Time-Dependent Changes in the Pharmacokinetics and Renal Excretion of Xanthine Derivative Enprofylline Induced by Bacterial Endotoxin in Rats

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Time-dependent changes in the pharmacokinetics and renal handling of enprofylline induced by bacterial endotoxin (Klebsiella pneumoniae LPS) were investigated in rats. To evaluate the early effect of LPS on kidney functions and the renal excretion of enprofylline, which is an organic anion drug excreted primarily by an active tubular secretion, LPS (250 μg/kg) was infused for 5 min under constant infusion at rates of 2.3 and 23 μg/min/kg for inulin and enprofylline, respectively. LPS caused a drop in the glomerular filtration rate (GFR), estimated as the renal clearance of inulin, to 65–75% of that observed in the control rats within 30 min after the LPS treatment. The renal clearance (CL) of enprofylline decreased in conjunction with GFR, while the percentage of decrease in the CL was slightly greater than that in GFR. LPS-induced decreases in the CL for enprofylline and GFR continued over the testing period of 120 min. The time-dependent effect of LPS on the pharmacokinetics of enprofylline was examined by a single injection of enprofylline (2.5 mg/kg) to rats pretreated 2, 10 or 24 h earlier with or without LPS. The pharmacokinetic parameters of enprofylline were determined by a model-independent method. Significant changes in the systemic clearance for enprofylline were observed in rats pretreated 2 and 10 h earlier with LPS, but no such changes were observed in rats pretreated 24 h earlier with LPS. These findings indicate the existence of a time-dependent effect of LPS on the pharmacokinetics of enprofylline, and suggest that LPS at a dose of 250 μg/kg, at least, does not induce cytotoxicity to kidney cells.

Key words Klebsiella pneumoniae endotoxin; enprofylline; time-dependent pharmacokinetics; renal handling

Endotoxin (LPS), a cell wall component of gram-negative bacteria, exhibits various physiological, immunological and pharmacological activities in the body. LPS has also shown strong nephrotoxicity and has induced decreases in the glomerular filtration rate (GFR) and renal plasma flow (RPF).1-6 Several studies have been undertaken to evaluate the effect of LPS on the pharmacokinetics and renal handling of drugs that are primarily excreted into the urine.7-11 Bergeron and colleagues7 reported that LPS potentiates the nephrotoxic effect of aminoglycoside antibiotics by increasing renal tubular reabsorption through adsorptive pinocytosis and/or adsorptive endocytosis. Previous studies in our own laboratory have demonstrated that LPS decreases the tubular secretion ability of anionic drugs in addition to decreasing glomerular filtration.12,13

Studies on the mechanism involved in these time-dependent and dose-dependent effects of LPS on nephrotoxicity are limited. We previously reported the existence of a dose-dependent effect of LPS on the pharmacokinetics of tobramycin in rats.14 More specifically, since LPS is released into biological fluids from the bacterial cell wall following treatment with antibiotics and is known to induce a decrease in renal functions, it is important to examine the time-dependent changes associated with LPS-induced nephrotoxicity and renal handling of drugs. Kikkeri et al.15 demonstrated that GFR drops immediately and progressively after an LPS treatment of 20–40 mg/kg and reverses spontaneously back to normal levels within 7–9 d. However, it has also been reported that chronic endotoxemia induced by continuous intravenous infusions of low doses of LPS (1 mg/kg per day for 7 d) enhances the renal cortical uptake of gentamicin and its adverse effects on the kidney in rats.10 And yet, no information is available on the time-dependent effects of LPS on the pharmacokinetics and renal handling of anionic drugs that are excreted into the urine by active tubular secretion.

One of our previous studies demonstrated that the pharmacokinetics of enprofylline, which is excreted into the urine by an active anion transport system,15,16 was dramatically modified 2 h after treatment with LPS.12 The present study aims to evaluate the time-dependent effects of LPS on the pharmacokinetics of enprofylline in rats.

MATERIALS AND METHODS

Chemicals Enprofylline and 3-butylxanthine were synthesized in our laboratory and were identical to those previously used.12,16,17 Inulin was purchased from Nacalai Tesque (Kyoto, Japan). All other chemicals used were commercially available and of analytical grade.

LPS was isolated from a cultured supernatant of Klebsiella pneumoniae LEN-1 (O3 : K1-), a decapsulated mutant strain derived from the Klebsiella pneumoniae strain of Kasuya (O3 : K1),19 as described previously.19,20 LPS was dissolved in saline.

Animal Experiments Eight- to nine-week-old male Wistar rats (Japan SLC, Hamamatsu, Japan) weighing 280–300 g each, were used in all experiments.

In experiments on the early effects of LPS on the pharmacokinetics of enprofylline, rats were anesthetized with sodium pentobarbital (25 mg/kg) and cannulated with polyethylene tubing in the right jugular vein, the left carotid artery, and the urinary bladder for drug infusion, blood sampling and urine collection, respectively. After surgical preparation, rats received constant-rate infusions of saline solution containing 23 μg/min/kg of enprofylline and 2.3 mg/min/kg of inulin, with loading doses of 0.5

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mg/kg of enprofylline and 50 mg/kg of inulin, respectively. The solution for infusion was adjusted to pH 7.4. Thirty min after starting the infusion, urine was collected at 10 min intervals up to 20 min to determine the initial values of GFR and the renal clearance (CLr) for enprofylline. Next, 250 μg/kg of LPS or saline were infused for 5 min, and urine was subsequently collected at 15 min intervals over a period of 120 min after the LPS treatment. About 0.25 ml of blood was taken at the midpoint of each urine collection period. Urine volume was measured gravimetrically assuming a specific gravity of 1.0.

In experiments on the late effects of LPS, the right jugular vein was cannulated with polyethylene tubing while rats were under pentobarbital anesthesia. On the following day, 250 μg/kg of LPS were infused for 5 min. Two, 10 and 24 h after the LPS treatment, 2.5 mg/kg of enprofylline were injected intravenously. The dosage of LPS was identical to that used in our previous studies.12,14

In the control rats, saline was administered in place of LPS. Blood samples of about 0.25 ml each were collected at appropriate times after the enprofylline administration. Urine samples were also taken over a period of 24 h after dosing rats in their metabolic cages. Plasma samples obtained by centrifugation and urine samples were stored at −40°C until analysis.

**Drug Analyses** Concentrations of enprofylline in plasma and urine were measured by HPLC as described previously.12,16 Briefly, 50 μl of plasma or urine samples were deproteinized by the addition of 350 μl of methanol containing 1.0 μg/ml of 3-butyxanthine as an internal standard and were centrifuged. The supernatant was evaporated under a nitrogen gas stream at 50°C. The residue was reconstituted in the mobile phase and was applied to HPLC. The HPLC apparatus was a Shimadzu LC-6A HPLC system (Shimadzu Co., Kyoto, Japan), and the UV spectrophotometric detector was set at 274 nm. The column was a Cosmosil 5C⋅18 column (Nacalai Tesque, Kyoto, Japan) and was heated to 50°C in a column oven. The mobile phase was made up of 30 mM KH2PO4 buffer (pH 5.0): methanol (80:20, v/v), and the flow rate was set at 1.5 ml/min. Inulin was measured by the standard colorimetric method described by Dische and Borenfreund.21

**Data Analyses** The plasma concentration-time data of enprofylline were analyzed on the basis of noncompartmental analysis. Area under the curve (AUC) and area under the first moment curve (AUMC) for enprofylline were calculated by the trapezoidal rule with extrapolation to infinity. The systemic clearance (CLsys) was calculated as the dose divided by AUC. The mean residence time (MRT) was calculated as MRT = AUMC/AUC, while the volume of distribution at steady state (Vss) was set at Vss = CLsys × MRT. The renal clearance (CLr) for enprofylline during each urine collection period was determined as the amount of drug excreted into the urine during the specified urine collection period divided by its plasma concentration at midpoint. The glomerular filtration rate (GFR) was set as the CLr of inulin. All computer analyses were performed by the nonlinear least squares regression program, MULTI, written by Yamaoka et al.22

**Statistical Analyses** Values are expressed as the mean ± S.E. for the indicated number of experiments. Statistical differences in the GFR and CLr for enprofylline were analyzed by the Student’s t-test. For comparisons of the late effects of LPS on the pharmacokinetics of enprofylline, statistical differences were examined by the analysis of variance (ANOVA), and the Tukey test was used to detect differences among individual groups.

**RESULTS**

Mean values of the CLr for enprofylline and GFR before and after the LPS administration are shown in Fig. 1. Values for the control rats were nearly constant throughout the experiments, and were used for comparisons with the LPS-treated groups before LPS treatment. The CLr for enprofylline and the GFR both immediately and gradually decreased with LPS treatment. Figure 2

![Fig. 1. Time-Dependent Changes in the GFR (○) and CLr for Enprofylline (△, ▲) in Control (Open Symbol) and LPS-Treated (Closed Symbol) Rats, Respectively](image1)

Each plot represents mean ± S.E. (n=4). △ Significantly different from control (p<0.05).

![Fig. 2. Changes in Relative Percentages of the GFR (○) and CLr for Enprofylline (△, ▲) Measured against Their Initial Mean Values in the Control (Open Symbol) and LPS-Treated (Closed Symbol) Rats, Respectively](image2)

Each plot represents mean ± S.E. (n=4). △ Significantly different from control (p<0.05).
Fig. 3. Mean Semilogarithmic Plots of the Plasma Concentration-Time Data for Enprofylline after a Single Intravenous Administration of Enprofylline at 2.5 mg/kg in Control Rats (○) and Rats Pretreated with LPS at 2 (●), 10 (▲) and 24 h (■) Earlier
Each plot represents mean ± S.E. (n=4–5).

Table 1. Time-Dependent Changes in the Pharmacokinetic Parameters of Enprofylline in Control Rats and Rats Pretreated with LPS at 2, 10 and 24 h Earlier

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$V_{ds}$ (l/kg)</th>
<th>$CL_{sys}$ (l/h/kg)</th>
<th>$MRT$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.294 ± 0.015</td>
<td>0.919 ± 0.066</td>
<td>0.302 ± 0.033</td>
</tr>
<tr>
<td>LPS</td>
<td>0.455 ± 0.062*</td>
<td>0.547 ± 0.053*</td>
<td>0.776 ± 0.090*</td>
</tr>
<tr>
<td>2h</td>
<td>0.307 ± 0.008*</td>
<td>0.639 ± 0.077*</td>
<td>0.483 ± 0.076*</td>
</tr>
<tr>
<td>10h</td>
<td>0.343 ± 0.017</td>
<td>0.935 ± 0.056*</td>
<td>0.348 ± 0.014*</td>
</tr>
<tr>
<td>24h</td>
<td>0.343 ± 0.017</td>
<td>0.935 ± 0.056*</td>
<td>0.348 ± 0.014*</td>
</tr>
</tbody>
</table>

Enprofylline was administered intravenously at a dose of 2.5 mg/kg. Each value represents mean ± S.E. (n=4–5). a) Significantly different from control. b) Significantly different from 2h after the LPS treatment. c) Significantly different from 10h after the LPS treatment.

shows the time-course for these changes in relative percentages with $CL_r$ for enprofylline and GFR measured against the mean initial values before the LPS administration. The relative percentages for both $CL$ and GFR were within 90—110% in the control rats. In the LPS-treated rats, GFR dropped significantly to 65—75% of the control within 30 min after the LPS administration, and a corresponding decrease in the $CL_r$ of enprofylline was also observed, although the decrease in the $CL_r$ was slightly larger than GFR. LPS-induced decreases in the $CL_r$ and GFR continued at nearly constant for 120 min after the LPS treatment.

Figure 3 shows the mean semilogarithmic plasma concentration-time data for enprofylline following a single intravenous injection in rats pretreated with LPS at 2, 10 and 24 h earlier and in the control rats which underwent no such pretreatment. The disappearance of enprofylline from the plasma was markedly delayed in the rats pretreated with LPS 2 h before, and plasma concentrations in this group were higher than in the controls throughout the entire experimental period. However, plasma concentrations for enprofylline in rats pretreated with LPS at 10 and 24 h earlier were lower than those in rats pretreated with LPS at 2 h earlier. The corresponding pharmacokinetic parameters for enprofylline are summarized in Table 1. Significant decreases in the $CL_{sys}$ and increases in $V_{ds}$ of enprofylline were observed in rats pretreated with LPS 2 h before, although the $CL_{sys}$, $V_{ds}$ and MRT for enprofylline in rats pretreated with LPS 24 h before did not differ from the controls. There were no changes among the groups with regard to the urinary recovery of enprofylline (>0.9).

DISCUSSION

Our recent studies demonstrated that K. pneumoniae LPS at a dose of 250 μg/kg dramatically modifies the pharmacokinetics and renal handling of various drugs, including enprofylline, without any histological changes in the kidneys. Direct evidence revealing the existence of a time-dependent effect of LPS on the renal functions and pharmacokinetics of drugs is sparse. For these reasons, in the present study, the time-dependent effect of LPS on the renal functions and pharmacokinetics of enprofylline was investigated with an LPS dose of 250 μg/kg. The present study suggested that LPS at a dose of 250 μg/kg has a time-dependent effect on the renal functions and pharmacokinetics of enprofylline in rats. The greatest decreases in the GFR, and in the $CL_{sys}$ and $CL_r$ for enprofylline, which is primarily secreted into the urine by an active anion transport system, were observed in rats pretreated with LPS 2 h earlier; however, these decreases returned to control levels in rats pretreated with LPS 24 h earlier. Such findings indicate that LPS-induced reductions in the GFR and renal secretion ability for enprofylline are transient events, and that LPS at a dose of 250 μg/kg, at least, has no lethal effect on renal function.

It has been reported that LPS is rapidly distributed in the kidney in rabbits, 5 min after an intravenous administration at a dose of 250 μg/kg, and that most of the LPS was cleared from the blood within 10 to 20 min after an intravenous injection of 20 mg/kg in rats. These findings suggest that LPS induces renal failure and hemodynamic changes, which can modify the pharmacokinetics and renal handling of various drugs in the early period following the administration of LPS. Kikeri et al. reported that GFR dropped following an intravenous administration of either 20 or 40 mg/kg of E. coli LPS, and that this decline continued for 210 min after the LPS treatment. Knight et al. also recently reported that E. coli LPS at doses of 1 and 20 mg/kg produced marked reductions in both the GFR and RPF for 120 min after an LPS treatment without causing any changes in the systemic hemodynamics. In the present study, a rapid decrease in GFR was noted within 30—45 min after the administration of K. pneumoniae LPS, and this decrease in GFR continued until the end of the experiment (120 min), which reflects the findings of Kikeri et al. and Knight et al.; however, the dose used in our current study was lower. This discrepancy may be explained by differences in LPS preparations derived from different bacterial species.

Bergeron et al. and Tardif et al. reported that marked changes in the pharmacokinetics and intrarenal
accumulation kinetics for gentamicin were induced in rats pretreated with LPS 2 h earlier. One of our previous studies also demonstrated that, when performed under the same experimental schedule, LPS causes a significant decrease in GFR and in the $CL_{sys}$ of enprofylline without inducing any changes in protein binding behavior, which affects the renal excretion of drugs. In this study, there were no differences among any of the groups in the urinary recovery for the amount of unchanged enprofylline. The time-dependent effects of LPS on the pharmacokinetics of enprofylline were thus evaluated by estimating the $CL_e$ or $CL_{sys}$ for total enprofylline. We found that the time-course for the decline in the $CL_e$ of enprofylline in the early period of LPS-induced renal failure closely resembled the decrease in GFR, although the $CL_e$ drop was slightly greater. It has been demonstrated that the LPS-induced decreases in GFR were greater than those in RPF. Although RPF was not measured in our current study, we previously demonstrated that RPF is less involved in any LPS-induced changes in the renal secretion clearance of enprofylline when a low extraction drug is used, even though hypoxia induced by a decrease in RPF may lead to tubular cell damage. Findings from our present study, whereby LPS decreased both the $CL_e$ of enprofylline and the GFR to the same degree, may be supported by evidence from another study showing that LPS reduces the renal secretion clearance of enprofylline by decreasing tubular cell ability.

A study on the duration of LPS-induced decreases in renal functions has been published. Auclair et al. reported the effects of LPS, following long-term infusions, on the renal accumulation of gentamicin. However, no studies have been undertaken to evaluate the time required to restore the changes in the pharmacokinetics of drugs induced by a single administration of LPS. For evaluating these time-dependent effects of LPS on the pharmacokinetics and renal excretion of certain drugs, we administered enprofylline to rats pretreated with LPS at 2, 10 or 24 h earlier. Marked changes in the plasma concentrations of enprofylline were observed in rats pretreated with LPS 2 h before, and the extent of the decrease in the $CL_{sys}$ and the increase in the $V_{adm}$ of enprofylline was comparable to that found in our previous studies. However, a delay in the elimination of enprofylline from plasma observed in rats pretreated with LPS 2 h earlier disappeared in rats pretreated at 10 and 24 h earlier. The corresponding pharmacokinetic parameters also indicated that LPS-induced changes in the renal excretion and tissue distribution of enprofylline had returned to normal with time. From this we conclude that the time-dependent alterations in the $CL_{sys}$ for enprofylline among LPS-treated rats may reflect changes in the renal tubular secretion of enprofylline and in the glomerular filtration, and that LPS-induced decreases in renal function are transient events. It should be noted, however, that Kamiya et al. reported that the maximum velocity for anion tubular secretion of sulfamethizole decreased to 55% of their control value on 3 and 7 d after the first injection of neomycin sulfate, a typical nephrotoxic drug that acts against tubular cells, whereas the decrease in GFR was only 20%. The impairment in renal handling of sulfamethizole was gradually recovered within 2 weeks after the cessation of any neomycin administration. They also demonstrated that mercuric chloride, a nephrotoxic compound, decreased the renal excretion of sulfamethizole as a function of time for 72 h after the treatment. In this study, the decreased $CL_{sys}$ for enprofylline observed in rats pretreated 2 h before with LPS rapidly recovered to control levels within 24 h. Our preliminary study showed that the decrease in GFR also recovered to within normal levels 24 h after an LPS administration (data not shown). Such observations suggest that LPS at a dose of 250 µg/kg, at least, does not induce any cytotoxicity to the renal tubular cells. Actually, our more recent studies showed that there was no histological change in the kidneys following an administration of LPS at a dose of 250 µg/kg. A separate study, on the other hand, demonstrated that a decrease in renal function induced by 20—40 mg/kg of LPS, which is 80—160 times higher than that used in this study, continued for 3—4 d after the LPS administration and that GFR recovered to normal ranges within 7—9 d. Further studies using larger doses of LPS will be needed to better understand the dose-dependent differences in the LPS effects on kidney function.

The precise mechanism responsible for LPS-induced acute renal failure is not yet completely understood. The contribution of oxygen radical production with conjugated diene formation by LPS to endothelial cell cytotoxicity has been widely discussed. However, reports have shown that reactive oxygen metabolites are not important mediators in LPS-induced acute renal failure, since superoxide dismutase (SOD) or catalase had no effect in protecting against LPS-induced renal failure. Rapid recovery of the $CL_{sys}$ for enprofylline, as observed in this study, may suggest that the LPS-induced decreases in renal functions are not caused by any cell cytotoxicity. However, isolated rat kidney perfused experiments have shown that LPS has no direct effect on renal functions, including GFR, Na⁺ reabsorption or tissue K⁺ content. It was concluded from this same study that mediators, released extrarenally by LPS treatment, may be required to initiate any changes in renal function. In addition, the primary mechanism for LPS-induced renal failure is most likely caused by vasoconstriction, which may be mediated by thromboxane, prostaglandins, leukotrienes, platelet activating factors (PAF) and endothelin. Previous studies by us and by Knight et al. demonstrated that adenosine may play a role in LPS-induced acute renal failure. On the other hand, it has been demonstrated that intravenous administration of tumor necrosis factor (TNF) to mice, which is released from macrophages and neutrophils during LPS stimulation, induces all of the reactions observed in an endotoxin shock state. One report showed that the TNF level in plasma peaked 1 h after the LPS application, but could not be detected 5 h later in guinea pigs. Based on such observations, we may surmise that TNF and its related cytokines play an important role in the mechanism of LPS-induced acute renal failure; however, further studies will be needed to clarify the precise mechanism for such LPS-induced renal failure.

In summary, we found that Klebsiella LPS at a dose of
250 μg/kg induces time-dependent decreases in both the GFR and renal excretion of an anionic drug namely, enprofylline, in rats, and that such decreased renal functions return to normal within 24 h after the LPS treatment.

REFERENCES