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Endotoxin impairs biliary transport of sparfloxacin and its glucuronide in rats

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Abstract

The effect of endotoxin on glucuronidation and hepatobiliary transport of quinolone antimicrobial agents was investigated in rats using sparfloxacin and *p*-nitrophenyl glucuronide as model drugs. The biliary clearance experiments were performed 24 h after a single intraperitoneal injection of endotoxin (1 mg/kg). Endotoxin significantly delayed the disappearance of sparfloxacin from plasma and increased plasma concentration of its glucuronide after intravenous injection of sparfloxacin (10 mg/kg). Significant decreases in the systemic clearance of sparfloxacin and the biliary clearance of sparfloxacin and the glucuronide were observed. Endotoxin had no effect on in vitro glucuronidation activity using *p*-nitrophenol as a substrate. When *p*-nitrophenyl glucuronide (8 mg/kg) was administered in endotoxin-pretreated rats, significant decreases in the systemic clearance, biliary clearance and renal clearance of *p*-nitrophenyl glucuronide were observed. These findings suggest that endotoxin decreases the biliary excretion of sparfloxacin and its glucuronide probably due to impairment of their hepatobiliary transport systems and renal handling.

Keywords: Endotoxin; Quinolone antimicrobial agent; Sparfloxacin; P-glycoprotein; Biliary excretion; Canalicular multispecific organic anion transporter; (Rat)

1. Introduction

Endotoxin, an active component in the outer membrane of the Gram-negative bacteria, is well known to induce damage to numerous organs including the liver (Hirata et al., 1980; Durham et al., 1990; Hewett and Roth, 1993). Among numerous organs, liver has an important function for the detoxication (phase I and phase II metabolism) and excretion (hepatobiliary excretion) of a variety of drugs and their metabolites. The effect of endotoxin and inflammatory cytokines released by endotoxin on phase I metabolism is well known. Studies in our laboratories with antipyrine as a substrate showed that endotoxin treatment induces time-dependent reduction in hepatic metabolism in rats and the reduction is due to overproduction of nitric oxide (NO) in plasma (Nadai et al., 1998; Kitaichi et al., 1999). Furthermore, we previously reported that endotoxin treatment impairs biliary and renal excretion of various organic anion drugs by changing the ability of the biliary and tubular secretory systems (Nadai et al., 1993a,b, 1996; Hasegawa et al., 1994; Haghgoo et al., 1994). However, less is known about the effect of endotoxin treatment on phase II metabolism and hepatobiliary transport systems.

It is well known that several drug transporters contribute to the hepatobiliary transport of drugs; for example, canalicular multispecific organic anion transporter (mrp2/cMOAT) and P-glycoprotein, which belong to ATP-binding cassette (ABC) transporter superfamily, present in the bile canalicular membrane and are considered to play a central role in the excretion of numerous xenobiotics from the liver. There are a number of papers suggesting that organic anions, including glucuronide conjugates, can be secreted into the bile by mrp2/cMOAT (Hirohashi et al., 1999; Kamisako et al., 1999; Fukumura et al., 1998; Seitz et al., 1998; Keppler et al., 1997).

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New quinolone antimicrobial agents are widely used for the treatment of patients with Gram-negative bacterial infection. Many new quinolone antimicrobial agents (enoxacin, levofloxacin, ciprofloxacin, sparfloxacin and grepafloxacin) are known to primarily excrete into the urine. Among them, sparfloxacin and grepafloxacin are typical groups of drugs excreted into the bile (Matsunaga et al., 1991; Akiyama et al., 1995). It has been suggested that grepafloxacin and its glucuronide are exported into the bile by mrp2/cMOAT and that grepafloxacin glucuronide has higher affinity with the transporter than the parent drug (Sasabe et al., 1998). The hepatobiliary transport mechanism of sparfloxacin remains to be elucidated. We consider that sparfloxacin may be also actively excreted into the bile by the same transporter as grepafloxacin or by P-glycoprotein since sparfloxacin is suggested to be a substrate for P-glycoprotein (Cormet-Boyaka et al., 1998; Dautrey et al., 1999). However, the effect of endotoxin on the phase II metabolism and hepatobiliary transport of sparfloxacin has not yet been fully clarified.

The aim of the present study is to clarify the effect of *Klebsiella pneumoniae* endotoxin on the hepatobiliary excretion of quinolones using sparfloxacin and *p*-nitrophenyl glucuronide as model drugs.

2. Materials and methods

2.1. Materials

Endotoxin was isolated from *K. pneumoniae* LEN-1 (O3: K1$^-$), which was identical to that used in previous studies (Nadai et al., 1998; Kitaichi et al., 1999). Sparfloxacin was kindly donated from Dainippon Pharmaceutical (Tokyo, Japan). β-Glucuronidase, UDP–glucuronic acid, *p*-nitrophenol and *p*-nitrophenyl glucuronide were purchased from Sigma (St. Louis, MO). All other reagents are commercially available and were of analytical grade.

2.2. Animals and experiments

Eight- to nine-week-old male Wistar rats weighing 280–300 g (Japan SLC, Hamamatsu, Japan) were used for all experiments. The rats were housed under controlled environmental conditions (temperature 23 ± 1 °C and humidity $55 \pm 5\%$) with a commercial food and water freely available. All animal experiments were carried out according to the guidelines of Nagoya University School of Medicine or Faculty of Pharmaceutical Sciences, Meijo University for the care and use of laboratory animals and the European Community guidelines for the use of laboratory animals.

Just before the experiments, rats under light anesthesia with pentobarbital (25 mg/kg) were cannulated with polyethylene tubes, in the left jugular artery and the femoral vein for blood sampling and drug administration, respectively. The bile duct was also cannulated with polyethylene tube for bile collection. In the clearance experiment of *p*-nitrophenyl glucuronide, urinary bladder was cannulated with polyethylene tube for urine sampling. In all experiments, the rats were placed in plastic metabolic cages (Natsume, Tokyo, Japan) under anesthesia with pentobarbital. Body temperature was maintained at 37 °C throughout the experiments with the assistance of heat lamps.

The rats received an intraperitoneal injection of *K. pneumoniae* endotoxin (1 mg/kg) 24 h before intravenous injection of each drug. The control group was treated with isotonic saline in place of endotoxin. To elucidate the effect of endotoxin on the biliary excretion of sparfloxacin and the glucuronide, the rats received a bolus injection of sparfloxacin (10 mg/kg). Blood samples of approximately 0.25 ml were collected at appropriate intervals after the administration of sparfloxacin (5, 10, 20, 30, 45, 60, 90, 120, and 180 min). Bile samples were collected in preweighed tubes at designated intervals (0 to 10 min and thereafter 20-min intervals) for 180 min.

To elucidate the effect of endotoxin on the biliary excretion of *p*-nitrophenyl glucuronide, the rats received a bolus injection of *p*-nitrophenyl glucuronide (8 mg/kg). The dose of *p*-nitrophenyl glucuronide used in this study was chosen on the basis of a report (Machida et al., 1982). After injection of *p*-nitrophenyl glucuronide, blood samples were collected at designated intervals (5, 10, 15, 20, 25, 30, 45, and 60 min after injection). Bile and urine samples were collected at three consecutive 20-min time points for 60 min.

Blood samples in all experiments were immediately centrifuged at $6000 \times g$ for 10 min to yield plasma samples. The volume of bile and urine was measured gravimetrically, with specific gravity assumed to be 1.0. Plasma, bile and urine samples were stored at -40 °C until analysis.

2.3. Protein binding experiments

To estimate the difference in the protein binding of *p*-nitrophenyl glucuronide between the control and endotoxin-treated rats, the protein binding experiment was done by equilibrium dialysis using a cellulose membrane (Visking Sheet, Sanplatec, Osaka, Japan) with molecular weight cut-off set at 10–20 kDa. Under light anesthesia with ethyl ether, blood samples were obtained from the abdominal aorta of control rats (saline) and rats 24 h after injection of endotoxin, and plasma samples were immediately obtained by centrifugation. Four-hundred microliters of pH 7.4 phosphate buffered saline (PBS) solution containing 5 μg/ml of *p*-nitrophenyl glucuronide was dialyzed against an equal volume of plasma samples at 37 °C for 8 h to attain equilibrium. The concentration of *p*-nitrophenyl glucuronide was chosen on the basis of plasma concentration

data obtained in vivo experiments. Concentrations of *p*-nitrophenyl glucuronide on both sides of the membrane were measured by high-performance liquid chromatography (HPLC).

2.4. Glucuronidation of p-nitrophenol in liver homogenates

Approximately 1 g of rat liver treated earlier 24 h with endotoxin or saline was suspended in 5-ml cold 0.15 M KCl solution and homogenized with a loose homogenizer on ice. Liver homogenate equivalent to about 100 mg/ml in incubation medium (pH 7.4) was incubated with 50 μM *p*-nitrophenol, 2 mM UDP–glucuronic acid. The incubation medium contained 131 mM NaCl, 5.2 mM KCl, 0.9 mM $MgSO_4$ $7H_2O$, 0.12 mM $CaCl_2 \cdot 2H_2O$, 3.0 mM NaH_2PO_4, and 10.0 mM Tris–ethanolamine. The final pH was adjusted to 7.4 with HCl. Incubation was carried out for 30 min at 37 °C and was stopped immediately by adding 10% perchloric acid into the incubation mixture. Concentrations of *p*-nitrophenol glucuronide in the samples were analyzed by HPLC.

2.5. Drug analysis

The concentration of sparfloxacin and *p*-nitrophenyl glucuronide in plasma and bile was measured by HPLC. Bile samples were measured by appropriate dilution with distilled water. Free sparfloxacin, released by hydrolysis of glucuronide conjugate in the plasma and bile, was measured. Briefly, 50 μl of plasma and bile samples were mixed with 100 μl of acetic acid buffer (pH 5.0) containing β-glucuronidase (250 unit/ml) for 15 h at 37 °C to hydrolyze the conjugate completely. The solution was added to 150 μl of 66 mM phosphate buffer solution (pH 7.4).

For measuring sparfloxacin and the glucuronide, 50 μl of each sample treated with or without treated with β-glucuronidase, were mixed with 300 μl of methanol containing norfloxacin (0.1 μg/ml) as an internal standard. After centrifuging the mixture, the supernatant was evaporated to dryness with N_2 gas stream at 50 °C. The residue was reconstituted with 200 μl of the mobile phase and the solution was subjected to HPLC.

For measuring *p*-nitrophenyl glucuronide in plasma or bile, 50 μl of each sample was mixed with 10% perchloric acid (100 μl) containing *p*-fluorophenol (35 mM) as an internal standard. After centrifuging the mixture, the supernatant was subjected to HPLC. Bile samples were diluted adequately with distilled water. After centrifuging the mixture, the supernatant was subjected to HPLC.

The apparatus used for HPLC was Shimadzu LC-6A system (Kyoto, Japan) consisting of a LC-6A liquid pump and an SIL-6A autoinjector. Sparfloxacin was separated on a Cosmocil $5C_{18}$ column (4.6 × 150 mm, Nacalai Tesque, Kyoto, Japan) with a mobile phase [20 mM Na_2SO_4/acetonitrile = 80:20 (vol/vol) solution containing 0.1% H_3PO_4] at 40 °C with a column oven (OTC-6A), and was detected by a RF-535 fluorescence detector (operated at emission λ 540 nm and excitation λ 310 nm). PN-G was separated on a Cosmocil $5C_{18}$ column with a mobile phase [water/methanol/acetic acid = 685:300:15 (vol/vol) solution containing 0.5 g/l of KNO3 and 30 mg/l of tetrabutylammonium bromide] at 40 °C; and was detected by an SPD-6A UV spectrophotometric detector (operated at 300 nm). The flow rate was 1.0 ml/min for sparfloxacin and 1.2 ml/min for *p*-nitrophenyl glucuronide. These assays were shown to be linear for the concentrations measured with a correlation coefficient of 0.999. The detection limit was 0.01 μg/ml for sparfloxacin and 0.05 μg/ml for *p*-nitrophenyl glucuronide. No interference with the peak of each drug was observed in any samples. The within-day and between-day coefficients of variation for this assay were less than 6%.

2.6. Pharmacokinetic analysis

Plasma concentration–time data for sparfloxacin, sparfloxacin glucuronide and *p*-nitrophenyl glucuronide were analyzed using noncompartmental model. The area under the curve (AUC) and the area under the first moment curve (AUMC) were calculated by the trapezoidal rule with extrapolation to infinity. The systemic clearance (CL_{SYS}) was determined as dose/AUC. The mean residence time (MRT) was calculated as AUMC/AUC. The volume of distribution at steady state was calculated as $CL_{SYS} \times MRT$.

The biliary clearance and renal clearance was calculated by dividing the total amount of drug excreted into the urine and bile, within the respective collection period (60 or 180 min) by the corresponding AUC (AUC_{60} or AUC_{180}).

2.7. Statistical analysis

The results are expressed as means ± S.E. for the indicated numbers of experiments. Statistical analysis was performed by one-way analysis of variance (ANOVA). When F ratios were significant ($P < 0.05$), Scheffe's post-hoc tests between the two groups were done and P values of < 0.05 were considered statistically significant post-hoc differences.

3. Results

3.1. Effect of endotoxin on the pharmacokinetics of sparfloxacin and the glucuronide

We investigated the effect of endotoxin on the plasma concentration and biliary excretion of sparfloxacin and the

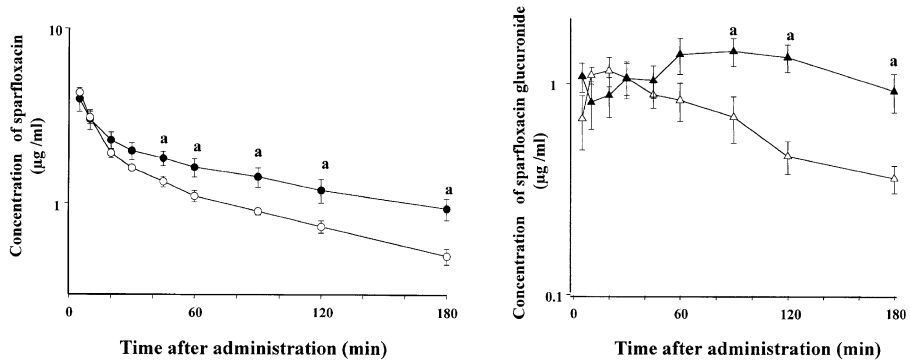

Fig. 1. Mean semilogarithmic plots of plasma concentration–time data for sparfloxacin (left) and sparfloxacin–glucuronide (right) in untreated rats (○) and in rats treated with endotoxin (●). Sparfloxacin (10 mg/kg) was administered intravenously 24 h after a single intraperitoneal injection of endotoxin (1 mg/kg). Each symbol represents the mean ± S.E.M. of five rats. a: Significantly different from control ($P < 0.05$).

glucuronide in rats. Fig. 1 shows mean plasma concentration–time curves of sparfloxacin and the glucuronide in the control and endotoxin-treated rats following an intravenous injection of sparfloxacin (10 mg/kg). As illustrated in Fig. 1, endotoxin dramatically delayed the disappearance of sparfloxacin from plasma and significantly increased the plasma concentrations of sparfloxacin glucuronide compared to the control rats. Table 1 shows the corresponding pharmacokinetic parameters of sparfloxacin and the glucuronide. The percentage of the dose of sparfloxacin and the glucuronide excreted in the bile during experimental period (0 to 180 min) following administration of sparfloxacin in normal rats was 4.0% and 30.2%, respectively. Endotoxin significantly decreased the systemic clearance and biliary clearance of sparfloxacin (2.1–1.3 and 0.1–0.06 l/h/kg, respectively). However, no significant difference in the steady state volume of distribution of sparfloxacin was observed between the control and endotoxin-treated rats. On the other hand, endotoxin significantly increased the area under plasma concentration–time curve (2.0 to 3.5 μg h/ml) and decreased the biliary clearance of sparfloxacin glucuronide (1.6 to 0.7 l/h/kg).

Table 1
Effect of endotoxin on the pharmacokinetic parameters of sparfloxacin and its glucuronide

	Control	Endotoxin
Sparfloxacin		
CL_{SYS} (l/h/kg)	2.06 ± 0.17	1.33 ± 0.18[a]
V_{SS} (l/kg)	4.37 ± 0.22	4.29 ± 0.50
CL_{BILE} (l/h/kg)	0.12 ± 0.02	0.06 ± 0.01[a]
Sparfloxacin glucuronide		
CL_{BILE} (l/h/kg)	1.64 ± 0.27	0.72 ± 0.17[a]

Each value represents the mean ± S.E.M. of results from five animals. Sparfloxacin (10 mg/kg) was administered intravenously in rats 24 h after injection of endotoxin. CL_{SYS}, systemic clearance; V_{SS}, volume of distribution at steady state; CL_{BILE}, biliary clearance.

[a] Significantly different from control ($P < 0.05$).

3.2. Effect of endotoxin on hepatic glucuronidation of p-nitrophenol as a substrate

To confirm whether the endotoxin-induced increase in plasma concentration of sparfloxacin glucuronide was due to an increased activity of the enzyme, we investigated the effect of endotoxin on the glucuronidation of *p*-nitrophenol as a substrate using liver homogenates from rats pretreated with or without endotoxin. No significant difference in the glucuronidation of *p*-nitrophenol was observed between the control (22.9 ± 0.4 μM/min/g liver) and endotoxin-treated (22.5 ± 0.4 μM/min/g liver) rats.

3.3. Effect of endotoxin on the biliary and renal excretion of p-nitrophenyl glucuronide

To confirm whether the endotoxin-induced decrease in the systemic clearance of sparfloxacin and biliary clear-

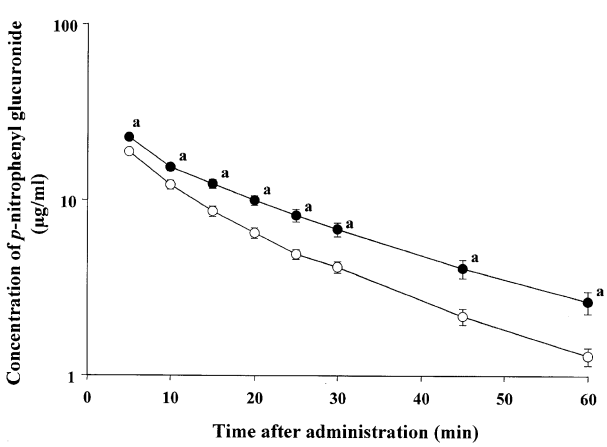

Fig. 2. Mean semilogarithmic plots of plasma concentration–time data for *p*-nitrophenyl glucuronide (*p*-nitrophenyl glucuronide) in untreated rats (○) and in rats treated with endotoxin (●). *p*-Nitrophenyl glucuronide (8 mg/kg) was administered intravenously 24 h after a single intraperitoneal injection of endotoxin (1 mg/kg). Each symbol represents the mean ± S.E.M. of five rats. a: Significantly different from control ($P < 0.05$).

Table 2
Effect of endotoxin on the pharmacokinetic parameters of *p*-nitrophenyl glucuronide

	CL_{SYS} (l/h/kg)	V_{SS} (l/kg)	CL_{BILE} (l/h/kg)	CL_R (l/h/kg)
Control	1.09 ± 0.06	0.42 ± 0.04	0.07 ± 0.01	0.82 ± 0.04
Endotoxin	0.74 ± 0.06[a]	0.37 ± 0.04	0.04 ± 0.01[a]	0.55 ± 0.04[a]

Each value represents the mean ± S.E.M.of results from five animals. *p*-Nitrophenyl glucuronide (8 mg/kg) was administered intravenously in rats 24 h after injection of endotoxin. CL_{SYS}, systemic clearance; V_{SS}, volume of distribution at steady state; CL_{BILE}, biliary clearance; CL_R, renal clearance.

[a] Significantly different from control ($P < 0.05$).

ance of sparfloxacin glucuronide was due to a decreased renal excretion, we investigated the effect of endotoxin on the biliary and renal excretion of *p*-nitrophenyl glucuronide as a model drug. The mean plasma concentration–time data for *p*-nitrophenyl glucuronide in the control and endotoxin-treated rats, following an intravenous injection of *p*-nitrophenyl glucuronide (8 mg/kg), are illustrated in Fig. 2. Endotoxin significantly delayed the disappearance of *p*-nitrophenyl glucuronide from plasma. The corresponding pharmacokinetic parameters of *p*-nitrophenyl glucuronide are summarized in Table 2. Endotoxin significantly decreased the systemic clearance, biliary clearance and renal clearance of *p*-nitrophenyl glucuronide by approximately 30%, 40% and 30%, respectively. However, no significant difference in the volume of distribution at steady state for *p*-nitrophenyl glucuronide was observed between the control and endotoxin-treated rats. The protein binding of *p*-nitrophenyl glucuronide was weak (less than 10%). No significant difference in the unbound fraction to plasma protein was observed between the control and endotoxin-treated rats (0.95 ± 0.06 and 0.86 ± 0.05, respectively).

4. Discussion

Our interest in the biliary transport of quinolones was stimulated by a report that the transport of grepafloxacin and the main metabolite, grepafloxacin glucuronide, across the bile canalicular membrane is mediated by mrp2/cMOAT and that the glucuronide has a much higher affinity for mrp2/cMOAT than the parent drug (Sasabe et al., 1998). Recently, Tamai et al. (2000) reported on the possible involvement of P-glycoprotein in the blood/brain distribution of grepafloxacin and sparfloxacin. Recently, we reported that some quinolones reverse P-glycoprotein-mediated drug resistance and that grepafloxacin is actively excreted into the bile by P-glycoprotein and/or mrp2/cMOAT (Zhao et al., in press). A series of experiments, therefore, was designed in our laboratories to investigate the effect of *K. pneumoniae* endotoxin on the biliary transport systems for organic anions using sparfloxacin as a model drug.

The data of the biliary clearance experiment of sparfloxacin demonstrate that endotoxin significantly delays the disappearance of sparfloxacin and the glucuronide from plasma and that it significantly decreases the systemic clearance and biliary clearance of the parent drug. Endotoxin was also shown to decrease the biliary clearance of sparfloxacin glucuronide. On the basis of the recovery of sparfloxacin from bile, it was concluded that the biliary excretion of the parent drug is only minor. Hence, it is unlikely that endotoxin-induced decrease in the systemic clearance of sparfloxacin and increase in the plasma concentrations of the parent drug are due to a decrease in the biliary clearance. Rather, the endotoxin-induced decrease in the systemic clearance of the parent drug may be due to a reduction of its glucuronidation in the liver and/or renal clearance. On the other hand, the endotoxin-induced decrease in the clearance of sparfloxacin glucuronide appears to be due to impairment of the biliary transport of sparfloxacin glucuronide.

To further clarify this phenomenon, we examined the effects of endotoxin on the formation of glucuronide conjugate and its biliary excretion of *p*-nitrophenyl glucuronide as a substrate, which is mainly excreted into the urine and bile. On the basis of the extent of protein binding and the normal value of glomerular filtration rate (approximately 3 ml/min), the clearance ratio (renal clearance/glomerular filtration rate) for *p*-nitrophenyl glucuronide in normal rats was calculated to be about 1.4, indicating that *p*-nitrophenyl glucuronide is at least partly secreted into the urine by organic anion transport mechanism. It is likely that endotoxin decreases the tubular secretion of *p*-nitrophenyl glucuronide, expressed as renal clearance for unbound drug minus glomerular filtration rate. The data from the in vivo experiments using *p*-nitrophenyl glucuronide demonstrated similar results to those observed with sparfloxacin. These results suggest that endotoxin-induced decrease in the systemic clearance of sparfloxacin and delay in the disappearance of sparfloxacin from plasma are caused by a decrease in renal excretion and/or rate of glucuronidation, but not by a decrease in the biliary excretion.

To clarify whether endotoxin increases the plasma concentrations of sparfloxacin by changing the glucuronidation, we examined the effect of endotoxin on the glucuronidation of *p*-nitrophenol in vitro. Endotoxin did not change the rate of formation of *p*-nitrophenol to *p*-nitrophenyl glucuronide, suggesting that endotoxin has no direct effect on hepatic glucuronidation. These results confirm the study by Roelofsen et al. (1994), who reported that intravenous injection of *E. coli* endotoxin induces a decrease in biliary transport of bilirubin and the impaired transport of bilirubin is not due to impairment of conjugation of bilirubin. They also reported that ATP levels in hepatocytes were slightly decreased 2 h after intravenous

injection of endotoxin (1 mg/kg) but returned to normal levels within 18 h after injection (Roelofsen et al., 1995). It is unlikely therefore that endotoxin-induced reduction of ATP-dependent biliary transport of sparfloxacin glucuronide and *p*-nitrophenyl glucuronide might be due to a decrease in ATP levels.

There is a possibility that the reduction of biliary excretion of sparfloxacin glucuronide and *p*-nitrophenyl glucuronide might be due to a decrease in the ability and/or capacity of transporters related to their biliary excretion. It has been suggested that sparfloxacin, lomefloxacin and a newly developed quinolone, HSR-903, are actively secreted into the bile via mrp2/cMOAT (Murata et al., 1999; Sasabe et al., 1998). Quinolones, which are significantly excreted into the bile, appear to be actively secreted into the bile by mrp2/cMOAT. There is a possibility that the biliary transport of quinolones is mediated by other transporters distinct from mrp2/cMOAT. For example, there is evidence for P-glycoprotein-mediated transport of quinolones (Rabbaa et al., 1995; Ito et al., 1997; Tamai et al., 2000). Indeed, it has been suggested that sparfloxacin is a substrate for P-glycoprotein (Cormet-Boyaka et al., 1998; Dautrey et al., 1999; Tamai et al., 2000; Zhao et al., in press). These observations suggest that quinolones, which are excreted into the bile, are, at least in part, excreted into the bile by mrp2/cMOAT and P-glycoprotein.

There are many reports suggesting that the biliary transport of substrates for mrp2/cMOAT is decreased by endotoxin injection (Roelofsen et al., 1994, 1995; Bolder et al., 1997). Trauner et al. (1997) have reported that reduction of the expression of mrp2/cMOAT mRNA was observed in rat liver 18 h after injection of endotoxin. Vos et al. (1998) have reported that *E. coli* endotoxin largely decreased the expression of mrp2/cMOAT at the mRNA and protein levels in rat liver 6 h after injection, whereas the expression did not return to normal until 48 h. Yet, they also reported that the expression of mdr1a mRNA did not change until at least 48 h after injection (Vos et al., 1998). To the contrary, Tang et al. (2000) have reported that *E. coli* endotoxin induced significant reductions in the protein and mRNA expression of mrp2/cMOAT and P-glycoprotein in rat liver 12 h after injection. There is evidence that cytokine induction in different bacterial strains and endotoxin preparations vary (Flad et al., 1993). The discrepancy in mdr1a regulation between the study of Vos et al. (1998) and the study of Tang et al. (2000) may be due to differences in bacterial strains (*E. coli* serotype O127:B8 and *E. coli* serotype O55:B5, respectively) and/or doses of endotoxin (2 and 5 mg/kg, respectively). The present in vivo results are, at least, supported by the study of Tang et al. (2000). On the basis of these findings, we assume that the endotoxin-induced decrease in the biliary excretion of sparfloxacin and glucuronide is due to the impairment of both mdr1a- and mrp2/cMOAT-mediated transport of sparfloxacin.

It has been shown that endotoxin induces increased levels of cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1, interleukin-2 and interleukin-6. The elevation of these cytokines might play an important role in endotoxin-induced changes in certain transporter-mediated biliary excretion mechanisms (Simpson et al., 1997; Hirsch-Ernst et al., 1998). Nakamura et al. (1999) reported that anti-interleukin-1 or anti-TNF-α antibodies restores endotoxin-induced reduction in the expression of cMOAT/MRP2, whereas IL-1 and TNF-α decrease the expression. On the other hand, there is evidence that TNF-α induces up-regulation of the expression of MRP1 in human colon carcinoma cells and mdr1 in rat hepatoma cell line (Chapekar et al., 1991; Stein et al., 1997). We have found that a nonselective inhibitor of TNF-α production, pentoxifylline, significantly inhibits endotoxin-induced reduction of biliary transport of rhodamine 123, a substrate for P-glycoprotein (unpublished data). On the basis of these observations, we speculate that the endotoxin-induced decrease in mrp2/cMOAT- or P-glycoprotein-mediated transport mechanism is due to the release of such endogenous cytokines. However, further studies are needed to confirm this idea.

In conclusion, in the present study we have found that *K. pneumoniae* endotoxin significantly decreases the systemic and biliary clearances of sparfloxacin and its glucuronide. We conclude that the endotoxin-induced decrease in the biliary excretion of sparfloxacin may be caused by a lower ability and/or capacity of transporters involved in the biliary and renal excretion of sparfloxacin.

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References

Akiyama, H., Abe, Y., Kusumoto, N., Odomi, M., 1995. Pharmacokinetics of grepafloxacin (II): absorption, distribution and excretion after oral administration of [^{14}C]-grepafloxacin in rats. Chemotherapy 43, 107–124.

Bolder, U., Ton-Nu, H.T., Schteingart, C.D., Frick, E., Hofmann, A.F., 1997. Hepatocyte transport of bile acids and organic anions in endotoxemic rats: impaired uptake and secretion. Gastroenterology 112, 214–225.

Chapekar, M.S., Huggett, A.C., Cheng, C., 1991. Dexamethasone prevents the growth inhibitory effects of recombinant tumor necrosis factor in a rat hepatoma cell line Reuber-RC-3: an association with the changes in the messenger RNA levels for multidrug resistance gene. Biochem. Biophys. Res. Commun. 181, 1524–1531.

Cormet-Boyaka, E., Huneau, J.F., Mordrelle, A., Boyaka, P.N., Carbon,

C., Rubinstein, E., Tome, D., 1998. Secretion of sparfloxacin from the human intestinal Caco-2 cell line is altered by P-glycoprotein inhibitors. Antimicrob. Agents Chemother. 42, 2607–2611.

Dautrey, S., Felice, K., Petiet, A., Lacour, B., Carbon, C., Farinotti, R., 1999. Active intestinal elimination of ciprofloxacin in rats: modulation by different substrates. Br. J. Pharmacol. 127, 1728–1734.

Durham, S.K., Brouwer, A., Barelds, R.J., Horan, M.A., Knook, D.L., 1990. Comparative endotoxin-induced hepatic injury in young and aged rats. J. Pathol. 162, 341–349.

Flad, H.D., Loppnow, H., Rietschel, E.T., Ulmer, A.J., 1993. Agonists and antagonists for lipopolysaccharide-induced cytokines. Immunobiology 187, 303–316.

Fukumura, S., Takikawa, H., Yamamaka, M., 1998. Effects of organic anions and bile acid conjugates on biliary excretion of pravastatin in the rat. Pharm. Res. 15, 72–76.

Haghgoo, S., Hasegawa, T., Nadai, M., Wang, L., Nabeshima, T., Kato, N., 1994. Effect of a bacterial lipopolysaccharide on biliary excretion of a β-lactam antibiotic, cefoperazone, in rats. Antimicrob. Agents Chemother. 39, 2258–2261.

Hasegawa, T., Nadai, M., Wang, L., Takayama, Y., Kato, K., Nabeshima, T., Kato, N., 1994. Renal excretion of famotidine and role of adenosine in renal failure induced by bacterial lipopolysaccharide in rats. Drug Metab. Dispos. 22, 8–13.

Hewett, J.A., Roth, R.A., 1993. Hepatic and extrahepatic pathobiology of bacterial lipopolysaccharides. Pharmacol. Rev. 45, 381–411.

Hirata, K., Kanako, A., Ogawa, K., Hayasaka, H., Onoe, T., 1980. Effect of endotoxin on rat liver: analysis of acid phosphatese isozymes in the liver of normal and endotoxin-treated rats. Lab. Invest. 43, 165–171.

Hirohashi, T., Suzuki, H., Sugiyama, Y., 1999. Characterization of the transport properties of cloned rat multidrug resistance-associated protein 3 (MRP3). J. Biol. Chem. 274, 15181–15185.

Hirsch-Ernst, K.I., Ziemann, C., Foth, H., Kozian, D., Schmitz-Salue, C., Kahl, G.F., 1998. Induction of mdr1b mRNA and P-glycoprotein expression by tumor necrosis factor alpha in primary rat hepatocyte cultures. J. Cell. Physiol. 176, 506–515.

Ito, Y., Yano, I., Tanaka, K., Inui, K., 1997. Transport of quinolone antibacterial drugs by human P-glycoprotein expressed in a kidney epithelial cell line, LLC-PK1. J. Pharmacol. Exp. Ther. 282, 955–960.

Kamisako, T., Leier, I., Cui, Y., Konig, J., Buchholz, U., Hummel-Eisenbeiss, J., Keppler, D., 1999. Transport of monoglucuronosyl and bisglucuronosyl bilirubin by recombinant human and rat multidrug resistance protein 2. Hepatology 30, 485–490.

Keppler, D., Leier, I., Jedlitschky, G., 1997. Transport of glutathione conjugates and glucuronides by the multidrug resistance proteins MRP1 and MRP2. Biol. Chem. 378, 787–791.

Kitaichi, K., Wang, L., Takagi, K., Iwase, M., Shibata, E., Nadai, M., Takagi, K., Hasegawa, T., 1999. Decreased antipyrine clearance following endotoxin administration: in vivo evidence of the role of nitric oxide. Antimicrob. Agents Chemother. 43, 2697–2701.

Machida, M., Morita, Y., Hayashi, M., Awazu, S., 1982. Pharmacokinetic evidence for the occurrence of extrahepatic conjugative metabolism of *p*-nitrophenol in rats. Biochem. Pharmacol. 31, 787–791.

Matsunaga, Y., Miyazaki, H., Oh-e, Y., Nambu, K., Furukawa, H., Yoshida, K., Hashimoto, M., 1991. Disposition and metabolism of ^{14}C-sparfloxacin in the rat. Arzneim-Forsch/Drug Res. 41, 747–759.

Murata, M., Tamai, I., Kato, H., Nagata, O., Tsuji, A., 1999. Efflux transport of a new quinolone antibacterial agent, HSR-903, across the blood–brain barrier. J. Pharmacol. Exp. Ther. 290, 51–57.

Nadai, M., Hasegawa, T., Kato, K., Wang, L., Nabeshima, T., Kato, N., 1993a. Alterations in pharmacokinetics and protein binding behavior of cefazolin in endotoxemic rats. Antimicrob. Agents Chemother. 37, 1781–1785.

Nadai, M., Hasegawa, T., Kato, K., Wang, L., Nabeshima, T., Kato, N., 1993b. The disposition and renal handling of enprofylline in endotoxemic rats by bacterial lipopolysaccharide (LPS). Drug Metab. Dispos. 21, 611–616.

Nadai, M., Hasegawa, T., Wang, L., Haghgoo, S., Okasaka, T., Nabeshima, T., Kato, N., 1996. Alterations in renal uptake kinetics of the xanthine derivatives enprofylline in endotoxaemic mice. J. Pharm. Pharmacol. 48, 744–748.

Nadai, M., Sekido, T., Matsuda, I., Wang, L., Kitaichi, K., Itoh, A., Nabeshima, T., Hasegawa, T., 1998. Time-dependent effects of *Klebsiella pneumoniae* endotoxin on hepatic drug-metabolizing enzyme activity in rats. J. Pharm. Pharmacol. 50, 871–879.

Nakamura, J., Nishida, T., Hayashi, K., Kawada, N., Ueshima, S., Sugiyama, Y., Ito, T., Sobue, K., Matsuda, H., 1999. Kupffer cell-mediated down regulation of rat hepatic CMOAT/MRP2 gene expression. Biochem. Biophys. Res. Commun. 255, 143–149.

Rabbaa, L., Dautrey, S., Colas-Linhart, N., Carbon, C., Farinotti, R., 1995. Stereoselectivity of ofloxacin intestinal transport in the rat. Drugs 49, 333–334.

Roelofsen, H., Van der Veere, C.N., Ottenhoff, R., Schoemaker, B., Jansen, P.L.M., Oude Elferink, R.P.J., 1994. Decreased bilirubin transport in the perfused liver of endotoxemic rats. Gastroenterology 107, 1075–1084.

Roelofsen, H., Schoemaker, B., Bakker, C., Ottenhoff, R., Jansen, P.L.M., Oude Elferink, R.P.J., 1995. Impaired hepatocanalicular organic anion transport in endotoxemic rats. Am. J. Physiol. 269, G427–G434.

Sasabe, H., Tsuji, A., Sugiyama, Y., 1998. Carrier-mediated mechanism for the biliary excretion of the quinolone antibiotic grepafloxacin and its glucuronide in rats. J. Pharmacol. Exp. Ther. 284, 1033–1039.

Seitz, S., Kretz-Rommel, A., Oude Elferink, R.P., Boelsterli, U.A., 1998. Selective protein adduct formation of diclofenac glucuronide is critically dependent on the rat canalicular conjugate export pump (Mrp2). Chem. Res. Toxicol. 11, 513–519.

Simpson, K.J., Lukacs, N.W., Colletti, L., Strieter, R.M., Kunkel, S.L., 1997. Cytokines and the liver. J. Hepatol. 27, 1120–1132.

Stein, U., Walther, W., Laurencot, C.M., Scheffer, G.L., Scheper, R.J., Shoemaker, R.H., 1997. Tumor necrosis factor-α and expression of the multidrug resistance-associated genes LRP and MRP. J. Natl. Cancer Inst. 89, 807–813.

Tamai, I., Yamashita, J., Kido, Y., Ohnari, A., Sai, Y., Shima, Y., Naruhashi, K., Koizumi, S., Tsuji, A., 2000. Limited distribution of new quinolone antibacterial agents into brain caused by multiple efflux transporters at the blood–brain barrier. J. Pharmacol. Exp. Ther. 295, 146–152.

Tang, W., Yi, C., Kalitsky, J., Piquette-Miller, M., 2000. Endotoxin downregulates hepatic expression of P-glycoprotein and MRP2 in 2-acetylaminofluorene-treated rats. Mol. Cell Biol. Res. Commun. 4, 90–97.

Trauner, M., Arrese, M., Soroka, C.J., Ananthanarayanan, M., Koeppel, T.A., Schlosser, S.F., Suchy, F.J., Keppler, D., Boyer, J.L., 1997. The rat canalicular conjugate export pump (Mrp2) is down-regulated in intrahepatic and obstructive cholestasis. Gastroenterology 113, 255–264.

Vos, T.A., Hooiveld, G.J., Koning, H., Childs, S., Meijer, D.K., Moshage, H., Jansen, P.L., Muller, M., 1998. Up-regulation of the multidrug resistance genes, Mrp1 and Mdr1b, and down-regulation of the organic anion transporter, Mrp2, and the bile salt transporter, Spgp, in endotoxemic rat liver. Hepatology 28, 1637–1644.

Zhao, Y.L., Cai, S.H., Wang, L., Kitaichi, K., Tatsumi, Y., Nadai, M., Yoshizumi, H., Takagi, K., Takagi, K., Hasegawa, T., 2001. Possible involvement of P-glycoprotein on biliary excretion of grepafloxacin. Clin. Exp. Pharmacol. Physiol., in press.