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# Influence of Endotoxin on the Intracortical Accumulation Kinetics of Gentamicin in Rats

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**The mechanism by which endotoxin (lipopolysaccharide [LPS]) modifies the intrarenal distribution and the nephrotoxic potential of gentamicin is unknown. We studied the influence of LPS on the intracortical accumulation kinetics of gentamicin in rats infused intravenously for 6 h, during which time steady-state levels of the antibiotic in serum were achieved. We compared gentamicin accumulation rates ($V$) in normal rats and in rats receiving LPS (0.5 and 5 mg/kg) as levels in serum ($S$) varied from 0.5 to 130 μg/ml. The pharmacokinetic parameters of gentamicin were previously measured in the three groups of rats that were studied in order to reach and maintain in each rat the desired levels of antibiotic in serum during the 6 h of infusion. Two hours before the infusion of gentamicin, LPS was injected intravenously over a period of 15 min. In normal rats, the increase in $S$ was associated with a nonlinear increase in $V$. The Michaelis-Menten kinetics, which was the best-fitting function, gave an apparent $V_{max}$ (maximal capacity of uptake) of 195.03 ± 9.75 μg/g per h and an apparent $K_m$ (concentration in serum at $V_{max}/2$, an index of affinity) of 34.91 ± 4.45 μg/ml (linear transformation of the experimental data by the Hanes-Woolf plot: $r = 0.93$, $n = 85$). In the rats that received LPS, the increase in $S$ was associated with a linear increase of $V$: for LPS at 0.5 mg/kg, $V = 27.00 + 1.50\ S$ ($r = 0.94$, $n = 80$); for LPS at 5 mg/kg, $V = 22.72 + 1.48\ S$ ($r = 0.94$, $n = 75$). We conclude that endotoxin modifies the accumulation kinetics of gentamicin in the kidney cortices of rats.**

The nephrotoxic potential of gentamicin has been studied extensively. Following glomerular filtration, this aminoglycoside is reabsorbed into the proximal tubular cells by pinocytosis and is accumulated in lysosomes (11, 25, 31), where it interferes with the normal catabolism of phospholipids, leading to cellular disturbance and necrosis (14).

Previous studies done in our laboratory have shown that endotoxin which is liberated during antibiotic therapy (24) disturbs the intrarenal pharmacokinetics of gentamicin (2). In fact, endotoxin-treated animals had higher levels of drug in serum and accumulated a greater amount of gentamicin in their renal cortices and medullae than normal rats did (2). Bergeron et al. (3) have shown, using autoradiography, an increased uptake of labeled tobramycin by renal proximal tubular cells in endotoxemic animals compared with that in normal rats. More recently, it has been observed that endotoxin increased the nephrotoxic potential of gentamicin and of the combination vancomycin-gentamicin (M. Ngeleka, D. Beauchamp, D. Tardif, P. Auclair, P. Gourde, and M. G. Bergeron, J. Infect. Dis., in press).

Numerous systemic effects have been observed following endotoxin injection: disturbance of the hemodynamic status of the host (7, 27), induction of vasoconstriction (26), and activation of the immunological and inflammatory processes (5). On the other hand, endotoxin can act directly on cells and organelles (16). Whether endotoxin modifies the pharmacokinetics, renal handling, and toxicity of drugs by acting directly on kidney cells or indirectly through one or several mechanisms remains to be elucidated.

The purpose of the present study was to evaluate the effect of endotoxin on the intracortical accumulation kinetics of gentamicin in rats.

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## MATERIALS AND METHODS

**Experimental model: accumulation kinetics studies.** Female Sprague-Dawley rats (weight, between 175 and 225 g; Charles-River Breeding Laboratories, Inc., Montreal, Quebec, Canada) were used in this study. They were housed individually and had free access to food and water throughout the experiment. They were infused intravenously over a 6-h period with gentamicin, achieving individual steady-state levels in serum that ranged from 0.4 to 130 μg/ml, as described previously by Giuliano et al. (8).

Three groups of animals were studied. Normals rats (control) and animals previously infused with *Escherichia coli* O127:B8 endotoxin (lipopolysaccharide) (trichloroacetic acid extract; Sigma Chemical Co., St. Louis, Mo.) at doses of 0.5 and 5 mg/kg. Control rats were infused with saline (NaCl, 0.9%). The day before the infusion, animals were anesthetized with chloral hydrate (350 mg/kg, intraperitoneally; Fisher Scientific Co., Quebec, Quebec, Canada) and a polyethylene catheter (PE 50; Intramedic; Clay-Adams, Parsippany, N.J.) was inserted into the left jugular vein. The other end was guided subcutaneously until it emerged through a hole made in the skin of the neck and was secured. After recovery from anesthesia, animals were returned to their cages and left undisturbed overnight.

The experiment was started by infusing saline and endotoxin over a period of 15 min. Exactly 2 h later, the infusion of gentamicin was started by injecting through the catheter a loading dose of the antibiotic in order to achieve the desired levels in serum instantaneously. Catheters were then connected to 20-ml plastic syringes (Becton-Dickinson and Co., Rutherford, N.J.) containing the appropriate solution and mounted on a continuous-flow compact infusion pump (model 975; Harvard apparatus; Ealing Scientific Ltd., St. Lau-

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rent, Quebec, Canada). The rate of infusion was 20 μl/min. Since animals remained quiet during the infusion, no restraining device or anesthesia was needed.

The loading dose (LD) and infusion dose used to obtain and maintain the desired levels in serum were adjusted to the body weight of the animals and were calculated from the apparent volume of distribution ($V$), serum half-life, and elimination rate constant ($k_{el}$) of gentamicin in rats [loading dose = desired level in serum × $V$ and infusion dose = (LD × $k_{el}$)/rate of infusion]. The pharmacokinetic parameters were previously determined for the three groups of rats as described below.

Blood samples were taken from the tip of the tail 3 h after the beginning of gentamicin infusion and at the time of sacrifice. Animals were killed by decapitation and bled exactly 6 h after the beginning of gentamicin infusion. Blood was centrifuged and the serum was immediately frozen (−20°C) for further antibiotic determination. The right kidney was removed from each rat. No difference in gentamicin concentration was previously observed between the left and the right kidneys (8). The cortex of each kidney was dissected and immediately frozen for further antibiotic assay.

The concentrations of gentamicin in serum and the renal cortex were determined by a standard microbiological assay, with *Bacillus subtilis* ATCC 6633 used as the test organism (4). Samples of the renal cortex were weighted, homogenized in distilled water by using a Tissue-Tearor RTM (Biospec Products, Bartlesville, Okla.), sonicated with a sonicator (model W-375; Bionetics Ltd., Montreal, Quebec, Canada), and, if necessary, diluted to obtain a concentration in the range of the standard curve (0.4 to 100 μg/ml) for the gentamicin assay. The limit of sensitivity was 10 μg/g of tissue. A good linearity of the assay was obtained from concentrations of 0.4 to 100 μg/ml, with a coefficient of correlation of 0.993. All assays were done in triplicate on tryptic soy agar (Difco Laboratories, Detroit, Mich.), and standard curves were prepared in normal rat serum for serum and in blank rat cortex for cortical renal tissue. The recovery of gentamicin after a known amount of this drug was added to antibiotic-free homogenates was 89.3 ± 15.5% (standard deviation). The interday coefficients of variation of the assay were 10.1% at a low concentration (1 μg/ml) and 13.1% at a high concentration (50 μg/ml) of gentamicin.

Since the relationship between the concentrations of gentamicin in the renal cortices and the time of infusion is linear for normal rats (8), the gentamicin concentrations in the cortices were expressed as renal cortical gentamicin accumulation rates (in micrograms per gram per hour) by dividing the cortical concentration measured at the end of the infusion by the duration of the infusion (6 h). The cortical accumulation rates of gentamicin ($V$) were analyzed as a function of levels in serum ($S$) by linear (least-squares regression analysis) and nonlinear regression analyses. For the nonlinear regression analysis, the Michaelis-Menten kinetic $\{V = V_{max}/[1 + (K_m/S)]\}$ was used as described by Giuliano et al. (8). The initial estimates for the $K_m$ and $V_{max}$ values were obtained from the linear transformation of the experimental data by the Hanes-Woolf plot ($S/V$ versus $S$) (33). To discriminate between the linear and nonlinear correlations, the coefficient of correlation ($r$) of the least-squares regression analysis of the experimental data ($V$ versus $S$) was compared with the correlation coefficient of the transformation of the same data by the Hanes-Woolf plot ($S/V$ versus $S$) for each group. For the nonlinear correlations, $V_{max}$ and $K_m$ values were optimized by using a nonlinear regression analysis (20) adapted for the Michaelis-Menten kinetic; this program also calculated the standard deviations for the optimized parameters. For the linear and nonlinear correlations, the comparisons were done by using analysis of variance as specified by Neter and Wasserman (19). A value of $P < 0.05$ was considered significant.

TABLE 1. Elimination rate constant, apparent volume of distribution, area under the serum curve, and total clearance of gentamicin in normal and endotoxin-treated rats

| Group[a] | Elimination rate constant ($h^{-1}$) | Apparent vol of distribution (ml/kg) | $AUC_{0-\infty}$ (μg · h/ml)[b] | Total clearance (ml/min per kg) |
|---|---|---|---|---|
| Normal | 1.98 ± 0.14[c] | 371 ± 68 | 16.2 ± 1.7 | 11.2 ± 1.7 |
| Endotoxin treated | | | | |
| 0.5 mg/kg | 1.17 ± 0.15 | 432 ± 63 | 28.2 ± 3.6[d] | 6.3 ± 0.7[e] |
| 5 mg/kg | 1.54 ± 0.46 | 365 ± 22 | 36.7 ± 3.4[e] | 4.7 ± 0.4[e] |

[a] There were six rats in each group.
[b] $AUC_{0-\infty}$, Area under the concentration-time curve.
[c] Values are means ± standard errors of the mean.
[d] Different from the normal group at $P < 0.05$.
[e] Different from the normal group at $P < 0.01$.

**Pharmacokinetics.** Before the beginning of the experiment, the pharmacokinetic parameters were evaluated in six animals per group. Briefly, animals were prepared as described above, except that the constant infusion was replaced by an intravenous bolus of 10 mg of gentamicin per kg. A total of eight samples of blood were taken from 5 min to 4 h postinjection. The elimination rate constant, the apparent volume of distribution, the area under the curve ($AUC_{0-\infty}$), and the total plasma clearance were determined for each group by fitting serum profiles for each animal to a sum of exponentials by using an iterative extended least-squares modeling program (MK model version 3.13; N. Holford, School of Medicine, University of Auckland, Auckland, New Zealand, 1986). This package estimates the initial and final parameters and determines the most appropriate compartment model for the concentration versus time data. The log likelihood indicates how well the parameter values in the model describe the data. The standard deviation of each predicted value was calculated from the square root of the predicted variance. The predicted variance was calculated as follows: $\text{Var}(y) = \text{SD}^2 \times (V_0 + y^{\text{PWR}})$, where Var ($y$) is the predicted variance, $y$ is the predicted value, PWR is the power parameter, $V_0$ is the expected variance, and SD is the variance scale parameter. PWR and $V_0$ are the model parameters of error. Statistical analysis of the differences between groups for each parameter was performed first by analysis of variance by a least-squares method. If the $P$ value was <0.05, a group comparison was done by the Waller-Duncan multiple range test with the adjustment for unequal frequencies described by Kramer (13). A $P$ value lower than 0.05 was considered significant. Calculations were made by using SAS software (SAS Institute Inc., Cary, N.C.).

## RESULTS

**Gentamicin pharmacokinetics.** The pharmacokinetic parameters of gentamicin measured in the three groups of animals used in the present study are shown in Table 1. No significant difference was observed for the elimination rate constant and the apparent volume of distribution between the endotoxemic groups of rats given endotoxin at either 0.5 and 5 mg/kg and the normal group of rats. The area under the concentration-time curve was significantly higher and the

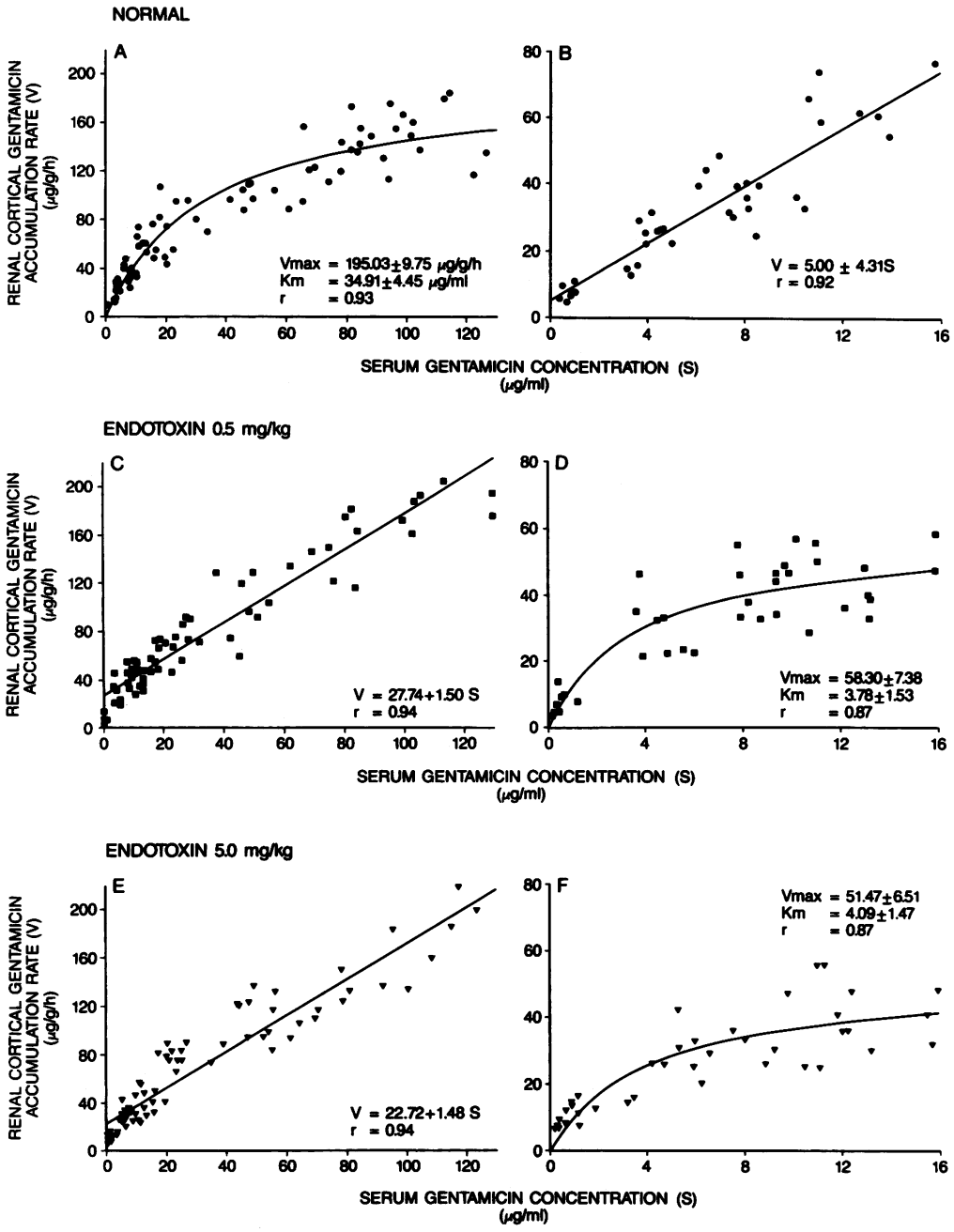


FIG. 1. Intrarenal accumulation kinetics of gentamicin in normal rats (A and B) and rats treated with 0.5 mg of endotoxin per kg (C and D) and 5 mg of endotoxin per kg (E and F). Gentamicin was infused over 6 h and achieved individual steady-state levels in serum ranging from 0.5 to 130 μg/ml. The initial accumulation kinetics of gentamicin was also illustrated over the therapeutic range of levels in serum (0.5 to 16 μg/ml) (B, D, and F for the three groups of rats described for panels A, C, and E, respectively). Each point represents one rat. For each animal, the corresponding level in serum was the mean of the gentamicin level in serum measured after 3 and 6 h of infusion.

total plasma clearance was significantly lower in the two endotoxemic groups of rats compared with that in the normal group of rats. These changes were not influenced by the lipopolysaccharide doses.

**Accumulation kinetics.** The correlation between levels of gentamicin in serum measured after 3 and 6 h of infusion over the whole range of levels in serum was as follows: in normal rats, $A = 0.014 + 0.969\,B$ ($r = 0.97$; $n = 85$); in the endotoxin (0.5 mg/kg)-treated group, $A = 2.880 + 0.839\,B$ ($r = 0.96$; $n = 80$); and in the endotoxin (5.0 mg/kg)-treated group, $A = 1.090 + 0.989\,B$ ($r = 0.93$; $n = 75$), where $A$ is 3 h, $B$ is 6 h, and $n$ is the number of rats in each group.

The relationship between the steady-state levels of gentamicin in serum and the gentamicin accumulation rate for the normal and the two endotoxemic groups are shown in Fig. 1A to F. The corresponding kinetic parameters ($V_{max}$ and $K_m$) or the linear equation and the correlation coefficient for each group are shown on the corresponding figures.

The steady-state elevation of gentamicin levels in serum was associated with a nonlinear increase of cortical genta-

micin accumulation in normal rats, suggesting a saturable process (Fig. 1A). These results are in accordance with those previously published by Giuliano et al. (8). The initial rate of cortical uptake (levels in serum below 16 μg/ml) which represents the therapeutic range of levels in serum followed a first-order kinetic process (Fig. 1B).

For the two groups of rats that received endotoxin (0.5 and 5 mg/kg), the relationship between levels in serum and the cortical accumulation rates of gentamicin was approximated by first-order kinetics, suggesting a nonsaturable process (Fig. 1C and E, respectively). There was no significant difference between the regression curves ($P = 0.15$). At low levels in serum (lower than 16 μg/ml), the renal cortical uptake showed a nonlinear correlation for these two groups (Fig. 1D and F, respectively). The regression curves for these two groups were significantly different ($P = 0.045$). A higher $V_{max}$ was observed for the group receiving 0.5 mg of endotoxin per kg.

## DISCUSSION

Little is known about the influence of endotoxin on the kinetics of uptake of antibiotics by kidney tissue. In the present study, we showed, using a model of constant infusion, that endotoxin modifies the intracortical accumulation kinetics of gentamicin in the kidney cortex of rats.

Many investigators have suggested a saturable mechanism of gentamicin uptake by the kidneys of normal rats by using in vitro (1, 12, 15) and in vivo models (8, 10, 30). Giuliano et al. (8), using the same model of antibiotic infusion but a different strain of rats, have also found a saturable mechanism of uptake of gentamicin in normal rats, but their $V_{max}$ and $K_m$ values were slightly lower than our values. In this model, $V_{max}$ represents the maximal capacity of gentamicin uptake by the kidney cortex, while $K_m$ is the concentration in serum at half the maximal rate of drug uptake ($V_{max}/2$) and represents an index of affinity of the drug toward the renal tubular cells.

It has previously been shown that endotoxin may influence the pharmacokinetics of antibiotics. In fact, Wilson et al. (35) have observed a decrease in the apparent and central volumes of distribution of gentamicin in horses treated intravenously with *E. coli* (O55:B5) endotoxin. In similarly treated ewes, Wilson et al. (34) did not observe a decrease in the apparent volume of distribution but noted an increase of the central volume of distribution and a decrease of the elimination rate constant. Halkin et al. (9) have noted a significant increase in the central and peripheral volume of distribution and a significant decrease of the elimination rate constant of gentamicin in endotoxin-treated rabbits.

In the present study, endotoxemia resulted in a significant increase in the area under the curve and a significant decrease in the total plasma clearance, but no significant difference in the elimination rate constant or the apparent volume of distribution between normal and endotoxemic animals was observed. Bergeron and Bergeron (2) also observed an increase in the area under the curve and a decrease in total clearance following endotoxin treatment. The reduced glomerular filtration rate and renal plasma flow might have been responsible for the higher levels in serum previously observed in endotoxemic animals (2, 35). By increasing the levels of gentamicin in serum in animals, endotoxin could have directly or indirectly allowed more drug to be taken up by the tubular cells, explaining, in part, the observation that endotoxin increases the nephrotoxic potential of gentamicin and the combination vancomycin-gentamicin (Ngeleka et al., in press).

Following glomerular filtration, gentamicin is believed to be reabsorbed in the proximal tubular cells by pinocytosis (11, 25) and accumulated into lysosomes (11, 25, 31), where it interferes with the normal catabolism of phospholipids, leading to cellular disturbances and necrosis. It has been shown by using filtering and nonfiltering perfused kidneys (6) and autoradiographic techniques (11, 25) that the luminal side of the renal proximal tubules is the major route by which gentamicin gains entry into proximal tubular cells in normal rats. Fluid pinocytosis and adsorptive pinocytosis, also called carrier-mediated pinocytosis, are the two types of endocytosis that have been suggested as possible mechanisms of renal cellular uptake of aminoglycosides (8, 18). In fluid pinocytosis, there is no discrimination and the rate of uptake of aminoglycosides is directly proportional to the concentration of the drug in the tubular fluid. Adsorptive pinocytosis involves the adsorption of antibiotic on receptor sites that are most likely located on the brush border membrane, and in contrast to fluid pinocytosis, the kinetics of uptake is saturable. Based on the results of the present study and those of several other investigators (8, 10, 30), transport of aminoglycosides in normal animals is probably the result of adsorptive pinocytosis. Our results are consistent with the fact that gentamicin binds in a saturable manner to rat renal brush border membranes (21).

Endotoxin disturbs the mechanism of uptake of gentamicin. In fact, the kinetics of gentamicin accumulation is modified in endotoxemic animals. One possible mechanism could be a radical change in the tubular uptake and a massive diffusion of antibiotics through the basal membrane (vascular side) instead of the luminal membrane (brush border membrane). This is most unlikely, since we have shown, using autoradiography, that tobramycin binds first to the apical portion of the tubular cells and is then moved into organelles of the cells in endotoxemic animals. The route of entry of aminoglycosides appears to be the same in both normal and endotoxemic animals, but the amount of antibiotic entering the cell is greater in endotoxemic animals (3).

Several mechanisms may be responsible for the change in the kinetics of uptake of gentamicin in endotoxemic rats. It has been shown that endotoxin is distributed throughout the kidney (17). An autoradiographic study (32) showed that lipid A, the active component of endotoxin, can be recovered within the tubular cells of the renal cortex. Shands (23) has shown that endotoxin may interact with the biological membrane by edge attachment. By attaching to the brush border membrane, endotoxin might have directly interacted with the membrane receptors of gentamicin, interfering with its adsorptive pinocytosis. The saturable mechanism of uptake observed in endotoxin-treated rats (treated with 0.5 and 5 mg of endotoxin per kg) over the therapeutic levels of gentamicin in serum (Fig. 1D and F) supported this hypothesis.

Schuster et al. (22) have demonstrated that endotoxin could have induced the rupture of the lipid bilayer in vitro. Manny et al. (16) have observed changes in the cellular and subcellular structures of canine kidneys exposed to the direct action of endotoxin. These changes in brush border membrane integrity might have allowed passive diffusion of gentamicin into the tubular cells. Breaches within the cell membrane, combined with the other factors cited above, could have resulted in the linearization of the accumulation kinetics of gentamicin in endotoxemic animals over the total range of gentamicin levels in serum that was studied (0.5 to 130 μg/ml).

Although endotoxin could have affected the tubules di-

rectly, the possibility that mediators of inflammation liberated during endotoxemia could have damaged the tubules; thus, modification of the renal uptake of drug might be considered. Recently, tumor necrosis factor$_{alpha}$ (cachectin), which is produced in large amount by lipopolysaccharide-activated macrophages, has been found to be an important mediator during shock and tissue injuries induced by lipopolysaccharide (29). Moreover, Tracey et al. (28) have observed damage to the proximal elements of the nephron following cachectin infusion.

More remains to be done to better understand the mechanism by which endotoxin interacts with antibiotics and kidney cells to disturb the intrarenal distribution of these drugs and how endotoxin potentiates the nephrotoxic potential of gentamicin. Our results demonstrate that endotoxin modifies the pharmacokinetics and the intracortical accumulation kinetics of gentamicin in rats.

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