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Effects of lipopolysaccharide on P-glycoprotein expression and activity in the liver and kidneys

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ABSTRACT

There have been many reports that P-glycoprotein expression and activity are altered during sepsis, but few of them have examined such changes over 72 h. In this study, we examined the effect of lipopolysaccharide (LPS, 5 mg/kg, ip) on P-glycoprotein expression (Western blotting) and activity (rhodamine-123 (Rho123) pharmacokinetics) in liver and kidneys for 7 days. On day 1 after LPS administration, hepatic P-glycoprotein expression and activity significantly decreased. On day 3, hepatic P-glycoprotein expression significantly increased compared with the control group, while activity had returned to the control level. On day 7, hepatic P-glycoprotein expression returned to the control level. There were no significant changes in P-glycoprotein expression or activity in the kidneys after LPS administration. The amount of Rho123 excretion in urine remained unchanged with (4.2%) or without (4.0%) LPS administration, but the amount of Rho123 excretion in bile decreased from 2.0 to 0.7% with LPS administration. Our findings suggested that hepatic P-glycoprotein expression and activity decreased on day 1 but recovered within 3 days, but there were no significant differences in the kidneys after LPS administration. These results suggested that the change in P-glycoprotein activity might be due to change in P-glycoprotein expression in the liver rather than the kidneys.

1. Introduction

P-glycoprotein, an ATP-dependent efflux pump, is widely expressed in human and rodent tissues and transports many structural, pharmacological (e.g., anticancer drugs, immunosuppressants, steroid hormones, calcium channel blockers, and cardiac glycosides) and endogenous P-glycoprotein substrate compounds (endogenous steroids). It has been reported that P-glycoprotein activity is inhibited not only by P-glycoprotein inhibitors but also by sepsis, and that endotoxin induced by *Klebsiella pneumoniae* decreases the biliary excretion and renal clearance of rhodamine-123 (Rho123). Rho123 has been used extensively as a marker of P-glycoprotein-mediated transporter in both *in vitro* and *in vivo* (Ando et al., 2001; Parasrampuria and Mehvar, 2008). There have been many reports on P-glycoprotein expression and activity in the liver and kidneys during acute pathological conditions (Piquette-Miller et al., 1998; Vos et al., 1998; Huang et al., 2000; Hidemura et al., 2003; Cherrington et al., 2004; Hartmann et al., 2005; Miyoshi et al., 2005; Sun et al., 2006), but few have examined such changes over 72 h. In our previous study, we examined levels of intestinal P-glycoprotein expression and activity using a rat sepsis model induced by lipopolysaccharide (LPS, from *Escherichia* (*E*) *coli*) for 7 days after LPS administration (Moriguchi et al., 2007). We found that intestinal P-glycoprotein recovered within 3 days after LPS administration.

In this study, we examined the effects of LPS on P-glycoprotein expression and activity in the liver and kidneys for 7 days after LPS administration.

2. Materials and methods

2.1. Chemicals

LPS (*E. coli*: 055: B5; SIGMA, MO, USA), urethane (SIGMA, MO, USA), and Rho123 were purchased from Sigma-Aldrich, Co. (MO, USA). Other reagents were commercially available extra-pure grade chemicals.

2.2. Animals

Male Wistar/ST rats (Japan SLC Inc., Shizuoka, Japan) weighing 205–300 g were used. The animals were housed for at least one week under controlled environmental conditions with free access to solid food and water. They were fasted for at least 12 h with free access to water before and during the experiment. All experimental procedures were performed in accordance with the Osaka University of Pharmaceutical Sciences Guidelines for the care and use of laboratory animals.

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2.3. Treatment with LPS

LPS was dissolved in saline at a concentration of 2 mg/ml. In the LPS-treated group, rats were given a single 5 mg/kg intraperitoneal injection of LPS (Moriguchi et al., 2007), while animals not treated with LPS were used as the control group. These animals were used for experiments at 1, 3, or 7 days after LPS administration.

2.4. Preparation of liver and renal P-glycoprotein fractions

Rat liver and kidneys were excised quickly under light anesthesia using ether. To obtain the hepatic crude membrane, approximately 2 g of liver was homogenized in 6 ml of lysis buffer (leupeptin 1 mg/ml, pepstatin A 1 mg/ml, phenylmethylsulfonyl fluoride (PMSF) 50 mg/ml, 0.1 M Tris–HCl (pH 7.5)). The homogenates were centrifuged at 1500×g for 10 min at 4 °C. The supernatants were centrifuged at 37,000×g for 60 min at 4 °C. The pellets were resuspended in lysis buffer and stored at −80 °C until use. To obtain the renal brush border membrane, approximately 1 g of kidneys was homogenized with 10 ml of solution A (50 mM mannitol and 2 mM Tris–HCl (pH 7.1)). The homogenates were mixed with 100 μl of 1 M $CaCl_2$ by stirring and allowed to stand at 4 °C for 20 min. The homogenates were then centrifuged at 3000×g for 15 min at 4 °C. The supernatants were centrifuged at 27,000×g for 30 min at 4 °C. The pellets were then resuspended in solution B (10 mM mannitol and 10 mM HEPES–Tris (pH 7.5)) and centrifuged again at 27,000×g for 30 min at 4 °C. The pellets were resuspended in solution C (100 mM mannitol and 10 mM MES–Tris (pH 6.5)) and stored at −80 °C until use. Protein concentrations were measured with the Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, CA, USA) using bovine serum albumin as a standard.

2.5. P-glycoprotein expression evaluated by Western blot analysis

Protein levels for liver and kidney samples were 40 μg/g (liver) and 50 μg/g (kidneys), respectively. Liver samples (80 μg) and kidney samples (50 μg) were separated by electrophoresis on 7.5% SDS-PAGE gel and transferred onto a polyvinylidene difluoride (PVDF) membrane, which was blocked in Tris-buffered saline containing 0.1% Tween 100 and 3% nonfat dry milk. The PVDF membrane was then incubated with mouse monoclonal antibody C-219 (Signet, MA, USA) in 1:1000 dilution for 2 h at 37 °C. The membrane was washed and incubated with horseradish peroxidase anti-mouse IgG (Cell Signaling, MA, USA) in 1:1000 dilution for 1 h at room temperature. After washing, immunoreaction was detected by the enhanced chemiluminescence detection system (GE Healthcare Bio-Sciences Co., Ltd, Buckinghamshire, UK).

2.6. In vivo clearance studies

Each rat was anesthetized with intraperitoneal injection of urethane (1.5 g/kg) and fixed in supine position. Body temperature was maintained using an incandescent lamp. The bile duct and bladder were cannulated for collection of bile and urine. After 40 min for stabilization, Rho123, which had been dissolved in 5% mannitol, was administered at 0.19 mg/kg via the jugular vein. Blood samples were collected via the jugular vein into heparinized microtubes at 2, 5, 15, 35, 65, and 95 min after Rho123 administration. The bile and urine were collected at 10 min intervals for 100 min after Rho123 administration. The blood samples were centrifuged at 2100×g for 10 min, and the supernatants were used as plasma samples. The perfusates and plasma samples were stored at 4 °C after collection. For deproteinization, 100 μl of the plasma sample was mixed with an equal volume of methanol. The mixture was vortexed for 10 s and centrifuged at 12,000×g for 10 min at 4 °C. The supernatant (150 μl) was then derived and placed onto 96-well microplates, and the concentration of Rho123 was measured using a spectrofluorometer (Hitachi, Ltd., Tokyo, Japan) at an excitation wavelength of 485 nm and an emission wavelength of 546 nm. The bile and urine samples were loaded directly on 96-well microplates and Rho123 was analyzed as described above.

2.7. Data and statistical analysis

The area under the plasma Rho123 concentration–time curve from 2 to 95 min (AUC) was calculated by the trapezoidal approximation method. Total body clearance (CLtot) was calculated as dose/AUC. Biliary (CLbile) and renal (CLurine) clearances were calculated by dividing the total amount of Rho123 excreted into the bile duct (EXbile) and urine (EXurine) by AUC. Differences between the control and LPS-treated groups were assessed using analysis of variance (One-factor ANOVA). Where statistical differences were noted, Fisher's exact test was used to determine differences among the groups. All data are presented as mean ± S.D. $P<0.05$ was statistically significant.

3. Results

3.1. Effects of LPS on P-glycoprotein expression

Figs. 1 and 2 show effects of LPS on liver and kidney P-glycoprotein, respectively. P-glycoprotein expression in the liver significantly decreased on day 1, and then increased on day 3 after LPS administration. On day 7, P-glycoprotein expression in the liver recovered to the control level. P-glycoprotein expression in the kidneys was not affected by LPS administration.

3.2. Effects of LPS on P-glycoprotein activity

Figs. 3 and 4 show the effects of LPS on the amount of Rho123 excretion in bile and urine. Rho123 excretion in bile significantly decreased on day 1 and then increased on day 3 after LPS administration. There was no significant difference between the control and LPS-treated groups in the amount of Rho123 excretion in urine. Table 1 shows each parameter measured to determine effects of LPS on amounts of Rho123 excretion in bile and urine. On day 1, EXbile and CLbile significantly decreased, compared with the control group. There were no significant changes in EXurine or CLurine, but CLtot significantly decreased. On day 3, only AUC significantly decreased compared with the control group, while no other parameter exhibited significant changes. Furthermore, the percentage of total amount of Rho123 excretion mediated by P-glycoprotein in the liver and kidneys, calculated from the dosage

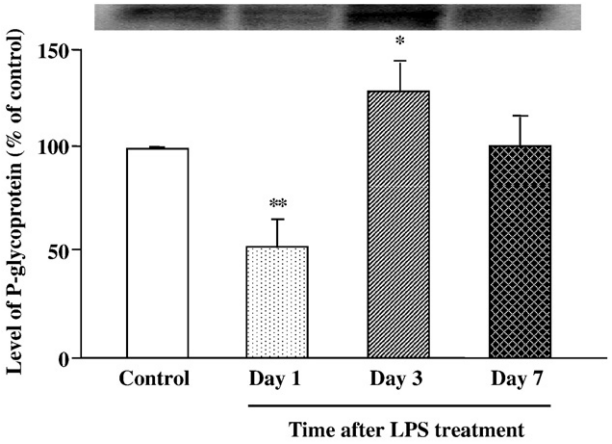

Fig. 1. Effects of lipopolysaccharide (LPS) on P-glycoprotein expression in the liver. Liver samples were prepared on days 1, 3, and 7 after LPS administration (5 mg/kg, ip). Each bar indicates the standard deviation (Mean ± S.D., $n=4$). $^{*}P<0.05$ and $^{**}P<0.01$, compared with the control group.