

Protective Effect of Cephalothin Against Gentamicin-Induced Nephrotoxicity in Rats

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The possibility that the nephrotoxicity of gentamicin may be potentiated by the concomitant administration of cephalothin was examined in a rat model. Cephalothin given once daily in dosages up to 800 mg/kg per day for 10 days produced no renal damage. Gentamicin, at 6 to 50 mg/kg per day, caused pathological changes which were dosage related and affected primarily the proximal tubular cells. Administration of the two drugs simultaneously resulted in a significant protective effect of cephalothin against gentamicin-related nephrotoxicity ($P < 0.01$). When the daily injections of the two agents were separated by an interval of 6 h, the protective effect was lost, and the resultant damage was the same as that due to gentamicin alone. The protective effect of cephalothin was reproduced by the administration of equimolar amounts of sulfate (sodium sulfate), suggesting that the phenomenon might be related to the presence of nonresorbable anion in the urine. These studies indicate that, in the rat, cephalothin does not potentiate, but, in fact, may prevent the nephrotoxic effects of gentamicin.

Cephalothin and gentamicin are frequently administered concomitantly. Recently, there have been reports of renal failure in some patients receiving this combination (5, 8, 11, 15, 20). This finding has led to the suggestion that nephrotoxicity may be substantially more frequent in these individuals than in those receiving either agent alone. Cephalothin itself appears to cause renal damage only rarely (3, 7, 9, 21-23, 26). Nephrotoxicity due to gentamicin, though uncommon, is believed to occur in about 2 to 5% of recipients of the drug (10, 14, 24, 29).

The true incidence of renal injury due to cephalothin, gentamicin, or their combination is difficult to determine in humans because of the multiplicity of variables present; these include underlying illnesses and concurrent administration of other drugs which may predispose to renal damage (14). The purpose of the present study was to examine the nephrotoxicity of cephalothin and gentamicin, alone and in combination, using an animal model in which other variables could be controlled.

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January 1975; and at the Ninth International Congress of Chemotherapy, London, England, 13-18 July 1975.)

MATERIALS AND METHODS

Male Fischer 344 rats (Microbiological Associates, Bethesda, Md.) 10 to 12 weeks old, weighing 200 to 300 g, were housed six per cage and fed commercial rat chow and water. Antibiotics were administered subcutaneously in the abdominal wall in single daily injections for 10 days. When the two drugs were given simultaneously, they were delivered at separate sites. Control animals were given comparable volumes of 0.85% sodium chloride.

The following solutions were injected: sodium cephalothin (Eli Lilly and Co., Indianapolis, Ind.), 200 mg/ml of isotonic saline; gentamicin sulfate injectable (Schering Corp., Kenilworth, N.J.), 40 mg/ml; sodium sulfate (J. T. Baker Chemical Co., Phillipsburg, N.J.), 71.85 mg/ml of distilled water; and isotonic saline (0.85% sodium chloride in distilled water).

Samples of blood for the assay of antibiotics were obtained 1 h after the last injection on the tenth day of therapy. Approximately 1 ml of blood was aspirated from the retro-orbital plexus, with the animals under light ether anesthesia. An agar diffusion bioassay was performed as described previously (2). For the assay of gentamicin in the presence of cephalothin, a potent cephalosporinase (Eli Lilly and Co.) was incorporated into the agar (2).

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The animals were lightly anesthetized with ether, and bilateral nephrectomy was performed 3 days after the last injection of drugs. Blood was obtained from the aorta at this time, and the concentrations of urea nitrogen and serum creatinine were determined by use of an Autoanalyzer.

Each kidney was trisected longitudinally and placed in buffered 10% formalin. The specimens were submitted under numerical code to the pathologist, who was unaware of the treatment given to any particular animal. The kidneys were routinely processed and sectioned at 4 μ m, and the sections were stained with hematoxylin and eosin or with periodic acid Schiff's reagent.

Histologic evaluation. From observations made in preliminary studies, the following scoring system ("tubular necrosis score") was devised: 0, normal; 1, rare foci of swelling ("ballooning") of tubular epithelial cells, or periodic acid Schiff-positive globular changes in the cytoplasm of tubular cells; 2, focal patchy swelling of tubular epithelial cells; 3, same as 2 with rare foci of tubular cell necrosis; 4, extensive patchy tubular necrosis with loss of tubular epithelial cells, eosinophilic granular casts, and tubular cell degenerative and/or regenerative changes; 5, nearly complete cortical necrosis.

Design of individual experiments. Preliminary studies demonstrated that the F344 strain was highly susceptible to the nephrotoxic effects of gentamicin and that renal damage was substantially more severe when the antibiotic was administered for 10 rather than for 5 days. Although histologic abnormalities were evident for at least 1 week, they were more striking after an interval of 3 days after the last dose of gentamicin. For this reason, in the experiments to be described, a 10-day course of therapy was given, and the rats were killed 3 days later. Each group contained five rats, except in the experiment with sodium sulfate. The volume of isotonic saline injected into the control animals was the same as that used to administer the antibiotics.

Cephalothin alone. Cephalothin alone was administered in dosages of 200, 400, or 800 mg/kg per day.

Gentamicin alone. Gentamicin alone was administered in dosages of 6, 12, 25, or 50 mg/kg per day.

Checkerboard. A "checkerboard" format was used to study the interaction between cephalothin and gentamicin, administered simultaneously. Twenty groups of rats were given cephalothin alone in doses of 0 (saline), 200, 400, and 800 mg/kg per day, or together with gentamicin in doses of 0 (saline), 6, 12, 25, or 50 mg/kg per day.

Dose separation. The effects of separating the daily injections of the two agents by an interval of 6 h were studied. The drugs were given in the following way: cephalothin alone, 400 mg/kg per day; gentamicin alone, 12 mg/kg per day; the combination given simultaneously; gentamicin followed 6 h later by cephalothin; cephalothin followed 6 h later by gentamicin; and isotonic saline (controls).

Sulfate. Because cephalothin excreted in the urine acts as a nonresorbable anion, the effects of

another nonresorbable anion, sulfate, were studied. Five groups of rats received the following substances; isotonic saline (5 rats); sodium sulfate (10 rats); gentamicin, 12 mg/kg per day (6 rats); gentamicin, 12 mg/kg per day, and cephalothin, 400 mg/kg per day, given simultaneously (5 rats); gentamicin, 12 mg/kg per day, and sodium sulfate given simultaneously (10 rats). The solutions of cephalothin and sulfate were equiosmolar with respect to cephalothin and sulfate anions (0.506 osmol/liter), and they were administered in the same doses (2 ml/kg of body weight). There was a total of 1.303 osmol/liter of sodium cephalothin solution (sodium, cephalothin, and chloride) and 1.518 osmol/liter of sodium sulfate (sodium and sulfate).

RESULTS

Pathological changes were found only in the cortex of the kidney. The most striking abnormality was tubular necrosis after injection of gentamicin, affecting primarily the proximal tubules. This change was often accompanied by tubular mitoses and less commonly by an interstitial infiltrate of mononuclear cells. The glomeruli were spared even in severely damaged kidneys. Control animals (isotonic saline) frequently exhibited mild abnormalities of renal histology, although these rarely exceeded a tubular necrosis score of 1. The mean score for control groups was, therefore, generally 0.5 to 1.0.

Cephalothin alone. Cephalothin in doses of 200, 400, or 800 mg/kg per day for 10 days produced a mean tubular necrosis score that was not significantly different from that after the injection of the same volume of isotonic saline (Fig. 1, top).

Gentamicin alone. Gentamicin, in amounts ranging from 6 to 50 mg/kg per day, produced tubular necrosis scores which increased linearly with increasing dosage of the antibiotic (Fig. 1, bottom).

Checkerboard. The results with either drug alone were identical to those observed in the two preceding studies (Fig. 2). Animals receiving a particular dosage of cephalothin exhibited increasing renal damage with progressively higher doses of gentamicin. Most striking, however, was the finding that, for any constant dose of gentamicin, tubular necrosis was diminished by the simultaneous administration of cephalothin. Analysis of variance showed that the interaction between cephalothin and gentamicin was highly significant ($P < 0.01$). Cephalothin appeared, therefore, to protect against the nephrotoxic effects of gentamicin.

Blood urea nitrogen and serum creatinine concentrations, measured at the time of death, were similar (mean = 20-25 and 0.5-0.8 mg/100 ml, respectively) in all groups except the one

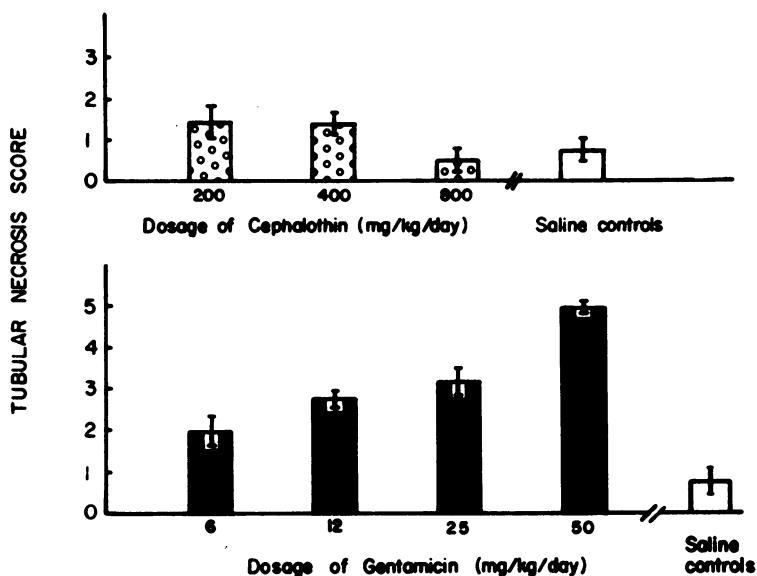


FIG. 1. Top, Pathologic changes (tubular necrosis score) in rats receiving 200, 400, or 800 mg/kg per day of cephalothin for 10 days. Bottom, Tubular necrosis scores in rats receiving gentamicin in dosages of 6 to 50 mg/kg per day for 10 days. Each bar indicates the mean and standard error of values for five animals.

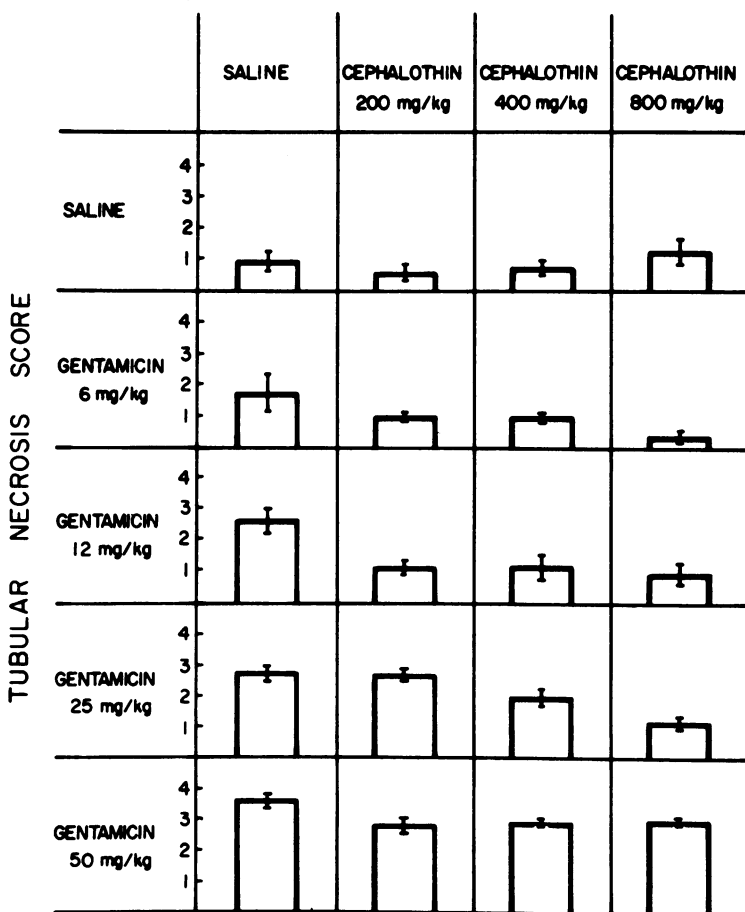


FIG. 2. Tubular necrosis scores in animals receiving varying doses of cephalothin alone, gentamicin alone, or the combination. Each bar represents the mean and standard error of results in five rats.

given gentamicin, 50 mg/kg per day with no cephalothin (mean = 52 and 1.6 mg/100 ml, respectively). Both indices of renal injury were significantly higher ($P < 0.05$) in animals receiving gentamicin (50 mg/kg) alone than in groups given the same quantity of gentamicin together with any dosage of cephalothin.

The levels of cephalothin and gentamicin in serum drawn 1 h after the last injection of antibiotic showed considerable variation within each group. There was no significant effect of concomitant administration of cephalothin on the levels of gentamicin (Table 1), when these values were subjected to an analysis of variance.

Dose separation. As before, the injection of cephalothin (400 mg/kg per day) concomitantly with gentamicin (12 mg/kg per day) produced a significant reduction in the mean tubular necrosis score compared to that of gentamicin alone ($P < 0.01$) (Fig. 3). The histologic changes in animals receiving the two antibiotics simul-

taneously were not significantly different from those in rats receiving cephalothin alone, or from controls. When the injections of cephalothin and gentamicin were separated by an interval of 6 h, however, the protective effect was abolished and the resultant changes were similar to those due to gentamicin alone. This was true irrespective of which agent was given first.

Sulfate. There appeared to be greater variability within groups of animals in the sulfate experiment than in the previous ones, for reasons which are not clear. Rats receiving sodium sulfate alone showed minimal histologic changes similar to those observed in controls, and markedly less than those in animals given gentamicin alone ($P < 0.01$) (Fig. 4). As in previous experiments, simultaneous injection of cephalothin and gentamicin produced a mean tubular necrosis score lower than that resulting from gentamicin alone; in this case, however, the differences were not quite significant ($P = 0.1$ to 0.2), presumably because of the greater intragroup variability. The most striking result was the finding that the mean tubular necrosis score in animals given gentamicin with sodium sulfate was almost identical to that in rats given gentamicin with cephalothin. When compared with gentamicin alone, the protective effect of sulfate was of borderline statistical significance ($P = 0.05$ to 0.1).

TABLE 1. Mean concentrations of gentamicin in serum 1 h after the last injection

Substance administered	Saline	Cephalothin		
		200 mg/kg	400 mg/kg	800 mg/kg
Saline	0	0	0	0
Gentamicin				
6 mg/kg	7.9	7.3	6.7	6.3
12 mg/kg	17	15	12	11
25 mg/kg	34	36	29	35
50 mg/kg	63	57	61	50

DISCUSSION

The rat has proven to be a useful model in which to study the potential nephrotoxicity of a number of agents, including the cephalo-

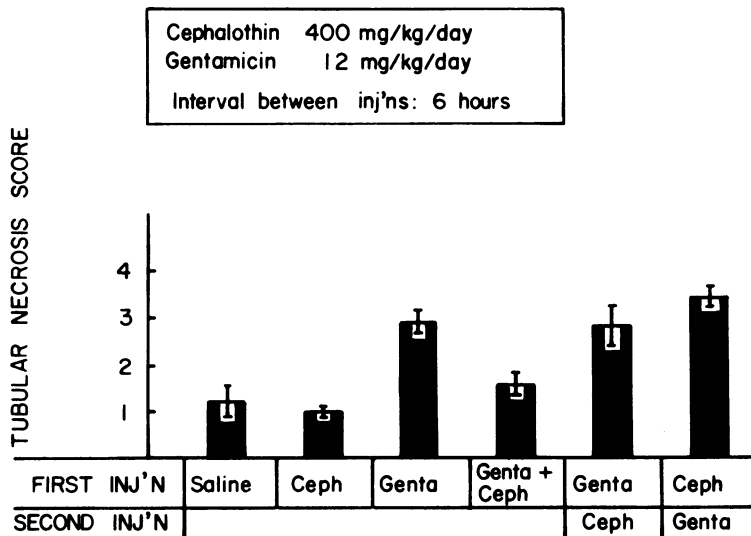


FIG. 3. Effect of temporal separation of injections on tubular necrosis scores. Second injection given 6 h after the first. Each bar indicates the mean and standard error of results in five rats.

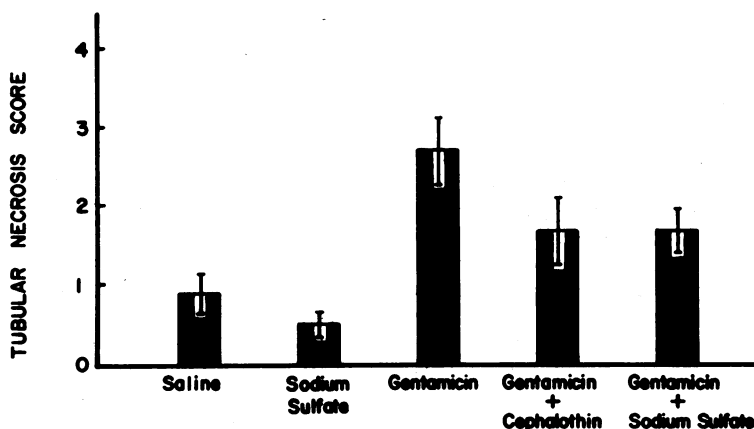


FIG. 4. Comparison of effects of sodium sulfate with those of cephalothin on nephrotoxicity due to gentamicin. Each of ten animals was given sulfate or gentamicin plus sulfate; other groups consist of five to six rats. Bars represent mean and standard error of tubular necrosis scores.

sporins (25, 27, 28) and aminoglycosides (4, 12, 13, 16). Combinations of drugs—furosemide and cephalothin (17, 18) and methoxyfluorane and gentamicin (1)—have also been examined. Cephalothin itself appears to be essentially free of nephrotoxicity in this animal (25). The extent of renal damage produced by gentamicin depends upon the age, sex, and species of rat, as well as on the dose and duration of therapy (4, 12, 16). It has recently been noted that the concurrent administration to rats of gentamicin (20 mg/kg) and cephalothin, cephaloridine, or cefazolin (500 mg/kg) daily for 4 weeks produced renal damage no more severe than that found with gentamicin alone (13). A protective effect of the cephalosporins was not mentioned.

The results of the present study support the notion that cephalothin is not intrinsically nephrotoxic in the rat, and that the effects of gentamicin are dose related and are manifested primarily in the proximal renal tubular cells. A striking result was the finding that the simultaneous administration of cephalothin and gentamicin, in a variety of dosages, produced a significant diminution in the nephrotoxicity of the aminoglycoside. This phenomenon did not appear to be related to a difference in the peak serum level of gentamicin. We did not, however, examine the effect of one drug upon the rate of urinary elimination or half-life of the other. When the daily injections of the two antibiotics were separated by an interval of 6 h, rather than being administered simultaneously, the protective effect of cephalothin was lost, and the resultant histologic damage was the same as that caused by gentamicin alone.

The possibility was considered that the protective influence of cephalothin might be re-

lated to alterations in the transport of sodium and water in the proximal tubule. This change could result from the effects of volume expansion on the proximal tubular reabsorption of sodium, or from the presence of cephalothin as a nonresorbable anion in the proximal nephron. The administration of this antibiotic in a dosage of 400 mg/kg to a rat weighing 250 g would impose an osmotic load equivalent to that contained in 2.25 ml of isotonic saline. Since about 3 ml of saline is required to produce a demonstrable effect on the reabsorption of salt and water by the proximal renal tubule in the rat (6), it seemed unlikely that the findings were due simply to the effects of volume expansion. The other possibility is that cephalothin, once excreted into the proximal tubule, acts as a nonresorbable anion and that its presence, in some manner, affects the intrarenal transport and/or accumulation of gentamicin. To test this hypothesis, we administered sodium sulfate so that the amount of anion was equiosmolar to that of cephalothin. The results, although not quite statistically significant, strongly suggested that sulfate protected against gentamicin nephrotoxicity to the same extent as did cephalothin.

Gentamicin is known to accumulate in the renal cortex of the rat in concentrations greatly exceeding peak levels in the serum, and to exhibit a much longer half-life in the former than in the latter site (19). We have demonstrated that the concurrent administration of cephalothin results in a significant reduction in the renal cortical concentrations of gentamicin (Dellinger et al., manuscript submitted for publication). One interpretation of these data is that the presence of nonresorbable anion

within the proximal tubule somehow reduces the renal accumulation and, hence, the nephrotoxicity of gentamicin. The precise relations of these various findings, however, await further definition.

The results of these studies indicate that, rather than potentiating, cephalothin actually protects against the nephrotoxicity of gentamicin in this experimental model if the two agents are given simultaneously. When injections of the two drugs are separated by an interval of 6 h, the resultant histologic change is consistent with that seen with gentamicin alone. Although not necessarily applicable to humans, particularly with underlying renal disease, these findings lend no support to the suggestion that cephalothin may potentiate the nephrotoxic effects of gentamicin.

ADDENDUM

Since this manuscript was submitted, two controlled studies in man have been reported.

Gaya, Klastersky, and Schimpff (Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 15th, Washington, D.C., Abstr. 401, 1975) reported that they had observed a significantly higher incidence of nephrotoxicity among patients receiving cephalothin and gentamicin than among individuals receiving carbenicillin plus gentamicin or carbenicillin plus cephalothin.

In the second study, Fanning, Jick, and Gump (Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 15th, Washington, D.C., Abstr. 402, 1975) reported no increased renal impairment produced by the combination as compared to either agent alone.

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