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Endotoxin from various gram-negative bacteria has differential effects on function of hepatic cytochrome *P*450 and drug transporters

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Abstract

The differential effects of endotoxin derived from *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli* on hepatic cytochrome *P*450 (CYP)-dependent drug-metabolizing enzyme activity and on the expression of hepatic CYP3A2, CYP2C11, P-glycoprotein and multidrug resistance-associated protein 2 (Mrp2) was investigated in rats. Endotoxin from all three different pathogens significantly decreased the systemic clearance of antipyrine, reflecting reduced hepatic drug-metabolizing enzyme activity 24 h after intravenous injection (0.5 mg/kg). The degree of the decreased systemic clearance by *P. aeruginosa* endotoxin was smaller than that by both *K. pneumoniae* and *E. coli* endotoxin. Western blot analysis revealed that the down-regulation of CYP3A2 by *K. pneumoniae* and *E. coli* endotoxin was greater than that by *P. aeruginosa* endotoxin. However, the down-regulation of CYP2C11 by all three different endotoxin was almost the same. Both *K. pneumoniae* and *P. aeruginosa* endotoxin significantly down-regulated P-glycoprotein, but did not down-regulate Mrp2. *E. coli* endotoxin had no effect on the expression of either P-glycoprotein or Mrp2, probably due to the low dose used. The down-regulation of CYP3A2 by endotoxin was parallel to the decreased systemic clearance of antipyrine. These results suggest that endotoxin has a differential effect on the hepatic CYP-mediated drug-metabolizing enzyme activity, and on the protein levels of hepatic CYP3A2 and P-glycoprotein, probably due to bacterial source-differences in the production of some proinflammatory mediators. Endotoxin appears to regulate coordinately CYP3A2, CYP2C11 and P-glycoprotein, but not Mrp2.

Keywords: Endotoxin; CYP3A2; CYP2C11; P-glycoprotein; Antipyrine clearance; (Rat)

1. Introduction

Endotoxin, an active component in the outer membrane of gram-negative bacteria, indirectly secretes various inflammatory cytokines (mediators) such as platelet activating factor (PAF), tumor necrosis factor-α (TNF-α), interleukin-1β, interleukin-6, and interferons (Cassatella et al., 1993; Crawford et al., 1997; Evans et al., 1993) from activated Kupffer cells (Bertini et al., 1989; Freudenberg et al., 1986), which are resident macrophages in the liver. These inflammatory cytokines are believed to induce various pathophysiological changes in the body, such as damage to the liver and kidney (Hewett and Roth, 1993; Hirata et al., 1980).

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The liver plays a major role in the detoxication (phase I and phase II metabolism) and elimination of various hydrophobic drugs and their metabolites. Endotoxin decreases hepatic cytochrome *P*450 (CYP)-dependent metabolism in experimental animals, including rats, as well as humans (Shedlofsky et al., 1994). Among various mediators, the release of nitric oxide (NO) is enhanced after endotoxin injection, subsequent to the expression of inducible NO synthase (iNOS) (Bredt and Snyder, 1994; Khatsenko and Kikkawa, 1997; Khatsenko et al., 1993; Moncada et al., 1991; Sewer et al., 1998), suggesting that NO may be involved in reducing hepatic CYP-dependent drug-metabolizing enzyme activity by endotoxin. In fact, *Escherichia coli* endotoxin was reported to reduce CYP-dependent drug metabolism and a reduction possibly due to the overexpression of various inflammatory cytokines and/or NO (Minamiyama et al., 1998; Morgan, 1997; Sewer et al., 1996). Previously we also found that *Klebsiella pneumoniae* endotoxin reduces hepatic drug-metabolizing enzyme activity, due in part to the over-production of NO in plasma (Kitaichi et al., 1999; Nadai et al., 1998). Otherwise, endotoxin has been reported to induce cholestasis and hyperbilirubinemia by down-regulating multidrug resistance-associated protein 2 (Mrp2) for bile acids and bilirubin, due to secretion of some cytokines from activated Kupffer cells (Green et al., 1996; Nakamura et al., 1999; Trauner et al., 1997). Like Mrp2, the ATP-binding cassette transport protein, P-glycoprotein, is expressed in many eliminating organs such as the liver and kidney (Cordon-Cardo et al., 1990; Thiebaut et al., 1987) and acts as efflux transport protein for endogenous and exogenous toxic substances (Thiebaut et al., 1987; Schinkel et al., 1996, 1997). These two drug transport proteins, P-glycoprotein and Mrp2, might have a protective function of excluding various lipophilic substrates from the liver as well as CYP. Considering that the numerous substrates of CYP3A, P-glycoprotein and Mrp2 overlap (Mayer et al., 1995; Oude Elferink et al., 1995; Wacher et al., 1995), and that some drugs such as rifampicin, dexamethazone and cyclosporin up-regulate both P-glycoprotein and CYP3A in the liver (Jette et al., 1996; Schuetz et al., 1996), CYP3A, P-glycoprotein and Mrp2 might be regulated coordinately.

There are several reports on the effect of endotoxin on the expression and function of P-glycoprotein and Mrp2 in the liver. For example, *E. coli* endotoxin down-regulates both P-glycoprotein and Mrp2 (Nakamura et al., 1999; Hartmann et al., 2001, 2002; Tang et al., 2000; Vos et al., 1998). Recent studies in our laboratories reported that *K. pneumoniae* endotoxin impaired the P-glycoprotein-mediated transport of P-glycoprotein substrates (Ando et al., 2001; Nadai et al., 2001; Zhao et al., 2002). These findings suggest the possibility that endotoxin might down-regulate simultaneously hepatic P-glycoprotein, Mrp2 and CYP3A. However, to our knowledge, there is no information confirming whether endotoxin simultaneously regulates the expression of CYP3A4, P-glycoprotein and Mrp2. In addition, the differential effects of endotoxin derived from various gram-negative bacteria on the expression of CYP3A, P-glycoprotein and Mrp2 is not fully understood.

Endotoxin derived from various gram-negative bacterial families shares a common architecture (Rietschel et al., 1993). The molecule consists of the O-antigenic polysaccharide, which is linked to the core oligosaccharide (R-core), which in turn is linked to the lipid portion lipid A (Rietschel et al., 1993; Westphal et al., 1983). The structure of the O-antigenic polysaccharide moiety is known to vary among species and strains of bacteria, whereas that of the core oligosaccharide is similar. In a series of our studies using *K. pneumoniae* endotoxin, Kato and colleagues demonstrated that *K. pneumoniae* endotoxin (O3 lipopolysaccharide) exhibits much stronger adjuvant activity in augmenting antibody responses and delayed-type hypersensitivity to protein antigens than other kinds of endotoxin from *E. coli* O55, O111, O127, and *Salmonella* enteritidis (Kato et al., 1984, 1985; Ohta et al., 1982a,b). They suggested that the extraordinarily strong adjuvant activity of *K. pneumoniae* endotoxin is due primarily to the binding of mannan as the O-specific polysaccharide moiety of *K. pneumoniae* endotoxin to the mannose-binding protein on the surface of macrophages (Kato et al., 1985). Considering that endotoxin has differential cytokine-inducing activity (Flad et al., 1993; Frieling et al., 1997; Mathiak et al., 2003; Netea et al., 2001), and that the mannose receptor is present on Kupffer cells (Magnusson and Berg, 1993), we presume that *K. pneumoniae* endotoxin, which possesses mannan as the O-specific polysaccharide moiety, might induce stronger down-regulation of the hepatic CYP3A, P-glycoprotein and Mrp2 than other endotoxin, including *E. coli* endotoxin.

The aim of the present study was to investigate the differential effects of endotoxin derived from *K. pneumoniae*, *Pseudomonas aeruginosa* and *E. coli*, which are the most frequent gram-negative bacterial pathogens in patients with sepsis, on the systemic antipyrine clearance, which represents the entire capacity of its hepatic CYP-dependent drug-metabolism (Kitaichi et al., 1999; Nadai et al., 1998), and on the expression of hepatic CYP3A2, CYP2C11, P-glycoprotein and Mrp2 by Western blot analysis.

2. Methods

2.1. Chemicals

Endotoxin was isolated from *K. pneumoniae* LEN-1 (O3:K1$^-$), which was identical to that used in previous studies (Ueyama et al., 2004; Nadai et al., 1998; Ando et al., 2001; Zhao et al., 2002). Endotoxin from *E. coli*

O55:B5 (lot 073K116) and *P. aeruginosa* serotype 10 (lot 50K4151) was purchased from Sigma (St. Louis, MO, USA). Antipyrine and phenacetin (an internal standard) were also purchased from Sigma. All other reagents were commercially available and of analytical grade. Endotoxin was dissolved in saline at a concentration of 0.5 mg/ml.

2.2. Animals

Eight-week-old male Wistar rats (Japan SLC, Hamamatsu, Japan) were used in this study. The rats were housed under controlled environmental conditions (temperature of 22–24 °C and humidity of 55±5%) with a commercial food diet and water freely available to animals for at least 3 days before the experiment and surgery. The procedures involving animals and their care conformed to the international guidelines, Principles of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) and Guiding Principles for the Care and Use of Laboratory Animals of Nagoya University, Japan.

2.3. Animal experiments

One day before the start of the experiments, rats were anesthetized with an intraperitoneal administration of sodium pentobarbital (25 mg/kg of body weight), and the right jugular vein was cannulated with polyethylene tubes (Natsume, Tokyo, Japan) for blood collection and drug administration. The rats were intravenously administered endotoxin (0.5 mg/kg), and control animals received an equivalent volume of saline in place of endotoxin. The dose of endotoxin was set at a level which does not cause mortality within 24 h after injection. For analysis of its systemic clearance, antipyrine (20 mg/kg of body weight) was administered intravenously in rats treated with endotoxin or saline (control rats). Blood samples were collected at designated intervals of 30, 60, 90, 120, 180, 240 and 300 min after injection of antipyrine. Plasma samples were immediately obtained by centrifugation at 1200×*g* for 5 min at 4 °C, and were stored at −40 °C until analyzed.

2.4. Drug assay

Concentrations of antipyrine in plasma were determined by high-performance liquid chromatography (HPLC) according to a method reported previously (Ueyama et al., 2004). The apparatus used for HPLC was a Shimadzu LC-10A system (Kyoto, Japan) consisting of an LC-10A liquid pump and an auto injector SIL-10Advp, and equipped with a UV–VIS detector (SPD-10 AV) set at a wavelength of 254 nm. The assay conditions were as follows: column, a LiChroCART (Merck, Tokyo, Japan); mobile phase, 30% (v/v) methanol in purified water (18 MΩ); temperature, 40 °C; flow rate, 1.0 ml/min.

2.5. Western blot analysis

Microsomal samples were prepared from liver homogenized samples as previously described (Omura and Sato, 1964). Western blot analysis of CYP3A2 and CYP2C11 was performed according to methods reported previously (Ueyama et al., 2004). For Western blot analysis of P-glycoprotein and Mrp2, the livers were washed out with a sufficient volume of ice-cold saline. Each liver was suspended in 10-fold volumes of 10 mM Tris–HCl buffer (pH 8.0) containing complete protease inhibitor, 1.5 μg/ml of aprotinin and 1 mM phenylmethylsulfonyl fluoride (Sigma, St. Louis, MO, USA). The suspension was homogenized with a tight homogenizer (20 strokes up and down) and centrifuged at 2000×*g* for 15 min at 4 °C. The supernatant was centrifuged at 80,000×*g* for 60 min at 4 °C. The pellet was dissolved in Laemmli buffer and incubated at 37 °C for 15 min.

The protein concentration in the solution was measured with Bio-Rad Protein Assay (Bio-Rad Laboratories, Richmond, CA, USA) using bovine serum albumin (Sigma, St. Louis, MO, USA) as a standard. The protein (40 μg) was separated by electrophoresis on 8% polyacrylamide gel containing 0.1% sodium dodecyl sulfate (SDS) and transferred to a polyvinylidene difluoride (PVDF) membrane. The membrane was blocked in phosphate-buffered saline containing 0.1% Tween 20 and 5% nonfat dry milk, and detected by C219 mouse monoclonal antibody to P-glycoprotein (Dako A/S, Glostrup, Denmark) and human monoclonal antibody to Mrp2 (Alexis, Biochemicals, San Diego, CA, USA); the membrane was then washed and incubated in a solution containing a 2000-fold diluted solution of horseradish peroxidase-conjugated anti-mouse IgG (Amersham Bioscience Co., NJ, USA) for 2 h at room temperature. After washing, the immunoreaction was detected by the enhanced chemiluminescence detection system (ECL, Amersham Bioscience).

To quantify the relative levels of CYP3A2, CYP2C11, P-glycoprotein and Mrp2 in each gel, the intensity of the stained bands was measured by the NIH image program (Bethesda, MD, USA). The levels were expressed as 100% of that of the control group.

2.6. Data analysis

Concentration–time data for antipyrine in each rat were individually analyzed by a noncompartmental model. The area under the plasma concentration–time curve (AUC) and the area under the first moment curve (AUMC) were calculated by the trapezoidal method up to the last measured concentration in plasma and were extrapolated to infinity. Systemic clearance (CL_{SYS}) was calculated by dividing the dose by the AUC. The steady-state volume of distribution (V_{SS}) was calculated as $V_{SS}=CL_{SYS}\times MRT$, where MRT represents the mean residence time and was calculated as MRT=AUMC/AUC.

2.7. Statistical analysis

All data are expressed as means±S.E.M. The statistical analyses were conducted with Excel (Microsoft, Redmond, DC, USA) and StatView (ABACUS, Berkeley, CA, USA) software. Analysis of variance (ANOVA) was used to determine the statistical significance of differences between experimental groups. When F ratios were significant, Scheffe's post hoc tests between two groups were performed. The 0.05 level of probability was used as the criterion of significance.

3. Results

3.1. Effects of endotoxin derived from K. pneumoniae, P. aeruginosa and E. coli on hepatic drug-metabolizing enzyme activity

Antipyrine is widely used as a tool to evaluate the capacity of drug metabolism in various pathological animal models since it is almost completely metabolized by the hepatic CYP isozymes in rats and humans (Balani et al., 2002; Carcillo et al., 2003). Mean semilogarithmic plots of plasma concentration–time data for antipyrine after intravenous injection (20 mg/kg) in control rats and rats pretreated with *K. pneumoniae*, *P. aeruginosa* and *E. coli* endotoxin are illustrated in Fig. 1. *K. pneumoniae* endotoxin showed the most delayed disappearance of antipyrine from plasma. Fig. 2 shows the effects of different endotoxin on the systemic clearance of antipyrine in rats 24 h after intravenous injection of endotoxin (0.5 mg/kg). The systemic clearance of antipyrine in rats treated with *K. pneumoniae*, *P. aeruginosa* and *E. coli* endotoxin significantly decreased to 50%, 73% and 57% of the control rats, respectively, without any change in the volume of distribution. The decreased systemic clearance of antipyrine in rats treated with *P. aeruginosa* endotoxin was significantly smaller than that in rats treated with either *K. pneumoniae* or *E. coli* endotoxin.

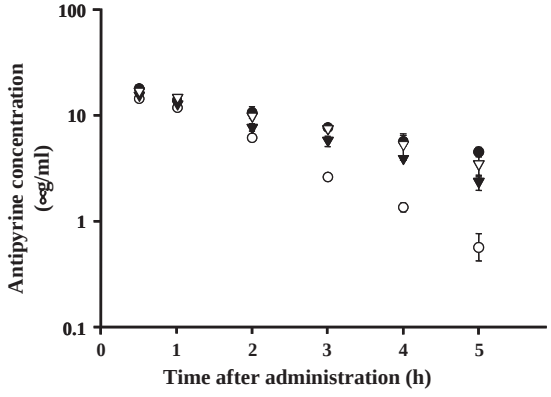

Fig. 1. Effects of different endotoxin on plasma concentration–time curves of antipyrine in rats 24 h after intravenous injection (0.5 mg/kg). (○) Control; (●) *K. pneumoniae* endotoxin; (▼) *P. aeruginosa* endotoxin; (▽) *E. coli* endotoxin. Each point shows the mean±S.E.M. (n=3–4).

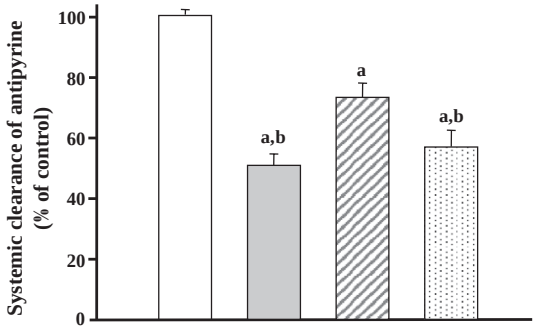

Fig. 2. Effects of different endotoxin on systemic clearance of antipyrine. Twenty-four hours after intravenous injection of endotoxin (0.5 mg/kg), antipyrine (20 mg/kg) was injected intravenously and blood samples were collected at the designated intervals. □, Control rats; ▒, *K. pneumoniae* endotoxin; ▨, *P. aeruginosa* endotoxin; ⁝⁝, *E. coli* endotoxin. Each bar shows the mean±S.E.M. (n=3–4). (a) and (b) indicate values that are significantly different from the control (P<0.01) and *P. aeruginosa* endotoxin (P<0.05).

3.2. Effects of endotoxin derived from K. pneumoniae, P. aeruginosa and E. coli on protein levels of CYP3A2 and CYP2C11

Fig. 3 shows changes in the protein levels of hepatic CYP3A2 and CYP2C11 in rats 24 h after injection of endotoxin (0.5 mg/kg). All kinds of endotoxin significantly decreased the protein levels of both hepatic CYP3A2 and CYP2C11. The down-regulation of CYP3A2 by *K. pneumoniae* and *E. coli* endotoxin was significantly greater than that by *P. aeruginosa* endotoxin. Contrary to CYP3A2, all kinds of endotoxin significantly decreased the expression of CYP2C11 by approximately 50%. As shown in Fig. 4, the extent of the down-regulation of CYP3A4 and CYP2C11 by endotoxin significantly correlated with endotoxin-induced decreases in the systemic clearance of antipyrine.

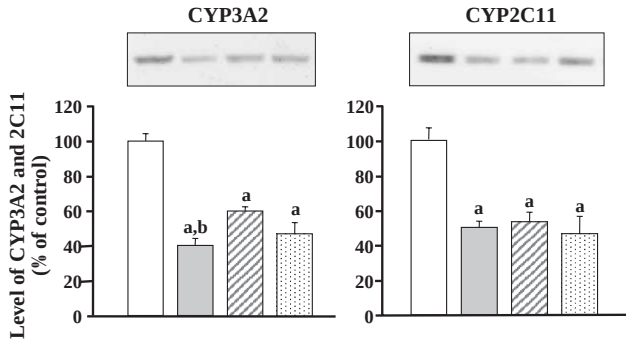

Fig. 3. Effects of different endotoxin on expression of hepatic CYP3A2 and CYP2C11. Liver samples were taken 24 h after intravenous injection of endotoxin (0.5 mg/kg). □, Control rats; ▒, *K. pneumoniae* endotoxin; ▨, *P. aeruginosa* endotoxin; ⁝⁝, *E. coli* endotoxin. Each bar shows the intensity ratio compared to the value for the control rats; values are the mean±S.E.M. (n=3–4). [a]Significantly different from control rats (P<0.01). [b]Significantly different from *P. aeruginosa* endotoxin (P<0.05).

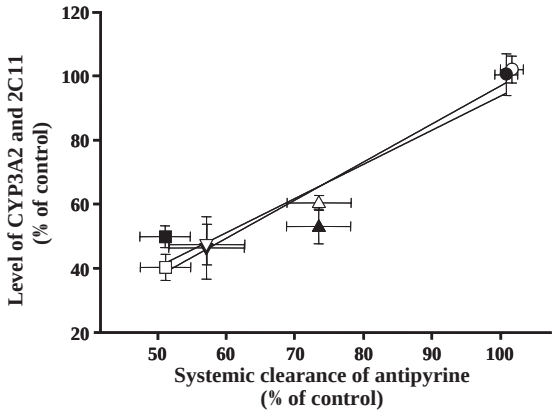

Fig. 4. Correlation between endotoxin-induced down-regulation of CYP3A2 and CYP2C11 and decreased systemic clearance of antipyrine. Open symbol, CYP3A2; closed symbol, CYP2C11. (○) and (●), Control; (□) and (■), *K. pneumoniae* endotoxin; (△) and (▲), *P. aeruginosa* endotoxin; (▽) and (▼), *E. coli* endotoxin.

3.3. Effects of endotoxin derived from K. pneumoniae, P. aeruginosa and E. coli on protein levels of P-glycoprotein and Mrp2

The effects of endotoxin on the expression of P-glycoprotein and Mrp2 in rats 24 h after injection are shown in Fig. 5. *K. pneumoniae* and *P. aeruginosa* endotoxin significantly decreased the protein levels of hepatic P-glycoprotein and the degree of decreased expression by *K. pneumoniae* endotoxin was significantly greater than that by *P. aeruginosa* endotoxin. However, *E. coli* endotoxin had no effect on the expression of P-glycoprotein. In contrast to P-glycoprotein, endotoxin did not change the expression of hepatic Mrp2.

4. Discussion

Hepatic CYP3A2 and CYP2C11 are major CYP subtypes and important for physiological function and homeostasis in the rat because these enzymes catalyze the oxidation of many steroids, xenobiotics and drugs (Souček and Gut, 1992; Waxman, 1988) and are sensitive to several cytokines (Sewer and Morgan, 1997). Most recently, we have reported that *K. pneumoniae* endotoxin down-regulates the hepatic CYP3A2 and CYP2C11 in rats 24 h following intraperitoneal injection (Ueyama et al., 2004). However, little data are available regarding the differential effects of endotoxin derived from various gram-negative bacterial pathogens on the expression of cytochrome *P*450 (CYP) subtypes.

First, we compared the effects of endotoxins derived from the major gram-negative bacterial pathogens (*K. pneumoniae*, *P. aeruginosa* and *E. coli*) on the hepatic CYP-mediated drug-metabolizing enzyme activity, which play a pivotal role in the elimination rate of hydrophobic drugs. Antipyrine clearance experiments showed that all kinds of endotoxin significantly decreased the systemic clearance of antipyrine, which reflects hepatic drug-metabolizing enzyme activity. The degree of the decreased systemic clearance of antipyrine by *K. pneumoniae* and *E. coli* endotoxin was similar, but stronger than that by *P. aeruginosa* endotoxin. In any case, it was confirmed that endotoxins from various gram-negative bacteria play a key role in the impairment of the hepatic drug metabolism.

Second, we investigated whether endotoxin has differential effects on the expression of CYP3A2 and CYP2C11 in the liver. Under our experimental conditions, bacterial source-related differences in the expression of CYP3A2, but not CYP2C11, were observed. It is most likely that the decreased hepatic CYP-mediated drug-metabolizing enzyme activity induced by endotoxin correlates well with the decreased expression of CYP3A2. The levels of decreased hepatic CYP3A2 and CYP2C11 induced by *P. aeruginosa* endotoxin were lower than those by *K. pneumoniae* and *E. coli* endotoxin. It has been reported that nitric oxide (NO) is not involved in the down-regulation of CYP3A2 and CYP2C11 in endotoxemia (Sewer and Morgan, 1997, 1998). In the present study, no significant differences in the plasma levels of NO were observed among endotoxins (data not shown). On the other hand, TNF-α is reported to be of major importance in the down-regulation of CYP3A2 and CYP2C11 in endotoxemia (Monshouwer et al., 1996; Sewer and Morgan, 1997). Based on these findings, the results of the present study are supported to some degree by a report which demonstrates that the levels of TNF-α in whole blood stimulated by *P. aeruginosa* endotoxin were lower than those stimulated by *E. coli* and *K. pneumoniae* endotoxin (Mathiak et al., 2003). It is possible that differences in the down-regulation of CYP3A2 and CYP2C11 by endotoxin are due to differences

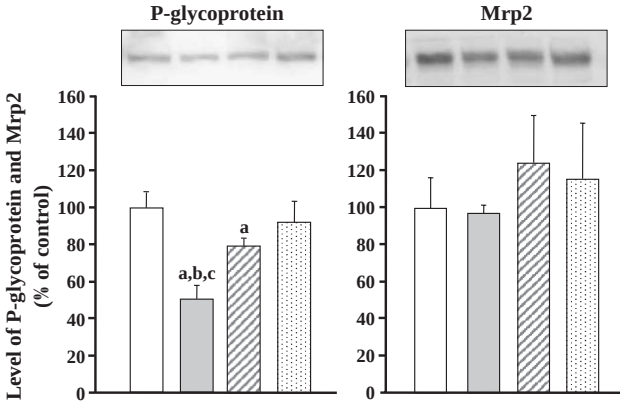

Fig. 5. Effects of different endotoxin on expression of hepatic P-glycoprotein and Mrp2. Liver samples were taken 24 h after intravenous injection of endotoxin (0.5 mg/kg). □, Control rats; ▨, *K. pneumoniae* endotoxin; ▨, *P. aeruginosa* endotoxin; ▨, *E. coli* endotoxin. Each bar shows the intensity ratio compared to the value for the control rats; values are the mean±S.E.M. (n=3). [a]Significantly different from control rats (P<0.01). [b,c]Significantly different from *P. aeruginosa* endotoxin and *E. coli* endotoxin (P<0.05).

in the production of TNF-α, although plasma levels of TNF-α were not measured in this study.

Finally, we investigated whether endotoxin has differential effects on the expression of P-glycoprotein and Mrp2. Interestingly, both *K. pneumoniae* and *P. aeruginosa* endotoxin significantly decreased the protein levels of P-glycoprotein, but unexpectedly, not Mrp2. On the other hand, *E. coli* endotoxin did not change the protein levels of both P-glycoprotein and Mrp2. Nakamura and colleagues demonstrated that interleukin-1 is responsible for endotoxin-induced suppression of the expression of hepatic Mrp2 mRNA (Nakamura et al., 1999). The effect of *K. pneumoniae* endotoxin on the production of interleukin-1 has been reported to be very similar to that of *E. coli* endotoxin (Mathiak et al., 2003). It is possible that the dose of endotoxin used in this study was not enough to produce interleukin-1. We could not exclude the possibility that no change in the expression of Mrp2 in the liver by endotoxin may be due to its protective function against endotoxin-induced damage to the liver by serving to flush out endogenous toxic substances such as bilirubin from the liver. Results of the present study are not consistent with the reports which demonstrate that *E. coli* endotoxin significantly down-regulated P-glycoprotein and Mrp2 (Hartmann et al., 2001, 2002; Tang et al., 2000; Vos et al., 1998). Although the reason for the discrepancy is not clear at present, it may be related to the differences between the high dose in their studies (5 mg/kg) and the low dose in ours (0.5 mg/kg). On the basis of these observations, the effect of *K. pneumoniae* endotoxin on the regulation of P-glycoprotein may well be much stronger than that of *E. coli* endotoxin. Moreover, the difference in the effects of *E. coli* and *K. pneumoniae* endotoxin on the expression of P-glycoprotein may be due to differences in their chemical structures consisting of mannose, as the O-specific polysaccharide chains might be taken into consideration since, mannan as the O-specific polysaccharide of *K. pneumoniae* endotoxin plays an important role in the expression of the adjuvant activity (Kato et al., 1985; Ohta et al., 1987).

In conclusion, the present study is the first to report the differential effects of endotoxin derived from the three major gram-negative bacterial pathogens, *K. pneumoniae*, *P. aeruginosa* and *E. coli*, on the protein levels of the CYP subtypes CYP3A2 and CYP2C11, as well as the drug transporters P-glycoprotein and Mrp2. From the present findings, it is suggested that *K. pneumoniae* endotoxin induces stronger down-regulation of CYP3A2, CYP2C11 and P-glycoprotein compared with *P. aeruginosa* and *E. coli* endotoxin, although the precise mechanisms are not fully understood. Further studies are needed to investigate the structure–activity relationship between endotoxin using different gram-negative bacterial sources. Finally, more detailed studies are also needed to elucidate the mechanisms by which endotoxin down-regulates the expression of CYP subtypes and drug transporters in consideration of the differential effects of endotoxin.

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