Gentamicin Nephrotoxicity: Failure of Three Cephalosporins to Potentiate Injury in Rats

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The possibility that gentamicin and cephalosporin antibiotics may act synergistically to produce nephrotoxicity was evaluated in an experimental model. Necrosis of the proximal tubules occurred when rats were treated with 60 to 120 mg/kg of gentamicin for 5 days but not when 15 to 20 mg/kg per day was given for up to 4 weeks. In all gentamicin-treated animals lysosomes of proximal tubules were increased in size and number and the lumens of many tubules contained a granular deposit. Examination by electron microscopy revealed that the abnormal lysosomes contained membranous whorls. The luminal deposits consisted of similar material; identical bodies were also present in the urinary sediment. To determine whether concurrent administration of a cephalosporin would augment the nephrotoxic potential of gentamicin, additional rats were treated for 4 weeks with daily injections of gentamicin (20 mg/kg) and either cephaloridine, cephalothin, or cefazolin (500 mg/kg). None of the combination regimens produced any more injury than did gentamicin alone.

The treatment of serious infections often requires the concurrent use of more than one antibiotic. Combined therapy is given in an attempt to broaden the spectrum of initial antibacterial coverage, to take advantage of possible synergistic activity against microorganisms, and to retard the emergence of resistant strains. The physician contemplating combined therapy must weigh these potential advantages against the possibility that the drugs might interact in a disadvantageous manner and be more toxic than either drug used alone.

It is sometimes difficult, however, to document the adverse effects of drug combinations. For example, although it has recently been reported that the simultaneous use of gentamicin and a cephalosporin antibiotic caused renal injury (6, 9, 12, 13, 20), these studies have not clearly distinguished damage due to the combination of drugs from the nephrotoxicity of the individual agents (4, 5, 8, 19, 25).

Because the combination of gentamicin and a cephalosporin is often selected for the initial treatment of life-threatening infections, we deemed it important to determine whether the simultaneous use of these agents does in fact enhance the risk of nephrotoxicity. This problem was studied in a rat model and was done concurrently with an investigation of cephalosporin nephrotoxicity reported previously (24). The results of these studies in animals indicate that combined therapy does not enhance the risk of nephrotoxicity.

**Animals and medications.** Female Sprague-Dawley rats weighing 240 to 260 g were used throughout the investigation. They were fed commercial chow and allowed water ad libitum. Antibiotics were dissolved in 0.9% sodium chloride solution and injected intramuscularly. Animals received various amounts of gentamicin, cephaloridine, cephalothin, or cefazolin, alone or in combination (Table 1). A total of 87 rats was used to delineate possible nephrotoxic effects.

**Tissue processing.** The kidneys were preserved by antemortem intravascular perfusion with half-strength Karnovsky's fixative (M. J. Karnovsky, J. Cell Biol. 27:137A–138A), according to the method of Griffith et al. (11). Subsequent processing for light and electron microscopy was accomplished by cutting 1-mm slices from the right and left kidneys of each animal with a razor blade so that the entire depth from cortex to papilla was included. These slices were placed in Karnovsky fixative at 4 C for an additional 4 h and washed in a solution of 0.1 M cacodylate buffer with 7% sucrose for at least 24 h. After being washed, the tissue was immersed in 1% OsO₄ buffered with

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TABLE 1. Nephrotoxic effects of gentamicin and three cephalosporins, administered individually and in combination to rats

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg per day)</th>
<th>No. of rats</th>
<th>Renal morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tubular necrosis</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>15</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Cephaloridine</td>
<td>1,100</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>1,100</td>
<td>10</td>
<td>0</td>
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<tr>
<td>Cefazolin</td>
<td>1,100</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>20</td>
<td>13</td>
<td>0</td>
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<tr>
<td>plus cephaloridine</td>
<td>500</td>
<td>13</td>
<td>0</td>
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<tr>
<td>Gentamicin</td>
<td>20</td>
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<td>plus cefazolin</td>
<td>500</td>
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</tr>
</tbody>
</table>

* Proximal tubules showed increased numbers of cytoplasmic dense bodies and granular deposit in lumen.

s-collidine, dehydrated in a graded series of ethanol, and embedded in Epon 812. Sections of this tissue, measuring approximately 6 by 10 mm, were cut on a Sorvall JB-4 microtome (Ivan Sorvall Inc., Norwalk, Conn.) using 1-cm-wide glass knives. The cut sections, each about 1 μm thick, were stained with toluidine blue and coded for examination by light microscopy. Retrieval of interesting areas for examination by electron microscopy was accomplished by methods described previously (23). Ultrathin sections were cut with a diamond knife, using a Reichert OM-2 ultramicrotome (William J. Hacker and Co., West Caldwell, N.J.). They were placed on copper grids coated with parlodion and carbon. The sections were stained with uranyl acetate and lead tartrate and photographed in an AEI 801 electron microscope (AEI Scientific Apparatus Inc., Elmsford, N.Y.).

Examination of urinary sediment by electron microscopy. The urine of one rat was collected over an 8-h period during the third week of therapy with gentamicin, 20 mg/kg of body weight. The sample was centrifuged and the sediment was then fixed for 2 h in Karnovsky solution. After an overnight wash in 0.1 M sodium cacodylate, the sediment was dispersed in melted agar and the mixture was placed in cylindrical molds. The agar casts were then processed for electron microscopy in a routine manner.

Gentamicin treatment alone. To determine the morphological appearance and the dose response of nephrotoxicity attributable to gentamicin, 24 rats were given daily injections of gentamicin in doses ranging from 15 to 120 mg/kg of body weight. Treatment was maintained for 4 weeks or until the animals appeared moribund. All animals given 15 or 20 mg/kg survived for 4 weeks. At lower doses (15 to 20 mg/kg) there was no evidence of cell necrosis, but the kidneys of all these animals had a striking accumulation of dense bodies in the renal epithelial cells (Fig. 1). Although the largest number of these structures occurred in the proximal tubules, they were also present in the cells of the cortical segments of collecting ducts, distal tubules, and in glomerular epithelial cells. A granular deposit was present in the lumen of many proximal tubules (Fig. 1). Occasionally an area of the cortex was seen where the interstitium was infiltrated by inflammatory cells. This was particularly evident around the interlobar arteries (Fig. 2).

Higher amounts of gentamicin (60 and 120 mg/kg) caused necrosis of the proximal tubules, in addition to the changes attributed to the lower doses (Fig. 3). Necrosis was limited to the middle portion of the proximal tubule in most animals; in the most severely injured kidneys, however, all three portions were involved. Every animal given 120 mg/kg was moribund within a week. Those that received 60 mg/kg survived longer and regenerating epithelium was seen within the residual basement membranes of necrotic tubules.

When examined by electron microscopy, the abnormal dense bodies were seen to contain...
Fig. 1. Photomicrograph of a kidney from a rat which received gentamicin (20 mg/kg) daily for 4 weeks. Proximal tubules contain increased numbers of cytoplasmic dense bodies (circle) and a granular deposit in their lumen (arrow) (×400).

Fig. 2. Photomicrograph of a kidney from a rat which received gentamicin (20 mg/kg) daily for 4 weeks. The interstitial space between the artery (A) and the venous sinusoid (V) is infiltrated with many inflammatory cells (arrow) (×250).

whorls of membranous material resembling myelin figures (Fig. 4), but otherwise resembled heterolysosomes seen in normal rats. Similar membranous whorls were present in the lumen of proximal tubules (Fig. 5) as well as the urinary sediment (Fig. 6). The results of gentamicin treatment are summarized in Table 1.

Cephalosporin treatment alone. Necrosis of the proximal tubules was seen in four of ten rats injected daily with 1,100 mg/kg of cephaloridine for 4 weeks (Table 1). In a fifth rat, proximal tubules were dilated and their brush border was missing in large stretches. No morphological evidence of renal injury was noted, however, in
Fig. 3. Photomicrograph of a kidney from a rat which received gentamicin (120 mg/kg) daily for 5 days. There is extensive necrosis of the proximal tubules. A glomerulus (G) and some profiles of distal convoluted tubules (DT) appeared spared (×250).

Fig. 4. Electron microscopy of a portion of a renal proximal tubule cell from a rat which received 20 mg/kg daily for 4 weeks. Heterolysosomes (L) contain osmophilic membranous whorls. Nucleus (N), microvilli (MV), mitochondria (M) (×14,150).
Fig. 5. Electron micrograph of the apical portion of a proximal tubule cell from a rat which received gentamicin (20 mg/kg) daily for 4 weeks. Membranous whorls (MW) similar to those present in the heterolysosomes depicted in Fig. 4 can be seen in the tubular lumen of this cell. Arrow points to additional membranous material, possibly just disgorged from the cell by exocytosis (×14,700).

Fig. 6. Electron micrograph of the urinary sediment from a rat which received gentamicin (20 mg/kg) daily for 4 weeks. The entire field consists of membranous material which appears similar to the contents of the heterolysosomes and tubular lumen of proximal tubules from gentamicin-treated rats (Fig. 4 and 5) (×5,440).
five rats treated with 500 mg of cephaloridine per kg. Cephalothin and cefazolin appeared much less nephrotoxic and no animal treated with 1,100 mg of either drug per kg daily for 4 weeks had any renal damage.

Combined cephalosporin and gentamicin therapy. To determine whether the risk of renal damage was enhanced by simultaneous administration of subnephrotoxic amounts of gentamicin plus a cephalosporin antibiotic, animals were treated with daily injections of 20 mg of gentamicin per kg of body weight and 500 mg of either cephaloridine, cephalothin, or cefazolin per kg for 4 weeks. The amount of renal damage seen with each combination regimen did not differ from the degree of injury attributable to gentamicin alone (Table 1).

Recent clinical reports have suggested that concurrent administration of gentamicin plus a cephalosporin antibiotic enhances the risk of nephrotoxicity due to these agents (6, 9, 12, 13, 20). The results of the present study indicate, however, that in the rat, concurrent administration of a cephalosporin antibiotic does not potentiate the nephrotoxicity of gentamicin. Although our evidence is entirely morphological, the technique used to fix the kidneys, antemortem intravascular perfusion, insures optimal preservation of renal structure and allows subtle deviations from normal to be detected. There are several possible explanations for our failure to document such a synergistic interaction. Firstly, it could be argued that had we used still higher concentrations of the drugs we might have demonstrated a synergistic effect. This study, however, was not designed to rigorously exclude the possibility of drug interaction at any dose, but was intended to duplicate, in experimental animals, the clinical situation in which high doses of these agents are given to patients for a prolonged time. Information obtained with still higher antibiotic concentrations, at or near the nephrotoxic dose we believe, would have less biological relevance for man.

Secondly, it is possible that rats do not respond to these agents in the same manner as humans. Although it is true that information gained from an animal model may have only limited applicability to man, indirect evidence cited below suggests that aminoglycosides may cause changes in the human kidney similar to those reported here. In any event, this question could only be truly answered by a well-controlled, prospective study of patients on combined therapy. Finally, we suggest that one source of the discrepancy between our results and the published clinical observations lies in the difficulty of identifying a specific cause of deteriorating renal function with retrospective studies. Frequently, more than one potentially nephrotoxic agent is used. For example, both gentamicin (5, 8, 19) and cephaloridine (4, 25) have been reported to damage the kidney. Furthermore, during the course of therapy, patients may experience a complication, such as shock, which in itself can produce tubular necrosis. In addition, some drugs have been shown to potentiate the nephrotoxicity of antibiotics. Two such synergistic combinations are the anesthetic methoxyflurane and gentamicin (3, 18) and the diuretic furosemide and cephaloridine (17). In the present experiment, an animal model was used to minimize the possibility that these uncontrolled variables would hinder recognition of changes caused by the drugs under investigation.

The changes in the ultrastructure associated with gentamicin described in this study are similar to those recently reported by Kosek et al. (16). These authors suggested that the myelin figures which accumulated in renal epithelial cell lysosomes were the residues of cell organelles injured by gentamicin and subsequently autophagocytosed by the lysosomes. We feel that this interpretation is unlikely as no organelle besides the lysosome appeared injured nor were there any partially digested organelles within the renal lysosome as is the case when autophagy occurs (1). As an alternative explanation we suggest that the myelin figures resulted from an effect of gentamicin directly on the lysosomes themselves, perhaps caused by gentamicin's polycationic nature. Similar myelin figures have been seen in the renal lysosomes of rats treated with other basic substances such as neutral red (14), chloroquin (21) or the herbicide paraquat (10). The mechanism whereby these changes occur is not known. Lysosomes are known, however, to concentrate basic dyes and other cationic molecules, and Koenig has suggested that these basic substances interact with acidic lipoproteins present in the lysosomal matrix, changing the conformation of the lipids to form concentric lamellae (15). In support of the hypothesis that gentamicin acts directly on the lysosome we have recently observed myelin figures in a lysosome-rich fraction of kidney homogenate incubated with 1,000 μg of gentamicin per ml (Silverblatt, unpublished observations).

In some cells, lysosomes release their contents into the extracellular environment by exocytosis (7). This process is probably responsible for the presence of myelin figures in the lumens of proximal tubules and in the urinary sediment of
rats treated with gentamicin. There is indirect evidence that a similar event may occur in patients treated with a related aminoglycoside, kanamycin. In a previous study we reported that urinary levels of the enzyme beta-glucuronidase rose promptly when kanamycin was given to patients with chronic pyelonephritis and returned to normal when therapy was stopped (22). Since this enzyme is known to be a constituent of proximal tubule lysosomes (2), this observation suggests that kanamycin, like gentamicin, may cause renal lysosomes to release their contents into the urine. Measuring the level of lysosomal enzymes in the urine of patients being treated with aminoglycoside antibiotics may, therefore, facilitate early detection of the nephrotoxic effects of these agents. We are currently evaluating the usefulness of this procedure in man.

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LITERATURE CITED