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The Effect of Lipopolysaccharide on the Disposition of Xanthines in Rats

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Abstract—The effect of lipopolysaccharide (LPS) isolated from *Klebsiella pneumoniae* O3 on the pharmacokinetic behaviour and metabolism of the xanthines, theophylline and 1-methyl-3-propylxanthine (MPX), which are mainly metabolized by the liver, was investigated in rats. LPS was infused at 0·25 mg kg^{-1} over a period of 20–30 min, 2 h before the administration of theophylline (10 mg kg^{-1}) or MPX (2·5 mg kg^{-1}). Concentrations of both xanthines in plasma and concentrations of the parent drug and metabolites in urine were measured by HPLC. Model-independent methods were applied to estimate the pharmacokinetic parameters for both xanthines. No significant changes in the pharmacokinetic parameters or metabolism of theophylline were observed in rats pretreated with LPS. However, the total body clearance and volume of distribution of MPX were significantly increased by pretreatments with LPS. Significant decreases in the binding capacity and number of binding sites on the albumin molecule were observed in the presence of LPS. Changes occurring in the protein binding behaviour as a result of the introduction of LPS is a primary factor which not only increases the volume of distribution but also increases total body clearance. These results indicate that LPS has no effect on the pharmacokinetics and metabolic pathway of theophylline although it changes the disposition of MPX due to decreases in the extent of the protein binding of MPX which is highly bound to protein.

Theophylline is one of the preferred drugs of choice for the treatment of bronchial asthma in patients with chronic obstructive airway diseases, who also sometimes experience accompanying respiratory infections from Gram-negative bacteria.

Lipopolysaccharide (LPS), a component of the cell wall of Gram-negative bacteria, has various immunological activities, including adjuvant effects, antitumour activity and macrophage activation. LPS has been reported to influence the pharmacokinetics and pharmacodynamics of various antibiotics, including aminoglycosides and cephalosporins (Halkin et al 1981; Wilson et al 1983; Bergeron & Bergeron 1986; Ngeleka et al 1989; Auclair et al 1990; Tardif et al 1990). In our previous studies, we found that *Klebsiella pneumoniae* O3 LPS exhibits strong adjuvant activity (Ohta et al 1982; Kato et al 1985; Kido et al 1985) and antitumour activity (Miyamoto et al 1984; Hasegawa et al 1985a), and that its adjuvant activity is much stronger than any other known adjuvants including LPSs from *Escherichia coli* O111 and *Salmonella* species (Ohta et al 1982; Kato et al 1985; Kido et al 1985). Our recent studies (Nadai et al unpublished data) have demonstrated that *Klebsiella* O3 LPS dramatically changes the pharmacokinetic behaviour and renal handling of the bronchodilator, enprofylline (Persson & Kjellin 1981), which is almost completely excreted into the urine (Apichartpichean et al 1991; Nadai et al 1991). Thus, LPS may induce clinical complications due to changes which occur in the pharmacokinetics and pharmacodynamics of certain drugs, thus increasing the risk for developing serious side-effects.

The present study was conducted as part of a programme directed towards developing guidelines for the safe use of xanthine derivatives in asthma patients with Gram-negative bacterial infections. We investigated the possibility that LPS may affect the pharmacokinetics of theophylline and 1-methyl-3-propylxanthine (MPX), which has been shown to be highly bound to plasma proteins and to be also completely metabolized in the liver (Apichartpichean et al 1991).

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Materials and Methods

Chemicals

The xanthines, 1-methyl-3-propylxanthine (MPX), 1-methyl-3-butylxanthine (MBX) and enprofylline were synthesized in our laboratory and were identical to those used previously (Hasegawa et al 1990, 1991a, b; Apichartpichean et al 1991). Theophylline and its metabolites, 1-methyluric acid (1-MU) and 1,3-dimethyluric acid (1,3-DMU), were purchased from Sigma Chemical Company (St Louis, MO, USA). All other reagents were commercially available and of analytical grade. Lipopolysaccharide (LPS) was isolated from a culture supernatant of *Klebsiella pneumoniae* LEN-1 (O3:K1$^-$), which is a decapsulated mutant strain derived from the *K. pneumoniae* Kasuya (O3:K1) (Ohta et al 1981), as described previously (Hasegawa et al 1983, 1985b). Theophylline and MPX were suspended in isotonic saline, and sodium hydroxide (2 M) was added in drops to create a clear solution. LPS was dissolved in isotonic saline by ultrasonification.

Pharmacokinetic experiments

Male Wistar rats (Japan SLC Inc., Hamamatsu, Japan), 8–9 weeks old, 250–290 g, were given free access to food and water. One day before the experiment, rats were anaesthetized with an intraperitoneal injection of sodium pentobarbitone (25 mg kg^{-1}) and cannulated with polyethylene tubing in the right jugular vein, then allowed to recover. After a

night with free access to food and water, rats were placed in individual metabolic cages, also with free access to food and water, and either theophylline (10 mg kg^{-1}) or MPX (2·5 mg kg^{-1}) was administered intravenously to rats pretreated with isotonic saline (control group). The dose of the two drugs was the same as previously reported (Hasegawa et al 1990, 1991a; Nadai et al 1990). The method of drug administration in this study was the same as that reported by Bergeron and colleagues (Bergeron & Bergeron 1986; Bergeron et al 1989; Ngeleka et al 1989); that is, LPS was infused at 0·25 mg kg^{-1} over a period of 20–30 min, 2 h before the administration of theophylline or MPX (LPS-treated group). In a preliminary histochemical study, the dose of LPS did not induce any histological changes in the kidneys, where LPS became well distributed. Blood samples (about 0·3 mL) were collected at designated intervals (10, 20, 30, 45, 60, 90, 120, 180, 240 and 300 min for theophylline and 10, 20, 30, 45, 60, 90, 120 and 150 min for MPX) and were immediately centrifuged at 6000 *g* for 5 min to yield plasma samples, which were stored at −40°C until analysis. Urine was collected over a period of 24 h after the administration of theophylline. Concentrations of theophylline and its major metabolites in urine were measured immediately.

Protein binding

Because the binding behaviour of MPX to albumin is concentration-dependent (Hasegawa et al 1991b), the effect of LPS on the plasma protein binding of MPX was examined by equilibrium dialysis using a cellulose membrane (Visking Sheet, Sanplatec Corp., Osaka, Japan) with molecular cut-off set at 10 000–20 000 Da. Blood samples were obtained from the control and LPS-treated groups by exsanguination from the abdominal aorta under light ether anaesthesia, and plasma samples were obtained by centrifugation. Plasma samples containing desired concentrations (0·5–85 $\mu g\ mL^{-1}$) of MPX were immediately dialysed against an equal volume of isotonic phosphate buffer (pH 7·4) at 37°C for 6 h to attain equilibrium. Concentrations of MPX on both sides of the membrane were measured by HPLC. Assuming that only one binding site exists for MPX in plasma, protein binding data were fitted according to equation 1 using the nonlinear least-squares method program MULTI (Yamaoka et al 1981)

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

where C_b and C_u are the concentrations of the bound drug and the unbound drug, respectively, nP is the binding capacity of the first class of binding sites, and K_d is the dissociation constant.

HPLC analysis

The HPLC apparatus was a Shimadzu LC-6A system (Shimadzu Co., Kyoto, Japan) consisting of an LC-6A liquid pump, an SPD-6AV UV-VIS spectrophotometric detector and an SIL-6A autoinjector. The column was a Cosmosil $5C_{18}$ packed column (4·6 × 150 mm; Nacalai Tesque, Kyoto, Japan). The mobile phases were 30 mM KH_2PO_4 (pH 4·0)–methanol (90:10; v/v) for theophylline, 30 mM KH_2PO_4 (pH 3·0)–methanol (60:40; v/v) for MPX and phosphoric acid–10 mM KH_2PO_4 solution (pH 2·5)–methanol (96:4; v/v) for theophylline and its metabolites in urine. The eluent from the column was monitored at 274 nm. The detection limit for each drug was 0·05 $\mu g\ mL^{-1}$, with a linear detection range of up to 50 $\mu g\ mL^{-1}$. The coefficients of variation for the HPLC assay were less than 6%.

Concentrations of theophylline and MPX in plasma were determined using a slight modification of the HPLC methods described previously (Ogura et al 1983; Hasegawa et al 1990). Fifty microlitres of a plasma sample was deproteinized by adding 0·35 mL of methanol containing an internal standard (enprofylline 1·0 $\mu g\ mL^{-1}$ and MBX 1·0 $\mu g\ mL^{-1}$ for theophylline and MPX, respectively). The supernatant obtained by centrifugation was evaporated to dryness under a gentle stream of nitrogen at 40°C. The residue was reconstituted with 0·2 mL of the mobile phase, and the reconstituted solution (120 μL) was injected into the HPLC.

Analyses of theophylline and its major metabolites, 1-MU and 1,3-DMU, in urine, were determined by HPLC as previously reported (Nadai et al 1990). Urine (0·1 mL) and distilled water (2·0 mL) were vortexed and filtered through Millipore filters (HV; 0·45 μm; Nihon Millipore Kogyo, Yonezawa, Japan). The filtrate was injected directly onto the column. For calculation, standard curves for all compounds were measured over a range of 1–15 $\mu g\ mL^{-1}$ and shown to be linear. Recoveries for all compounds were more than 95%, with the coefficient of variation being less than 5%. Blank urine samples showed no interference with any of the peaks corresponding to each compound.

Pharmacokinetic analysis

Plasma concentration-time data for each drug was analysed using model-independent methods and a nonlinear least-squares method program (Yamaoka et al 1981). The area under the plasma concentration-time curve (AUC) and the area under the first moment curve (AUMC) were calculated by the trapezoidal rule with extrapolation to infinity. Total body clearance (CL_T) was calculated as the dose divided by AUC. The volume of distribution at steady-state (Vd_{ss}) was calculated by $Vd_{ss} = CL_T \times MRT$. Mean residence time (MRT) was calculated by MRT = AUMC/AUC. The corresponding pharmacokinetic parameters (CL_{Tu}, Vd_{ssu} and MRT_u) for the unbound drug were estimated in the same manner as that for total concentrations, whereby the unbound concentration was calculated from total plasma concentration data and binding parameters obtained in the protein binding experiments in a rearrangement of equation 1. The renal clearance of theophylline was calculated as $CL_R = CL_T \times f_e$ and the formation clearance of theophylline metabolites as $CL_m = CL_T \times f_m$, where f_e and f_m represent the fractions of the administered theophylline dose excreted either as unchanged drug or as a specific metabolite, respectively.

Data analysis

Values are expressed as mean ± s.e. for the indicated number of experiments. Statistical comparisons between the control and treated groups were assessed using the unpaired Student's *t*-test. Statistical significance was defined at $P < 0·05$.

Results

Fig. 1 shows the mean plasma theophylline concentration-

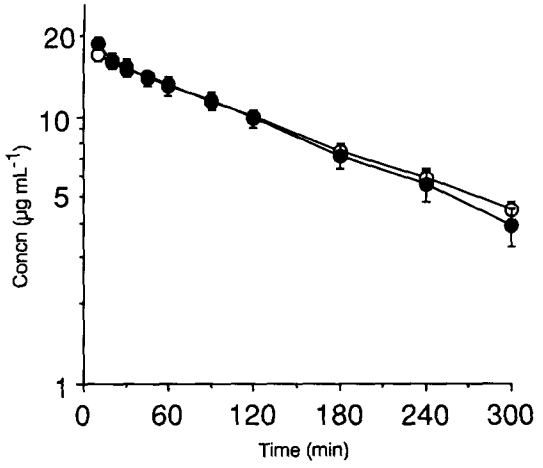

FIG. 1. Mean semilogarithmic plots of plasma concentration-time data of theophylline in control (○) and lipopolysaccharide pretreated (●) rats after a single intravenous administration. Each point represents mean ± s.e. (n = 5). When the s.e. was small, it was included in the symbol.

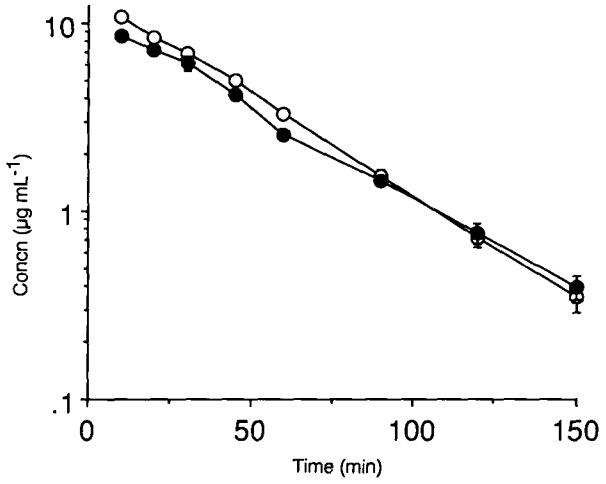

FIG. 2. Mean semilogarithmic plots of plasma concentration-time data of 1-methyl-3-propylxanthine in control (○) and lipopolysaccharide-pretreated rats (●) after a single intravenous administration. Each point represents mean ± s.e. (n = 4–5). When the s.e. was small, it was included in the symbol.

Table 1. Effect of lipopolysaccharide (LPS) on the pharmacokinetic parameters of theophylline in rats.

Treatment	Vd_{ss} (L kg^{-1})	CL_T (L h^{-1} kg^{-1})	MRT (h)
Control	0·569 ± 0·026	0·165 ± 0·009	3·468 ± 0·172
LPS	0·536 ± 0·023	0·180 ± 0·024	3·104 ± 0·397

Values represent mean ± s.e. (n = 5). No significant difference was noted between the control and the treated rats. LPS was infused at 0·25 mg kg^{-1} 2 h before intravenous administration of theophylline 10 mg kg^{-1}.

time profiles after a single intravenous administration of theophylline (10 mg kg^{-1}) and after pretreatment with LPS (0·25 mg kg^{-1}). No significant differences in the theophylline concentration were observed between treatments. Estimated model-independent pharmacokinetic parameters are summarized in Table 1. No significant changes in the pharmacokinetic parameters were observed between the control and treated groups. There were also no significant differences in total urinary recovery of unchanged theophylline and individual metabolites or in renal clearance of theophylline and formation clearance to its metabolites in a comparison of LPS-pretreated and non-pretreated rats (Table 2).

Mean plasma concentration-time profiles of MPX for the control and treated groups are shown in Fig. 2. Table 3 summarizes the corresponding pharmacokinetic parameters of MPX. The total body clearance (CL_T) and volume of distribution (Vd_{ss}) of MPX significantly increased after pretreatment with LPS (28 and 15%, respectively), but no statistical differences in these pharmacokinetic parameters (CL_{Tu}, Vd_{ssu} and MRT_u) for the unbound drug could be observed between the control and the LPS-pretreated groups. The protein binding parameters for MPX are summarized in Table 4. There was no significant difference in the binding affinity (K_d) of MPX either in the presence or absence of LPS, but the binding capacity (nP) and number of binding sites (n) on the albumin molecule significantly decreased in the presence of LPS.

Discussion

It has been reported that LPS induces various haemodynamic changes including decreases in glomerular filtration, renal plasma flow rate, and blood pressure (Gilbert 1960; McKay et al 1966; Cavanagh et al 1970; Bradley 1979; Siegel 1981), and that LPS becomes well distributed in the kidney tissues (Braude et al 1955; Mathison & Ulevitch 1979). Its active substance, lipid A, has also been shown to dramatically accumulate in the renal tubular cells (Westenfelder et al 1975; Bergeron & Bergeron 1986). Following these reports, Bergeron and colleagues (Bergeron & Bergeron 1986; Ber-

Table 2. Effect of LPS on urinary excretion of theophylline and its metabolites in rats.

	% recovery in urine			Metabolic clearance (mL h^{-1} kg^{-1})		Renal clearance of theophylline (mL h^{-1} kg^{-1})
Treatment	1-MU	1,3-DMU	Theophylline	1-MU	1,3-DMU	
Control	32·39 ± 0·93	39·66 ± 1·55	19·82 ± 1·73	53·45 ± 1·25	65·80 ± 1·80	32·61 ± 2·15
LPS	28·83 ± 1·32	38·89 ± 1·18	24·25 ± 4·50	51·95 ± 2·63	69·77 ± 2·54	44·85 ± 10·59

Each value represents mean ± s.e. (n = 5). No significant difference was noted between the control and the treated rats.

Table 3. Effect of LPS on the pharmacokinetic parameters of total and unbound 1-methyl-3-propylxanthine in rats.

Treatment	Vd_{ss}	Vd_{ssu}	CL_T	CL_{Tu}	MRT	MRT_u
	(L kg^{-1})		(L h^{-1} kg^{-1})		(h)	
Control	0·174±0·006	1·502±0·052	0·260±0·008	2·313±0·075	0·671±0·025	0·651±0·024
LPS	0·222±0·019*	1·728±0·170	0·300±0·007*	2·419±0·070	0·739±0·044	0·717±0·044

Values represent mean±s.e. (n=4–5). Data (Vd_{ssu}, CL_{Tu} and MRT_u) were obtained from calculated unbound concentration-time curves using total plasma concentrations and binding parameters. *Significant difference was noted between the control and the treated rats ($P<0·05$).

Table 4. Effect of LPS on the protein binding of MPX in rats.

Treatment	nP (μM)	K_d (μM)	n
Control	457·71±17·18	54·44±4·36	0·76±0·03
LPS	380·24±9·81*	50·55±9·58	0·64±0·02*

Each value represents mean±s.e. (n=4) and was calculated on the basis of human serum albumin with a mol. wt 69000 using equation 1. Significant difference was noted between the control and the treated rats ($P<0·05$).

geron et al 1989; Ngeleka et al 1989; Tardif et al 1990) extensively studied the influence of *E. coli* LPS on the renal handling and uptake of the antibiotics gentamicin and vancomycin into the kidney tissue of rats. They concluded that *E. coli* LPS changes the renal handling of vancomycin and the accumulation of gentamicin in the kidney cortices, and that LPS increases the potential nephrotoxicity of aminoglycosides, including gentamicin, by increasing renal uptake. However, since there is little information available on the influence of LPS on the metabolism of drugs and there is no information relating to the effect of LPS on the differential metabolic pathways of theophylline in animals and man, we decided to investigate the effect of LPS on the pharmacokinetic behaviour of highly metabolized drugs, specifically theophylline and MPX, in rats.

The biotransformation of methylxanthines, including theophylline, has been found to be catalysed by the cytochrome P450 mono-oxygenase system and to lead to the formation of demethylated compounds, uric acids and diaminouracils (Bortolotti et al 1985). The metabolic pathway of MPX, despite its resemblance to theophylline, has been unclear up to now. Our previous studies, however, showed that MPX with its rapid elimination is useful as a tool for clarifying the precise mechanism of metabolic interaction in the liver (Hasegawa et al 1990, 1991a). The results of the present study clearly show that *Klebsiella* O3 LPS has little or no effect on the pharmacokinetics and differential metabolic pathways of theophylline in rats, indicating that LPS has no effect on the hepatic microsomal drug metabolizing enzyme activities.

The clearance of drugs with a high hepatic extraction ratio is affected by changes in hepatic blood flow although a low hepatic extraction ratio has no effect on the changes in hepatic blood flow but is dependent upon the degree of plasma protein binding (Rowland & Tozer 1989). The results obtained from this study using theophylline are consistent with theophylline's low hepatic extraction ratio and weak binding to plasma proteins (approx. 40%).

In contrast, the pharmacokinetic behaviour of MPX was affected by pretreatment with LPS. Unbound drug can diffuse across biological membranes and alterations in the degree of protein binding can influence their distribution and elimination kinetics. MPX binds strongly to albumin and exhibits concentration-dependent characteristics in protein binding (Apichartpichean et al 1989; Hasegawa et al 1991b). It is possible that changes in the protein binding behaviour of MPX induced by LPS are due to changes in the conformation of the albumin molecule; further studies, however, are required to clarify this. The pharmacokinetic parameters for unbound MPX, calculated using the unbound drug concentration-time data, were unaffected after treatment with LPS, indicating that increases in total body clearance and volume of distribution for the total drug are apparently due to the protein binding behaviour but that the distribution of MPX in the tissue is not altered by LPS. On the basis of these data, we conclude that changes which occur in the protein binding of MPX after pretreatment with LPS are a primary factor influencing the disposition of MPX and that the difference in the degree of the effect of LPS on the pharmacokinetic behaviour between theophylline and MPX is due to differences in the protein binding between the two drugs.

The present data has shown that pretreatment with LPS enhances the elimination of MPX but not theophylline in rats. In contrast to the results reported for aminoglycoside antibiotics, which are mainly excreted by the kidneys, *Klebsiella* O3 LPS had no dramatic effects on the pharmacokinetics or metabolism of theophylline and MPX, which are solely metabolized by the liver. The increases in the total body clearance and volume of distribution for MPX observed by pretreatments with LPS might be due to the decreased extent of plasma protein binding of MPX. These results suggest that LPS may affect the pharmacokinetics of drugs, particularly those drugs which are highly bound to albumin. The results reported here on the pharmacokinetic interaction between LPS and xanthines may provide useful clinical information regarding drugs with hepatic clearance and higher protein binding. They also indicate that it would be safe to administer theophylline to asthma patients with infections from Gram-negative bacteria.

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