>
<td>0.016 ± 0.008*</td>
<td>0.261 ± 0.119**</td>
</tr>
<tr>
<td>100 g</td>
<td>0.005 ± 0.001**</td>
<td>0.179 ± 0.032**</td>
<td>0.403 ± 0.041**</td>
<td>0.010 ± 0.0078**</td>
<td>0.101 ± 0.069**</td>
</tr>
<tr>
<td>24 hr 50 g</td>
<td>0.021 ± 0.004</td>
<td>0.316 ± 0.033</td>
<td>0.968 ± 0.045</td>
<td>0.032 ± 0.015</td>
<td>0.938 ± 0.341</td>
</tr>
<tr>
<td>100 g</td>
<td>0.040 ± 0.004</td>
<td>0.359 ± 0.047</td>
<td>0.893 ± 0.066</td>
<td>0.042 ± 0.012</td>
<td>1.001 ± 0.400</td>
</tr>
</tbody>
</table>

Values are means ± S.E. (n = 6 - 10 for each condition).
*p < 0.05 and **p < 0.01 represent significantly different from control and 24 hr values.

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Expression levels of the renal brush border membrane fraction was observed at any time after reperfusion (Fig. 2b).

Effects of cyclosporine and verapamil on CL_{bile} and CL_{r} of rhodamine123 at 3 hr and 24 hr after reperfusion: Decrease in CL_{bile} and CL_{r} at 3 hr after reperfusion represented by open column was observed in the intestinal I/R group compared with normal rats, respectively. These results correspond well with the relative activity of P-gp (Liver: CTRL: 1.07 vs 50 g: 0.362, 100 g: 0.173; Kidney: CTRL: 1.45 vs 50 g: 0.215, 100 g: 0.105). Cyclosporine and verapamil represented by closed column had no inhibitory effect on the above CL_{bile} and CL_{r} (Fig. 3a and b). In contrast, cyclosporin A had inhibitory effect on CL_{bile} of rhodamine123 at 24 hr after reperfusion in the intestinal I/R groups (Fig. 4a). Significant difference in CL_{bile} observed between the control and 100 g load groups was not observed between the control and 50 g load groups (Fig. 4a open column), indicating that the excretion ability of liver in the 50 g load group returned to normal. Also, no marked change in CL_{r} was observed in the intestinal I/R group compared with the control (Fig. 4b open column). CL_{r} of rhodamine123 in the presence of verapamil decreased compared with the absence of verapamil, although the difference failed to reach the 10% level of statistical significance in control and 50 g load groups (Fig. 4b). Significant difference in verapamil efficacy was seen in the 100 g load group (Fig. 4b). Comparing the relative activity of P-gp between 3 hr and 24 hr after reperfusion, the recovery of relative activity of P-gp at 24 hr after reperfusion was observed (Liver: 50 g: 0.362 vs 1.79, 100 g: 0.173 vs 2.80, Kidney: 50 g: 0.215 vs 1.31, 100 g: 0.105 vs 1.33) (Fig. 3 and Fig. 4). Decrease in CL_{bile} and CL_{r} at 1 hr after reperfusion was observed compared with the control, and cyclosporine and verapamil did not have an inhibitory effect on decrease in CL_{bile} and CL_{r}, respectively (data not shown).

Effects of intestinal I/R on expression level of iNOS and nNOS in liver and kidney tissues: Time-dependent changes in the expression of iNOS and nNOS as a mediator after intestinal I/R are shown in Figure 5. At 3 hr after reperfusion, iNOS mRNA in liver increased in a load depending manner during ischemia (Fig. 5a). These increased expression levels of iNOS mRNA
Fig. 2. Effects of intestinal I/R on expression levels of P-gp in liver bile canaliculicular membrane fraction (a) and renal brush border membrane fraction (b). Data represent means and S.E. (n = 4–6 for each condition). # significantly different from control value (p < 0.1, t-test). N.S.: not significant from control value.

Discussion

The present study focuses on the effects of intestinal I/R on P-gp mediated biliary and renal excretion of the P-gp substrate, rhodamine123. No change in the level of P-gp protein in kidney was observed in rats at 3 hr and 24 hr after reperfusion (Fig. 2b). However, CLr of rhodamine123 significantly decreased in rats at 3 hr after reperfusion (Table 2) and verapamil had no inhibitory effect on decrease in CLr of rhodamine123 (Fig. 3b). In contrast, recovery to the control level of CLr (Table 2) and inhibition by verapamil were observed in rats at 24 hr after reperfusion compared with the absence of verapamil (Fig. 4b). The verapamil had significantly inhibitory effect on CLr of rhodamine123 in normal rats (Fig. 5a). No marked changes in iNOS were observed in kidney at 3 hr or 24 hr after reperfusion (Fig. 5b). The expression of nNOS mRNA could not be found in liver or kidney, at 3 hr or 24 hr (data not shown).
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1b), indicating rhodamine123 to be excreted into the urine by active tubular secretion. Intestinal I/R decreased the tubular secretion of rhodamine123 at 3 hr after reperfusion, suggesting renal P-gp-mediated secretory function to be reduced by intestinal I/R in the brush border membrane of renal proximal tubular cells continues up until at least 3 hr after reperfusion.

Some data on whether the biliary excretion of rhodamine123 is mediated by P-gp are available.23) The present study using in vivo clearance experiments shows that the P-gp substrate, cyclosporine A, inhibits the biliary excretion of rhodamine123 (Fig. 1a), whereas cimetidine and TEA, not P-gp substrates, do not (Fig. 1a). These results suggest that rhodamine123 is mainly excreted into the bile by P-gp, supporting the report that biliary excretion of P-gp substrates was noted to decreased in Mdr1a knockout mice.26) In our experiments, significant reduction of P-gp mediated biliary clearance of rhodamine123 was observed at 3 hr but not at 24 hr after reperfusion together with change in of the protein level of P-gp (Table 2 and Fig. 2a). P-gp-mediated biliary secretory function in the bile canalicular membrane of hepatocytes thus appears to be impaired at 3 hr after reperfusion and to recover to the control level by 24 hr (Table 2 and Fig. 2a).

The relative role of P-gp in permeability may be evaluated as described in Materials and Methods.10) The relative activity decreased in both the 50 g and 100 g load groups compared with the control in both liver and kidney at 3 hr after reperfusion (Fig. 3). These relative activities of P-gp were significantly lower in the 100 g load group than the 50 g load group in both liver and kidney,
indicating decrease in relative activity of P-gp by I/R to be dependent on loading force during ischemia (Fig. 3). In contrast, the relative activity of P-gp in liver and kidney at 24 hr after reperfusion improved (Fig. 4). The relative activities of P-gp in both liver hepatocyte and kidney were higher in both I/R groups compared with the control (Fig. 4), indicating that the relative activity of P-gp increased.

We have shown that decrease in P-gp function observed in the previous in vitro and in vivo study corresponds with the production level of thiobarbituric application of acid reactive substance (TBA-RS) as an indicator of lipid peroxidation. Increases in iNOS, including superoxide anion, hydrogen peroxide, hydroxyl radical etc., induces increase in the level of lipid peroxidation and the elevation of lipid peroxidation may play an important role in intestinal I/R-induced change in certain transporter-mediated biliary and urinary excretion systems. Iseki et al. reported increase in IL6 and TNFα to induce decrease in the level of mdr1a mRNA. Evidence has been given that TNFα induces up-regulation of transporter genes or MRP1 protein in human colon carcinoma cells and Mdr1 in rat hepatoma cells. Anti-IL-1 or anti-TNFα antibody has been found to restore down-
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Fig. 5 Effects of intestinal I/R on level of iNOS mRNA in liver (a) and kidney (b) at 3 hr and 24 hr after reperfusion
Data represent means and S.E. (n = 4 for each condition). # significantly different from control value (p < 0.1, t-test). N.S.; not significantly different from control value.

regulation in expression of cMOAT/MRP2.28) However, the roles of these cytokines and some mediators, such as nitric oxide (NO) and platelet-activating factor, in the physiological function of the drug transporters remain unclear. We therefore focused on the role of iNOS in intestinal I/R-induced decrease in the biliary and urinary clearance of rhodamine123. Increase in iNOS at 3 hr after reperfusion and recovery to the control level at 24 hr after reperfusion were observed in liver, but not in kidney (Fig. 5). This suggests the possible involvement of iNOS in decreased P-gp-mediated biliary transport of rhodamine123. It is possible that some factor other than iNOS is involved in functional impairment of the renal rhodamine123 excretion.

The effects of intestinal I/R on the expression of mRNA of other transporters located in canalicular membranes of hepatocytes remain unclear, although it has been reported that intestinal I/R may induce the down-regulation of mrp2 mRNA in liver and kidney at 6 hr after reperfusion.25) Also, biliary excretion of P-gp substrates decreases in mdr1a knockout mice.29) We thus investigated the effects of intestinal I/R on the expression of the level of P-gp protein in liver and kidney. Western blot analysis revealed that the expression of P-gp protein did not change in kidney at any time after reperfusion compared with the control. In liver the expression of P-gp protein decreased at 3 hr and returned at 24 hr after reperfusion, respectively (Fig. 2). Iseki et al. reported that intestinal I/R decreased the expression of mdr1a mRNA in rat liver and kidney at 6 hr after reperfusion. The discrepancy between their results and ours is not clear at present, although it may be a result of the different methods including ischemia preparation, of different time such as 3 hr and 6 hr, and/or differences in the protein level of P-gp and the level of its encoding mRNA. P-gp expression level was examined by western blot analysis using C219. Three multi-drug resistance (mdr) genes have been reported in rodents: mdr1a (or mdr3), mdr1b (mdr1), and mdr2, where mdr1a and mdr1b genes encode a phospholipid translocator.30) C219 used as anti-P-gp antibody cross-reacts with products of these three mdr genes. Also in the western blotting, C219 recognizes sister P-gp, a major bile canalicular salt export pump (bsep) of mammalian liver closely related to the P-gp family.31) It is reported that sister P-gp is expressed in the intestine of rats.32) Therefore, not only P-gp but also sister P-gp may change in rat bile canalicular membrane of hepatocyte and kidney by intestinal I/R in the present study.

It has been suggested that several endogenous modulators of P-gp exist in human plasma.32) There is a possibility that the production of endogenous P-gp substrates in plasma of rats caused by intestinal I/R is associated with intestinal I/R-induced decrease in P-gp-mediated biliary and renal excretion of rhodamine123.

In conclusion, the present study is the first to use in vivo clearance experiments and western blot analysis to reveal that intestinal I/R decreases P-gp-mediated biliary excretion and renal excretion of rhodamine123. Decrease in the biliary excretion is partly elucidated by decreasing the protein level of P-gp, but that in the renal excretion is not. iNOS may be at least one component associated with intestinal I/R-induced decrease in excretion in the biliary excretion of rhodamine123. Further studies are needed to clarify the roles of other factors besides iNOS in reduced renal excretion of rhodamine123 and in the level of P-gp in intestinal I/R.

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References


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