Comparative Nephrotoxicity of Gentamicin and Tobramycin in Rats

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Received for publication 10 May 1977

A rat model was utilized to compare the nephrotoxic potential of gentamicin and tobramycin. Gentamicin, 40 mg/kg per day, predictably produced renal failure and morphological evidence of proximal tubular necrosis over 14 days of treatment. An identical dosage of tobramycin was associated with only minimal morphological changes and normal concentrations of serum creatinine and blood urea nitrogen. Similar results were obtained even after the tobramycin dosage was tripled to 120 mg/kg per day. A decrease in urine osmolality, mechanism unknown, was observed in all aminoglycoside-treated rats, but the lowest osmolalities were found in the gentamicin-treated rats. According to both histological criteria and renal function measurements, gentamicin was more nephrotoxic than tobramycin in this animal model.

This study was undertaken as a result of our interest in determining whether there were any important differences between the aminoglycoside antibiotics gentamicin and tobramycin. A review of the literature indicated only minor differences in in vitro antibacterial activity (1, 6, 8, 14, 20, 31). Gentamicin is slightly more active against Serratia species, and tobramycin is slightly more active against isolates of Pseudomonas aeruginosa. In vivo animal studies of antibacterial activity again show similar degrees of activity, except that tobramycin is more active against P. aeruginosa infections (5, 30). The pharmacokinetics of the two drugs in humans are felt to be virtually identical (9, 12, 22, 28). Comparative toxicity studies of the effects of these two drugs in humans have not been reported.

In a rat model, tobramycin was found to have a lower lethal dose than gentamicin (5). Tobramycin was found to have a lower lethal dose than gentamicin (5). Tobramycin was found to be less ototoxic than gentamicin in guinea pigs (2). Tobramycin was considered less nephrotoxic than gentamicin in rats on the basis of subtle but consistent histological changes and differences in drug-induced urinary lysosomal enzymes (12, 21). Since a clear difference in nephrotoxic potential would have clinical importance, studies were designed to more precisely detect any abnormalities in renal structure or function.

Initially, a prospective randomized double-blind study in humans was considered. A recent report describes such a study comparing gentamicin and amikacin (26). However, it appears virtually impossible to determine the incidence of aminoglycoside-induced nephrotoxicity in humans. There is no way for the investigator to separate disease-induced toxicity from drug-induced toxicity. Also, preillness measurements of renal structure and function are rarely available. Because of these concerns, we chose to use a rat model of aminoglycoside nephrotoxicity. Nephrotoxicity was quantitated by histological changes and alterations in renal function. The results demonstrate that, in this model, tobramycin is less nephrotoxic than gentamicin.

MATERIALS AND METHODS

Animals. Adult male Fischer 344 rats, weighing 175 to 275 g, were used. The rats were kept singly in metabolic cages, allowed free access to water, and fed ad libitum a standard Purina rat diet. Screens were placed below the animal living spaces to avoid fecal or debris contamination of urine specimens.

Aminoglycoside administration. Groups of 16 to 25 rats were used for each dosage. All animals in a group were weighed, and the average group weight was determined. The volume of drug solution required for each dose was calculated, based on the average weight. This method of dosage determination was considered valid as a coefficient of variation of the weights was 6% for the 40 mg of gentamicin per kg per day group, 5% for the 40 mg of tobramycin per kg per day group, and 16% or less for the 120 mg of tobramycin per kg per day group as well as
the lower-dose gentamicin groups. Control animals received an equivalent volume of antibiotic-free saline. The daily dosage was divided into two equal parts and given subcutaneously at 8:00 a.m. and 4:00 p.m. The aminoglycosides used were obtained from the hospital pharmacy in vials containing 40 mg/ml. A single dose of approximately 0.1 ml was drawn into a tuberculin syringe with sterile water.

Because preliminary studies indicated that blood sampling during treatment affected the animal's vascular volume, blood specimens were obtained only at time of sacrifice.

The serum level experiments were carried out over 2 days, whereas the comparative nephrotoxicity experiments were conducted over 14 days. To determine the serum levels achieved, rats were injected twice daily for 2 days. On the morning of the day 3, animals were sacrificed in groups of at least four, just before dose 5, and at 15-, 30-, 45-, and 60-min intervals after subcutaneous injection. Serum and renal tissue were obtained for measurement of aminoglycoside concentration.

In the nephrotoxicity studies, three to four drug-treated and two control rats were sacrificed 1 h after their last dose on days 3, 7, 10, and 14 of treatment. At sacrifice, blood was obtained for determination of blood urea nitrogen and serum creatinine concentration. Both kidneys were perfused with 0.1 M, 300 mosM, phosphate buffer, pH 7.1, containing 1% glucose and 1% procaine, until the kidneys blanched and the renal vein effluents were clear. One kidney was processed for electron microscopy, and the other kidney was processed for aminoglycoside content and light microscopy.

Histological studies. After the specimen for aminoglycoside concentration was obtained, one kidney from each animal was fixed in buffered Formalin for light microscopy. The other kidney of each animal was perfused for 5 to 10 min with 1% gluteraldehyde in 0.1 M phosphate buffer. Sections of renal cortex were removed and immersed in the same fixative for 2 h. Tissue cut into 1-mm3 blocks was postfixed in 2% osmium tetroxide–3% sucrose–0.05 M cacodylate buffer, pH 7.4. After dehydration in graded ethanol solutions, tissue blocks were embedded in Araldite 502 as described by Luft (15) and thin-sectioned with glass knives on a Porter-Blum MT2-B ultramicrotome. Sections were stained with uranyl acetate and lead citrate (23) and examined at 60 kV in a Philips EM-200 electron microscope.

Electron microscopic examination was conducted on renal tissue from all animals given 40 mg of gentamicin or tobramycin and from one animal of each treatment duration in the rats given 120 mg of tobramycin per kg per day. Tissue specimens were number coded and examined by the microscopist without prior knowledge of the animal's treatment group or treatment duration. Light microscopy was used to determine the extent and distribution of frank tubular necrosis, tubular regeneration, tissue inflammation, and cortical scarring. Electron microscopy was used to detect, define, and quantify prenecrotic cell injury. The parameters used for grading the severity of changes observed were ranked on a 0 to 4+ scale (Table 1). The grades for each animal were based on the light microscopic appearance of one coronal or transverse section of kidney and the electron microscopic changes in three to five blocks of renal cortical tissue. The grades for each interval of each treatment group were combined as an average score.

Aminoglycoside concentrations. Gentamicin and tobramycin serum concentrations were determined with an adenylating radioenzymatic assay (25). Because an inhibitor of the adenylating enzyme was found in renal eluates, renal tissue concentrations were determined by radioimmunoassay. Control experiments gave no indication of an enzyme inhibitor in serum.

A wedge of renal cortex and medulla was weighed and then homogenized in 0.2 M tris(hydroxymethyl)aminomethane buffer, pH 8.0. The homogenized tissue was centrifuged at 2,000 × g for 10 min, and the eluate was stored at 4°C until assayed. The radioimmunoassay materials were obtained from Diagnostic Products, Los Angeles, Calif. The same method was used for gentamicin and tobra-

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**TABLE 1. Schema for grading light and electron microscopic proximal tubule changes**

<table>
<thead>
<tr>
<th>Microscopy</th>
<th>Grade</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light</td>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Desquamation of tubular epithelial cells in small foci only (less than 1% of total tubule population involved)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Areas of focal granulovascular epithelial cell degeneration and granular debris in tubular lumen with or without evidence of desquamation</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Tubular epithelial necrosis and desquamation prominent but involving less than one-half of cortical tubules</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>More than one-half of proximal tubules are undergoing necrosis and desquamation, but intact tubules are easily identified</td>
</tr>
<tr>
<td>Electron</td>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Numerous myeloid bodies but no other evidence of cell injury</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Numerous myeloid bodies; mitochondrial swelling but no disruption; myeloid bodies in tubular lumen</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Mitochondrial swelling and disruption; marked cell edema and cytoplasmic disorganization</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Disintegration and desquamation of epithelial cells</td>
</tr>
</tbody>
</table>
mycin, but only the gentamicin assay procedure is published (27). Each sample was assayed in duplicate. Renal concentrations are expressed in micrograms per gram of tissue, whereas serum concentrations are expressed as micrograms per milliliter.

Other studies. Blood urea nitrogen and serum creatinine concentrations were determined by standard methodologies (3, 10). Osmolality was determined by freezing-point depression, using a Fisk osmometer. Statistical analysis of variance utilized Student's two-tailed t-test.

RESULTS

Under the conditions employed, all animals survived the 14-day experimental period.

Aminoglycoside concentrations. Initial experiments were designed to determine the dosage of gentamicin that, given twice daily, would be predictably toxic to the Fischer rat. The dosage was gradually increased from 10 to 20 and then to 40 mg/kg per day. The renal concentrations increased as the daily dose increased. The renal concentration also increased over time with the 10- to 20-mg/kg per day dosage groups. In the 40-mg/kg per day rats, the renal concentration was greatest at 7 days, then decreased despite continued gentamicin administration.

There was no evidence of drug-induced renal toxicity with the two lower dosages of gentamicin. Renal failure (see below) was consistently induced by the 40-mg/kg per day dosage of gentamicin. For this reason, we compared an initial gentamicin dosage of 40 mg/kg with a similar dosage of tobramycin. When renal failure was not produced with tobramycin at this dosage, the tobramycin dosage was empirically tripled in an attempt to produce toxicity.

The renal concentrations of gentamicin and tobramycin were similar in rats given 40 mg/kg per day (Fig. 1). The only difference of statistical significance occurred on day 7 of treatment ($P < 0.01$). Rats given 120 mg of tobramycin achieved renal concentrations approximately threefold greater than rats given 40 mg of tobramycin per kg per day. The high-dose tobramycin rats achieved the greatest renal concentration of the drug after 10 days of treatment. Then, as in the gentamicin-treated rats, the renal concentration tended to decrease ($P > 0.1$) after 14 days despite continued tobramycin administration.

Aminoglycoside serum concentrations. With the gentamicin and tobramycin doses used, the peak serum concentrations were determined after 2 full days of treatment. Two days were considered adequate time for equilibration of pharmacokinetics, but inadequate time for any functional manifestation of nephrotoxicity. Maximal drug concentrations occurred after 15 to 30 min of subcutaneous injection (Fig. 2). No sharp peak concentration was observed. At 30, 45, and 60 min postinjection, the serum concentrations after 40 mg of gentamicin were significantly higher ($P < 0.01$) than the serum concentrations of 40 mg of tobramycin per kg per day. As anticipated, the 120 mg of tobramycin dosage produced serum concentrations three to four times greater than the 40 mg of tobramycin per kg per day.

Histological results. The light and electron microscopic appearances of the renal tissue of control animals were essentially as described by Rouille (24) and by Ericsson and Trump (7).

The results of light and electron microscopic examination of rats treated with 40 mg of gentamicin and 40 or 120 mg of tobramycin per kg per day are summarized in Table 2.

As detailed in previous studies by ourselves and others (11, 13, 29, 32), renal tubular epithelial cells of the gentamicin-treated rats displayed progressive light and electron microscopic changes, which resulted in nearly total

![Fig. 1. Renal concentrations (mean ± 2 standard error of the mean) of rats given either gentamicin or tobramycin.](http://aac.asm.org/Downloaded from http://aac.asm.org/ on October 16, 2017 by guest)
proximal tubular necrosis. On the treatment day 3, light microscopic changes were minimal, but electron microscopy revealed large lysosomal vacuoles, some containing myeloid bodies and autophagic debris in proximal tubular epithelial cells. By day 7 of gentamicin treatment, light microscopy showed extensive proximal tubular necrosis. Electron microscopy on these tissues revealed increased numbers of myeloid bodies, cytoplasmic edema, and mitochondrial swelling. Within 10 days of initiation of gentamicin administration, proximal tubular epithelial destruction was nearly total in examination by both light and electron microscopy. However, on day 14, despite continuous gentamicin therapy, the proximal tubules were repopulated almost entirely by squamous-regenerating epithelial cells. These cells had hematoxiphilic cytoplasm and possessed no brush borders. Their nuclei were large and vesicular with occasional mitotic figures. Only rare proximal tubules were undergoing acute necrosis.

The early tubular epithelial changes observed in the animals of the treatment group on 40 mg of tobramycin per kg per day resembled those of the gentamicin group. Myeloid bodies and autophagic vacuoles were numerous by day 3 and had become generally more prominent and complex by day 7. These structures, however, were not accompanied by the other progressive changes of cell injury that were observed in the gentamicin-treated group. Moreover, little further change in the appearance of the proximal tubular epithelial cells had occurred by day 10. Autophagic vacuoles and myeloid bodies had become very numerous, but the cells remained generally intact. By light microscopy, tubular necrosis was identified in a very small number of proximal tubules, and even after 14 days of treatment no acute tubular changes were identified.

In animals treated with 120 mg of tobramycin per kg per day, the histological evaluation was limited to only one rat at each time period. Nevertheless, there was surprisingly minor renal tubular damage. At 3 and 7 days the

Fig. 2. Serum concentrations of gentamicin and tobramycin (mean ± 2 standard error of the mean) at timed intervals just before and after the fifth dose.

Table 2. Microscopic proximal tubule changes in rats given gentamicin or tobramycin

<table>
<thead>
<tr>
<th>Drug and dosage (per day)</th>
<th>Duration of therapy (days)</th>
<th>No. of rats studied</th>
<th>Grade of proximal tubule changes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Light microscopy</td>
</tr>
<tr>
<td>Gentamicin 40 mg/kg</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>3</td>
<td>3-4</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>3</td>
<td>1-2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Extensive regeneration)</td>
</tr>
<tr>
<td>Tobramycin 40 mg/kg</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>3</td>
<td>1</td>
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<tr>
<td></td>
<td>14</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Focal regeneration)</td>
</tr>
<tr>
<td>Tobramycin 120 mg/kg</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Focal regeneration)</td>
</tr>
<tr>
<td>All control rats</td>
<td></td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

* ND, Not done.
light and electron microscopic changes were essentially the same as those at the same intervals in animals treated with 40 mg of tobramycin per kg per day. After 10 days of treatment, foci of acute necrosis were identified in approximately 5% of proximal tubules. By electron microscopy, features of cell injury, such as cytoplasmic edema and vacuolization and mitochondrial distention and disruption, were found only rarely. Tubular alterations were the same after 14 days as after 10 days of treatment, except that infrequent small foci of proximal tubule regeneration and perivasculary inflammatory infiltrates were identified.

Myeloid bodies were present in distal tubular epithelial cells in very small numbers in all gentamicin and tobramycin treatment groups by 10 days of drug administration, but there was never evidence of accompanying cell injury.

Urine osmolality. Urine osmolality decreased in both the gentamicin- and tobramycin-treated animals (Fig. 3). No differences were apparent after 3 days of treatment. By 7 days of treatment, animals given 40 or 120 mg of tobramycin per kg per day had a significant \( P < 0.02 \) decrease in urine osmolality as compared with controls. In comparison with controls, rats treated for 7 days with 40 mg of gentamicin per kg per day had a highly significant decrease \( P < 0.001 \) in urine osmolality. By 10 days of treatment, the osmolality values in the 40 mg of gentamicin per kg per day rats were significantly less than control animals \( P < 0.001 \), the 40 mg of \( P < 0.01 \) tobramycin per kg per day animals and the 120-mg/kg per day dose \( P < 0.001 \) tobramycin-treated animals. At no time were there significant differences in urine osmolality between the low-dose and high-dose tobramycin animals.

Blood urea nitrogen and serum creatinine. The blood urea nitrogen and serum creatinine results were similar, and, hence, only the creatinine results are presented (Fig. 4). In the animals given 40 mg of gentamicin per kg per day, the mean blood urea nitrogen was increased compared with all other treatment groups after 10 days of treatment, and the increase was statistically significant \( P < 0.01 \) after 14 days of treatment. The mean serum creatinine concentration of the gentamicin group was significantly higher \( P < 0.01 \) than all other groups as early as day 10. The values for the low- and high-dose tobramycin animals were similar to control values.

DISCUSSION

The results of this study support the hypothesis that for a specific aminoglycoside antibiotic the renal parenchymal accumulation of drug is related to the development of nephrotoxicity. Increasing doses of gentamicin led to increasing renal concentrations, which were associated with increasing evidence of nephrotoxicity.

The animals given tobramycin showed a similar increase in renal drug concentration with an increase in dosage. However, there was no
corresponding gross renal toxicity other than minimal morphological changes and a lower urine osmolality. Overt toxicity may require a tobramycin dosage greater than 120 mg/kg per day or a continuation of the 120-mg/kg per day dosage for longer than 14 days.

The pattern of renal accumulation of gentamicin and tobramycin suggests that, for a given dosage, there is gradual increase in renal concentration that reaches a maximum value despite continued drug administration. Similar observations have been reported by others for both gentamicin and netilmicin (18, 19). An understanding of this process will require much more information on the renal handling of aminoglycosides. Indeed, even though the proximal tubule cells are prime suspects, the intrarenal site of gentamicin accumulation is unknown. It is also intriguing that the renal concentration of gentamicin fell between days 10 and 14 of treatment, despite continued drug administration. It is tempting to speculate that this fall in concentration was the result of necrosis and subsequent urinary loss of gentamicin-laden proximal tubular cells. Finally, the slightly lower renal concentrations of tobramycin, as compared with rats given equal gentamicin, may be due to the shorter renal half-life of tobramycin, reported by Luft and Kleit (16).

The renal concentrations of high-dose tobramycin were twice those of gentamicin and yet renal failure occurred only in the gentamicin animals. Thus, comparisons of different aminoglycosides show no correlation between renal concentration and toxicity. Similar results were described when gentamicin and netilmicin were compared in rats (19).

Our studies were not designed to evaluate the potential relationship between aminoglycoside serum levels and nephrotoxicity. Our goal was to establish a model of gentamicin nephrotoxicity that could be used to compare the nephrotoxic potential of gentamicin and other aminoglycosides. Thus, the gentamicin dosage of 40 mg/kg per day was selected for comparison with tobramycin because this gentamicin dosage predictably produced renal failure. Once the toxic dosage was derived, the serum levels were measured. Furthermore, Luft and colleagues have pointed out that, based on body surface area, the dosage employed would correspond to a dosage in humans of approximately 8 mg/kg per day (17).

We have no explanation of why equal-dosage regimens of gentamicin and tobramycin produced lower tobramycin serum concentrations. In humans, with intramuscular and intravenous administration, the pharmacokinetics of the two drugs were virtually identical. The reason for the differences may be related to the obvious species differences or to the subcutaneous route of administration. The differences in serum concentrations after 40 mg/kg per day seem of little consequence in comparing the nephrotoxic potential of gentamicin and tobramycin. The rats given 120 mg of tobramycin per kg per day achieved serum levels much higher than the gentamicin animals with virtually no evidence of toxicity.

Low urine osmolalities in rats given aminoglycoside antibiotics have been reported by others (4, 18, 19, 20). Whether this change represents a true inability to concentrate the urine, a change in thirst mechanisms, or some other abnormality will require further study.

The morphological evidence of proximal tubular cell regeneration during continued gentamicin therapy deserves emphasis. Several questions arise from this observation. Would the tubular cells return to normal even if the gentamicin were continued longer than the 14 days of this study? At what point is the gentamicin-induced toxicity irreversible? It should be possible to answer these and other related questions with the rat model described.
ACKNOWLEDGMENTS

We acknowledge the technical assistance of K. Bare, J. DeFehr, and J. Kimsey.

This work was supported by Veterans Administration grant no. 646-0001. Public Health Service grant no. R01 GM-22929 from the National Institute of General Medical Sciences, and a grant from Eli Lilly and Co., Indianapolis, Ind. C. Plamp was supported in this work by a fellowship grant from the Oregon Heart Association.

LITERATURE CITED


