Influence of Endotoxin and Lipid A on the Renal Handling and Accumulation of Gentamicin in Rats

Takaaki Hasegawa,*,a Masayuki Nadai,a Li Wang,a Soheila Haghigh,a Toshitaka Nabhima,a and Nobuo Kato b

Department of Hospital Pharmacy,a and Department of Bacteriology,b Nagoya University School of Medicine, 65 Tsuruma-cho, Showa-ku, Nagoya 466, Japan. Received May 2, 1994; accepted September 8, 1994

The contribution of lipid A, an active component of endotoxin (LPS), to changes in the pharmacokinetics, renal handling and intrarenal accumulation of gentamicin induced by Klebsiella pneumoniae LPS was investigated in rats. Either LPS (250 μg/kg) or lipid A (equivalent to dose of LPS) was infused 2 h before the administration of gentamicin (10 mg/kg). The effects of LPS and lipid A on the intrarenal accumulation of gentamicin were also evaluated. Significant increases in the levels of plasma creatinine and blood urea nitrogen were observed in both the LPS and lipid A groups. Both LPS and lipid A induced significant decreases in the glomerular filtration rate (by approximately 30%) and systemic clearance of gentamicin (by approximately 25%). No changes in the fraction of urinary excretion (>0.9) or steady-state volume of distribution of gentamicin were observed between either the control, LPS or lipid A groups. There were no significant differences among the three groups in the tubular reabsorption or intrarenal accumulation of gentamicin. The degree of effect of lipid A on the pharmacokinetics of gentamicin observed in this study was nearly equal to that of LPS. These results suggest that lipid A plays a major role in changes in the pharmacokinetics and renal handling of gentamicin induced by LPS.

Keywords Klebsiella pneumoniae endotoxin; lipid A; gentamicin; renal accumulation; reabsorption; pharmacokinetics

It has been widely accepted that bacterial endotoxin (LPS), an active component in the outer membrane of gram-negative bacteria, consists of a polysaccharide (specific O antigen) which is linked to the R core (oligosaccharide), which in turn is linked to the lipid portion termed lipid A. It is also known that LPS has various biological and immunological activities. In our earlier studies, we found that Klebsiella pneumoniae LPS has potent adjuvant13 and antitumor activities.4,5 Studies in progress in this laboratory have shown that K. pneumoniae LPS dramatically modifies the pharmacokinetics and renal handling of drugs, which are primarily excreted into the urine.69 It has also been found that LPS induces adverse and nephrotoxic effects on the kidneys and causes physiological changes in glomerular filtration rate, renal plasma flow and blood pressure.107 The renal failure and hemodynamic changes induced by LPS can modify the pharmacokinetics and renal handling of various drugs which are primarily excreted by the kidney.

Aminoglycoside antibiotics, including gentamicin, are widely used as the first choice in treating serious gram-negative bacterial infections. They should be used with great caution because of their predisposition towards nephrotoxicity, which is closely related to their transport and accumulation in the proximal tubular cells. Much useful information on this topic, that LPS potentiates the nephrotoxicity of gentamicin16 and that LPS induces changes in the pharmacokinetics and renal handling of aminoglycoside antibiotics, is available.16–20 The nephrotoxic effects of LPS may cause clinical problems with aminoglycoside antibiotic therapy. The precise mechanisms involved in the potentiation effect of LPS on the nephrotoxicity and LPS-induced changes in the pharmacokinetics and renal handling of aminoglycoside antibiotics, however, have not yet been clarified.

It is well known that the lipid A portion of LPS, an active component, is responsible for the biological activities of LPS. In a series of earlier studies, we observed that neither a polysaccharide fraction nor a lipid A fraction derived from K. pneumoniae LPS could reproduce potent adjuvant activities in mice, that a single mixture of these two fractions also failed to do so, and that both lipid A and polysaccharide moieties must be combined in the form of LPS for expressing adjuvant activity.23 These findings suggest that the polysaccharide portion of LPS contributes to the action of LPS in some immunological activities by modifying the action of lipid A. Westenfelder et al. have reported that lipid A is well distributed in the tubular cells of the renal cortex in dogs.24 In contrast, Mathison and Ulevitch have reported that LPS is distributed in the kidney in rabbits.25 The possibility exists that lipid A, which is probably liberated from the LPS form in the body following the administration of LPS, is a major determinant in LPS-induced renal failure. Detailed information concerning whether lipid A contributes to LPS-induced alterations in renal handling and the intrarenal distribution of aminoglycoside antibiotics, therefore, remains necessary.

The present study aims to clarify the role of lipid A in changes which occur in the pharmacokinetics, renal handling and intrarenal distribution of gentamicin in endotoxemic rats by K. pneumoniae LPS.

MATERIALS AND METHODS

Materials Gentamicin was prepared for injection using a commercial preparation (Gentacin, Schering-Plough Co., Osaka, Japan) and was diluted to the desired concentration with isotonic saline immediately prior to injection. Inulin was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). All other chemicals were of the highest quality available. Inulin was dissolved in isotonic saline for intravenous injection. Rat serum albumin, for obtaining good water solubility of lipid A, was purchased...
from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

LPS was isolated from a cultured supernatant of *Klebsiella pneumoniae* LEN-1 as described previously, \(^{26-28}\) which was identical to that used previously. \(^{6-9,29}\) Lipid A was isolated from the LPS according to methods described previously. \(^{27-28}\) Lipid A was dissolved in a 2% rat serum albumin-saline solution, which corresponds to 250 \(\mu\)g/ml of LPS.

**Animal Experiments** Eight- to nine-week-old male Wistar strain rats (Nippon SLC, Inc., Hamamatsu, Japan), weighing 250 to 300 g, were used in all the experiments.

For evaluating the effects of LPS or lipid A on the pharmacokinetics and renal handling of gentamicin, rats were divided into three treatment groups: (1) a control group (2% rat serum albumin-saline solution was infused in place of LPS or lipid A); (2) an LPS-treated group (250 \(\mu\)g/kg); (3) a lipid A-treated group (lipid A was infused at dose corresponding to 250 \(\mu\)g/kg of LPS). They were maintained on commercial food and water *ad libitum*. One day before the experiments, the rats were anesthetized with sodium pentobarbital (25 mg/kg), cannulated in the right jugular vein with polyethylene tubing for administering drugs and blood sampling, and allowed to recover. On the following day, either LPS or lipid A was dissolved in 2% rat serum albumin-saline solution at a dose of 250 \(\mu\)g/kg or lipid A corresponding to 250 \(\mu\)g/kg of LPS was infused for a period of 20 to 30 min (3.93 \(\mu\)g/min/kg), 2 h before administering both gentamicin (10 mg/kg) and insulin (100 mg/kg) as described previously. \(^{9}\) In the control rats, 2% rat serum albumin-saline solution was infused in the same manner.

Blood samples of approximately 0.25 ml were collected at designated intervals (5, 10, 20, 30, 45, 60, 90, 120, 150, and 180 min after administration) and plasma samples were obtained by centrifugation. Urine was also collected for 24 h after injection of gentamicin for determining urinary recovery. All samples were stored at \(-40^\circ\)C until analysis.

In other experiments for determining the effect of both LPS and lipid A on the intrarenal distribution of gentamicin, rats in each group were exsanguinated at 1 h after the administration of gentamicin. For estimating the effects of LPS or lipid A on the renal clearance of gentamicin, urine samples were collected over a period of 60 min and residual urine in the urinary bladder was also collected. Urine volume was measured gravimetrically assuming a specific gravity of 1.0. Both kidneys were removed and the wet weights were recorded. The plasma samples and both kidneys were immediately frozen at \(-40^\circ\)C until gentamicin, BUN and plasma creatinine assays were performed. Right kidneys were homogenized with an adequate volume (10 ml) of isotonic saline, and the resulting homogenates were centrifuged at 9000 \(\times\) g for 5 min at \(5^\circ\)C. The concentrations of gentamicin in the supernatants were measured in the same manner.

**Drug Analysis** Concentrations of gentamicin in plasma, urine and tissue were measured by fluorescent polarization immunoassay using the TDX-analyzer (Dainabot Co., Ltd., Tokyo, Japan). The intra- and inter-day coefficients of variation were less than 7% in plasma samples spiked with desired concentrations of gentamicin (2 and 10 \(\mu\)g/ml). The quantitative limit of this assay was 0.5 \(\mu\)g/ml. Recovery of gentamicin from the urine was 96% and the coefficients of variation ranged from 3 to 5%. The recovery of gentamicin ranged from 94 to 98% when known concentrations of gentamicin were spiked in the supernatants obtained from normal rat kidney homogenates. The coefficients of variation of this assay were less than 8%. Insulin was measured by the standard colorimetric method as described by Dische and Borenfreund. \(^{39}\) Concentrations of blood urea nitrogen (BUN) and plasma creatinine were measured using commercially available kits (Blood Urea Nitrogen B-Test Wako and Creatinine-Test Wako, respectively, Wako Pure Chemical Industries, Ltd., Osaka, Japan).

**Data Analysis** The plasma concentration–time data for gentamicin in each group were analyzed on the basis of model-independent methods. The area under the curve (AUC) and area under the first moment curve (AUMC) were determined by the trapezoidal rule method with extrapolation to infinity. The systemic clearance (\(CL_{sys}\)) was calculated as the dose divided by the AUC. The mean residence time (MRT) and the volume of distribution at steady-state (\(V_{dss}\)) were calculated as \(MRT = AUMC / AUC\) and \(V_{dss} = CL_{sys} \times MRT\), respectively. The glomerular filtration rate (GFR) was determined as the \(CL_{sys}\) of insulin.

**Statistical Analysis** Values are expressed as the mean \(\pm\) S.E. Statistical analysis of the experimentally estimated parameters of gentamicin in the control, LPS and lipid A groups was performed using the Student’s *t*-test, where \(p<0.05\) was defined as statistically significant.

**RESULTS**

Mean semilogarithmic plasma concentration–time curves for gentamicin after a single intravenous administration at a dose of 10 mg/kg in the control, LPS and lipid A groups are shown in Fig. 1. Both LPS and lipid A significantly delayed the disappearance of gentamicin from plasma. The data were obtained from separate experiments and were independently analyzed, statistically significant changes in the \(CL_{sys}\) and MRT of gentamicin were observed between the control and the LPS or lipid A groups: the \(CL_{sys}\) values were reduced by 26% and 24%.
TABLE I. Pharmacokinetic Parameters of Gentamicin in Control, LPS- and Lipid A-Pretreated Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>( V_{\text{dss}} ) (l/kg)</th>
<th>( CL_{\text{tot}} ) (l/h/kg)</th>
<th>MRT (h)</th>
<th>GFR (l/h/kg)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>0.267 ± 0.013</td>
<td>0.446 ± 0.020</td>
<td>0.580 ± 0.018</td>
<td>0.601 ± 0.033</td>
</tr>
<tr>
<td>LPS</td>
<td>0.284 ± 0.011</td>
<td>0.330 ± 0.020( ^a )</td>
<td>0.832 ± 0.048( ^a )</td>
<td>0.409 ± 0.037( ^a )</td>
</tr>
<tr>
<td>Lipid A</td>
<td>0.258 ± 0.011</td>
<td>0.339 ± 0.017( ^a )</td>
<td>0.744 ± 0.039( ^a )</td>
<td>0.429 ± 0.011( ^a )</td>
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Each value represents mean ± S.E. for seven control rats and for five rats receiving LPS or lipid A treatments. \( ^a \) A significant difference was noted between the control and the treated rats.

Fig. 2. Effects of LPS and Lipid A on Concentrations of Plasma Creatinine (White Bars) and BUN (Black Bars)
Each bar represents mean ± S.E. of five control and six LPS- and lipid A-treated rats. \( ^a \) Significantly different from control, \( p < 0.05 \).

Fig. 3. Effect of LPS and Lipid A on Urinary Excretion of Gentamicin During the First Hour
Each bar represents mean ± S.E. of five rats. \( ^a \) Significantly different from control, \( p < 0.05 \).

respectively. Neither LPS nor lipid A affected either the \( V_{\text{dss}} \) or the fraction of urinary excretion as an unchanged drug (> 0.9). As shown in Table I, the GFR estimated as inulin clearance in both the LPS and lipid A groups also significantly decreased to approximately 70% of that in the control group (0.6 l/h/kg).

Three hours after the administration of LPS or lipid A, the concentrations of BUN and plasma creatinine significantly increased to 26.8 ± 1.8 and 0.60 ± 0.03 mg/dl in the LPS-treated rats and 23.2 ± 1.0 and 0.62 ± 0.04 mg/dl in the lipid A-treated rats, respectively, as compared with 13.2 ± 0.8 and 0.47 ± 0.01 mg/dl in the control rats (Fig. 2).

As shown in Fig. 3, significant differences were observed in the amount of gentamicin excreted into the urine for 60 min after the administration of gentamicin between the control (2.3 ± 0.1 mg) and lipid A-treated groups (1.9 ± 0.1 mg), but there was no significant difference between the control and LPS-treated groups (2.1 ± 0.3 mg). Further, the urine volumes were not significantly different between the control (3.0 ± 0.5 ml) and the treated groups (2.6 ± 0.3 and 2.3 ± 0.2 ml for LPS- and lipid A-treated groups, respectively).

The effects of LPS and lipid A on the intrarenal accumulation of gentamicin during the first hour after the administration of gentamicin are shown in Fig. 4. The concentrations of gentamicin in the kidney were: 47.6 ± 5.4 μg/g (control rats); 68.4 ± 9.6 μg/g (LPS-treated rats); and 55.0 ± 10.0 μg/g (lipid A-treated rats). There was no significant difference in the intrarenal accumulation of gentamicin either in the presence or absence of LPS or lipid A. The ratio of kidney weight to body weight was significantly increased in the LPS- and lipid A-treated groups (3.5 ± 0.1 and 3.4 ± 0.1 g/kg) when compared with the control group (3.0 ± 0.1 g/kg), although there were no significant differences in kidney weight between the three groups (Fig. 5).

DISCUSSION

Direct evidence revealing whether lipid A, an active
component of LPS, contributes to changes in the pharmacokinetics, renal handling and intrarenal distribution of drugs in endotoxemia is sparse. In the present study, using the aminoglycoside antibiotic gentamicin, we demonstrated that lipid A derived from *K. pneumoniae* LPS decreased GFR and the CL_{sys} for gentamicin, whereas it had no effect on the tubular reabsorption or intrarenal accumulation of gentamicin; also, the changes induced by lipid A were nearly equal to those induced by LPS.

Recent studies in our laboratory illustrated that the volume of distribution of the aminoglycoside antibiotic tobramycin and extracellular fluid volume of rats did not change in the presence of *K. pneumoniae* LPS, whereas it increased the volume of distribution of drugs which are highly bound to plasma protein due to decreases in the extent of protein binding. These experiments further suggested the possibility that LPS modifies either drug-to-tissue or drug-to-extracellular protein binding properties, and induces increases in both the tissue perfusion rate and membrane permeability. In the present study, no effect of the *K. pneumoniae* LPS on the V_{ao} of gentamicin was observed. It has been reported that the V_{ao} of aminoglycosides approaches extracellular fluid volume when their pharmacokinetic parameters were analyzed by a two-compartment model. Indeed, the V_{ao} for gentamicin following pretreatment with LPS observed in this study was nearly equal to that of tobramycin and the extracellular fluid volume in rats. In contrast to our results, it has been reported that a decrease in the volume of distribution of gentamicin in endotoxemic horses was induced by *E. coli* LPS but that an increase in the volume of distribution in endotoxemic rabbits was also induced by *E. coli* LPS. Conversely, Tardif and colleagues have observed no change in the volume of distribution of gentamicin in endotoxemic rats induced by *E. coli* LPS, a finding that concurs with those of this study and our previous experiments regarding tobramycin. Knowing that there are differences in sensitivity among different animal species to LPS, discrepancies in the volume of distribution may be explained by species differences in the tissue perfusion rate and in membrane permeability, resulting in fever. Further studies on this point are indicated.

The present study shows that both LPS and lipid A decrease the CL_{sys} of gentamicin and that the fraction of urinary excretion of gentamicin for 24 h following the administration of gentamicin alone was not significantly different from that in the presence of either LPS or lipid A (>0.9). These results indicate that the renal clearance of gentamicin was decreased by administering either LPS or lipid A. The present findings are supported by the earlier findings of Tardif and colleagues using *E. coli* LPS.

A significant decrease in the GFR was also observed in both the LPS and lipid A groups. However, the precise mechanism for this decrease is unclear at this stage. It is known that the renal clearance of aminoglycosides is dependent on renal function, especially GFR. Either LPS- or lipid A-induced decreases in GFR lead to decreases in the CL_{sys} of gentamicin, a finding which was supported by our previous studies which revealed that *K. pneumoniae* LPS at doses of 50 and 250 μg/kg dramatically decreased the CL_{sys} for tobramycin. However, Halkin and colleagues have reported that pyrexia induced by the intravenous administration of *E. coli* LPS at a dose of 35 μg/kg lower than that used in this study does not change the systemic clearance of gentamicin in rabbits. Bergeron and Bergeron and Bergeron et al. have also found that there were no changes in the systemic clearance of gentamicin among female Sprague-Dawley rats treated with *E. coli* LPS at the same dose as used in this study. No discussion regarding this point, however, was included. Thereafter, they found decreases in the systemic clearance of gentamicin in rats treated with LPS at doses of 0.5 and 5 mg/kg. On the basis of these findings, we suggest that LPS- and lipid A-induced decreases in the CL_{sys} for gentamicin may be caused primarily by a reduction in GFR, and that lipid A, an active component of LPS, is closely related to LPS-induced nephrotoxicity.

It is well known that the renal excretion of aminoglycoside antibiotics is made up of both glomerular filtration and tubular reabsorption, and is dependent upon the GFR. The mechanism for the renal accumulation of aminoglycosides in humans and in laboratory animals involves various transport processes, including renal tubular reabsorption in the proximal tubular cells by adsorptive pinocytosis (binding to phospholipids on the brush-border membranes) and transport into renal tubular epithelial cells by adsorptive endocytosis (uptake into liposomes). Our previous studies have reported that LPS increases the renal tubular reabsorption of tobramycin, although the precise mechanism is unclear.

The present study demonstrated no significant differences in the clearance ratio (CL_{sys}/GFR) of gentamicin among the three groups, although the CL_{sys} and GFR significantly decreased in rats treated with LPS and lipid A (Table I). We then evaluated the effects of LPS and lipid A on the renal handling of gentamicin. No significant differences in the intrarenal accumulation of gentamicin for the collection period (60 min) were observed among the three groups (Fig. 4), although lipid A decreased the urinary excretion of gentamicin (Fig. 3). To ensure the action of LPS and lipid A on the tubular reabsorption of gentamicin, the fraction of renal tubular reabsorption of gentamicin was calculated. As the renal clearance (CL_{r}) of a drug can generally be calculated by the amount of drug excreted into the urine during the collection period (A_{t}) divided by the corresponding AUC (CL_{r} = A_{t}/AUC), the amount of gentamicin filtered at the glomeruli within the first hour after administration can be calculated as GFR multiplied by the corresponding AUC, since the protein binding of gentamicin is negligible. The fraction of reabsorption (FR) is calculated as FR = [1 – A_{t}/GFR · AUC], and the resulting values are 0.27, 0.27 and 0.29 for the control and treated rats with LPS and lipid A, respectively. These observations indicate that both LPS and lipid A have no effect on the tubular reabsorption and/or transport into renal tubular epithelial cells of gentamicin, which were different from our previous findings that LPS increases the tubular reabsorption of tobramycin. This discrepancy may be explained by evidence that the affinity of gentamicin for phospholipids
in rat renal brush-border membranes, which possess a negative charge, is lower than that of tobramycin. However, Bergeron and Bergeron\(^1\)\(^3\) and Tardif \textit{et al.}\(^2\) have reported results which are contradictory to the present study in some ways. First, they observed a significant increase in the intrarenal accumulation of gentamicin in rats pretreated with \textit{E. coli} LPS. Second, despite marked increases in the intrarenal uptake of gentamicin in the LPS-treated rats as compared with the control rats, no changes were noted in any of the pharmacokinetic parameters, including \textit{GFR}, \textit{PAH} clearance and systemic clearance of gentamicin. An explanation for these discrepancies is unclear at this stage, but these discrepancies may be caused by differences in LPS preparations derived from different bacterial species (\textit{K. pneumoniae} and \textit{E. coli}).

Otherwise, both LPS and lipid A gave rise to increases in kidney weight, thereby significantly increasing the ratios of kidney to body weight (Fig. 5). The reason for this phenomenon is also unclear from the present study, but the possibility exists that LPS and lipid A induce hypertrophy of the kidney as a result of functional and structural damage to one or both of the kidney tissues and membranes during the early stage. Such damage would likely be due to inflammation by LPS- or lipid A-induced cytokines.

In summary, lipid A derived from \textit{K. pneumoniae} LPS primarily decreases the systemic clearance of gentamicin without inducing changes in the steady-state volume of distribution, reabsorption and intrarenal accumulation of gentamicin. The major cause for changes in the pharmacokinetics of gentamicin may be due to the decrease in \textit{GFR} by either LPS or lipid A. The data presented herein indicate that the lipid A portion of LPS contributes to the action of LPS in the pharmacokinetics of gentamicin. The findings of this study appear to provide further information regarding the renal excretion of aminoglycoside antibiotics in endotoxemia and in the treatment of patients with gram-negative bacterial infections.

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