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Influence of a Bacterial Lipopolysaccharide on the Pharmacokinetics of Tobramycin in Rats

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Abstract—The effects of *Klebsiella pneumoniae* O3 lipopolysaccharide on the renal handling and distribution characteristics of the aminoglycoside tobramycin were investigated in rats. Tobramycin (2 mg kg^{-1}) and inulin (100 mg kg^{-1}) were administered intravenously 2 h after administration of 50, 250 or 500 μg kg^{-1} lipopolysaccharide. Lipopolysaccharide delayed the disappearance of tobramycin from plasma in a dose-dependent manner. A dose-dependent decrease in systemic clearance of tobramycin was observed, although the elimination rate constant and fraction of urinary recovery of unchanged drug were not significantly different in any group. Lipopolysaccharide significantly decreased the central compartment volume of distribution of tobramycin, but did not influence the steady-state volume of distribution. A dose-related increase in the ratio of the rate constant of transfer to the peripheral compartment to the rate constant of transfer from peripheral compartment to central compartment was observed. The glomerular filtration rate was significantly decreased by pretreatment with 250 μg kg^{-1} lipopolysaccharide and the clearance ratio was decreased by 20%, indicating that lipopolysaccharide increases the tubular reabsorption of tobramycin. Our findings suggest that *K. pneumoniae* O3 lipopolysaccharide modifies the glomerular filtration rate and tubular reabsorption without change in the terminal half-life and that drug distribution characteristics from the rapidly-distributing compartment to the peripheral compartment were altered without expansion of the extracellular fluid volume.

Lipopolysaccharide, a cell component of the Gram-negative bacterial cell wall, is released into the body by cell lysis as a result of the use of antibiotics. It is known that lipopolysaccharide has various biological and immunological activities, including adjuvant effects, antitumour activities and macrophage activation. It has also been reported that physiological changes induced by lipopolysaccharide in several organs affect the pharmacokinetics of drugs (Lodefoged 1977; Bergeron & Bergeron 1986; Ganzinger et al 1986; Ngeleka et al 1989; Auclair et al 1990; Tardif et al 1990). The nephrotoxic effects of lipopolysaccharide which produce decreases in glomerular filtration rate and renal plasma flow (Hinshaw et al 1959; Gilbert 1960; Cavanagh et al 1970; Kikeri et al 1986; Churchill et al 1987), may cause clinical problems with antibiotic therapy as most antibiotics are excreted by the kidney. It has been reported, for example, that renal handling and pharmacokinetics of aminoglycoside antibiotics gentamicin, tobramycin and amikacin are affected by lipopolysaccharide (Bergeron & Bergeron 1986). Increases in the cortical accumulation of these antibiotics have also been reported (Bergeron et al 1982; Auclair et al 1990; Tardif et al 1990).

Our previous studies (Wang et al 1993) have demonstrated that lipopolysaccharide has no affect on the pharmacokinetics and metabolism of theophylline, although it has been shown to change the pharmacokinetics of 1-methyl-3-propylxanthine (Apichartpichean et al 1991). Lipopolysaccharide has also been reported to modify the renal handling of enprofylline by inducing reductions in the maximum velocity and the Michaelis–Menten constant for tubular secretion. Additionally lipopolysaccharide increases the volume of distribution of enprofylline without altering protein binding (Nadai et al 1992). The effect of lipopolysaccharide on drug disposition is therefore complicated by simultaneous changes in excretion and distribution.

Halkin et al (1981) and Bergeron & Bergeron (1986) have reported that lipopolysaccharide increased the volume of distribution of gentamicin in rabbits and rats, but did not induce total clearance. Aminoglycoside antibiotics are not bound to plasma proteins, are distributed throughout the extracellular fluid and are excreted from the kidney, and their renal clearance is dependent upon the glomerular filtration rate (Pechere & Dugal 1979; Wenk et al 1984). A detailed explanation which can account for changes in the pharmacokinetics of aminoglycosides, therefore, remains necessary.

The present study aims to clarify the mechanism by which lipopolysaccharide changes the pharmacokinetics of the aminoglycoside tobramycin in rats.

Materials and Methods

Chemicals

Tobramycin was prepared for injection using a commercial preparation (Tobracin, Shionogi & Co., Osaka, Japan) and was diluted to the desired concentration with isotonic saline. Inulin was purchased form Nacalai Tesque Inc. (Kyoto, Japan). All other chemicals were of the highest quality available. Inulin was dissolved in isotonic saline for intravenous injection.

Lipopolysaccharide was isolated from a cultured supernatant of *Klebsiella pneumoniae* LEN-1 (O3:K1$^-$), a decapsulated mutant strain derived from the *K. pneumoniae* strain of Kasuya (O3:K1) (Ohta et al 1981), as previously described (Hasegawa et al 1983, 1985).

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Animal experiments

Eight- to nine-week-old male Wistar rats (Japan SLC Inc., Shizuoka, Japan) were used in all experiments. One day before the experiments, rats were anaesthetized with sodium pentobarbitone (30 mg kg^{-1}) and the right jugular vein was cannulated with polyethylene tubing for administration of drugs and for blood sampling. On the following day, lipopolysaccharide was dissolved in isotonic saline and infused at doses of 50, 250 or 500 $\mu g\ kg^{-1}$, for a period of 20–30 min 2 h before 2 mg kg^{-1} tobramycin and 100 mg kg^{-1} inulin were administered intravenously (Nadai et al 1992; Wang et al 1993). In the control rats, saline was infused in the same manner. Blood samples of approximately 0·25 mL were collected at designated intervals and plasma samples were obtained by centrifugation. In the tobramycin experiment, urine was also collected for 24 h after injection. All samples were stored at −40°C until analysis.

Drug analysis

Concentrations of tobramycin in plasma and urine were measured using the fluorescence polarization immunoassay method with a TDX-analyzer (Abbott, USA). In plasma, the intra-day and inter-day coefficients of variation ranged from 4 to 6% in plasma samples with the desired concentrations (0·04–10 $\mu g\ mL^{-1}$) of tobramycin added. The limit of quantitation was 0·05 $\mu g\ mL^{-1}$. Recovery of tobramycin from urine was 98%. Coefficients of variation ranged from 3 to 5%. Inulin was measured by the standard colorimetric method (Dische & Borenfreund 1951).

Data analysis

The pharmacokinetic parameters of a two-compartment model from a computer-fitted biexponential function describing the plasma concentration-time curves for tobramycin were analysed by a nonlinear least-squares regression analysis using the computer program MULTI (Yamaoka et al 1981):

$$C = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

where C is the plasma concentration of tobramycin at time t, and A, α, B and β are the biexponential equation constants. The half-life of the terminal phase was calculated as $t_{\frac{1}{2}}$ $0{\cdot}693/\beta$. The volume of the initial distribution space (Vd) was calculated by dividing the dose by the plasma concentration at zero time. The area under the plasma concentration-time curve (AUC) for tobramycin and inulin was determined by the trapezoidal rule with extrapolation to infinity. The systemic clearance (CL) was calculated as dose/AUC. The mean residence time (MRT) and the volume of distribution at steady-state (Vd_{ss}) were calculated as:

$$MRT = AUMC/AUC \quad (2)$$

$$Vd_{ss} = CL \times MRT \quad (3)$$

where AUMC represents the area under the first moment curve. The systemic clearance of inulin was designated as glomerular filtration rate (GFR). The clearance ratio was determined by dividing CL for tobramycin by GFR, since tobramycin is mainly excreted into the urine in the unchanged form and is not bound to plasma protein (Pechere & Dugal 1979; Wenk et al 1984).

Statistical analysis

Statistical analysis of tobramycin pharmacokinetic parameters in the control group and lipopolysaccharide-treatment groups was performed using one-way analysis of variance. Tukey's test was used to detect differences among groups. In experiments analysing inulin pharmacokinetics, Student's *t*-test was used. In all statistical testing, a *P* value of $< 0{\cdot}05$ was considered statistically significant.

Results

The mean plasma concentration-time curves for tobramycin in the control and treated rats are shown in Fig. 1. The elimination profiles of tobramycin from plasma were characterized as biexponential equations for all groups studied. Plasma concentrations of tobramycin in the lipopolysaccharide-treated groups at any given time, however, were higher than those of the control groups. Moreover, lipopolysaccharide delayed the disappearance of tobramycin from plasma in a dose-dependent manner. The pharmacokinetic parameters, as estimated using a two-compartment open model, are listed in Table 1. The central compartment volume of distribution (Vd) decreased significantly as the dose of lipopolysaccharide increased, although the steady-state volume of distribution (Vd_{ss}) was not affected. A dose-dependent decrease in systemic clearance (CL) was also found in the treated rats. The terminal elimination half-life of tobramycin, however, was not significantly different in any group. The rate constant of transfer to the peripheral compartment (k_{12}) was significantly raised by administering lipopolysaccharide. There were no significant differences among groups of rats in the urinary recovery of tobramycin ($> 75\%$).

Fig. 2 and Table 2 show the effect of 250 $\mu g\ kg^{-1}$ lipopolysaccharide on the plasma concentration and pharmacokinetic parameters of inulin. The plasma concentration of inulin in the treated rats was higher than that of the control rats. No significant difference in the steady-state volume of distribution of inulin was noted between the control rats and

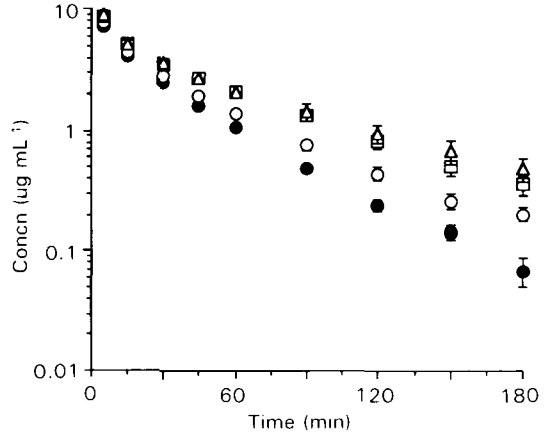

FIG. 1. Mean semilogarithmic plots of plasma concentration-time curves of tobramycin in control (●) and lipopolysaccharide-pretreated (○, 50; □, 250; △, 500 $\mu g\ kg^{-1}$) rats after a single intravenous administration of tobramycin, 2 mg kg^{-1}. Each plot represents mean ± s.e. for five rats. When the standard error is small, it is included in the symbol.

Table 1. Pharmacokinetic parameters of tobramycin (2 mg kg^{-1}, i.v.) in control rats and in rats given different doses of lipopolysaccharide.

Parameter	Control	Lipopolysaccharide ($\mu g\ kg^{-1}$) 50	250	500
Vd ($L\ kg^{-1}$)	0·206 ± 0·019	0·183 ± 0·013	0·157 ± 0·006	0·141 ± 0·006[a]
Vd_{ss} ($L\ kg^{-1}$)	0·272 ± 0·018	0·300 ± 0·014	0·271 ± 0·015	0·289 ± 0·007
CL ($L\ h^{-1}\ kg^{-1}$)	0·484 ± 0·013	0·405 ± 0·023	0·298 ± 0·020[a]	0·306 ± 0·039[a,b]
k_e (h^{-1})	2·526 ± 0·261	2·317 ± 0·154	1·963 ± 0·163	2·270 ± 0·335
$t_{\frac{1}{2}}$ (h)	0·534 ± 0·051	0·597 ± 0·039	0·733 ± 0·061	0·768 ± 0·096
k_{12} (h^{-1})	1·502 ± 0·413	2·453 ± 0·350	3·787 ± 0·454[a]	3·936 ± 0·472[a]
k_{21} (h^{-1})	3·091 ± 0·592	3·712 ± 0·336	4·586 ± 0·337	3·675 ± 0·235
MRT (h)	0·569 ± 0·038	0·719 ± 0·051	0·929 ± 0·817[a]	0·938 ± 0·136[a]

Each value represents mean ± s.e. for five rats. [a] Significantly different from the control rats. [b] Significantly different from the lipopolysaccharide-treated rats (50 $\mu g\ kg^{-1}$).

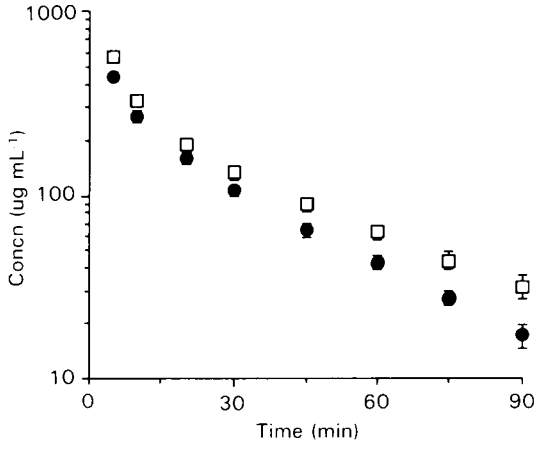

FIG. 2. Mean semilogarithmic plots of plasma concentration-time of inulin in control (●) and lipopolysaccharide-pretreated (□) rats after a single intravenous administration of inulin, 100 mg kg^{-1}. Each plot represents the mean ± s.e. for five rats. When the standard error is small, it is included in the symbol.

Table 2. Pharmacokinetic parameters of inulin after intravenous administration of 100 mg kg^{-1} in control and lipopolysaccharide-treated (250 $\mu g\ kg^{-1}$) rats.

Treatment	Vd_{ss} ($L\ kg^{-1}$)	CL ($L\ h^{-1}\ kg^{-1}$)	MRT (h)
Control	0·228 ± 0·007	0·529 ± 0·027	0·342 ± 0·013
Lipopolysaccharide	0·200 ± 0·011	0·395 ± 0·023[a]	0·408 ± 0·059

Each value represents mean ± s.e. for five rats. [a] Significantly different from the control rats.

the treated rats. The systemic clearance of inulin, equated with the GFR, was significantly lower in the treated rats.

Discussion

This study clearly demonstrated that *K. pneumoniae* O3 lipopolysaccharide reduces GFR and that it dramatically modifies the pharmacokinetics of tobramycin in a dose-dependent manner. The finding that the pharmacokinetics and renal cortical accumulation of aminoglycosides were affected by treatments with lipopolysaccharide has been previously reported (Halkin et al 1981; Bergeron et al 1982; Bergeron & Bergeron 1986; Tardif et al 1990). Halkin et al (1981) have demonstrated that lipopolysaccharide induced an increase in the central and peripheral volume of distribution of gentamicin in rabbits. Bergeron & Bergeron (1986) and Tardif et al (1990) observed no change in Vd_{area} for gentamicin in rats, following description of the pharmacokinetics by a one-compartment model. It is well known, on the other hand, that aminoglycoside antibiotics distribute into extracellular fluid in the body in the absence of protein and tissue binding and that the pharmacokinetics of tobramycin is adequately described by a two-compartment model (Pechere & Dugal 1979; Wenk et al 1984). In addition, it is believed that using Vd_{area} as a parameter to quantify drug distribution can be misleading, because the value of Vd_{area} is influenced by the rate of elimination (Wagner 1975). In the present study, therefore, the effect of lipopolysaccharide on the distribution of tobramycin was determined using the value of volume of distribution at steady-state (Vd_{ss}), which more accurately reflects drug distribution in the body. The results of this study indicate that there was no change in Vd_{ss} among the treatment groups, although lipopolysaccharide produced a dose-dependent decrease in Vd_{ss} for tobramycin.

In confirming that Vd_{ss} was not affected by lipopolysaccharide, the effect of lipopolysaccharide on the pharmacokinetics of inulin, which distributes only in extracellular fluid, was also examined. As can be seen in Table 2, no differences in Vd_{ss} were observed in either control group or the lipopolysaccharide-treated rats.

In the present study, decreases in Vd for tobramycin were found in lipopolysaccharide-treated rats. The ratio of the rate constant (k_{12}) of transfer to the peripheral compartment from the central compartment to the rate constant (k_{21}) of transfer from the peripheral compartment to the central compartment was greater in the lipopolysaccharide-treated rats compared with the control group. These results indicate that drug distribution from the highly perfused central compartment to the peripheral compartment was altered by lipopolysaccharide without expanding extracellular fluid volume. It has been reported that some cytokines, when induced by lipopolysaccharide, increase vascular endothelial cell permeability (Royall et al 1989). Our previous study demonstrated an increase in Vd_{ss} of enprofylline in lipopolysaccharide-treated rats (Nadai et al 1992). It may be considered, therefore, that lipopolysaccharide alters either drug-to-tissue or drug-to-extracellular protein binding characteristics, and induces increases in both tissue perfusion rate and the membrane permeability. The former suggestion is probably not applicable to tobramycin because it is not

bound to plasma proteins. Moreover, lipopolysaccharide-induced pyrexia in the rabbit has been reported to produce an increase in the volume of distribution of gentamicin by inducing changes in the tissue perfusion rate or tissue binding of drug (Lodefoged 1977; Halkin et al 1981; Ganzinger et al 1986). However, Bergeron & Bergeron (1986) have reported that there was no fever response to *E. coli* lipopolysaccharide in rats. It is unlikely, therefore, that lipopolysaccharide-induced changes in the distribution of tobramycin observed in rats is caused by induced pyrexia.

The excretion mechanism for aminoglycoside antibiotics consists of both glomerular filtration and tubular reabsorption (Pechere & Dugal 1979; Wenk et al 1984).

Our results show that lipopolysaccharide treatment decreased the systemic clearance of tobramycin in a dose-dependent manner without causing significant changes in urinary excretion over 24 h, indicating that the renal clearance of tobramycin was decreased by administering lipopolysaccharide. Tardif et al (1990) also demonstrated that lipopolysaccharide at higher doses (0·5–5 mg kg^{-1}) decreased total clearance of gentamicin in a dose-dependent manner.

The ratio of systemic clearance to GFR (clearance ratio) in the control rats was 0·92, indicating that tobramycin is partially reabsorbed in the proximal tubular cells. A decrease in the clearance ratio was observed in the lipopolysaccharide-treated rats, suggesting an increased reabsorption of tobramycin.

Rowland & Tozer (1989) reported that only the initial half-life of gentamicin is altered in patients who suffer varying degrees of renal impairment, and that the terminal half-life is not affected, because gentamicin elimination from plasma is rate-limited by drug efflux from the peripheral compartment to the central compartment during the terminal phase. They concluded that the terminal half-life was unchanged until renal function was markedly impaired. In the present study, there were no significant differences in the elimination half-life of tobramycin between the control group and the lipopolysaccharide-treated groups, although a 25% decrease in GFR was found in rats receiving 250 $\mu g\ kg^{-1}$ lipopolysaccharide. These findings indicate that the decrease in renal function induced by high doses of lipopolysaccharide (500 $\mu g\ kg^{-1}$) was not enough to reduce the elimination half-life in rats. Tardif et al (1990) have also demonstrated that while lipopolysaccharide had no effect on the elimination rate constant of gentamicin in rats, it reduced the total clearance. The results obtained in this study support the theory of Rowland & Tozer (1989).

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