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Comparative Nephrotoxicities of Netilmicin and Gentamicin in Rats

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The relative nephrotoxicities of netilmicin (Sch 20569) and gentamicin were compared in rats at doses of 30, 60, 90, and 120 mg/kg per day for 15 days. Both drugs caused proteinuria and a decrease in urine osmolality; however, netilmicin produced significantly less changes at all doses than gentamicin. Whereas gentamicin resulted in a decline in creatinine clearance at all doses, netilmicin failed to cause a decline in creatinine clearance. Renal-cortical concentrations of antibiotic at sacrifice were similar in animals receiving either drug. Light-microscopic changes were less severe with netilmicin than gentamicin. Cytosegresomes with myeloid bodies were identified electron microscopically in the kidneys of animals receiving either netilmicin or gentamicin at all doses. Electron-microscopic manifestations were similar. The data indicate that in the rat, netilmicin is distinctly less nephrotoxic than gentamicin.

Netilmicin (Sch 20569) is a new semisynthetic aminoglycoside which has in vitro bactericidal efficacy against both gentamicin-sensitive and gentamicin-resistant microorganisms (11). In vitro studies have shown that the spectrum of activity of netilmicin is similar to that of gentamicin against most gram-negative bacteria and *Staphylococcus aureus*. Although it has been shown to be active against many gentamicin-resistant organisms, it is somewhat less active than gentamicin against strains of *Pseudomonas aeruginosa* (12). In molecular structure, it closely resembles gentamicin C_{1a}, a component of the gentamicin complex. Fourteen-day and ninety-day chronic studies in rats, dogs, and cats have suggested that netilmicin has significantly less oto- and nephrotoxicity than gentamicin (12). To compare their relative toxicities in greater detail and to define the ultrastructural morphological changes rendered by netilmicin in kidney, we studied the effects of repetitive injections of both drugs at several different doses in rats. Parameters of renal function and concentrations of drug in renal cortex at sacrifice, as well as light- and electron-microscopic changes were monitored.

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MATERIALS AND METHODS

Adult, male Sprague-Dawley rats weighing 200 to 225 g were selected, housed singly in metabolic cages, allowed free access to water, and fed a standard Purina rat diet ad libitum. The metabolic cages were equipped with screens below the animals' living space to avoid contamination of the urine specimens with feces or other debris. The design of the cages was such that the animals were unable to contaminate the specimens with drinking water. The 24-h urine samples were collected under mineral oil to preclude evaporation.

Four groups of 24 rats each, half of which received netilmicin and half of which received gentamicin, were studied. Eight control animals which received saline diluent accompanied each of the four experimental groups. Groups 1, 2, 3, and 4 received the drugs subcutaneously in 1 ml of saline diluent at doses of 30, 60, 90, and 120 mg/kg per day, respectively. Urine specimens were collected on days 3, 5, 8, 10, 12, and 15, at which time urine volume, urine protein excretion, and urine osmolality were measured.

Eight animals from each group, four receiving netilmicin and four receiving gentamicin, were sacrificed 24 h after a previous injection of antibiotic on days 5, 10, and 15. Four control animals for each group were sacrificed on days 5 and 15. Serum was collected for the measurement of urea nitrogen and creatinine clearance. The kidneys were removed and were examined by light and electron microscopy. In addition, homogenates were prepared for the measurement of antibiotic concentrations in renal tissue. These were compared to antibiotic concentrations in urine obtained on those same days. Urine specimens for antibiotic assay were obtained by direct needle puncture of the bladder.

The kidneys were halved through the hilus along the long axis to obtain a maximum area of light microscopy, fixed in buffered 10% formalin, embedded in paraffin, and stained with hematoxylin and

eosin or by the periodic acid Schiff reaction. The histological sections were coded and read blind by one of us (M.N.Y.). To assess the extent of the tubular necrosis, the entire cortical area was systematically examined using a mechanical stage at low magnification. Additional small samples of the kidneys were fixed in cold 4% glutaraldehyde solution in 0.1 M phosphate buffer (pH 7.4), postfixed in cold 1% phosphate-buffered osmium tetroxide solution, and embedded in Epon 812 epoxy resin. Ultrathin sections were stained with urnayl acetate and lead citrate and viewed under a Philips 300 electron microscope.

Antibiotic concentrations in urine and renal tissue were measured by an agar well diffusion method (1). *Bacillus subtilis* served as the marker organism. Renal tissue was weighed, homogenized, and diluted as necessary with phosphate buffer (pH 8). Those kidneys acquired on day 15 were further divided into cortex and medulla. The fractions were homogenized and similarly analyzed. Urine concentrations were measured after dilution in pH 8 phosphate buffer. Concentrations in renal tissue are expressed as micrograms per gram of tissue, whereas urine concentrations are expressed as micrograms per milliliter.

Urine protein was determined by a biuret method; serum urea nitrogen and creatinine were measured by standard autoanalyzer techniques (9). Osmolality was determined by freezing-point depression. A statistical analysis of variance was used to compare animals receiving netilmicin to those receiving gentamicin and to controls.

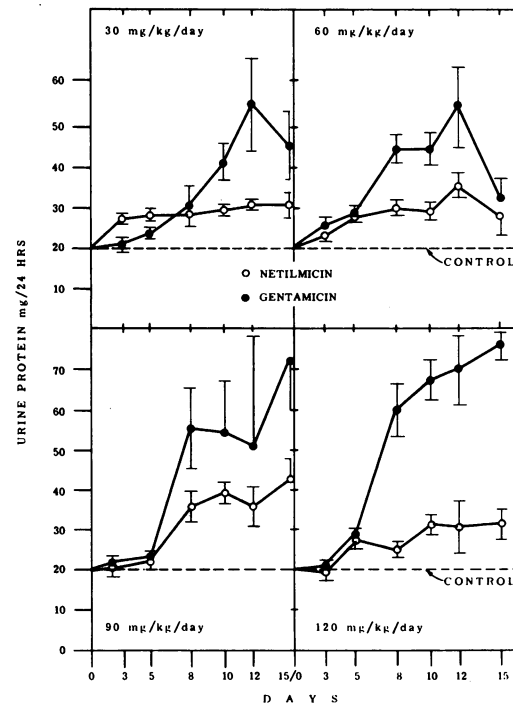

FIG. 1. *Mean values of controls are represented by a broken line. Bars represent standard error. Both drugs caused proteinuria at all doses tested, but netilmicin did so at a lesser degree.*

RESULTS

The administration of both drugs caused moderate increases in urine volume which are not graphically displayed. Significant differences between netilmicin and gentamicin were apparent only at the 30-mg/kg per day dose after 10 days of treatment. Proteinuria (Fig. 1) uniformly occurred with both regimens and was significantly increased above controls by day 5 at all doses ($P < 0.05$). The protein excretion of animals receiving gentamicin exceeded those receiving netilmicin by day 10 at 30 mg/kg per day ($P < 0.01$) and by day 8 at 60, 90, and 120 mg/kg per day, respectively ($P < 0.01$).

Urine osmolality (Fig. 2) decreased with both regimens at all doses. Gentamicin resulted in significant depression of urine osmolality compared with controls by day 8 at 30 and 60 mg/kg per day, by day 3 at 90 mg/kg per day, and by day 5 at 120 mg/kg per day ($P < 0.01$). Significant differences were observed between netilmicin and gentamicin by day 8 at 30, 60, and 120 mg/kg per day ($P < 0.05$) and by day 5 at 90 mg/kg per day ($P < 0.01$).

The serum urea nitrogen and creatinine clearance data are displayed in Tables 1 and 2. Significant serum urea nitrogen elevations compared to controls and to animals receiving netilmicin were observed in those receiving gentamicin at the 60-, 90-, and 120-mg/kg doses ($P < 0.05$). Netilmicin failed to cause a significant decrease in creatinine clearance at any of the doses tested ($P > 0.05$). Gentamicin resulted in a significant decrease by day 15 at 30 mg/kg per day, by day 10 at 60 and 90 mg/kg per day, and by day 5 at 120 mg/kg per day ($P < 0.05$). There was no mortality in netilmicin-treated animals, whereas three animals died with gentamicin treatment at 90 mg/kg per day and one animal died at 120 mg/kg per day.

Antibiotic concentrations in bladder urine at sacrifice ranged from 30 to 90 μg/ml and were similar with both regimens. Antibiotic concentrations in renal cortex at sacrifice are displayed in Fig. 3. Both drugs achieved high concentrations in renal cortex that exceeded those found in medulla fivefold or greater. There was no apparent differences among the regimens ($P > 0.05$).

Dose-related tubular damage involving primarily the convoluted portion of the proximal tubule was observed by light microscopy. In general, the changes with netilmicin were milder and occurred after a longer duration of treatment. At the 30-mg/kg per day dose,

cloudy swelling was seen in animals receiving netilmicin, whereas those receiving gentamicin exhibited, in addition, patchy areas of tubular necrosis. At the higher doses, tubular necrosis developed after 10 days of treatment with either drug, but was more extensive in animals receiving gentamicin (Table 3).

Electron-microscopic changes noted with both drugs at all doses were the formation of large numbers of cytosegresomes containing myeloid bodies within the proximal tubular cells. These bodies were also found within the lumina of the proximal tubules. Mild mitochondrial swelling and dilation of the cisternae of the rough endoplasmic reticulum were noted as well.

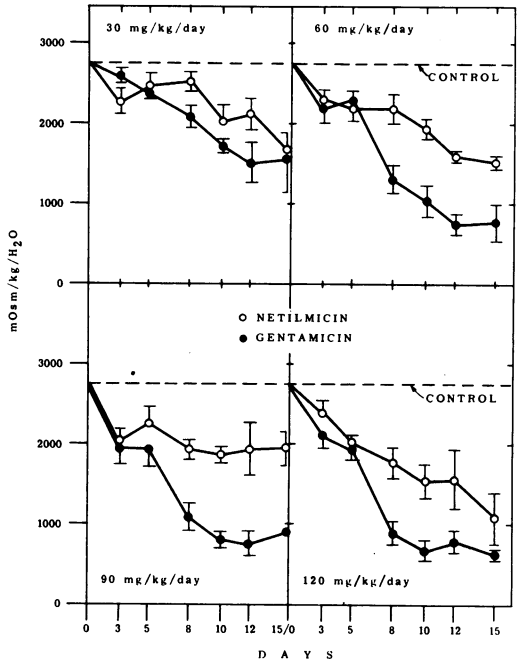

FIG. 2. *Mean values of controls are represented by a broken line. Bars represent standard error. Urine osmolality decreased with the regimens at all doses; however, the changes were more pronounced with gentamicin.*

DISCUSSION

Netilmicin appeared less toxic than gentamicin by all parameters of renal function measured. The drug resulted in less proteinuria, less depression in urine osmolality, and no decline in creatinine clearance at the doses studied. Increases in serum urea nitrogen observed with netilmicin were significantly less than those with gentamicin. These mild increases, not accompanied by significant decreases in creatinine clearance, may reflect "pre-renal" azotemia on the basis of mild dehydration since the animals were polyuric; however, no specific measurements of plasma volume or renal blood flow were done so the point remains conjectural. Serum urea nitrogen concentrations with netilmicin were significantly different from controls only on days 10 and 15 at the 90-mg/kg per day dose, a time at which the serum urea nitrogen concentrations of the controls were lower than generally seen. No consistent proteinuria occurred with netilmicin treatment.

The manifestations of netilmicin nephrotoxicity were similar to those ascribed to gentami-

TABLE 1. *Serum urea nitrogen (mg/100 ml ± standard error) determined at time of sacrifice.*

Dose	Controls (n = 4)	Netilmicin (n = 4)	Gentamicin (n = 4)
30 mg/kg per day			
5 days	15 ± 0.48	14 ± 0.48	16 ± 2.26
10 days		13 ± 0.7	15 ± 0.25
15 days	16 ± 1.56	15 ± 0.25	7 ± 0.85
60 mg/kg per day			
5 days	20 ± 0.65	26 ± 2.06	21 ± 0.61
10 days		23 ± 1.25	41 ± 12[a, b]
15 days	19 ± 0.48	23 ± 1.75	63 ± 11.7[a, b]
90 mg/kg per day			
5 days	13 ± 0.43	13 ± 0.78	13 ± 0.25
10 days		20 ± 0.75[c]	137 ± 16[a, b]
15 days	12 ± 0.29	18 ± 0.65[c]	240[a, b] (n = 1)
120 mg/kg per day			
5 days	20 ± 0.65	23 ± 0.48	22 ± 1
10 days		24 ± 1.7	138 ± 8[a, b]
15 days	19 ± 0.78	20 ± 1.47	194 ± 33[a, b] (n = 3)

[a] $P \leq 0.05$, gentamicin versus controls.
[b] $P \leq 0.05$, gentamicin versus netilmicin.
[c] $P \leq 0.05$, netilmicin versus controls.

TABLE 2. *Creatinine clearance (ml/min ± standard error) determined at time of sacrifice*

Dose	Controls (n = 4)	Netilmicin (n = 4)	Gentamicin (n = 4)
30 mg/kg per day			
5 days	1.47 ± 0.20	1.37 ± 0.07	1.41 ± 0.06
10 days		1.45 ± 0.24	1.07 ± 0.03
15 days	1.24 ±0.03	1.22 ± 0.06	0.84 ± 0.09[a, b]
60 mg/kg per day			
5 days	1.74 ± 0.09	1.42 ± 0.07	1.66 ± 0.09
10 days		1.37 ± 0.09	0.34 ± 0.04[a, b]
15 days	1.72 ± 0.08	1.50 ± 0.1	0.21 ± 0.03[a, b]
90 mg/kg per day			
5 days	1.62 ± 0.12	1.26 ± 0.17	1.56 ± 0.29
10 days		1.31 ± 0.35	0.45 ± 0.11[a, b]
15 days	1.78 ± 0.20	1.68 ± 0.11	0.1[a, b] (n = 1)
120 mg/kg per day			
5 days	1.60 ± 0.13	1.55 ± 0.04	1.07 ± 0.06[a, b]
10 days		1.29 ± 0.05	0.26 ± 0.025[a, b]
15 days	1.69 ± 0.16	1.62 ± 0.13	0.20 ± 0.02[a, b] (n = 3)

[a] $P \leq 0.05$, gentamicin versus controls.
[b] $P \leq 0.05$, gentamicin versus netilmicin.

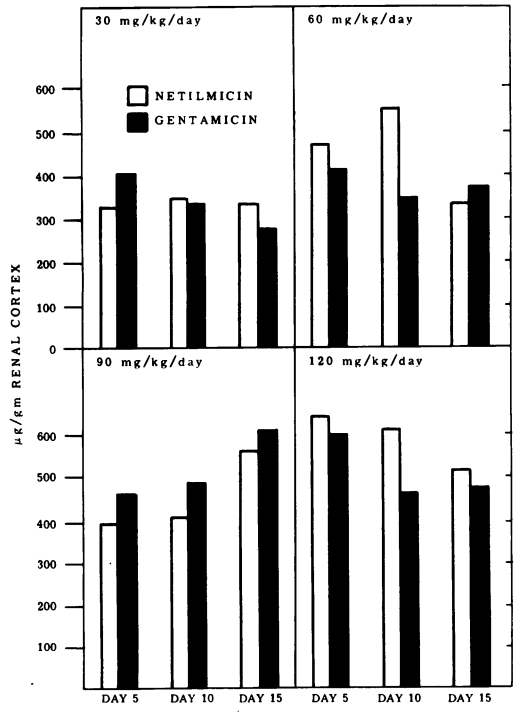

FIG. 3. *The concentrations of antibiotics in the renal cortex. No significant differences were apparent between the regimens.*

cin and other aminoglycosides (10). Proteinuria and a decrease in urine osmolality were early manifestations of toxicity, whereas alterations in glomerular filtration, observed only with gentamicin in the present study, occurred later. Histological changes observed with light microscopy were similar to those reported previously with gentamicin and other aminoglycosides (8, 10). This proved to be the case for the electron-microscopic changes as well. The formation of cytosegresomes with myeloid bodies was first described in the English literature by Kosek et al., who noted these changes in Fischer 344 rats with doses of gentamicin as low as 1 mg/kg per day after 48 h (6). Houghton et al. have recently published an elaborate series of photomicrographs which displayed the electron-microscopic changes seen with gentamicin at 40 mg/kg per day in Fischer 344 rats (5). Their observations are very similar to the findings in the present study. The origin and the significance of the electron-microscopic changes observed in proximal tubular epithelium remain unknown. Houghton et al. suggest that the cell may isolate and subsequently eject gentamicin-injured or possibly gentamicin-bound cytoplasmic structures in the form of myeloid bodies (5). Aminoglycosides are known to accumulate in renal cortex in which they have long half-lives (7). Since they impair protein synthesis in susceptible bacteria (4), it is conceivable that they impair protein or lipid synthetic processes in mammalian cells as well.

Whether or not renal-cortical concentrations of aminoglycosides correlate with the degree of nephrotoxicity is not established with certainty. In a previous publication, we reported that the renal-cortical concentrations achieved with an experimental aminoglycoside which proved to be most nephrotoxic were greater than a number of less toxic aminoglycosides (8). Dellinger et al. noted a protective effect of cephalothin against gentamicin nephrotoxicity (2)

TABLE 3. *Extent of tubular necrosis at 10 days of treatment (four rats in each group)*

Symptom	No. of rats receiving antibiotic[a]							
	Net (30 mg/kg)	Gen (30 mg/kg)	Net (60 mg/kg)	Gen (60 mg/kg)	Net (90 mg/kg)	Gen (90 mg/kg)	Net (120 mg/kg)	Gen (120 mg/kg)
Cloudy swelling without necrosis	4	4	4		2			
Necrosis involving 25% of total cortical area				1	2		4	
Necrosis involving 25–50% of total cortical area				3				
Necrosis involving 50–75% of total cortical area						1		
Necrosis involving 75–100% of total cortical area						3		4

[a] Net, Netilmicin; Gen, gentamicin.

and were able to demonstrate renal cortical concentrations of gentamicin that were significantly lower in rats given gentamicin and cephalothin simultaneously as compared with animals given gentamicin alone (3). In the present study, the renal cortical concentrations of netilmicin were not significantly different from those of gentamicin. Furthermore, the concentrations found with both drugs did not appear related to the dose. It is possible that the doses at which the drugs were given saturated all available binding sites and that with a lower dose, such as that used by Dellinger et al., a difference would have been apparent. Also, present bioassay techniques of aminoglycosides in homogenates of renal tissue are relatively crude. More sensitive assay systems may demonstrate a correlation between degree of toxicity and the renal-cortical concentration of aminoglycosides.

In summary, the present study presents evidence that netilmicin was significantly less nephrotoxic than gentamicin in the rat. The morphological changes engendered in proximal tubular epithelium by both drugs were similar in character, but less severe with netilmicin. The nephrotoxicity of the drugs could not be correlated with concentrations of the drugs in homogenates of renal cortex at sacrifice.

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