

## Comparison of the Nephrotoxicity of Netilmicin and Gentamicin in Rats

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The nephrotoxicity of netilmicin relative to that of gentamicin was examined in Sprague-Dawley rats. Balance studies were performed on rats injected with netilmicin or gentamicin (50 mg/kg per day for 14 days, 100 mg/kg per day for 8 days, and 150 mg/kg per day for 8 days). Control rats were injected with saline. Both drugs caused a dose-related decrease in urine osmolality and increases in urine volume, water intake, and serum creatinine; however, the magnitude of these changes was significantly less in netilmicin- than in gentamicin-injected rats. Light microscopy of renal tissue revealed less proximal tubular cell necrosis in netilmicin- than in gentamicin-injected rats. There was no significant difference between the renal cortical concentrations of the two drugs. Both drugs stimulated uptake of *p*-aminohippurate in rat renal cortical slices to the same degree. The data indicate that netilmicin is less nephrotoxic than gentamicin in rats, that the difference in nephrotoxicity cannot be explained by a difference in drug concentration in the renal cortex, and that the ability of aminoglycosides to stimulate the organic acid transport system of proximal tubular cells does not correlate with their nephrotoxic potential.

Renal failure is a well recognized but poorly understood complication of gentamicin therapy. Recognition of this problem has stimulated the development of semisynthetic analogs of gentamicin that possess equivalent antimicrobial efficacy along with a higher ratio of therapeutic effect to toxicity. Netilmicin (Schering 20569, Schering Laboratories, Bloomfield, N. J.) is one such semisynthetic analog of gentamicin (10). Recent reports indicate that the antimicrobial spectrum of netilmicin is not only similar to that of gentamicin but that this agent exhibits antimicrobial activity against many aminoglycoside-resistant strains of gram-negative bacteria (7, 8). Of equal interest is the recent report of Luft et al. (6) that states that netilmicin is less nephrotoxic than gentamicin in rats.

In the present study, we examined the effects of three dose schedules of netilmicin and gentamicin on renal function and morphology in rats to further establish the relative nephrotoxicity of these two agents.

### MATERIALS AND METHODS

Balance studies were performed in three groups of female Sprague-Dawley rats initially weighing 180 to 210 g. The rats were placed in individual metabolic cages and permitted free access to tap water and standard rat chow. A minimum of 10 days was allowed for acclimatization before measurements

were begun. After 3 days of control measurements of body weight, water intake, urine volume, urine osmolality, solute excretion, and protein excretion, the rats were injected with one of three agents. In group I, rats received a daily subcutaneous injection of gentamicin sulfate or netilmicin sulfate (40 mg of base per ml) at a dose of 50 mg of base per kg of body weight per day for 14 days. Control rats for each group were injected with an equivalent volume of 0.9% saline (1.25 ml/kg of body weight per day). In group II, rats were injected with drugs at the same concentration but at a dose of 100 mg/kg of body weight per day for 8 days. In group III, the dose was increased to 150 mg/kg of body weight per day for 8 days.

At the end of the study, the rats were sacrificed and the combined weight of both kidneys from each rat was determined. Blood was collected from the aorta and analyzed for urea nitrogen (BUN) and serum creatinine with a Technicon autoanalyzer. Urine was collected under oil, the daily volume was measured, and a portion was analyzed for solute concentration with a Precision osmometer. Urine protein was precipitated with 11% trichloroacetic acid and quantitated by the Lowry method (4).

In a separate group of experiments, rats were injected with gentamicin or netilmicin at the same doses and for the same durations as described above. At the end of the experiment, the kidneys were fixed by *in vivo* perfusion of the renal arteries (3) with 3% glutaraldehyde buffered to pH 7.4 with 0.1 M sodium phosphate. Immediately thereafter, the kidneys were removed and placed in 3% glutaraldehyde for 4 h and then placed in a solution of 0.1 M cacodylate

with 7% sucrose (pH 7.4). Subsequently, 1-mm blocks of cortex were embedded in Epon, and 1- $\mu$ m-thick sections were cut and stained with toluidine blue. The sections were coded and examined by blind study under light microscopy for evidence of tubular necrosis and lysosomal changes.

In a fourth group of experiments, rats were injected with gentamicin or netilmicin at a dose of 100 mg/kg of body weight per day for 2, 4, and 8 days. Twenty-four hours after the last injection, the rats were sacrificed, and the drug concentration in the renal cortex and medulla was measured by a microbiological assay technique (5). *Bacillus globigii* was used as the marker organism. The renal cortex and medulla from each rat were dissected, weighed, homogenized, and diluted as necessary with phosphate buffer (pH 8). Antibiotic standards were prepared with the same buffer.

In a fifth group of experiments, the effect of gentamicin and netilmicin on *p*-aminohippurate (PAH) uptake by renal-cortical slices was examined. Rats were injected with drugs at a dose of 100 mg/kg per day for 2 days. Twenty-four hours after the last injection, the kidneys were removed and sliced with a Stadie-Riggs microtome. Cortical slices were incubated for 120 min in a medium containing  $10^{-4}$  M PAH, after which the concentration ratio of cortical slice to medium PAH was determined as previously described (1).

The data in the text and figures are expressed as the mean  $\pm$  standard error. Analysis of variance was used to compare the effects of drug treatment within and between groups.

## RESULTS

Table 1 summarizes the balance data for the control period (day 0) and the last day of treatment for the three-dose schedules of each drug. The first sign of gentamicin or netilmicin nephrotoxicity was a decrease in urine osmolality followed by a rise in urine volume and water intake with no consistent changes in solute excretion. At 50 mg/kg per day, the changes in these variables were similar in gentamicin- and netilmicin-injected rats. At 100 and 150 mg/kg per day, they became more pronounced. However, compared with gentamicin-injected rats, netilmicin-injected rats exhibited significantly greater weight gain ( $P < 0.01$ ), less depression of urine osmolality ( $P < 0.01$ ), and no change in solute excretion during both higher dose schedules. Urinary protein excretion increased similarly in response to gentamicin or netilmicin.

Figure 1 summarizes the changes in BUN, serum creatinine, and kidney weight in experimental groups I, II, and III. At 50 mg/kg, no difference in BUN was evident among the three groups, but serum creatinine significantly increased in gentamicin ( $0.64 \pm 0.09$  mg/100 ml) and netilmicin-injected rats ( $0.52 \pm 0.02$  mg/100 ml) compared with that in the saline control rats ( $0.38 \pm 0.01$  mg/100 ml,  $P < 0.01$ ). In rats

injected with gentamicin at 100 and 150 mg/kg, BUN and serum creatinine increased sharply and significantly ( $P < 0.01$ ) above the levels observed in netilmicin- and saline-injected rats. In contrast, at each dose schedule, no difference in BUN was observed between netilmicin- and saline-injected rats ( $P > 0.1$ ). Although serum creatinine increased slightly in the netilmicin group as a function of drug dose, the increase was impressively less than that observed in gentamicin-injected rats. At 150 mg/kg, serum creatinine increased to  $0.84 \pm 0.02$  mg/100 ml in netilmicin-injected rats as compared with  $4.19 \pm 0.45$  mg/100 ml in gentamicin-injected rats ( $P < 0.01$ ).

Kidney weight was significantly greater in gentamicin- and netilmicin-injected rats than in saline-injected rats ( $P < 0.01$ ). At 100 and 150 mg/kg, kidney weight of gentamicin-injected rats exceeded that of rats injected with netilmicin ( $P < 0.01$ ). Expressing kidney weight as a function of body weight did not reveal any correlation between kidney weight, drug dosage, BUN, or serum creatinine. The increase in kidney weight most likely indicates interstitial edema reflecting drug-induced, tubular injury.

Light microscopic examination of renal tissue revealed dose-related injury characterized by increased lysosomal bodies, disruption of brush borders, cellular swelling, and frank necrosis of proximal tubular epithelium. The changes observed in renal tissue of netilmicin-injected rats were less severe than those found in renal tissue of gentamicin-injected rats.

The concentration of gentamicin or netilmicin in the renal cortex and medulla was measured after administration of 100 mg/kg per day for 2, 4, and 8 days. In all experiments, the cortical concentrations of the drugs exceeded that of the medulla ( $P < 0.01$ ). After 2 days of injections, the cortical concentrations of gentamicin and netilmicin were similar ( $1,029 \pm 114$  and  $1,067 \pm 124$   $\mu$ g/g of cortex, respectively;  $P > 0.4$ ) and increased to the same extent ( $2,230 \pm 275$  and  $2,142 \pm 222$   $\mu$ g/g of cortex, respectively;  $P > 0.4$ ) after 4 days of injections. After 8 days of injections, netilmicin measured  $1,705 \pm 151$   $\mu$ g/g of cortex, whereas gentamicin decreased to  $832 \pm 108$   $\mu$ g/g of cortex,  $P < 0.01$ . The decrease in the cortical concentration of gentamicin most likely reflects the extensive proximal tubular cell necrosis observed in this group of rats.

Both drugs similarly stimulated the uptake of PAH in renal cortical slices. The concentration ratios of cortical slice to medium PAH measured  $18.3 \pm 1.3$  and  $18.1 \pm 1.4$  in gentamicin- and netilmicin-injected rats, respectively,

TABLE 1. Summary of balance data before and at end of drug treatment<sup>a</sup>

Determination	Drug treatment group	50 mg/kg of body weight per day for 14 days			No. of rats		100 mg/kg daily × 8			150 mg/kg daily × 8			
		No. of rats	Day 0	Day 14	No. of rats	Day 0	Day 8	No. of rats	Day 0	Day 8	No. of rats	Day 0	Day 8
Body wt (g)	Saline	9	204 ± 3	222 ± 3 <sup>b</sup>	9	184 ± 5	196 ± 7 <sup>b</sup>	14	187 ± 2	207 ± 1 <sup>b</sup>	14	187 ± 2	207 ± 1 <sup>b</sup>
	Gentamicin	9	199 ± 3	208 ± 2 <sup>b</sup>	9	182 ± 5	173 ± 4 <sup>b</sup>	14	189 ± 3	186 ± 4	14	189 ± 3	186 ± 4
	Netilmicin	9	208 ± 2	219 ± 3 <sup>b</sup>	10	178 ± 5	189 ± 6	14	187 ± 2	197 ± 2 <sup>b</sup>	14	187 ± 2	197 ± 2 <sup>b</sup>
Water intake (ml/day)	Saline	9	34 ± 2	34 ± 2	9	30 ± 1	29 ± 1	14	28 ± 1	31 ± 2	14	28 ± 1	31 ± 2
	Gentamicin	9	27 ± 2	33 ± 3	9	30 ± 1	28 ± 5	14	33 ± 2	45 ± 8 <sup>b</sup>	14	33 ± 2	45 ± 8 <sup>b</sup>
	Netilmicin	9	29 ± 2	33 ± 2	10	30 ± 1	38 ± 3 <sup>b</sup>	14	28 ± 1	58 ± 9 <sup>b</sup>	14	28 ± 1	58 ± 9 <sup>b</sup>
Urine output (ml/day)	Saline	9	11 ± 2	14 ± 2	9	14 ± 1	12 ± 1	14	13 ± 1	15 ± 1	14	13 ± 1	15 ± 1
	Gentamicin	9	10 ± 2	21 ± 2 <sup>b</sup>	9	13 ± 1	20 ± 3 <sup>b</sup>	14	14 ± 1	39 ± 6 <sup>b</sup>	14	14 ± 1	39 ± 6 <sup>b</sup>
	Netilmicin	9	11 ± 1	17 ± 2 <sup>b</sup>	10	13 ± 1	22 ± 2 <sup>b</sup>	14	15 ± 2	42 ± 4 <sup>b</sup>	14	15 ± 2	42 ± 4 <sup>b</sup>
Urine osmolality (mosm/kg per liter)	Saline	9	1,913 ± 164	1,684 ± 99	9	1,968 ± 66	2,082 ± 79	14	1,702 ± 111	1,755 ± 94	14	1,702 ± 111	1,755 ± 94
	Gentamicin	9	1,958 ± 40	1,015 ± 103 <sup>b</sup>	9	2,012 ± 82	770 ± 80 <sup>b</sup>	14	1,603 ± 64	433 ± 30 <sup>b</sup>	14	1,603 ± 64	433 ± 30 <sup>b</sup>
	Netilmicin	9	1,904 ± 57	1,346 ± 91 <sup>b</sup>	10	2,005 ± 60	1,228 ± 86 <sup>b</sup>	14	1,632 ± 62	671 ± 66 <sup>b</sup>	14	1,632 ± 62	671 ± 66 <sup>b</sup>
Solute excretion (mosmol/kg per day)	Saline	9	21 ± 2	23 ± 2	9	27 ± 1	24 ± 1	14	21 ± 1	22 ± 1	14	21 ± 1	22 ± 1
	Gentamicin	9	19 ± 1	20 ± 1	9	25 ± 1	15 ± 2 <sup>b</sup>	14	21 ± 1	15 ± 2 <sup>b</sup>	14	21 ± 1	15 ± 2 <sup>b</sup>
	Netilmicin	9	20 ± 1	21 ± 1	10	26 ± 2	26 ± 1	14	21 ± 1	24 ± 3	14	21 ± 1	24 ± 3
Urine protein (mg/day)	Saline	9	6.9 ± 0.2	7.1 ± 0.3	9	8.0 ± 0.4	7.9 ± 0.5	14	6.0 ± 0.3	5.9 ± 0.2	14	6.0 ± 0.3	5.9 ± 0.2
	Gentamicin	9	6.6 ± 0.4	8.1 ± 0.4 <sup>b</sup>	9	7.9 ± 0.4	11.2 ± 1.6 <sup>b</sup>	14	6.3 ± 0.3	10.0 ± 1.1 <sup>b</sup>	14	6.3 ± 0.3	10.0 ± 1.1 <sup>b</sup>
	Netilmicin	9	6.9 ± 0.3	7.8 ± 0.3 <sup>b</sup>	10	8.1 ± 0.4	10.4 ± 0.4 <sup>b</sup>	14	6.1 ± 0.2	10.1 ± 0.6 <sup>b</sup>	14	6.1 ± 0.2	10.1 ± 0.6 <sup>b</sup>

<sup>a</sup> Data expressed as mean ± standard error.<sup>b</sup> Significantly different from day 0,  $P < 0.01$ .

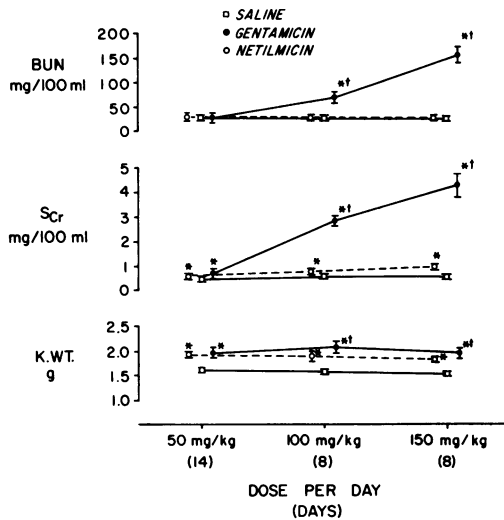


FIG. 1. Changes in BUN, serum creatinine ( $S_{Cr}$ ), and kidney weight (K.WT.) as a function of drug dose. Symbols: \*, significantly different from saline-injected rats ( $P < 0.01$ ); †, significantly different from netilmicin-injected rats ( $P < 0.01$ ).

and were significantly higher than that of the saline-injected rats ( $10.5 \pm 0.6$ ,  $P < 0.01$ ).

DISCUSSION

The objective of the present study was to compare the nephrotoxicity of netilmicin with that of gentamicin. Both drugs caused a similar pattern of nephrotoxicity characterized by a decrease in urine osmolality and an increase in urine volume, protein excretion, and serum creatinine. At 50 mg/kg of body weight per day for 14 days, the changes in these parameters were similar in gentamicin- and netilmicin-injected rats. However, at 100 and 150 mg/kg of body weight per day for 8 days, the severity of renal impairment caused by netilmicin was significantly less than that seen in gentamicin-injected rats. This conclusion is clearly evident from comparison of the changes in BUN and serum creatinine induced by each agent. Increasing the dose of gentamicin from 50 to 150 mg/kg per day was associated with a progressive rise in BUN and serum creatinine, whereas identical doses of netilmicin had no significant effect on BUN, and the rise in serum creatinine was strikingly less than that seen in gentamicin-injected rats. Moreover, examination of renal tissue by light microscopy also supports the conclusion that netilmicin is less nephrotoxic than gentamicin. Tubular necrosis was less severe and progressed to a lesser

extent as a function of drug dose than that observed in gentamicin-injected rats.

Luft et al. (6) also found that netilmicin was less nephrotoxic than gentamicin in Sprague-Dawley rats. These investigators observed a similar pattern of change in urine osmolality and urine volume. In contrast to our study, however, Luft et al. (6) observed a greater increase in proteinuria in gentamicin- than in netilmicin-injected rats. Moreover, they found no significant change in creatinine clearance in rats injected with 30 to 120 mg of netilmicin per kg per day for 15 days, although histological evidence of tubular injury was present.

In our study, creatinine clearance was not measured. However, the rise in serum creatinine from 0.52 to 0.84 mg/100 ml in netilmicin-injected rats (50 and 150 mg/kg, respectively) would suggest that creatinine clearance was depressed. To what extent the rise in serum creatinine reflects a decrease in glomerular filtration rate secondary to nephron loss or a possible decrease in extracellular volume consequent to decreased food and water intake remains uncertain.

Despite these obvious differences between the results of our study and those of Luft et al. (6), the main conclusion of both is the same, i.e., netilmicin is less nephrotoxic than gentamicin in rats.

The mechanism by which aminoglycoside antibiotics induce renal tubular injury remains unknown. Several studies have suggested that nephrotoxicity appears to correlate with the renal cortical concentration of the drug (2, 5). In the present study, however, we found no difference in the renal cortical or medullary concentrations of netilmicin and gentamicin after 2 and 4 days of injections at 100 mg/kg per day. After 8 days of injections, the cortical concentration of gentamicin was significantly less than that of netilmicin. These observations are evidence against tissue concentration of the drugs as the primary explanation for the difference in nephrotoxic potential of netilmicin and gentamicin.

In a previous study, we reported that gentamicin stimulated renal PAH transport in vivo and in vitro (1). No effect on PAH transport was seen with streptomycin (9). These observations led us to consider the possibility that stimulation of the organic acid transport system of renal proximal tubular cells might correlate with the nephrotoxic potential of aminoglycoside antibiotics. This hypothesis is no longer tenable in view of the finding that netilmicin stimulated PAH uptake to the same extent as

gentamicin, despite the demonstrated lower nephrotoxicity of this agent.

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