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Alterations in Pharmacokinetics and Protein Binding Behavior of Cefazolin in Endotoxemic Rats

MASAYUKI NADAI,[1] TAKAAKI HASEGAWA,[1]* KATSUYOSHI KATO,[1] LI WANG,[1] TOSHITAKA NABESHIMA,[1] AND NOBUO KATO[2]

Department of Hospital Pharmacy[1] and Department of Bacteriology,[2] Nagoya University School of Medicine, Nagoya 466, Japan

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The possible alterations in the pharmacokinetics and protein binding behavior of the β-lactam antibiotic cefazolin (CEZ) were investigated in endotoxemic rats induced by *Klebsiella pneumoniae* O3 lipopolysaccharide (LPS). LPS (250 μg/kg of body weight) was infused for 20 to 30 min 2 h before an intravenous administration of CEZ (20 mg/kg). Significant decreases in systemic clearance and renal clearance of CEZ were observed in LPS-treated rats without any changes in fraction of urinary excretion in unchanged CEZ (>0.8). The volume of distribution at steady state showed a tendency to increase. The protein binding parameters of CEZ, the binding capacity, and number of binding sites on the albumin molecule were decreased by LPS, whereas the dissociation constant did not change. Significant decreases in systemic and renal clearances for unbound CEZ were observed in LPS-treated rats. The glomerular filtration rate estimated as inulin clearance was also decreased by LPS. The ratio of renal clearance of unbound CEZ to glomerular filtration rate (clearance ratio) dropped to 70% of that in control rats, and the net tubular secretion of CEZ was also dramatically reduced. The present study suggests that LPS has an effect on the pharmacokinetics of CEZ by changes which occur in renal handling and protein binding.

Lipopolysaccharide (LPS), which is one of the gram-negative bacterial cell wall components, is known to have various biological and immunological activities. LPS has also been shown to induce nephrotoxicity in the kidney and reduce both the glomerular filtration rate (GFR) and renal plasma flow rate (3, 6, 9). In our previous studies, we found that *Klebsiella pneumoniae* O3 LPS possesses strong adjuvant activity and antitumor activity and its adjuvant activity is much stronger than that of any other known adjuvants, including LPSs from *Escherichia coli* O111 and *Salmonella* species (11, 15, 16, 20, 24).

Our recent studies have demonstrated that *K. pneumoniae* O3 LPS dramatically modified the pharmacokinetics of xanthine enprofylline, which is primarily excreted into the urine, by decreasing the renal tubular secretion ability and glomerular filtration (21). LPS has also been shown to modify the disposition of 1-methyl-3-propylxanthine, which binds strongly to plasma protein, because of changes in plasma protein binding behavior, despite no alterations in metabolism (31). Thus, LPS may induce clinical complications because of changes which occur in the pharmacokinetic behavior of certain drugs which possess high protein binding potency and are mainly excreted from the kidney.

In the present study, the disposition and renal excretion of drugs in endotoxemic rats induced by LPS were examined by using cefazolin (CEZ) as a model drug, since it is mainly excreted into the urine by tubular organic anion transport (4, 19, 33) and exhibits high protein binding potency (25, 30). Ordinarily, CEZ is one of the most widely used β-lactam antibiotics as the first choice for preoperative and postoperative chemotherapy to prevent gram-negative bacterial infections.

* Corresponding author.

MATERIALS AND METHODS

Chemicals. Cefazolin sodium was kindly donated by the Fujisawa Pharmaceutical Co. (Osaka, Japan). The internal standard, 8-chlorotheophylline, was obtained from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Inulin was purchased from Nacalai Tesque (Osaka, Japan). LPS was isolated from a culture supernatant of *K. pneumoniae* LEN-1 (O3:K1$^-$), which is a decapsulated mutant strain derived from *K. pneumoniae* Kasuya (O3:K1), as described previously (12, 13). All other chemicals used were of the highest purity available.

Animal experiments. Eight- to 10-week-old male Wistar rats (Japan SLC, Shizuoka, Japan), weighing 280 to 320 g, were used for all experiments. One day before the experiments, rats were anesthetized with sodium pentobarbital (25 mg/kg of body weight), cannulated with polyethylene tubes in the right jugular vein, and then allowed to recover. LPS (250 μg/kg) dissolved in isotonic saline was constantly infused over a period of 20 to 30 min (3.93 μg/min/kg) as described previously (21, 31). In the control group, rats were pretreated with isotonic saline in place of LPS. Two hours after LPS infusion, CEZ was administered intravenously at the dose of 20 mg/kg, and blood samples (0.2 ml) were collected at designated intervals (10, 20, 30, 45, 60, 90, 120, 150, 180, and 210 min after CEZ administration). In the separate experiments, 100 mg of inulin per kg was also administered to determine the GFR in the same manner as that for CEZ. The blood samples obtained were immediately centrifuged at 6,000 × *g* for 5 min to yield plasma samples. Urine samples were also collected over a period of 24 h after CEZ injection. Plasma and urine samples were stored at −40°C until analysis.

Protein binding. The effect of LPS on the plasma protein binding of CEZ was examined by the micropartition equilibrium dialysis method by using the cellulose membrane (Visking sheet; Samplatec Corp., Osaka, Japan) with a molecular cutoff of 10,000 to 20,000. Blood samples were

obtained from both groups by exsanguination from the abdominal aorta under light ether anesthesia, and plasma samples were obtained by centrifugation at 6,000 × *g* for 5 min. CEZ-spiked plasma samples of various concentrations (1 to 100 μg/ml) were immediately dialyzed against an equal volume of pH 7.4 isotonic phosphate buffer at 37°C for 6 h. The time required to reach equilibrium was determined beforehand. After dialysis, the concentration of CEZ in both sides of the membrane were measured. Assuming that only one binding site exists for CEZ, plasma protein binding data were fitted to the following equation by using the nonlinear least-squares method:

$$C_b = \frac{nP \cdot C_u}{K_d + C_u} \quad (1)$$

where C_b and C_u are concentrations of the bound and unbound drug, respectively, nP is the binding capacity of the first class of binding sites, and K_d is the dissociation constant. The binding site available on the albumin molecule (n) was calculated by dividing nP by the molar concentration of albumin, which was determined on the basis of human serum albumin with a molecular weight of 69,000.

Analytical procedure. Plasma and urine concentrations of CEZ were measured by the high-pressure liquid chromatography (HPLC) method as described previously (23). Briefly, the HPLC apparatus was a Shimadzu LC-6A system (Kyoto, Japan) consisting of an LC-6A liquid pump, an SPD-6A UV spectrophotometric detector, and an SIL-6A autoinjector. The UV detector was set at 274 nm and a Cosmosil $5C_{18}$-P column (Nacalai Tesque, Kyoto, Japan) was used with a column oven (OTC-6A) heated to 40°C. The mobile phase consisted of 0.03 M phosphate buffer (pH 5.0)-methanol (88:12 [vol/vol]), and the flow rate was 1.2 ml/min. Three hundred fifty microliters of methanol containing 8-chlorotheophylline (0.5 μg/ml) as an internal standard was added to 50 μl of plasma sample and urine sample diluted with distilled water and vortexed. After centrifugation at 6,000 × *g* for 5 min, 300 μl of supernatant was evaporated to dryness under an N_2 gas stream at 50°C. The residue was reconstituted with 250 μl of the mobile phase and injected into the HPLC system. Blank plasma and urine samples did not interfere with the peak corresponding to CEZ. The assay was shown to be linear for the concentrations that we studied. The within-day and between-day coefficients of variation for the assay were less than 8%. Inulin was measured by a standard colorimetric method (7). The concentration of albumin in plasma was determined by the bromcresol green method with a commercial kit (Albumin Kit; Iatron Laboratories, Tokyo, Japan).

Data analysis. The plasma concentration-time data for CEZ were analyzed on the basis of a noncompartment model. The area under the plasma concentration-time curve (AUC) was calculated by the trapezoidal rule method with extrapolation to infinity. Systemic clearance (CL_{SYS}) was calculated as the dose divided by AUC. The mean residence time (MRT) was calculated by MRT = AUMC/AUC, where AUMC represents the area under the first moment curve. The steady-state volume of distribution (V_{ss}) was determined by $V_{ss} = CL_{SYS} \times MRT$. The renal clearance ($CL_R$) was calculated by $CL_R = CL_{SYS} \times f_e$, where f_e represents the fraction of urinary excretion in unchanged form. The pharmacokinetic parameters for unbound drug were estimated in the same manner as that for total drug concentration, where the unbound concentration was calculated by the total plasma concentrations and binding parameters obtained from the protein binding experiments in rearrangement of equation 1.

All computer analyses were performed by a nonlinear least-squares regression program with no weight function (MULTI; written by Yamaoka et al. [32]).

Statistical analysis. Results are expressed as mean ± standard error for the indicated number of experiments. Statistical differences between the control and the LPS-treated groups were examined by the unpaired Student's t test. Statistical significance was defined at $P < 0.05$.

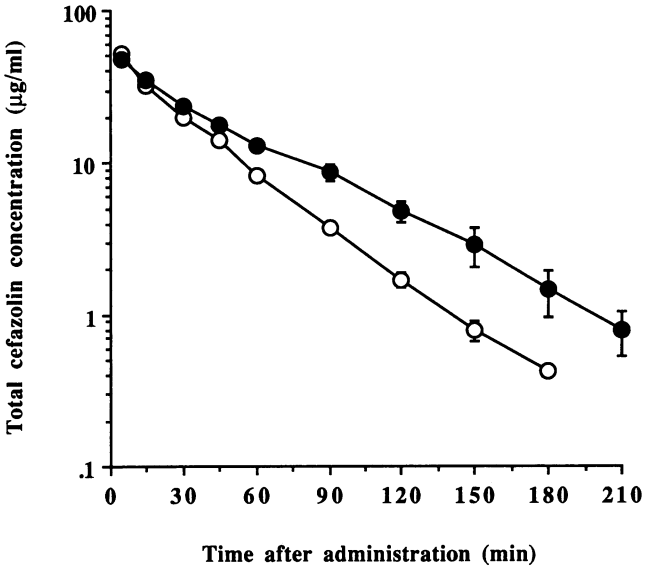

FIG. 1. Mean semilogarithmic plots of plasma concentration-time data for CEZ in control (○) and LPS-treated (●) rats after a single intravenous administration of CEZ (20 mg/kg). Each plot represents mean ± standard error (n = 4 or 5).

RESULTS

Mean semilogarithmic plasma concentration-time curves for CEZ in the control and LPS-treated rats are shown in Fig. 1. Pretreatment with LPS increased the level of CEZ in plasma and delayed the disappearance of CEZ from plasma. The corresponding pharmacokinetic parameters of CEZ are summarized in Table 1. CL_{SYS} and CL_R for CEZ significantly decreased with LPS pretreatment, and the MRT was also prolonged. The V_{ss} showed a tendency to increase. However, there was no significant difference in the f_e between the control rats (0.90 ± 0.02) and LPS-treated rats (0.86 ± 0.03). As shown in Fig. 2, the GFR as estimated by inulin clearance, significantly decreased in LPS-treated rats (0.432 ± 0.051 liters/h/kg) compared with that in control rats (0.566 ± 0.035 liters/h/kg).

TABLE 1. Pharmacokinetic parameters of CEZ in control and LPS-treated rats[a]

Treatment	V_{ss} (liters/kg)	CL_{SYS} (liters/h/kg)	CL_R (liters/h/kg)	MRT (h)
Control	0.183 ± 0.005	0.331 ± 0.009	0.297 ± 0.012	0.538 ± 0.018
LPS	0.216 ± 0.016	0.251 ± 0.015[b]	0.217 ± 0.016[b]	0.787 ± 0.058[b]

[a] Each value represents mean ± standard error (n = 4 or 5).
[b] Significantly different from control ($P < 0.05$).

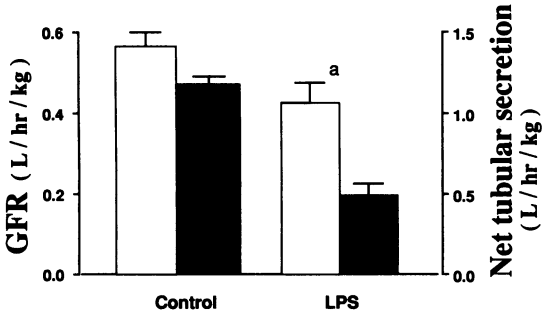

FIG. 2. GFR and net tubular secretion (CL_{Ru} − GFR) for CEZ in control and LPS-treated rats. Open and closed columns represent GFR and CL_{Ru} − GFR, respectively. Each column represents mean ± standard error (n = 4 or 5). a, significantly different from control ($P < 0.05$).

The plasma protein binding behavior of CEZ changed in the presence of LPS, although concentration dependency was observed in both groups (Fig. 3). The protein binding parameters for CEZ and plasma albumin concentrations are listed in Table 2. The binding capacity (nP) and number of binding sites (n) of CEZ significantly decreased in the presence of LPS. However, no significant changes in the K_d or albumin concentration were observed between the control rats and LPS-treated rats.

The mean unbound plasma concentrations of CEZ, as calculated by the total plasma concentration and binding parameters for each group, were plotted against time (Fig. 4). The elimination profile for unbound CEZ was still affected by LPS. The corresponding pharmacokinetic parameters for unbound drug are represented in Table 3. Significant differences in CL_{SYS}, CL_R, and MRT for the unbound drug (CL_{SYSu}, CL_{Ru}, and MRT_u, respectively) were observed between the control and LPS-treated rats. No significant differences in the distribution parameter for unbound drug, however, were observed between the two groups. The ratio of CL_R for unbound CEZ to GFR (CL_{Ru}/GFR) dropped

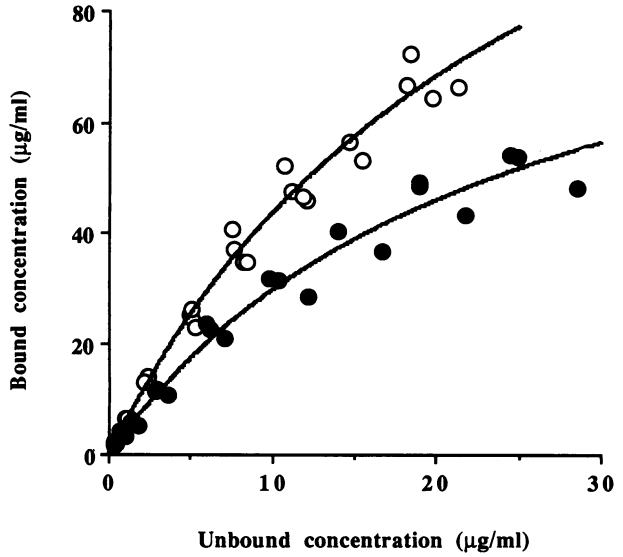

FIG. 3. Protein binding profiles of CEZ in fresh plasma obtained from control (○; n = 4) and LPS-treated (●, n = 3) rats. Solid lines represents computer-fitted curves taken from equation 1.

TABLE 2. Protein binding parameters of CEZ in control and LPS-treated rats[a]

Treatment	nP (μM)	K_d (μM)	n	P (μM)
Control	331.13 ± 22.48	54.61 ± 2.04	0.60 ± 0.05	554.94 ± 11.71
LPS	214.77 ± 6.26[b]	50.94 ± 3.43	0.41 ± 0.01[b]	526.67 ± 12.35

[a] Each value represents mean ± standard error (n = 3 or 4). Abbreviations: nP, binding capacity of the first class of binding sites; K_d, dissociation constant; n, number of binding sites on the albumin molecule; P, albumin concentration.
[b] Significantly different from control ($P < 0.05$).

to approximately 70% of that in control rats. Marked reduction in the net tubular secretion (CL_{Ru} − GFR) for CEZ was also observed in LPS-treated rats (Fig. 2).

DISCUSSION

A number of articles concerning the nephrotoxic effects of LPS on the pharmacokinetics of aminoglycosides, which are excreted into the urine by glomerular filtration with tubular reabsorption, have reported that LPS reduces the CL_R and increases the accumulation of drugs in the kidney as a result of both a reduction in the GFR and an increase in tubular reabsorption (1, 2, 10, 27). It is also known that β-lactam antibiotics are excreted into the urine and that their pharmacokinetics are affected by the renal functions (4, 14, 23). It has been reported that the renal excretion mechanism of CEZ consists of glomerular filtration and tubular anion secretion in the kidney (4, 19, 23, 33). There is the possibility that LPS also affects the pharmacokinetics of β-lactam antibiotics, including CEZ. However, there is little information available on the influence of LPS on the disposition of β-lactam antibiotics. We decided to investigate the effect of LPS on the pharmacokinetics of CEZ in rats.

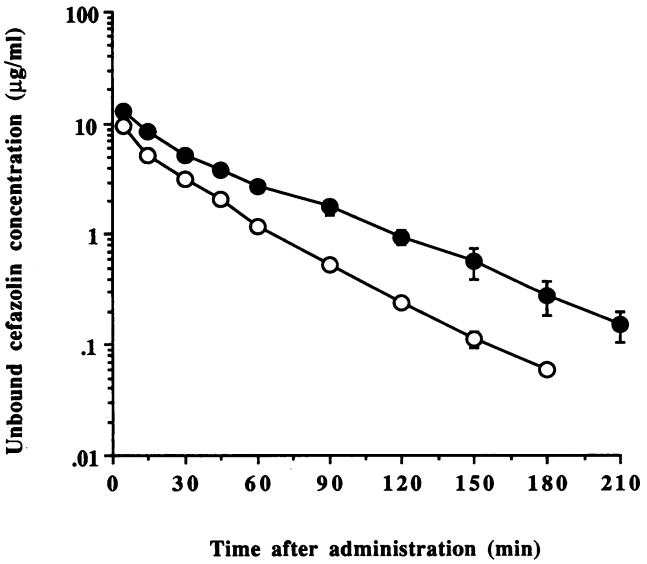

FIG. 4. Mean semilogarithmic plots of plasma concentration-time data for unbound CEZ in control (○) and LPS-treated (●) rats after a single intravenous administration of CEZ (20 mg/kg). Each plot represents mean ± standard error (n = 4 or 5). Data were calculated from total plasma concentrations and mean binding parameters in each group.

TABLE 3. Pharmacokinetic parameters of unbound CEZ in control and LPS-treated rats[a]

Treatment	V_{ssu} (liters/kg)	CL_{SYSu} (liters/h/kg)	CL_{Ru} (liters/h/kg)	MRT_u (h)
Control	1.020 ± 0.036	1.945 ± 0.064	1.750 ± 0.080	0.489 ± 0.016
LPS	0.864 ± 0.076	1.077 ± 0.063[b]	0.928 ± 0.066[b]	0.763 ± 0.082[b]

[a] Each value represents mean ± standard error (n = 4 or 5). Abbreviations: V_{ssu}, V_{ss} for unbound drug; CL_{SYSu}, CL_{SYS} for unbound drug; CL_{Ru}, CL_R for unbound drug; MRT_u, MRT for unbound drug. Data were obtained from calculated unbound concentration-time profiles by using total plasma concentrations and binding parameters.

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

It has been reported that the pharmacokinetics and renal handling of gentamicin change with LPS treatment at a dose of 250 μg/kg (1). Our recent studies also demonstrated that LPS (250 μg/kg) modifies the pharmacokinetics of both 1-methyl-3-propylxanthine (31) and enprofylline (21) without any histological changes in the kidneys. In the present study, therefore, the effect of LPS on the pharmacokinetics of CEZ was examined with an LPS dose of 250 μg/kg. LPS dramatically delayed the disappearance of CEZ from plasma and induced significant decreases in the CL_{SYS} and CL_R of CEZ. These results indicate that LPS of 250 μg/kg significantly modified the pharmacokinetics of CEZ, including renal excretion.

Plasma protein binding is known as a limiting factor in drug disposition, since only the unbound drug is distributed in the body and is subject to renal excretion and hepatic metabolism. CEZ has been shown to bind strongly to plasma protein in a concentration-dependent manner (25, 30). In the present study, this concentration-dependent protein binding was observed in both groups. The binding parameters observed in the control rats were identical to those reported by Tsuji et al. (30). In contrast, significant decreases in the binding capacity (nP) and number of binding sites (n) of CEZ were observed in LPS-treated rats. The results of this study support our previous findings whereby LPS decreased the binding capacity (nP) and number of binding sites (n) of 1-methyl-3-propylxanthine, which binds strongly to plasma albumin, the same as for CEZ (31). LPS may thus modify the pharmacokinetics of drugs which are highly protein bound by changing their protein binding behavior. It is possible that changes in the protein binding behavior of CEZ induced by LPS are caused by changes in the conformation of the albumin molecule. However, the precise mechanism whereby LPS alters drug protein binding remains unclear.

Several investigators have reported that the volume of distribution for gentamicin (10), trimethoprim (18), and cephalosporins (8) increased with pretreatments of LPS. Ganzinger et al. (8) demonstrated that LPS increases the volume of distribution of some cephalosporins as a result of specific changes in the peripheral compartment. In the present study, however, no such change in V_{ss} for unbound CEZ was observed with LPS treatment. Tsuji et al. (28–30) reported that CEZ is localized in the extracellular water space and binds to plasma protein in the intravascular and interstitial fluids in nondisposing organs, although it is distributed in intracellular fluid in the liver and kidney. It has also been shown that, in rats, CEZ distribution contributes little in the disposing organs to V_{ss} (26). In addition, our recent studies suggested that the extracellular fluid volume, which is estimated as V_{ss} of inulin, does not change with LPS treatment (22). From these observations, it may be considered that LPS decreases the binding potency of CEZ to albumin in the interstitial fluid as well as in plasma and that the actual distribution volume of CEZ does not change with LPS treatment.

It has been reported that most of the administered CEZ is excreted into the urine by active tubular secretion and glomerular filtration (4, 19, 23, 30, 33). The nephrotoxic effects of LPS, which may affect renal tubular functions and drug excretion, have already been reported (5, 17). The LPS-induced decreases in GFR, CL_{SYSu}, and CL_{Ru} for CEZ indicate that the renal handling of CEZ was affected by the nephrotoxic effect of LPS. The clearance ratio in LPS-treated rats dropped to approximately 70% of that in control rats. In fact, LPS reduced the net tubular secretion (CL_{Ru} − GFR) for CEZ by approximately 60% (Fig. 2). These results suggest that LPS affects not only glomerular filtration but also tubular secretion and that the percentage of decrease is greater in renal tubular secretion than in glomerular filtration.

Bergeron and Bergeron (1) reported that LPS has no effect on the tubular active transport of cepharothin, an organic acid β-lactam antibiotic which is handled by active tubular secretion similar to that for CEZ, and that the uptake of cepharothin into kidney cells is not affected by LPS. They also reported that LPS increases the plasma concentration of cephalothin and decreases the amount of urinary excretion in the first hour after injection (1). It is also generally accepted that organic anions, including β-lactam antibiotics, are concentrated intracellularly across the basolateral membrane and subsequently pass through the brush border membrane and are excreted into the urine by facilitated diffusion and that the active tubular secretion depends upon the drug concentration in the plasma. Such results may be interpreted to mean that LPS inhibits the uptake of cephalothin into the tubular cell across the basolateral membrane, since no change was reported in the amount of the renal accumulation of cephalothin in either group, although the plasma concentration was two times higher in LPS-treated rats compared with control rats. In addition, Kikeri et al. (17) have reported that the renal extraction ratio of *p*-aminohippuric acid decreased with LPS treatment. Our recent study also demonstrated that LPS induces both decreases in the Michaelis-Menten constant and maximum velocity of tubular secretion of xanthine enprofylline, which is mainly excreted by anion tubular secretion (21). The results obtained from the present study reflect these previous findings by us and other investigators. However, the precise mechanism for inducing this decrease in the tubular secretion of CEZ with treatment of LPS could not be clarified in the present study. Further investigations are needed to determine this.

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