The Protective Effect of Nitrendipine on Gentamicin Acute Renal Failure in Rats

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Aminoglycoside nephrotoxicity was produced in two groups of Fischer rats by intraperitoneal injection of gentamicin, 40 mg/kg/day for 2 weeks. Beginning 3 days prior to, and continuing throughout the 2-week treatment period, one of the groups (control) received the inert vehicle, polyethylene glycol, while the experimental group was given nitrendipine, a calcium channel blocker, in a dose of 25 mg/kg/day by gavage. Both groups received food and water ad libitum. Gentamicin with vehicle caused a marked decrease in inulin clearance (4.9 ml/min/kg) and paraaminohippurate (PAH) extraction (26%), and extensive renal tubular necrosis. In comparison, the nitrendipine-treated rats had a significantly increased clearance (9.8 ml/min/kg) and PAH extraction (48%), and less histopathologic damage. Renal tissue content of gentamicin was not influenced by nitrendipine after 4 days of dosing. Nitrendipine, a diisopyridine derived calcium channel blocker, offers significant functional and histologic protection against aminoglycoside nephrotoxicity in Fischer rats. Its mode of action in this regard is unknown.

INTRODUCTION

The nephrotoxic effects of the aminoglycoside antibiotics have been extensively described from a clinical and experimental standpoint (Humes et al., 1982b). Epidemiologic studies indicate that 10 to 20% of acute renal failure in hospitalized patients is associated with aminoglycoside administration, although other factors appear to enhance the risk of developing aminoglycoside nephrotoxicity. Gentamicin-induced renal injury in the rat has been utilized as the prototypal experimental model, although there are unexplained rat-strain differences in susceptibility, and the doses required to produce renal injury in rats are somewhat higher than those in common clinical usage. The pathologic changes and the natural history of nephrotoxicity in the rat are similar to man, and have been well described (Tuft et al., 1975; Cuppage et al., 1977).

In contrast to ischemic acute renal failure, where pharmacologic, dietary, and hemodynamic intervention has been demonstrated to modify the natural history of the acute renal failure, there is limited evidence for similar maneuvers protecting against aminoglycoside nephrotoxicity. Suppression of the renin–angiotensin system with volume loading or captopril partially reverses the hemodynamic changes associated with aminoglycoside nephrotoxicity, but does not prevent the histopathological abnormalities (Schor et al., 1981). Teixeira and co-workers (1982) have demonstrated that the diabetic state in rats confers significant protection against gentamicin nephrotoxicity. Recently, Humes and colleagues (1984) showed that dietary calcium supplementation prevented the decline in renal function and preserved renal cortical mitochondrial function in rats given gentamicin. The present study was undertaken to examine the effects of a calcium channel inhibitor, nitrendipine, in gentamicin-induced nephrotoxicity in the rat.
MATERIALS AND METHODS

Adult Fischer rats of either sex weighing 175–300 g were used in all studies. They were housed three to four per cage and permitted *ad libitum* access to food (Wayne Lab Blox) and water.

*Induction of Renal Failure*

Gentamicin sulfate (Schering) was injected subcutaneously in a dose of 40 mg/kg/day. The drug was given as a single daily dose.

*Nitrendipine Administration*

Animals were given nitrendipine in a dose of 25 mg/kg/day by gavage on a twice-daily schedule. Nitrendipine dosing was initiated 3 days prior to gentamicin and was continued throughout the period of gentamicin injections. The drug was prepared as a fine suspension using 0.05% polyethylene glycol (PEG) as the vehicle and given in a volume of 3 ml b.i.d. Fresh drug suspension was prepared every 3 days and was kept shielded from light at all times.

*Renal Function Studies*

Renal function was evaluated after the final day of gentamicin administration. Animals were not fasted or water depleted prior to functional studies. Anesthesia was induced with Inactin (Promonta, Hamburg, FRG) in a dose of 100 mg/kg by intraperitoneal injection. The rats were placed on a heated table and rectal temperature was monitored with a thermistor probe and maintained at 36–38°C. A tracheotomy was performed and catheters were placed in the left femoral artery, femoral vein, and bladder. The femoral artery was used for blood pressure measurement which was recorded continuously throughout the experiment. At the conclusion of the preparative surgery, animals received a loading dose (20 μCi/kg) of [14C]inulin and [3H]paraaminohippurate (PAH), and a sustaining solution of 12 μCi/hr administered in normal saline (25 ml/kg/hr). In animals with suspected renal failure, the sustaining doses of inulin and PAH were appropriately decreased.

After a 45-min recovery period following surgery, clearance measurements were initiated. Urine collection periods ranged from 15 to 30 min depending on flow rate and were bracketed by about 150 μl blood collections from the tail vein. At least four measurements were obtained in each experiment. At the conclusion of the clearance collections, the left spermatic and adrenal veins were clamped and the left renal vein punctured with a 27-gauge needle to collect a sample for calculation of PAH extraction.

*Biochemical Analysis*

Serum creatinine was determined by the method of Folin and Wu adapted for the Technicon autoanalyzer. Plasma and urine samples (20 μl) were analyzed for [14C]inulin and [3H]PAH. They were placed in Aquasol scintillation fluid (New England Nuclear, Boston, Mass.) and counted on a Packard Instrument Company (Downers Grove, Ill.) Model 3330 Tri-Carb liquid scintillation counter. There were only negligible numbers of 3H disintegrations counted in the 14C channel and no correction was made for them.

Gentamicin content of renal tissue was performed on whole kidney samples.
Following exsanguination of the rat, the kidney was removed, weighed, blotted dry, and stored at −180°C. Subsequently, the samples were homogenized and the gentamicin content was analyzed in triplicate samples by a homogeneous enzyme immunoassay using glucose-6-phosphate as the substrate.

**Histologic Methods**

Kidneys were removed and fixed by immersion in 10% buffered Formalin. Sections (5–6 µm) were stained with hematoxylin & eosin and examined in a blinded fashion. The tissues were evaluated by light microscopy for the presence of intratubular casts and debris, tubular cell vacuolation, loss of brush border, tubular necrosis, and regenerative changes. The severity of the pathologic findings was assigned a composite score based on an arbitrary scale of 0 to 4, 0 being normal and 4 being the most severely damaged.

**Experimental Protocol**

The study was conducted in three phases.

**Phase 1.** Two groups of six rats were designated. Both groups received gentamicin sulfate for a period of 12 days. The experimental group was dosed with nitrendipine, which was started 3 days prior to the gentamicin and continued for a total duration of 15 days. At the conclusion of the experimental period, the rats were sacrificed, blood was obtained for serum creatinine measurement, and the kidneys were rapidly removed for immersion fixation.

**Phase 2.** Two groups of six rats were designated. Both were injected with gentamicin sulfate 40 mg/kg/day for 4 days. A control group was given the vehicle 0.05% PEG in a dose of 3 ml b.i.d. The experimental group was dosed with nitrendipine 25 mg/kg/day beginning 3 days prior to the gentamicin. At the conclusion of the experimental period, the kidneys were removed, weighed, and stored frozen in preparation for tissue gentamicin assay.

**Phase 3.** Two groups of rats were designated, both of which received gentamicin sulfate 40 mg/kg/day by subcutaneous injection. A control group was dosed with the vehicle 0.05% PEG, 3 ml b.i.d. by gavage. The experimental group received nitrendipine 25 mg/kg/day by gavage in a volume of 3 ml b.i.d. The drug and vehicle dosing were initiated 3 days prior to starting the gentamicin. At the conclusion of the experimental period, the animals underwent renal functional measurements and the kidneys were subsequently removed for histologic evaluation.

The pathologic findings in the kidneys from rats in phases 2 and 3 were similar despite the fact they were dosed for 12 and 14 days, respectively. They are considered together for the purposes of histologic scoring in the results.

**Statistical Methods**

Results are expressed as means ± standard error of the mean. In the renal functional studies, differences among the groups were evaluated by one-way analysis of variance. In the case of a significant F value, the least significant difference test of Fisher was applied to identify the significantly different mean value. In situations where two groups of animals are being compared, the statistical significance of differences in means was established with Student’s t test for unpaired samples. The histopathological differences among the groups were compared using nonparametric statistics employing the Wilcoxon rank sum test.
RESULTS

Renal function: Gentamicin administration to Fischer rats for 12 days caused a significant decrease in effective glomerular filtration as evidenced by a serum creatinine of $3.54 \pm 0.19 \text{ mg/dl}$. Concurrent nitrendipine treatment ameliorated the severity of the gentamicin-induced renal dysfunction and serum creatinine was $1.64 \pm 0.08 \text{ mg/dl}$ in that group. The difference between the two groups was significant below the 0.001 level.

Renal function studies and hemodynamics are detailed in Table I, and confirmed the beneficial effect of nitrendipine. Inulin clearance was significantly reduced in the gentamicin-treated animals ($4.9 \pm 0.8 \text{ ml/min/kg}$) (Table I). Normal rats have an inulin clearance of $14 \pm 1 \text{ ml/min/kg}$ under similar experimental conditions. Nitrendipine administration was associated with a significant improvement in inulin clearance ($9.8 \pm 0.7 \text{ ml/min/kg}$), although it remained less than normal. PAH clearance was reduced in both groups of gentamicin-treated rats, and nitrendipine administration did not alter the calculated PAH clearance. This calculated clearance was derived utilizing PAH extraction because of the diminished tubular function. It was noted that the PAH extraction was significantly increased in the nitrendipine-treated group suggesting that tubular function was preserved in these animals. The relative kidney weight was decreased in the nitrendipine-treated group, but no differences were evident in hematocrit or body weight.

The tissue content of gentamicin as measured in homogenates of whole kidney was similar in both gentamicin groups and was not influenced by nitrendipine administration (Table II).

Histopathologic abnormalities in the kidneys of gentamicin-treated rats were similar in the groups dosed for 12 and 14 days and are considered together. There was widespread proximal tubular cell vacuolation. Intratubular casts were noted throughout the medulla, and contained both hyaline proteinaceous material and cellular debris. Extensive tubular necrosis was present in the renal cortex but was patchy in its distribution. Proximal tubules were predominantly involved and showed dilatation, loss of brush border, flattened desquamating epithelial lining, and loss of nuclear staining. Interstitial inflammatory cells were much in evidence, especially in the regions of tubular necrosis. Widespread regenerative changes were observed adjacent to the necrotic areas, with cellular basophilia and occasional mitotic figures. The severity and distribution of all the pathologic abnormalities was significantly reduced in the nitrendipine-treated animals. A semiquantitative analysis and comparison of the histopathology is shown in Table III.

DISCUSSION

In this study, we have demonstrated that nitrendipine, a slow calcium channel blocker, offered significant protection against the development of gentamicin nephrotoxicity in the rat. Simultaneous administration of nitrendipine to gentamicin-treated animals preserved effective glomerular filtration, tubular function, and renal tubular morphology.

The nephrotoxicity of the aminoglycoside antibiotics is related in part to their biochemical structure and electrochemical properties. Gentamicin, a polycationic molecule with five amino groups, is minimally protein bound in the circulation and is readily filtered at the glomerulus. Within the tubular lumen, it is taken up by the convoluted and straight portion of the proximal tubule, where it enters a
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<th></th>
<th>$C_{IN}$ (ml/min/kg)</th>
<th>$C_{PAH}$ (with extraction) ml/min/kg</th>
<th>Extraction (%)</th>
<th>MBP (mm Hg)</th>
<th>Hct (%)</th>
<th>BW (g)</th>
<th>KW/BW ($\times 10^{-3}$)</th>
</tr>
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<tbody>
<tr>
<td>Gentamicin–nitrendipine ($n = 6$)</td>
<td>9.8 ± 0.7</td>
<td>29.0 ± 4.0</td>
<td>48 ± 3</td>
<td>133 ± 2</td>
<td>49 ± 1</td>
<td>192 ± 5</td>
<td>9.2 ± 0.2</td>
</tr>
<tr>
<td>Gentamicin–polyethylene glycol ($n = 8$)</td>
<td>4.9 ± 0.8</td>
<td>29.2 ± 4.4</td>
<td>26 ± 3</td>
<td>119 ± 3</td>
<td>50 ± 2</td>
<td>201 ± 9</td>
<td>12 ± 1.0</td>
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Note. Data are means ± SEM. $C_{IN}$—inulin clearance; $C_{PAH}$—PAH clearance; MBP—mean blood pressure; Hct—hematocrit; BW—Body weight; KW—Kidney weight. Normal untreated rats have inulin clearance 14 ± 1 ml/min/kg, and PAH clearance 47 ± 4 ml/min/kg under similar conditions.
TABLE II
Renal Gentamicin Content in Rats Given Gentamicin and Treated with Nitrendipine or Polyethylene Glycol

<table>
<thead>
<tr>
<th>Gentamicin levels (µg gentamicin/mg kidney wet wt)</th>
<th>Gentamicin–nitrendipine</th>
<th>Gentamicin–polyethylene glycol</th>
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<tr>
<td></td>
<td>0.689 ± 0.08</td>
<td>0.543 ± 0.04</td>
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Note. Data are means ± SEM. Six animals in each group given gentamicin 40 mg/kg for 4 days.

poorly exchangeable storage pool with a prolonged half life. As a result of this proximal tubule storage phenomenon, renal cortical gentamicin levels may exceed plasma levels by 100-fold, and of the total renal gentamicin content, 80% is present in the cortex and 20% in the medulla (Luft and Kleit, 1974). It was of interest to note that nitrendipine treatment did not alter net accumulation of gentamicin in the kidney. However, it is possible that measurement of the “whole kidney” gentamicin could have masked subtle difference in renal cortical gentamicin content. In addition these rats received nitrendipine and gentamicin for only 4 days prior to the onset of renal dysfunction.

Approximately 40% of the filtered gentamicin is reabsorbed along the proximal tubule (Seriekjian et al., 1981). A high- and low-affinity phospholipid receptor for gentamicin has been identified on the brush border membrane. The initial step in the reabsorptive process is the charge interaction between the cationic aminoglycoside and the anionic phospholipid receptor (Schacht, 1979). Subsequent pinocytosis results in the appearance of gentamicin in apical vesicles within 10 min, and in secondary lysosomes (cytosegrosomes) after 1 hr (Silverblatt and Juehn, 1979). Pinocytosis, vesicle formation, and transport are energy-requiring processes which may be inhibited by anoxia or 2,4-dinitrophenol.

Gentamicin binding to its brush border receptor is competitively inhibited by other aminoglycosides and by divalent cations including calcium. This probably explains the protective effect of oral calcium loading on gentamicin nephrotoxicity (Humes et al., 1984). It has been postulated that the biochemical structure of the receptor site might be altered in experimental diabetes mellitus, with reduced binding and uptake, thus conferring protection against renal injury (Teixeira et al., 1982). The influence of nitrendipine on local calcium activity at the gentamicin-receptor interface is unknown, but could explain a decreased cellular uptake of the aminoglycoside.

The nephrotoxicity resulting from aminoglycoside administration leads to bio-

TABLE III
Renal Histopathology in Rats Given Gentamicin and Treated Concurrently with Nitrendipine or Polyethylene Glycol

<table>
<thead>
<tr>
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<th>Tubular necrosis</th>
<th>Tubular casts</th>
<th>Tubular regeneration</th>
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</thead>
<tbody>
<tr>
<td>Gentamicin–nitrendipine</td>
<td>1.25 ± 0.22</td>
<td>0.23 ± 0.12</td>
<td>1.58 ± 0.31</td>
</tr>
<tr>
<td>Gentamicin–polyethylene glycol</td>
<td>2.81 ± 0.11</td>
<td>2.12 ± 0.25</td>
<td>2.50 ± 0.14</td>
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<tr>
<td></td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
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</table>

Note. Data are means ± SEM. Twelve animals in each group. Results are based on a scale of 0 to 4, where 0 is normal. Statistical comparison based on Wilcoxon rank sum test.
chemical, functional, and pathological alterations, whose interrelationships are not completely understood. Attempts to modify gentamicin nephrotoxicity have included inhibition of angiotensin converting enzyme, which in fact improved renal hemodynamics and function, but did not offer histologic protection (Schor et al., 1981). These observations suggest a discordance between structure and function, a feature commonly noted in experimental and clinical acute renal failure.

One group of investigators has attributed the fall in glomerular filtration rate in gentamicin nephrotoxicity to a decrease in glomerular ultrafiltration coefficient (Baylis et al., 1977). However, in the setting of widespread tubular necrosis, it is difficult to ignore the contribution of impaired tubular integrity, tubular backleak, and obstruction. Nitrendipine, a potent vasodilator, did not in fact influence PAH clearance in this study and presumably did not mediate its beneficial protective effect by altering renal hemodynamics. The improved histopathologic appearance of the tubules would suggest that nitrendipine either decreased gentamicin uptake and/or prevented its destructive influence within the tubular cell.

Gentamicin becomes sequestered within ‘‘cytosegrosomes’’ in the proximal tubule cell and in addition causes significant mitochondrial injury. It interferes with magnesium-controlled ionic permeability at the inner mitochondrial membrane, and results in a partial uncoupling of oxidative phosphorylation (Humes et al., 1982a). In addition, lysosomal dysfunction is probably important in the cellular damage consequent on gentamicin administration. The sequestration of gentamicin with large lysosomes, and the relative stability of these ‘‘cytosegrosomes’’ suggests that the cell may be deprived of normal lysosomal function. There is no evidence to suggest that nitrendipine directly protects against the mitochondrial injury or alters lysosomal function in this situation.

We would postulate that nitrendipine acts by altering calcium activity at the brush border membrane of the proximal tubule, which in turn reduces gentamicin binding and uptake. It is possible that nitrendipine may decrease vesicle formation and the actual process of pinocytosis. Whether this protective effect of nitrendipine is common to all the calcium channel blockers remains to be determined. The observations reported in this study could have important clinical application.

ACKNOWLEDGMENTS

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REFERENCES