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Effect of lipopolysaccharide on P-glycoprotein-mediated intestinal and biliary excretion of rhodamine123 in rats

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ABSTRACT

The effects of lipopolysaccharide (LPS) on the ileal and biliary excretion of rhodamine123 were investigated in rats at different times after intraperitoneal (i.p.) injection (1 mg/kg and 5 mg/kg of body weight). P-gp protein decreased 8 h after injection of LPS and returned to the control level 24 h after i.p. injection of LPS in the ileum. There was a marked decrease in the expression level of mdr1a mRNA in the ileum and liver 8 h after i.p. injection of LPS when compared with the control condition. Also, the ileal and biliary clearance of rhodamine123 significantly decreased 8 h after i.p. injection of LPS, but returned to the control levels 24 h after i.p. injection of LPS. These results suggest that LPS-induced decreases in P-gp-mediated ileal and biliary excretion of rhodamine123 were probably due to impaired P-gp-mediated transport ability. The levels of iNOS and IL-1β mRNA in the ileum and liver increased 2 and 8 h after i.p. injection of LPS, respectively, and returned to the control levels 24 h after injection of LPS. These findings suggest that LPS markedly decreases P-gp-mediated ileal and biliary excretion of rhodamine123, probably by partly decreasing the expression of P-gp protein levels, likely due to increased lipid peroxidation levels through iNOS mRNA and inflammatory mediators such as IL-1β.

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1. Introduction

Endotoxin, which is an active component of the cell wall of Gram-negative bacteria, is well known to damage numerous organs, including the intestine and liver, which have crucial functions for the absorption and elimination of endogenous and exogenous substances. These are converted to more hydrophilic compounds by cytochrome P-450 and/or conjugating enzyme, and then excreted into the bile and urine. A series of studies suggested that endotoxin reduces biliary excretion of various organic anionic drugs as a result of changes in the biliary secretory systems (Hasegawa et al., 1985; Haghgoo et al., 1995; Nadai et al., 1996). Also, it has been demonstrated that endotoxin reduces hepatic cytochrome P-450-dependent drug-metabolizing enzymes 24 h after intraperitoneal (i.p.) injection in rats, due to the overproduction of nitric oxide in plasma (Kitaichi et al., 1999); however, less is known about the effect of endotoxin treatment on intestinal absorption and the hepatobiliary transport system.

It is well known that hepatobiliary excretion of organic anionic drugs is mediated by a bile canalicular multispecific organic anion transporter (cMOAT), which belongs to the ATP-binding cassette transporter superfamily (Ito et al., 1997). Another important transporter, P-glycoprotein (P-gp), acts as an ATP-dependent efflux pump for various drugs, such as vinca alkaloid and anthracycline anticancer drugs, calcium blocker, immunosuppressive agents, and macrolide antibiotics (Tsuruo et al., 1982; Yamashita et al., 1987; Wang et al., 2000), in normal tissues such as the brain, liver, intestine and kidney (Fojo et al., 1987; Thiebaut et al., 1987; Kamimoto et al., 1989; Hsing et al., 1992; Schinkel et al., 1994). Interestingly, there is evidence that the substrate specificities of P-gp, cytochrome P-450 3A4 (CYP3A4), and cMOAT overlap, and these proteins are located in hepatocytes and have similar functions for removing various drugs from the body (Mayer et al., 1995; Oude et al., 1995; Wacher et al., 1995; Kiso et al., 2000). On the basis of these observations, endotoxin may possibly modify the pharmacokinetics of drugs mediated by P-gp; however, there is little information regarding the effect of endotoxin on P-gp-mediated absorption and the biliary transport system and the expression of P-gp transport genes.

The aim of the present study was to clarify whether lipopolysaccharide (LPS) modifies P-gp-mediated intestinal absorption and the biliary transport systems in rats. Rhodamine123 is a substrate of P-gp and thus, it was chosen as the model drug in this study. This compound is primarily excreted into the intestinal lumen and bile in unchanged form (Kunihara et al., 1998). In order to evaluate the contribution of P-gp-mediated transport to the luminal and hepa-

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tobiliary excretion of rhodamine123, we used reverse transcriptase PCR (RT-PCR) to measure the expression of mRNA of the multidrug resistance proteins 1a and 1b (Mdr1a and Mdr1b), which are members of the P-gp subfamily, isolated from the ileum and liver at different times after i.p. injection of LPS.

2. Materials and methods

2.1. Materials

LPS was isolated from *Escherichia coli o111:B4*, which was identical to that used in this study. Rhodamine123 was purchased from Sigma Co. Ltd. (St. Louis, MO). All other reagents are commercially available and were of analytical grade or better.

2.2. Animal and experimental protocol

Eight nine-week-old male Wistar rats weighing 250–280 g (Japan SLC, Hamamatsu, Japan) were used for all experiments. The rats were housed under controlled environmental conditions (temperature, 23 °C ± 1 °C, humidity, 55% ± 5%) with a commercial food diet and water freely available. All animal experiments were carried out according to Tokyo University of Pharmacy and Life Sciences guidelines for the care and use of laboratory animals.

For rhodamine123 clearance experiments, control and rats treated 8 and 24 h earlier with an i.p. injection of LPS (1 mg/kg and 5 mg/kg of body weight) were anesthetized with pentobarbital (25 mg/kg) and cannulated with polyethylene tubes in the right jugular vein for intravenous (i.v.) administration and blood collection. The dose of LPS used in this study was chosen on the basis of previous study (Kitaichi et al., 1999). We examined three LPS doses, 1, 5, and 10 mg, and at all these doses, the significant dose-dependent decrease in mdr1a mRNA level was found in comparison with the control group. Also the decrease at 5 and 10 mg was not different, and thus, two doses of 1 and 5 mg were used. At these doses, no side effect was found. All experiments were performed under anesthesia, and body temperature was maintained at 37 °C with a heat lamp.

2.3. Excretion experiments to ileal perfusate from blood using the in situ single pass method

A cannula (silicone tubing, Silascon; Kaneka Medix Co.) was placed in the jugular vein for i.v. administration and sampling. Also, the lumen of the ileum (7 cm length) was flushed with saline pre-warmed to 37 °C, and the proximal end of the lumen was catheterized with an in-flow glass cannula, which was connected to the perfusion system. The distal end of the ileum was also catheterized with an out-flow glass cannula to collect intestinal effluents serially. The single pass perfusion of Krebs Henseleit bicarbonate buffer (pH 7.4) into the ileal lumen was started at a rate of 1 mL/min, as described previously (Tomita et al., 2008, 2009a). Rhodamine123 dissolved in saline (0.02 mg/mL) was administrated by i.v. injection, as a bolus with a volume of 2.8 mL/kg, followed by saline injection at the same volume, via the cannula inserted into the jugular vein. Intestinal effluent samples were then collected every 10 min. Excretion of rhodamine123 from blood to the ileal lumen was expressed as the total excreted amount for 60 min. The ileal luminal excretion clearance (CLlumen) was calculated by dividing the excretion rate by the plasma concentration in the middle of the collection period of ileal excretion.

2.4. Biliary excretion experiments

A cannula (silicone tubing, Silascon; Kaneka Medix Co.) was placed in a jugular vein for administration and sampling. The bile duct was also cannulated for bile collection. Bile was collected in pre-weighed tubes at 20 min intervals for 60 min throughout the experiment. Rhodamine123 dissolved in saline (0.02 mg/mL) was administrated by i.v. injection, as a bolus with a volume of 2.8 mL/kg, followed by saline injection at the same volume via the cannula inserted into the jugular vein. Blood samples were taken at the midpoint of the bile collection period. Plasma samples were obtained by centrifuging the blood samples at 3000 × *g* for 10 min. The volume of bile samples was measured gravimetrically. All plasma and bile samples were stored at −40 °C until analysis.

2.5. Pharmacokinetics analysis

Pharmacokinetics parameters, such as total clearance (CLtot), were calculated by dividing the dose by the area under the plasma concentration curve, which was obtained by the MULTI program. The intestinal (CLlumen) and biliary clearance (CLbile) of rhodamine123 during each luminal perfusate and bile collection period was calculated by dividing the luminal exsorption rate and biliary excretion rate by the plasma concentration obtained in the middle of the collection of bile excretion, respectively.

2.6. Western blotting

The intestine and liver were isolated in each rat at different times after LPS i.p. injection. An *in situ* ileal loop (60 cm length) was isolated in each rat at different times after LPS injection, and brush border membrane fractions were prepared using magnesium chloride precipitation (Tomita et al., 2008). Protein was determined by the method using a Micro BCA Protein Assay Reagent Kit (PIERCE). The protein expression levels of P-gp in the brush border membrane fraction were evaluated by Western blotting, which was performed as reported previously using C219 monoclonal antibody (Alexis) for P-gp (Tomita et al., 2008).

2.7. Preparation of RNA and cDNA synthesis

Total RNA was isolated from ileal and liver specimens using TRIzol reagents (Invitrogen Co. Ltd., Paisley, UK) according to the manufacturer's instructions. Complementary DNA (cDNA) was prepared from total RNA using GeneAmp™ PCR System 9600 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, 10 units RNaseOUT recombinant ribonuclease inhibitor.

2.8. Analysis of mdr1a and mdr1b gene expression in intestine and liver 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 was used. Assay on-Demand Gene Expression Products were purchased for GAPDH, mdr1a and mdr1b (Table 1). Reverse transcription was performed for 1 μg 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). The PCR conditions were 10 min at 90 °C, followed by 40 cycles of 15 s at 95 °C, and 1 min at 60 °C. All assays were RNA-specific (spanning exon–exon junctions) predesigned TaqMan Gene Expression Assays from Applied Biosystems (Table 1).

Table 1
Sequences of primers used for real-time RT-PCR.

Gene		Sequence (5′–3′)	Amplicon size
GAPDH	Forward	TGA GGT GAC CGC ATC TTC TTG	102 bp
	Reverse	TGG TAA CCA GGC GTC CGA TA	
mdr1a	Forward	GCC GCT GCT TCT TCC AAA	102 bp
	Reverse	CGT TAA GGT CTT CTT CGA GCT CC	
mdr1b	Forward	GGA GCA GGT AAG GTA CAG ATG TCC	101 bp
	Reverse	GGT CTC CTT CCT GAG CAA GAT G	
iNOS	Forward	CTG GGT GAA AGC GGT GTT CT	101 bp
	Reverse	CCG ACT TCC TTG TCT CAG TAG CA	
IL-1β	Forward	GGC GGT TCA AGG CAT AAC AG	101 bp
	Reverse	CAG TTG AGT TCA GGG ACA GTT GC	

2.9. Measurement of transepithelial transport of rhodamine123 and membrane resistance (R_m) in the in vitro diffusion chamber methods

The methods used in this study followed those previously described by Tomita et al. (2000). The transepithelial transport of rhodamine123 in the rat ileum was examined using diffusion chamber methods. The serosal and mucosal reservoirs were filled with 5 mL Krebs Henseleit bicarbonate buffer (KHBB) solution, which was continuously circulated and oxygenated by mixed gas (95% O_2/5% CO_2) to maintain tissue viability at pH 7.4 and 37 °C throughout the experiments. After 20 min, the KHBB solution was exchanged for 5 mL KHBB solution containing Rho123 on the serosal side (donor side) and 5 mL KHBB solution without rhodamine123 on the mucosal side (acceptor side). For the efflux experiments, Rho123 was added to the mucosal side (donor side) but not to the serosal side (acceptor side). To examine the effect of verapamil, the mucosal reservoir was filled with KHBB solution containing verapamil (100 μm). The pH of KHBB solution containing verapamil was adjusted to 7.4 using sodium hydroxide. The samples were taken from the acceptor side at intervals of 10 min. Permeation clearance (CLp) was obtained as follows:

$$\mathrm{CLp} = \frac{dQ/dt}{A \times C_0}$$

The dQ/dt is the transport rate (μg/min) and corresponds to the slope of the regression line between the transport amounts and time. C_0 is the initial concentration (0.001%) in the donor chamber (μg/mL) and A is the area of the membrane (0.64 cm^2). Simultaneously, the R_m of the rat ileum was calculated from the membrane potential difference measured under the load of a small external current (0.1 mA and 0.01 mA) according to Ohm's law (Yamashita et al., 1987).

2.10. Assay of rhodamine123 in ileum and liver

For the assay of rhodamine123, the fluorescence method was used. Before the assay, ileum and bile samples were diluted with saline solution 0–1000 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) (Tomita et al., 2008, 2009a,b).

2.11. Statistical analysis

All results were expressed as the mean ± standard error (mean ± S.E.). Statistical significance between two groups was analyzed using Dunnett's test, Tukey's test and Student's t-test. p value < 0.05 was considered significant.

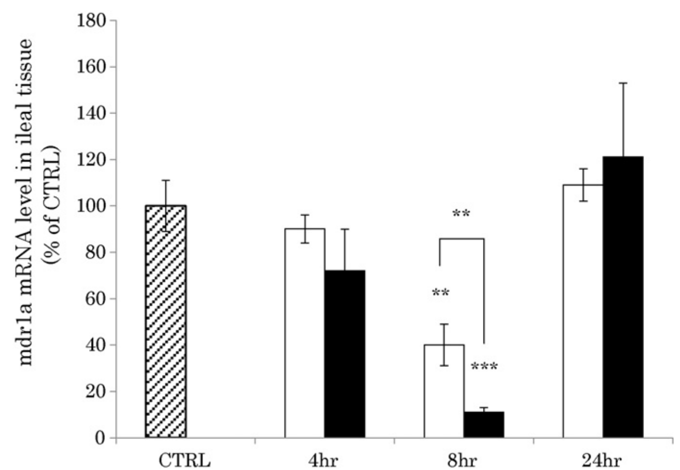

Fig. 1. Effects of LPS on expression level of mdr1a mRNAin ileal tissue at different times (4, 8 and 24 h) after LPS injection. Data represent the means and S.E. (n = 4–8 for each condition). Open and closed columns represent i.p. administration groups of 1 mg/kg and 5 mg/kg, respectively. **Significantly different from the control value and between the two dosing levels (p < 0.01, t-test). ***Significantly different from the control value (p < 0.001, t-test).

3. Results

3.1. Effects of LPS on the level of mdr1a mRNA expression in ileal tissue

We examined the level of mdr1a mRNA expression at different times after i.p. injection of LPS. The significant decrease in mdr1a mRNA expression level from the control value in ileal tissue 8 h after injection of LPS was observed in a dose-dependent manner, but the levels under both 1 mg/kg and 5 mg/kg LPS injection groups returned to the control 24 h after i.p. injection of LPS (Fig. 1). No marked changes were observed at 4 h after both doses of LPS when compared with the control condition (Fig. 1). Marked changes were not seen in the level of mdr1b mRNA expression 8 and 24 h after injection of LPS when compared with the control condition (data not shown).

3.2. Effects of LPS on the level of P-gp protein expression in ileal brush border membrane

A significant decrease in the level of P-gp protein 8 h after i.p. injection of LPS was observed in both 1 mg/kg group and 5 mg/kg group when compared with control conditions (Fig. 2). On the other hand, in both groups, complete recovery of the protein level of P-gp 24 h after i.p. injection of LPS was achieved (Fig. 2).

3.3. Effects of LPS on ileal excretion of rhodamine123 to lumen from blood after i.v. administration of rhodamine123

The ileal excretion clearance of rhodamine123 (CLlumen) was investigated in rats at different times after injection of LPS. After i.p. injection of LPS in ileal excretion of rhodamine123, a significant decrease was found in CLlumen evaluated by the excretion data from 8 to 9 h (abbreviated as 8 h) after injection of LPS when compared with the control. Recovery to the control level from the excretion data from 24 to 25 h (abbreviated as 24 h) was also observed (Fig. 3). The decreased excretion of rhodamine123 at 8 h after 5 mg/kg injection of LPS was greater than that after 1 mg/kg i.p. LPS (Fig. 3).

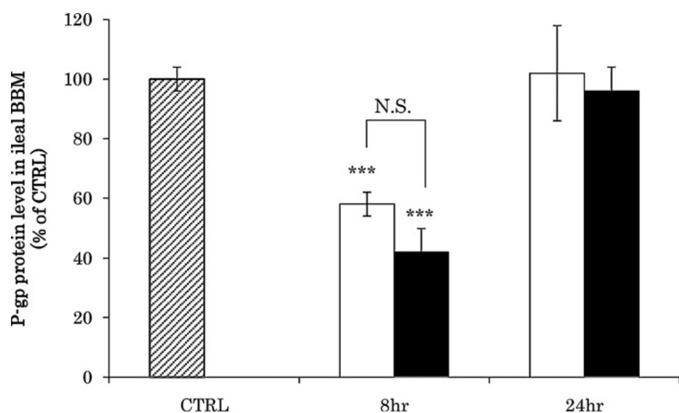

Fig. 2. Effects of LPS on expression levels of P-gp protein in ileal brush border membrane (BBM) fraction. Data represent the means and S.E. ($n = 4–8$ for each condition). Open and closed columns represent i.p. administration groups of 1 mg/kg and 5 mg/kg, respectively. ***Significantly different from the control value ($p < 0.001$, *t*-test). N.S.: not significantly different.

3.4. Comparative study of permeation clearance of rhodamine123 across isolated ileal mucosa at different times after injection of LPS

Time-dependent effects of i.p. injection of LPS on *in vitro* permeation clearance of rhodamine123 across isolated ileal mucosa are presented in Fig. 4. Significant reduction in permeation clearance (CLp) in the seresal-to-mucosal direction of rhodamine123 was observed in rats 8 h after i.p. injection of LPS (Fig. 4). In contrast, 24 h after i.p. injection of both 1 mg/kg and 5 mg/kg LPS, CLp of rhodamine123 returned to the control level (Fig. 4).

3.5. Effect of LPS on expression level of iNOS and IL-ip mRNA in ileal tissues by RT-PCR

The level of iNOS and IL-1β mRNA was obtained at different times after i.p. injection of LPS. The time course of the expression level of iNOS and IL-1β mRNA after i.p. injection of LPS is shown in Fig. 5. By 2 h after i.p. injection of LPS, iNOS mRNA in the ileum tended to increase (Fig. 5). The increased expression level returned to the control level 24 h after injection of LPS (Fig. 5). The dose-dependent pattern of the time course in the expression of IL-1β was similar to that of iNOS (data not shown).

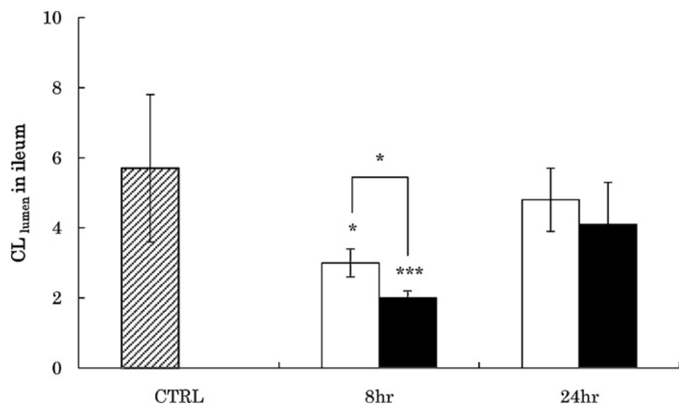

Fig. 3. Effects of LPS on ileal excretion clearance (CLlumen) of rhodamine123 evaluated from *in vivo* experiments. Open and closed columns represent i.p. administration groups of 1 mg/kg and 5 mg/kg, respectively. Data represent the means and S.E. ($n = 4–13$ for each condition). *Significantly different from the control value and between the two dosing levels ($p < 0.05$, *t*-test). ***Significantly different from the control value ($p < 0.001$, *t*-test).

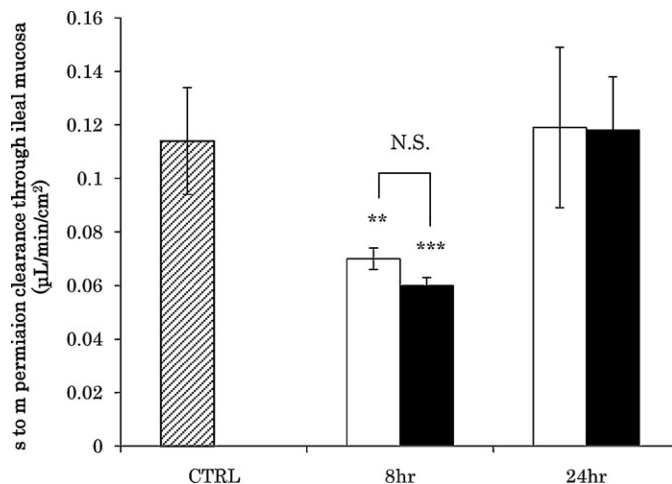

Fig. 4. Effects of LPS on ileal permeation clearance from serosal (s) to mucosal (m) direction (CLp) of rhodamine123 evaluated from *in vitro* experiments. Open and closed columns represent i.p. administration groups of 1 mg/kg and 5 mg/kg, respectively. Data represent the means and S.E. ($n = 5–9$ for each condition). **Significantly different from control value ($p < 0.01$, *t*-test). ***Significantly different from control value ($p < 0.001$, *t*-test). N.S.: not significantly different.

3.6. Effects of LPS on the level of mdr1a mRNA expression in liver

We examined the level of mdr1a mRNA expression in the liver at different times after i.p. injection of LPS. The significant decrease in mdr1a mRNA expression level from the control value in the liver 8 h after injection of LPS was observed in a dose-dependent manner, but the level in both 1 mg/kg and 5 mg/kg LPS injection groups returned to the control at 24 h (Fig. 6).

3.7. Effects of LPS on biliary excretion of rhodamine123

Time-dependent effects of LPS injection on the biliary excretion of rhodamine123 in rats after i.p. injection of LPS are listed in Table 2. No changes in CL_{bile} of rhodamine123 and the bile flow rate were observed 24 h after LPS injection when compared with the control condition (Table 2). In contrast, significant reductions in the values of CLbile and the bile flow rate were observed in rats 8 h after i.p. injection of LPS when compared with the control condition (Table 2).

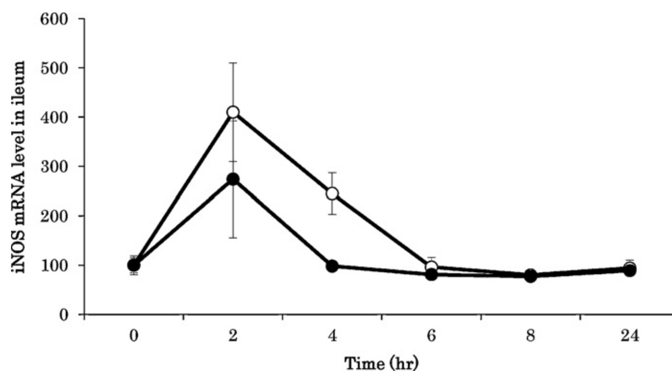

Fig. 5. Time courses of levels on iNOS mRNA in ileal fraction at different times after administration of LPS. Data represent the means and S.E. ($n = 4–6$ for each condition). Open and closed circles represent i.p. administration groups of 1 mg/kg and 5 mg/kg, respectively.

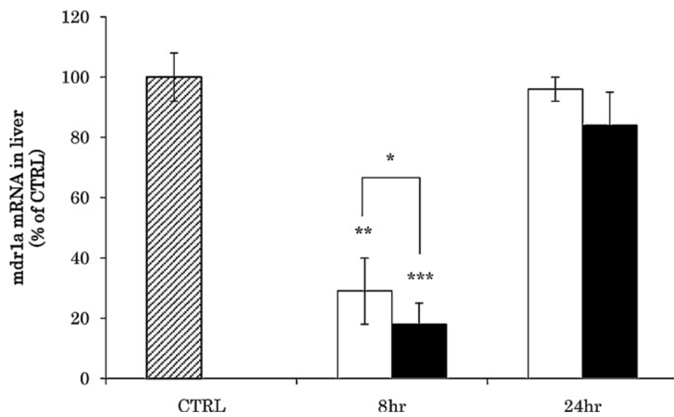

Fig. 6. Expression level mdr1a mRNA in liver 8 and 24 h after injection of LPS. Data represent the means and S.E. ($n = 5–8$ for each condition). Open and closed columns represent i.p. administration groups of 1 mg/kg and 5 mg/kg, respectively. **Significantly different from control value ($p < 0.01$, *t*-test). ***Significantly different from the control value ($p < 0.001$, *t*-test). *Significantly different between the two dosing levels ($p < 0.05$, *t*-test).

Table 2
Effect of LPS on biliary excretion of rhodamine123 in rats.

Time (h) after injection of LPS	i.p. dose	CL_{bile} (mL/min)	Bile flow rate (μL/min)
CTRL		1.46 ± 0.28	19.8 ± 1.35
8	1 mg/kg	0.59 ± 0.11**	12.0 ± 1.11**
	5 mg/kg	0.36 ± 0.1***	10.7 ± 1.31***
24	1 mg/kg	1.33 ± 0.14	19.1 ± 0.77
	5 mg/kg	1.24 ± 0.08	19.8 ± 0.94

CL bile: biliary excretion clearance.
** $p < 0.01$ (*t*-test) vs. the value at time 0 (before administration).
*** $p < 0.001$ (*t*-test) vs. the value at time 0 (before administration).

3.8. *Effects of LPS on expression level of iNOS and IL-ip in liver tissues*

Time-dependent changes in the expression of iNOS and IL-1β mRNA levels after i.p. injection of LPS are shown in Figs. 7 and 8. By 8 h after LPS injection, iNOS mRNA in the liver increased in a dose-depending manner (Fig. 7). The increased expression levels of iNOS mRNA returned to the control level 24 h after injection of LPS (Fig. 7). The pattern of the time course in the expression of IL-1β, including a dose-dependent increase, was similar to that of iNOS (Fig. 8). No marked increase in iNOS and IL-1β in the liver was observed 2 h after i.p. injection of LPS (data not shown).

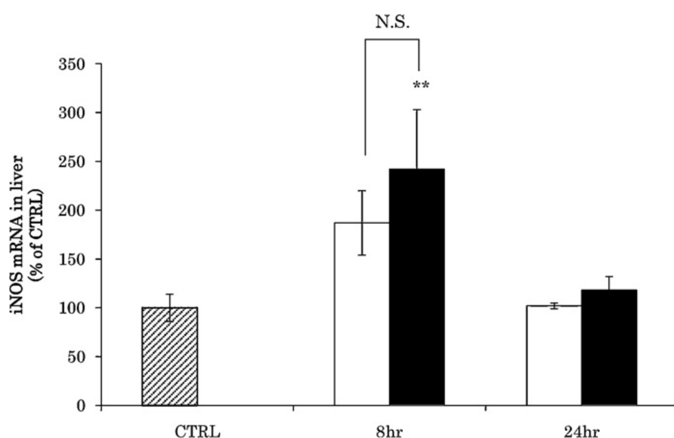

Fig. 7. Expression levels of iNOS mRNAin liver at 8 and 24 h after injection of LPS. Data represent the means and S.E. ($n = 6–8$ for each condition). Open and closed columns represent i.p. administration groups of 1 mg/kg and 5 mg/kg, respectively. **Significantly different from the control value ($p < 0.01$, *t*-test). N.S.: not significantly different.

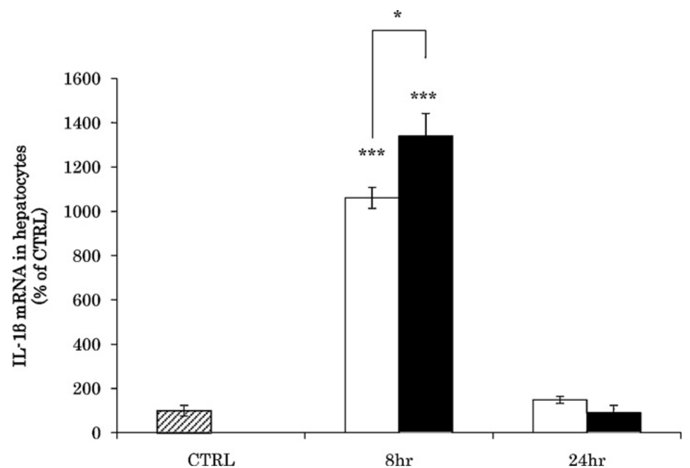

Fig. 8. Expression levels of IL-1β mRNA in hepatocytes 8 and 24 h after injection of LPS. Data represent the means and S.E. ($n = 4$ for each condition). Open and closed columns represent i.p. administration groups of 1 mg/kg and 5 mg/kg, respectively. ***Significantly different from control value ($p < 0.001$, *t*-test). *Significantly different between the two dosing levels ($p < 0.01$, *t*-test).

4. Discussion

The present study focused on the effect of i.p. injection of LPS on P-gp mediated ileal and biliary excretion of the P-gp substrate, rhodamine123, which has been used as a marker for evaluating the role of P-gp in anticancer drug-resistant cells and various normal tissues, such as the small intestine, liver, kidney and brain (Chin et al., 1989; Neyfakh et al., 1989; Lee et al., 1994; Wang et al., 1995; de Lange et al., 1998; Kunihara et al., 1998). According to our studies, it is considered that LPS-induced changes in the pharmacokinetics of drugs are not directly related to histopathological changes in the intestine and liver. We also investigated the effect of LPS on the levels of mRNA of mdr1a and P-gp protein in the ileal brush border membrane and bile canalicular membrane of hepatocytes.

It has been reported that rhodamine123 is actively secreted into the urine by P-gp and that glycerol-induced acute renal failure reduces P-gp-mediated renal tubular secretory clearance of rhodamine123 by impairment of P-gp (Kunihara et al., 1998). Also, we have already reported that exsorption of rhodamine123 from blood into the intestinal lumen was measured by the *in situ* single pass perfusion method using ileal segments to assess P-gp function (Tomita et al., 2008). In the present experiments, no change in the level of P-gp protein and mRNA of mdr1a was observed in rats 24 h after injection of LPS, although a marked decrease in these levels was seen 8 h after injection of LPS (Figs. 1 and 2). Also, the CLlumen and CLbile of rhodamine123 significantly decreased in rats 8 h after injection of LPS (Figs. 3 and 4 and Table 1). LPS decreased the intestinal exsorption and biliary excretion of rhodamine123 with changes in the level of P-gp protein 8 h after injection of LPS, suggesting that intestinal and biliary P-gp-mediated secretory function induced by LPS in the brush border membrane of intestinal epithelial cells and bile canalicular membrane in hepatocytes decreases by at least 8 h after injection of LPS.

Various data are available on whether the intestinal and biliary excretion of rhodamine123 is mediated by P-gp (Tomita et al., 2008, 2009a). From *in vivo* clearance experiments, we have already shown that the P-gp substrate, cyclosporine A, inhibited the intestinal and biliary excretion of rhodamine123, whereas cimetidine and tetraethylammonium, which are not P-gp substrates and are medi-

ated by the organic cation transport system, did not (Tomita et al., 2008, 2009b). These results suggest that rhodamine123 is mainly excreted into the lumen and bile by P-gp, supporting a report that intestinal and biliary excretion of P-gp substrates, such as doxorubicin and vinblastine, was decreased in Mdr1a knockout mice (van Asperen et al., 2000). In our experiments, significant reduction of P-gp mediated intestinal and biliary clearance of rhodamine123 was observed 8 h after injection of LPS together with decreases in the protein level of P-gp and mRNA level of mdr1a (Figs. 2 and 3 and Table 2). These results suggest that P-gp-mediated intestinal and biliary secretory function in the brush border membrane and bile canalicular membrane of hepatocytes is impaired until 8 h after injection of LPS and recovered to the control level by 24 h (Fig. 3 and Table 2).

We have already shown that the decrease in P-gp function observed in the previous *in vitro* and *in vivo* studies corresponded with the production level of thiobarbituric acid reactive substance (TBA-RS) as an indicator of lipid peroxidation (Tomita et al., 2008; Nagira et al., 2006). Increased inducible nitric oxide synthesis (iNOS), including superoxide anion, hydrogen peroxide, hydroxyl radical, etc., increases lipid peroxidation, the elevation of which might play an important role in intestinal disease-induced changes in certain transporter-mediated intestinal and biliary excretion systems (Tomita et al., 2009a,b). Regarding LPS administration, it is reported that increases in IL-6 and TNFα induced decreases in the level of mdr1a mRNA (Hirsch-Ernst et al., 1998). Evidence has been presented that TNFa induces the up-regulation of transporter genes or MRP1 protein in human colon carcinoma cells and Mdr1 in rat hepatoma cells (Chapekar et al., 1991; Stein et al., 1997). Also, it is reported that anti-IL-1 or anti-TNFa antibody restores the down-regulation of cMOAT/MRP2 expression (Nakamura et al., 1999). However, the roles of these cytokines and some mediators, such as nitric oxide (NO) and platelet-activating factor, remain unclear in the physiological function of drug transporters. We therefore focused on the role of iNOS and IL-1β in LPS-induced decreases in the intestinal and biliary clearance of rhodamine123. Increases in the level of iNOS and IL-1β 8 h after injection of LPS and recovery to the control level at 24 h after injection of LPS were observed in the liver, but not in the intestine (Figs. 5, 7 and 8). This suggests the possible involvement of iNOS and IL-1β in decreased P-gp-mediated biliary transport of rhodamine123. Regarding the intestine, increases in the level of iNOs and IL-1β were seen 2 h after injection of LPS, but not at 8 h. It is not clear which factors are involved in the functional impairment of intestinal rhodamine123 excretion; however, the increase in both mRNA of iNOS and IL-1β 2 h after injection of LPS may result in decreased intestinal rhodamine123 excretion 8 h after injection of LPS.

The effects of LPS on mRNA expression of other transporters located in bile canalicular membranes of hepatocytes remain unclear, although it has been reported that LPS appears to down-regulate mrp2 mRNA in the liver and kidney 6 h after injection of LPS (Roelofsen et al., 1995). It has also been reported that biliary excretion of P-gp substrates was decreased in mdr1a knockout mice (van Asperen et al., 2000). We then attempted to investigate the effect of LPS on the expression level of P-gp protein in both the intestine. Western blotting revealed that the P-gp protein expression decreased in the intestine 8 h after injection of LPS when compared with control conditions, and returned to control level after 24 h (Fig. 2).

In conclusion, the present study is the first to use *in vivo* clearance experiments, Western blotting and the mRNA level of mdr1a to reveal that LPS decreases P-gp-mediated ileal and biliary excretion of rhodamine123 by partly decreasing the protein level of P-gp and mRNA level of mdr1a. These results suggest that iNOS and IL-ip may possibly be at least components associated with the LPS-induced decrease in the ileal and biliary excretion of rhodamine123; however, further studies are needed to clarify the role of other factors besides iNOS and IL-1β in the reduced intestinal excretion of rhodamine123 and the reduced level of P-gp with LPS injection.

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