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Alterations in Renal Uptake Kinetics of the Xanthine Derivative Enprofylline in Endotoxaemic Mice

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Abstract

The pharmacokinetics and renal uptake of enprofylline, which is primarily excreted into the urine by an active tubular secretion mechanism, were investigated in endotoxaemic mice by lipopolysaccharide isolated from *Klebsiella pneumoniae*.

Lipopolysaccharide (1 mg kg^{-1}) was infused 2 h before starting the examination, thereby inducing a decrease in the systemic clearance and an increase in the steady-state volume of distribution of enprofylline while inducing no changes in the urinary recovery (> 90%). The protein binding of enprofylline significantly decreased in the presence of lipopolysaccharide. Both the systemic clearance for unbound enprofylline and glomerular filtration rate decreased in the treated mice. A nonlinear relationship was found in both groups between the steady-state unbound plasma concentration and renal uptake of enprofylline after constant infusion for 1 h. The renal uptake rate of enprofylline decreased in the treated mice. Lipopolysaccharide caused increases in the apparent maximum capacity for renal uptake (V_{max}) from 17·3 to 32·2 $\mu g\ h^{-1}\ g^{-1}$ of kidney and in the Michaelis–Menten constant (K_m) from 2·7 to 21·7 $\mu g\ mL^{-1}$ and decrease in the nonsaturable uptake rate constant (K_d) from 0·87 to 0·43 $mL\ h^{-1}\ g^{-1}$ of kidney.

These results indicate that lipopolysaccharide decreases the renal tubular secretion of enprofylline by inducing a decrease in the renal uptake ability.

Lipopolysaccharide, an active component of the Gram-negative bacterial cell wall, is well known to have various biological and immunological activities. Lipopolysaccharide has also been shown to induce nephrotoxicity in the kidney and decreases in both glomerular filtration rate (GFR) and renal plasma flow rate (Kikeri et al 1986; Hewett & Roth 1993). Several investigations have demonstrated that lipopolysaccharide potentiates the renal toxicity of aminoglycoside antibiotics by inducing an increase in tubular reabsorption (Bergeron & Bergeron 1986; Auclair et al 1990; Tardif et al 1990). However, there is a little information concerning the effect of lipopolysaccharide on the renal excretion mechanism of anions or cations by active transport in the tubules. Changes which occur in the uptake of such drugs into the tubular cells may influence their nephrotoxicity in the kidney.

Recent studies in our laboratory using rats have demonstrated that *Klebsiella pneumoniae* lipopolysaccharide dramatically modified the pharmacokinetics of the anionic drug enprofylline, which is mainly excreted into the urine by an anion tubular secretion mechanism in man (Borgå et al 1986) and in animals, including mice (Tsunekawa et al 1992; Haghgoo et al 1995) and rats (Apichartpichean et al 1991; Nadai et al 1991; Tsunekawa et al 1992), by decreasing both glomerular filtration and tubular secretion ability (Nadai et al 1993a,b). More recent studies have shown that lipopolysaccharide did not change the tubular secretion ability of the cationic drug famotidine (Hasegawa et al 1994b). It is widely known that anionic drugs are actively taken into the tubular cells across the basolateral membranes and pass through the brush-border membranes into the urine by facilitated diffusion. However, it is not clear whether lipopolysaccharide modifies the transport system for uptake into the tubule or for excretion from the cells.

The present study aims to clarify changes in the pharmacokinetics and renal uptake kinetics of enprofylline in endotoxaemic mice induced by *K. pneumoniae* lipopolysaccharide.

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Materials and Methods

Chemicals

Enprofylline (3-propylxanthine) and 3-butylxanthine used in this study were synthesized in our laboratory and were identical to those used in previous studies (Apichartpichean et al 1991; Tsunekawa et al 1992; Nadai et al 1993a). Inulin was obtained from Nacalai Tesque (Kyoto, Japan). All other reagents used were of the highest purity available. Enprofylline and inulin were simultaneously dissolved in an isotonic saline solution with sodium hydroxide.

Lipopolysaccharide was isolated from a cultured supernatant of *K. pneumoniae*, a decapsulated mutant strain derived from *K. pneumoniae* strain of Kasuya (Ohta et al 1981), as described previously (Hasegawa et al 1983, 1985). Lipopolysaccharide was dissolved in isotonic saline.

Animal experiments

Eight- to nine-week-old male ddY strain mice (Nippon SLC, Hamamatsu, Japan), 35–40 g, were used. One day before the experiments, mice were cannulated in the right jugular vein with polyethylene tubing under light anaesthesia with sodium pentobarbital (20 mg kg^{-1}). On the following day, lipopolysaccharide was infused at a dose of 1 mg kg^{-1} for a period of

about 5 min, 2 h before the administration of drugs. In the control mice, saline was infused in the same manner.

In the pharmacokinetic studies, enprofylline (2·5 mg kg^{-1}) and inulin (100 mg kg^{-1}) were then administered intravenously. At designated intervals (10, 20, 30, 45 and 60 min, and 90 min for inulin determination) after injection, mice were exsanguinated from the abdominal vein for collection of blood samples and the right kidneys were removed under light anaesthesia with sodium pentobarbital (20 mg kg^{-1}). The plasma samples were obtained by centrifugation (6000 *g*, 5 min). The kidneys were homogenized with pH 7·4 isotonic phosphate buffer and adjusted to a volume of 10 mL. In separate experiments, urine was collected for 24 h after injection. Plasma, urine and supernatants of the homogenates were frozen at −40°C until analysis.

The renal uptake experiments were performed using a constant-infusion method. For attaining various steady-state plasma enprofylline concentrations, both groups (68 controls and 50 lipopolysaccharide-treated mice) received constant-rate infusions of enprofylline for 1 h at the rate of 0·06–2·28 mg h^{-1} following a bolus loading dose. The infusion and loading doses of enprofylline were calculated from the pharmacokinetic parameters obtained in the pharmacokinetic studies. After infusion, blood and right kidneys were obtained as described above.

Plasma protein binding experiments

The binding of enprofylline to mouse plasma protein was measured by equilibrium dialysis as described previously (Apichartpichean et al 1991; Nadai et al 1991,1993a). Plasma samples containing various enprofylline concentrations (0·5–20 μg mL^{-1}), which were obtained from both groups of mice (n = 30 and 20 for control and lipopolysaccharide-treated mice, respectively) at the end of the renal-uptake experiments.

The albumin concentration was determined using the bromcresol green method (Iatron Albumin Kit, Iatron Laboratories, Tokyo, Japan) and was represented as molar concentration with a molecular weight of 69 kDa.

Drug analysis

The concentration of enprofylline in plasma, urine and kidney homogenate was measured by HPLC as described previously (Nadai et al 1991, 1993a). Briefly, either 50 μL plasma or 100 μL supernatant of kidney homogenate was deproteinized with 0·35 mL methanol containing 3-butylxanthine as an internal standard and centrifuged at 6000 *g* for 5 min. The resultant supernatant was then dried under a stream of nitrogen at 50°C. The residue was reconstituted in the mobile phase and subjected to HPLC. The HPLC apparatus was a Shimadzu LC-6A system (Shimadzu Co., Kyoto, Japan) consisting of an LC-6A liquid pump, an SPD-6A spectrophotometric detector and an SIL-6A autoinjector. A Cosmosil 5C18 packed column (4·6 × 150 mm; Nacalai Tesque, Kyoto, Japan) was maintained at 50°C. The mobile phase was 30 mM KH_2PO_4 (pH 5·0)-methanol (80 : 20) and the flow rate was 1·5 mL min^{-1}. The detection limit was 0·05 μg mL^{-1}, with a linear detection range of up to 80 μg mL^{-1}. No interference with the peak of enprofylline was observed in any samples. The standard colorimetric method was used for the measurement of inulin (Dische & Borenfreund 1951).

Pharmacokinetic analysis

The mean plasma concentration-time data for enprofylline and inulin was analysed using a noncompartmental method. 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 (Vd_{ss}) was calculated as $CL_{sys} \times MRT$.

The renal uptake rate of enprofylline was quantitatively analysed as a function of steady-state unbound plasma concentration according to the nonlinear regression principle of the Michaelis–Menten equation applying a term for nonsaturable uptake. It has been reported that tubular reabsorption of enprofylline is negligible in man (Lunell et al 1984) and in rats (Nadai et al 1991). By assuming that renal uptake rate of enprofylline is dependent upon its unbound plasma concentration, the Michaelis–Menten constant (K_m), maximum velocity for renal uptake (V_{max}) and nonsaturable uptake rate constant (K_d) of enprofylline were estimated using equation 1:

$$V = \frac{V_{max} C_u}{K_m + C_u} + K_d C_u \quad (1)$$

where V is the renal uptake rate of enprofylline, and C_u is the steady-state concentration of unbound enprofylline in plasma. Values of K_m, V_{max} and K_d were represented as the computer-estimated mean ± s.d. All computer analyses were performed by the nonlinear least squares regression program, MULTI (Yamaoka et al 1981), weighting the data with the reciprocal of the concentrations.

Statistical analysis

Statistical comparisons between controls and lipopolysaccharide-treated mice were assessed using the unpaired Student's *t*-test. Statistical significance was defined as $P < 0.05$.

Results

Semilogarithmic plots of the mean plasma concentration–time data of enprofylline in controls and lipopolysaccharide-treated mice after intravenous administration are shown in Fig. 1. Plasma disappearance of enprofylline was markedly delayed by pretreatment with lipopolysaccharide. The corresponding pharmacokinetic parameters for enprofylline are summarized in Table 1. The systemic clearance (CL_{sys}) of enprofylline in the lipopolysaccharide-treated mice dropped to half that of the control mice. Lipopolysaccharide also reduced the glomerular filtration rate from 1·06 to 0·47 L h^{-1} kg^{-1}. On the other hand, the steady-state volume of distrubution (Vd_{ss}) of enprofylline in lipopolysaccharide-treated mice increased to 1·4 times that of control mice. No significant differences in the fraction of urinary excretion of unchanged enprofylline were observed in either group (> 0·9). Treatment with lipopolysaccharide did not change the renal accumulation of enprofylline at 10, 20, 30, 45 and 60 min after injection (Fig. 2).

The effect of lipopolysaccharide on the plasma protein binding of enprofylline was examined using plasma samples obtained after 1 h constant infusion of enprofylline. A significant increase in unbound plasma fraction of enprofylline was found in the lipopolysaccharide-treated mice

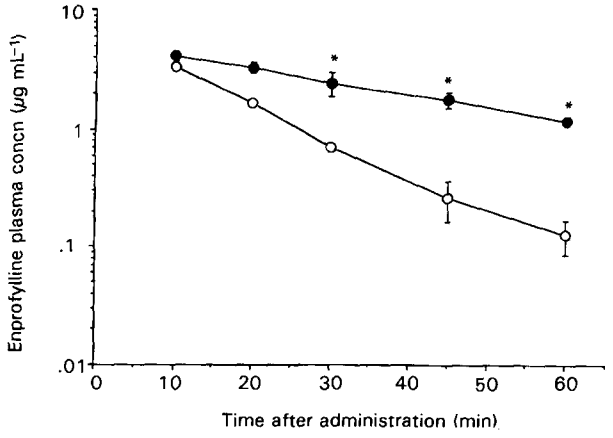

FIG. 1. Semilogarithmic mean plasma concentration–time data after a single intravenous administration of enprofylline at a dose of 2·5 mg kg^{-1} in control (○) and lipopolysaccharide-treated (●) mice. Each plot represents mean ± s.e. for three mice. *$P < 0.05$ compared with control mice.

Table 1. Pharmacokinetic parameters of enprofylline after intravenous administration in control and lipopolysaccharide-treated mice.

Treatment	Vd_{ss} (L kg^{-1})	CL_{sys} (L h^{-1} kg^{-1})	MRT (h)	GFR (L h^{-1} kg^{-1})
Control	0·323	1·502	0·215	1·063
Lipopolysaccharide	0·461	0·702	0·657	0·474

Each value was calculated from the mean plasma concentrations of three animals.

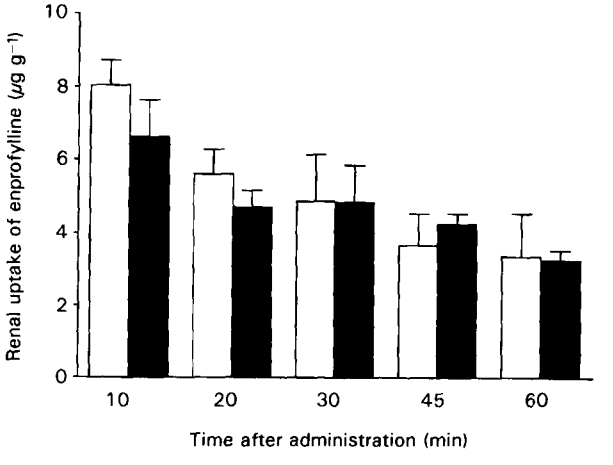

FIG. 2. Amount of enprofylline taken into the right kidneys after intravenous administration of enprofylline at a dose of 2·5 mg kg^{-1} in control (open column) and lipopolysaccharide-treated mice (closed column). Each column represents mean ± for three mice. No significant difference was noted between the control and the treated mice.

(0·866 ± 0·081) when compared with the control mice (0·718 ± 0·020). A significant decrease in albumin concentration was observed in the lipopolysaccharide-treated mice (341·3 ± 7·0 μM) when compared with the control mice (399·9 ± 5·4 μM).

The relationship between the steady-state unbound plasma concentration of enprofylline and renal uptake rate in both

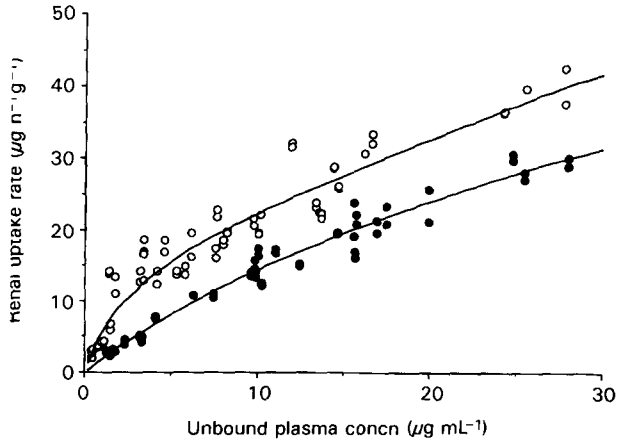

FIG. 3. Relationship between renal uptake rate of enprofylline and its steady-state unbound plasma concentration in control (○) and lipopolysaccharide-treated (●) mice. Solid lines represent computer-fitted curves taken from equation 1.

Table 2. Kinetic parameters for renal uptake of enprofylline in control and lipopolysaccharide-treated mice.

Parameter	K_m (μg mL^{-1})	V_{max} (μg h^{-1} g^{-1})	K_d (mL h^{-1} g^{-1})
Control	2·71 ± 0·74	17·34 ± 3·16	0·87 ± 0·15
Lipopolysaccharide	21·73 ± 13·87	32·16 ± 25·53	0·43 ± 0·36

Each value is the computer-estimated mean ± s.d.

groups is shown in Fig. 3. Concentration-dependent uptake of enprofylline was observed in the unbound concentration range chosen for the study (below 30 μg mL^{-1}). The uptake rate of enprofylline was significantly lower in the lipopolysaccharide-treated mice than in the control mice. The estimated kinetic parameters for renal uptake of enprofylline is summarized in Table 2. The net renal uptake ability for enprofylline as represented by V_{max}/K_m was decreased by lipopolysaccharide.

Discussion

The present study demonstrated that lipopolysaccharide markedly decreased the CL_{sys} value and increased the Vd_{ss} for enprofylline in ddY mice as seen in our previous studies using Wistar rats (Nadai et al 1993a). Renal uptake rate of enprofylline was decreased in lipopolysaccharide-treated mice when compared with the control mice, suggesting that lipopolysaccharide influences the tubular uptake of enprofylline rather than efflux from the cells and decreases the tubular secretion.

In the present study, the unbound fraction of enprofylline in the control mice was identical to that in ddY mice (Haghgoo et al 1995) and in ICR mice (Tsunekawa et al 1992) as reported previously and lipopolysaccharide significantly decreased protein binding of enprofylline due to decrease in albumin concentration in plasma. Therefore, the pharmacokinetic parameters for unbound enprofylline were recalculated using

the mean values of unbound fraction for both groups. Marked decreases in the CL_{sys} for unbound enprofylline (CL_{sysu}) (2·09 and 0·81 L h^{-1} kg^{-1} for controls and lipopolysaccharide-treated mice, respectively) and the net tubular secretion, CL_{sysu} – glomerular filtration rate (0·85 and 0·34 L h^{-1} kg^{-1} for controls and lipopolysaccharide-treated mice, respectively), were observed in the lipopolysaccharide-treated mice. These results indicate that lipopolysaccharide impairs both the glomerular filtration and tubular secretion processes of enprofylline, since the tubular reabsorption of enprofylline is negligible in man (Lunell et al 1984) and in rats (Nadai et al 1991) and renal clearance of low-extraction drugs, such as enprofylline (Nadai et al 1991), is not affected by changes in renal plasma flow rate induced by lipopolysaccharide. On the other hand, the volume of distribution for unbound enprofylline (Vd_{ssu}) in the lipopolysaccharide-treated mice (0·53 L kg^{-1}) was slightly larger than that of control mice (0·45 L kg^{-1}). It has been reported that the volume of distribution for gentamicin (Halkin et al 1981; Tardif et al 1990), trimethoprim (Lodefoged 1977) and some cephalosporins (Ganzinger et al 1986) increases with lipopolysaccharide pretreatment. The results of this study support these reports and our previous findings that lipopolysaccharide may induce increases in tissue binding and membrane permeability of enprofylline (Nadai et al 1993a). The decrease in the plasma albumin concentration caused by lipopolysaccharide may be explained by indications that lipopolysaccharide decreases albumin synthesis (Hewett & Roth 1993) and that some cytokines produced by lipopolysaccharide directly increase vascular endothelial membrane permeability (Royall et al 1989).

Bergeron & Bergeron (1986) have demonstrated that the renal cortical accumulation of the β-lactam antibiotic cephalothin, which is primarily excreted into the urine by active tubular secretion, was not modified by lipopolysaccharide treatment. In the present study, no significant changes in the amount of enprofylline taken into the kidney were observed in either group during the sampling periods following single intravenous administration, although its plasma concentrations were higher in the lipopolysaccharide-treated mice compared with control mice (Fig. 2). In addition, the decrease in the renal uptake rate of enprofylline was observed in lipopolysaccharide-treated mice over the same concentration range. It has also been found that the tubular secretion ability of the cationic drug famotidine, which is secreted into the urine by active transport at the basolateral membrane, is not affected by lipopolysaccharide (Hasegawa et al 1994b). From these findings, decrease in renal uptake of enprofylline in lipopolysaccharide-treated mice suggests that lipopolysaccharide influences the renal uptake system at the basolateral membrane rather than excretion at the brush border membrane.

From the renal-uptake experiments, it appears that enprofylline may be characterized by a high affinity uptake with low K_m (2·7 μg mL^{-1}) and a low capacity for renal uptake with low V_{max} (17·3 μg h^{-1} g^{-1} of kidney). The K_m value for enprofylline in the lipopolysaccharide-treated mice was 8 times that of control mice, although changes in the V_{max} were only two-fold. Westenfelder et al (1975) have reported that lipid A is well distributed in the tubular cells of renal cortex in dogs, and recently, we found that lipid A plays a major role in changes in the pharmacokinetics and renal handling of gentamicin induced by *K. pneumoniae* lipopolysaccharide (Hasegawa et al 1994a). Based on these findings, it is possible to speculate that enprofylline can share a common transport system with lipopolysaccharide or lipid A in the tubular cells and that enprofylline from renal tubular secretion competes with lipopolysaccharide or lipid A. The reduction of the K_d value in the presence of lipopolysaccharide may be caused by the inhibition of the basolateral membrane surface binding and also by the specific inhibition of intracellular uptake, since enprofylline has a relatively high hydrophobicity (Hasegawa et al 1991) and its affinity for phospholipids in mouse renal basolateral membranes, which possess a negative charge, may be low. These results provide indirect evidence of competition for secretion between enprofylline and lipopolysaccharide or lipid A in renal proximal tubular cells.

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