ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 1980, p. 346-348
0066-4804/80/08-0346/03$02.00/0

Vol. 18, No. 2

NOTES

Comparative Nephrotoxicities of High-Dose Netilmicin and Tobramycin in Rats

RICHARD A. PARKER,* DAVID N. GILBERT, DONALD C. HOUGHTON, GEORGE A. PORTER, AND WILLIAM M. BENNETT

Division of Nephrology, University of Oregon Health Sciences Center, Portland, Oregon 97201

The nephrotoxic potentials of netilmicin and tobramycin given in doses of 180 mg/kg per day for up to 14 days were compared in Fischer rats. At this dosage, tobramycin produced mortality and significant changes in renal structure and function; netilmicin evoked minimal nephrotoxicity.

Recent studies have shown that tobramycin and netilmicin are less nephrotoxic than gentamicin in various animal models (2, 3, 6, 7). As shown in an earlier study (8), daily doses of 120 mg of netilmicin or tobramycin per kg produce minimal nephrotoxicity in Fischer 344 rats. The current report compares the nephrotoxicities of these two drugs at daily doses of 180 mg/kg.

Male Fischer 344 rats weighing 220 to 250 g were housed in metabolic cages, fed a standard rat chow, and allowed free access to water. Tobramycin or netilmicin in a dose of 180 mg/kg per day was administered subcutaneously in two equal doses. Littermate controls received an equal volume of normal saline in two divided doses. The rats were weighed at 3-day intervals during the treatment periods, and appropriate adjustments in drug doses were made.

Animals were sacrificed in groups of six (four treated and two control) after 3, 7, 10, and 14 days of treatment. A total of 48 rats was ultilized during the study. Of these 16 received tobramycin, 16 received netilmicin, and 16 received normal saline only. At 12 h after the last injection, blood was collected for measurements of urea nitrogen (Technicon autoanalyzer), creatinine (modified Jaffe reaction), and electrolytes (flame photometry). One kidney was fixed in situ via cardiac perfusion for examination by light microscopy and measurement of aminoglycoside concentration by methods previously described (5). The remaining kidney was removed, weighed, and placed in ice-cold 0.9% saline for slice transport studies. Renal cortical slices were prepared freehand and processed by methods modified from Hook and Munro (4). The results were expressed as a slice/medium ratio (S/M), where S equals either the milligrams of *para*-aminohippurate (PAH) per gram of wet tissue or the counts per minutes of *n*-[^{14}C] methylnicotinamide ([^{14}C]NMN) activity per gram of wet tissue, and M equals the PAH or [^{14}C]NMN concentration per milliliter of medium. Renal tissue aminoglycoside concentrations were determined by radioimmunoassay and expressed as micrograms per gram of wet weight (8). Statistical evaluation was accomplished with Student's t test, with $P < 0.05$ chosen as the minimum level for statistical significance.

There was no mortality among netilmicin-treated animals. In contrast, only one of four tobramycin-treated rats survived the 10-day-treatment interval, and this survivor had a serum creatinine level of 5.3 mg/dl. The three animals that failed to survive died on day 8 and 9 of drug administration. In this 14-day-treatment study, one of four recipients of tobramycin died on day 11. Rats that failed to complete tobramycin treatment exhibited moderate nasal bleeding and staggering gait before death. There were no observed seizures or overt evidence of neuromuscular paralysis. Rats receiving tobramycin and surviving for 10 and 14 days were lethargic but mobile. There were essentially no physical manifestations of drug toxicity among the netilmicin-treated animals.

Renal function data are summarized in Fig. 1. By day 3, there were significant differences in the levels of serum creatinine in the two treatment groups ($P < 0.001$). By 14 days, animals that survived tobramycin treatment had a mean serum creatinine level of 3.2 mg/dl compared with a mean of 0.67 mg/dl in the netilmicin-treated group ($P < 0.01$). Urea nitrogen levels followed the same pattern.

The changes in slice accumulation of PAH and NMN after 90 min of incubation are also shown in Fig. 1. Significant differences between netilmicin- and tobramycin-treated animals were present at all treatment intervals. Com-

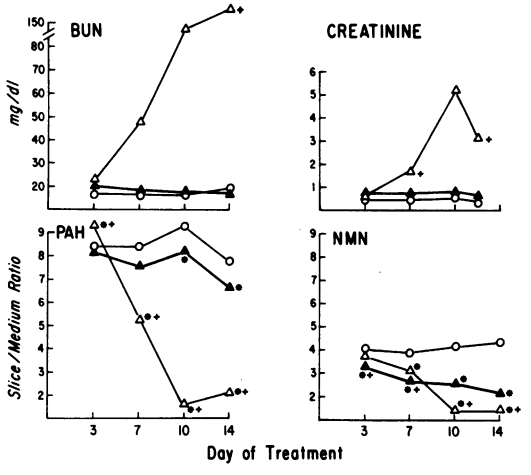

FIG. 1. *Summary of renal function and organic ion transport. Data from control and aminoglycoside-treated rats. Symbols: ○, control animals; △, tobramycin (180 mg/kg per day); ▲, netilmicin (180 mg/kg per day); +, significantly different versus netilmicin; *, significantly different versus control. n = 4 for netilmicin at each time point. n = 4 for tobramycin at 3 and 4 days, n = 1 at 10 days, and n = 3 at 14 days. n = 2 for control rats at each time point. BUN, Blood urea nitrogen.*

pared with controls, tobramycin induced an initial stimulation followed by a profound depression of PAH transport. Netilmicin did not stimulate PAH uptake and induced only moderate impairment of transport at 10 and 14 days. NMN transport was impaired by both drugs, with tobramycin producing the greater changes.

The concentrations of netilmicin in renal cortex exceeded those of tobramycin at all treatment intervals. Statistically significant differences were noted at 3 days (1,384 versus 696 μg/g, $P < 0.01$) and 14 days of drug administration (1,213 versus 376 μg/g, $P < 0.001$).

The animals treated with tobramycin developed progressive tubular necrosis such that, after 10 days, 50% of the outer cortical tubules exhibited destruction of the epithelium. At the conclusion of 14 days, necrosis was still apparent, but tubular regeneration predominated. Tubular necrosis was minimal among rats treated with netilmicin. Vacuolization of proximal tubular epithelial cells was present in each netilmicin treatment group; however, disintegration and sloughing were only rarely observed.

Previous studies in animal models have failed to demonstrate a significant difference in the nephrotoxicities of netilmicin and tobramycin (8, 9). However, a clear-cut differential develops when the drugs are administered in doses of 180 mg/kg per day: netilmicin produces minimal toxic damage, whereas tobramycin induces profound renal structural and functional alterations.

The changes in organic ion (PAH and NMN) transport seen in response to these drugs conform to the pattern that we have previously described for aminoglycoside antibiotics (1). Those drugs which are destined to produce overt renal failure induce an initial stimulation of the slice uptake of PAH, whereas those that do not produce significant nephrotoxicity fail to enhance PAH transport. The mechanism of this stimulation of PAH transport remains to be determined. Both drugs impaired organic base (NMN) transport, although the degree of impairment was greater after tobramycin administration. Drug-induced alteration of the organic base transport system represents one of the more subtle manifestations of aminoglycoside nephrotoxicity.

Both drugs were concentrated within the renal cortex, but, as has been the case in previous studies, the absolute cortical concentrations of the aminoglycosides failed to predict their ultimate nephrotoxicities.

We have previously shown that Fischer rats tolerate doses of tobramycin up to 120 mg/kg per day without mortality (8). Increasing the dose to 180 mg/kg per day exceeds the maximally tolerated dose in 50% of the animals. Netilmicin, on the other hand, is well tolerated in doses up to 180 mg/kg per day. Preliminary studies in our laboratory, however, have shown that 100% mortality ensues when netilmicin is given in a dose of 240 mg/kg per day. These animals die of apparent acute respiratory paralysis.

Extrapolation of these data comparing the nephrotoxicities of equal doses of netilmicin and tobramycin in rats to clinical practice is complicated by the current dosage recommendations in humans. The recommended maintenance dose of tobramycin is 4.5 mg/kg per day, whereas that of netilmicin is 6 mg/kg per day, a difference of 25%. Although the clinical relevance of the drug dosages employed in this study is questionable, the doses were chosen to produce changes sufficient to form a basis for comparison. It should be noted that, when factored for body surface area rather than for body mass, the doses employed in this study are only six to seven times the accepted human doses. The data demonstrate that, at 180 mg/kg per day, netilmicin is less nephrotoxic than tobramycin. In addition, in the doses employed in rats, netilmicin demonstrates a flat dose-response relationship as regards nephrotoxicity.

This work was supported by the Oregon Heart Association, the National Institutes of Health (Public Health Service grant RO1 GM22928), and Eli Lilly & Co.

We are indebted to Joan DeFehr for technical assistance and to Jennifer Paquet for excellent secretarial support.

LITERATURE CITED

1. **Bennett, W. M., C. E. Plamp, R. A. Parker, D. N. Gilbert, D. C. Houghton, and G. A. Porter.** 1979. Renal transport of organic acids and bases in aminoglycoside nephrotoxicity. Antimicrob. Agents Chemother. **16**:231–233.
2. **Bowman, R. L., F. J. Silverblatt, and G. J. Kaloyanides.** 1977. Comparison of the nephrotoxicity of netilmicin and gentamicin in rats. Antimicrob. Agents Chemother. **12**:474–478.
3. **Gilbert, D. N., C. E. Plamp, P. Starr, W. M. Bennett, D. C. Houghton, and G. Porter.** 1978. Comparative nephrotoxicity of gentamicin and tobramycin in rats. Antimicrob. Agents Chemother. **13**:34–40.
4. **Hook, J. B., and J. R. Munro.** 1968. Specificity of the inhibitory effect of uremic serum on p-aminohippurate transport. Proc. Soc. Exp. Biol. Med. **127**:289–291.
5. **Keeler, R.** 1968. Composition and function of rat kidneys after transient normothermic ischemia. Can. J. Physiol. Pharmacol. **46**:739–744.
6. **Luft, F. C., R. Bloch, R. S. Sloan, M. N. Yum, R. Costello, and D. Maxwell.** 1978. Comparative nephrotoxicity of aminoglycoside antibiotics in rats. J. Infect. Dis. **138**:541–545.
7. **Luft, F. C., M. N. Yum, and S. A. Kleit.** 1976. Comparative nephrotoxicities of netilmicin and gentamicin in rats. Antimicrob. Agents Chemother. **10**:845–849.
8. **Ormsby, A. M., R. A. Parker, C. E. Plamp, P. Stevens, D. C. Houghton, D. N. Gilbert, and W. M. Bennett.** 1979. Comparison of the nephrotoxic potential of gentamcin, tobramycin and netilmicin in the rat. Curr. Ther. Res. Clin. Exp. **25**:335–343.
9. **Soberon, L., R. L. Bowman, E. Pastorizu-Munoz, and G. J. Kaloyanides.** 1979. Comparative nephrotoxicities of gentamicin, netilmicin and tobramycin in the rat. J. Pharmacol. Exp. Ther. **210**:334–343.