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Protection from Gentamicin Nephrotoxicity by Cephalothin and Carbenicillin

RICHARD BLOCH,† FRIEDRICH C. LUFT,* LAURA I. RANKIN, REBECCA S. SLOAN, MOO NAHM YUM, AND DOUGLAS R. MAXWELL

Departments of Medicine and Pathology, Indiana University Medical Center, Indianapolis, Indiana 46223

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In rats, cephalothin exerts a protective effect upon the nephrotoxicity of gentamicin. To examine the possibility that this effect is also observed with carbenicillin, we gave the following (milligrams per kilogram) to rats daily for 14 days: gentamicin alone, 60; gentamicin plus cephalothin, 100, 500, or 1,000; gentamicin plus carbenicillin, 50, 100, 250, 500, or 1,000. A 500-mg/kg dose of cephalothin afforded significant partial protection from gentamicin nephrotoxicity, as did a 100-mg/kg dose of carbenicillin. Increasing doses of either drug failed to increase protection. The data suggest that in rats not only does carbenicillin afford some protection from gentamicin nephrotoxicity, but also that it does so at a lower dose than cephalothin. These findings may in part explain the divergent observations regarding the nephrotoxicity of cephalothin-gentamicin combination therapy in rats and humans.

In humans, the results of a retrospective (6) and a prospective (18) study showed that the combination of gentamicin and cephalothin was more nephrotoxic than gentamicin combined with a penicillin derivative. In rats, investigators reported that cephalosporins either had no effect on gentamicin nephrotoxicity (8) or actually ameliorated the nephrotoxicity observed with gentamicin (1, 4, 12, 17; A. Sugarman, R. S. Brown, and S. Roger, Abstr. Proc. Am. Soc. Nephrol. **9**:80, 1976). The studies in humans differed from those in experimental animals since no patients were included who received gentamicin as the sole antimicrobial agent. In the studies in rats, on the other hand, the results observed with cephalosporin-gentamicin combinations were compared with the results with gentamicin alone. We conducted additional experiments in rats in which the combination of cephalothin and gentamicin was compared with the combination of gentamicin and carbenicillin, as well as with gentamicin alone. We found that in rats carbenicillin also ameliorated gentamicin nephrotoxicity and that it did so at a lower dose than that of cephalothin.

MATERIALS AND METHODS

Adult male Sprague-Dawley rats (Cox Laboratories, Indianapolis, Ind.) weighing 200 to 225 g were housed and fed as described elsewhere (7). Ten groups of 12 rats each were studied (see Table 1 for group regimens). The animals received subcutaneous injections of the respective drugs dissolved in 1 ml of D5W diluent (Abbottt Laboratories, North Chicago, Ill.) or D5W alone daily for 14 days. The drugs were injected at the same time; however, each was given separately into a different site. Gentamicin was given at a dose of 60 mg/kg per day. This dose causes a fairly uniform acute renal failure, with a low mortality, in Sprague-Dawley rats (13). Three doses of cephalothin were chosen: 100, 500, and 1,000 mg/kg per day. Carbenicillin was given at doses of 50, 100, 250, 500, and 1,000 mg/kg per day. Cephalothin contains 2.6 meq of Na per g, whereas carbenicillin contains 4.7 meq of Na per g. The sodium in 100-, 500-, or 1,000-mg/kg cephalothin was approximated by 50-, 250-, or 500-mg/kg carbenicillin, respectively. The 100- and 1,000-mg/kg carbenicillin doses corresponded to the 100- and 1,000-mg/kg cephalothin doses on a weight basis.

On the day before the first injection (day 0), day 7, and day 15, 24-h urine specimens were collected, and volume, osmolality, and *N*-acetylglucosaminidase (NAG) were measured. Blood was obtained from the tail for blood urea nitrogen (BUN) determinations. On the day of sacrifice (day 15), creatinine was measured in serum and urine for the calculation of creatinine clearance. The kidneys were prepared for light microscopy by standard techniques. Histological changes were graded by a pathologist unaware of the regimens as follows: grade 0, normal; grade 1, necrosis <25% of the cortical area; grade 2, necrosis ≥25%, <50% of the cortical area; grade 3, necrosis ≥50%, <75% of the cortical area; and grade 4, necrosis ≥75% of the cortical area. Renal tissue was also obtained for measurement of gentamicin concentrations. The kidneys were weighed, snap frozen in liquid nitrogen, pulverized, and diluted with phosphate buffer (pH 8). A beta-lactamase (Whatman Biochemical Ltd., Kent, England) which inactivates both cephalothin and carbenicillin was added to the specimens. Gentamicin concentrations were measured by agar well diffusion, us-

† Present address: Arnett Clinic, Lafayette, IN 47902.

ing a strain of *Bacillus subtilis* as the marker organism (2). Standards were prepared in homogenates of rat kidney prepared in an identical fashion.

NAG in urine was measured by the technique of Patel et al. (16), BUN was measured by the method of March et al. (15), and creatinine was measured by the Autoanalyzer technique (Technicon, Tarrytown, N.Y.). The urine osmolalities were determined by freezing-point depression (Advanced Instruments, Newton Highlands, Mass.). Data were analyzed by one-way analysis of variance. Specific comparisons between pairs of regimens were made with Duncan's test. Orthogonal Fisher's exact tests were used to compare the pathological scores.

RESULTS

The effects of the regimens on enzymuria, urine osmolality, and BUN are shown in Table 1. All regimens except the D5W diluent produced significant decreases in urine osmolality by day 7 ($P < 0.05$). The regimens containing ≥500-mg/kg cephalothin or ≥250-mg/kg carbenicillin decreased urine osmolality to a lesser degree than did gentamicin alone ($P < 0.05$). This pattern was no longer evident by day 15, at which time urine osmolality had decreased to less than one-half of the pretreatment values in all groups receiving gentamicin. By day 7, the concentrations of NAG in urine increased at least fivefold in the groups receiving gentamicin ($P < 0.05$) (Table 1). Gentamicin alone, gentamicin plus 100-mg/kg cephalothin, and gentamicin plus the two lower doses of carbenicillin caused more enzymuria than did the other regimens ($P < 0.05$). On day 15, there was no longer a consistent pattern to the responses. The measurement of BUN revealed that animals receiving ≥500-mg/kg cephalothin or ≥250-mg/kg carbenicillin had BUN values significantly lower by day 7 than with other drug treatments ($P < 0.05$) (Table 1). These results remained consistent at day 15 ($P < 0.05$).

The results of creatinine clearance measurements, drug concentration in the kidney, and the pathological scores are shown in Table 2. A single specimen from group 9 was inadvertently destroyed and therefore could not be included in the analysis. Gentamicin alone, gentamicin plus 100-mg/kg cephalothin, and gentamicin plus 50-mg/kg carbenicillin caused a greater decrease in creatinine clearance than did any other regimen ($P < 0.05$). However, groups receiving ≥100-mg/kg carbenicillin and ≥500-mg/kg cephalothin in conjunction with gentamicin had lower creatinine clearance values than did controls ($P < 0.05$). Gentamicin concentrations in the kidney at sacrifice were not consistently affected by either beta-lactam antibiotic. The histological damage was less severe in animals receiving 1,000-mg/kg cephalothin, 500-mg/kg carbenicillin, or 1,000-mg/kg carbenicillin than in animals receiving gentamicin alone or combined with lower doses of these drugs ($P < 0.05$). However, the amelioration of renal injury was far from complete, and all antibiotic-containing regimens showed significant damage when compared with controls ($P < 0.05$).

DISCUSSION

These experiments indicate that both cephalothin and carbenicillin exert an ameliorative effect on gentamicin nephrotoxicity in rats. Carbenicillin produced this effect at a lower dose

TABLE 1. *Effects of the regimens on urine osmolality, enzymuria, and BUN (mean ± standard error of the mean)*

Regimen[a]	Osmolality (mosmol/kg of water)			NAG (mU/24 h)			BUN (mg/dl)		
	Day 0	Day 7	Day 15	Day 0	Day 7	Day 15	Day 0	Day 7	Day 15
G 60 mg/kg	2,469 ± 91	1137 ± 170	807 ± 78	229 ± 66	1,734 ± 212	628 ± 70	19 ± 1	35 ± 2	55 ± 5
G + K, 100 mg/kg	2,094 ± 118	1,310 ± 182	889 ± 173	236 ± 41	2,281 ± 387	641 ± 43	19 ± 1	29 ± 3	41 ± 2[b]
G + K, 500 mg/kg	2,288 ± 72	1,810 ± 64[b]	921 ± 108	182 ± 21	864 ± 100[b]	969 ± 124[b]	19 ± 1	26 ± 2[b]	31 ± 2[b]
G + K, 1,000 mg/kg	2,276 ± 120	1,568 ± 116[b]	1,342 ± 121[b]	183 ± 20	724 ± 85[b]	846 ± 69	21 ± 1	24 ± 1[b]	31 ± 2[b]
G + C, 50 mg/kg	2,253 ± 130	1,100 ± 70	698 ± 87	243 ± 27	1,772 ± 495	2,031 ± 223[b]	19 ± 1	35 ± 4	48 ± 4
G + C, 100 mg/kg	2,373 ± 163	1,331 ± 187	921 ± 108	153 ± 24	1,683 ± 440	1,098 ± 109[b]	19 ± 1	28 ± 2	30 ± 2[b]
G + C, 250 mg/kg	2,408 ± 113	1,716 ± 140[b]	856 ± 79	184 ± 18	828 ± 118[b]	1,029 ± 139[b]	19 ± 1	24 ± 1[b]	30 ± 3[b]
G + C, 500 mg/kg	2,310 ± 111	1,694 ± 78[b]	1,012 ± 95[b]	171 ± 21	726 ± 93[b]	686 ± 86	22 ± 1	26 ± 2[b]	27 ± 1[b]
G + C, 1,000 mg/kg	2,326 ± 125	1,937 ± 115[b]	702 ± 129	197 ± 19	798 ± 71[b]	1,079 ± 196[b]	18 ± 1	22 ± 1[b]	30 ± 2[b]
D5W/ml/day	2,392 ± 120	2,301 ± 149[b]	2,027 ± 119[b]	150 ± 23	228 ± 21[b]	269 ± 21[b]	23 ± 1[b]	21 ± 1[b]	20 ± 1[b]

[a] G, Gentamicin; K, cephalothin; C, carbenicillin; D5W, diluent.
[b] Compared with G, $P < 0.05$.

TABLE 2. *Regimens and their effects at sacrifice (mean ± standard error of the mean)*

Regimen[a]	Creatinine clearance (ml/min)	G in kidney (μg/g)	No. of rats with score:				
			0	1	2	3	4
G 60 mg/kg	0.29 ± 0.03	109 ± 6	0	0	0	6	6
G + K, 100 mg/kg	0.41 ± 0.04	190 ± 20	0	1	3	2	6
G + K, 500 mg/kg	0.89 ± 0.10[b]	168 ± 18	0	1	1	6	4
G + K, 1,000 mg/kg	0.99 ± 0.08[b]	156 ± 13	0	6	5	1	0[c]
G + C, 50 mg/kg	0.42 ± 0.06	188 ± 16	0	0	4	3	5
G + C, 100 mg/kg	0.83 ± 0.07[b]	170 ± 14	0	0	1	3	8
G + C, 250 mg/kg	0.86 ± 0.14[b]	203 ± 13	0	1	2	6	3
G + C, 500 mg/kg	0.89 ± 0.12[b]	104 ± 7	0	4	6	2	0[c]
G + C, 1,000 mg/kg	0.86 ± 0.13[b]	161 ± 10	0	7	3	1	0[c]
D5W, 1 ml/day	1.57 ± 0.07[d]		12	0	0	0	0[d]

[a] G, Gentamicin; K, cephalothin; C, carbenicillin, D5W, diluent.
[b] Compared with gentamicin alone, $P < 0.05$.
[c] Compared with other antibiotic regimens, $P < 0.05$.
[d] Compared with all other regimens, $P < 0.05$.

than did cephalothin. Once a given degree of protection was achieved, higher doses of either drug did not appear to afford additional protection. The protective effect resulted in not only improved glomerular filtration as reflected by BUN and creatinine clearance values, but also less severe renal structural damage. The results obtained from measurements of urine osmolality and NAG excretion, which were included in an attempt to monitor tubular function and integrity, were less conclusive. On day 7, these parameters suggested that the addition of increasing amounts of beta-lactam antibiotics provided protection; however, by day 15, a clear separation was no longer observed. Models of aminoglycoside nephrotoxicity are characterized by concomitant tubular necrosis and regeneration (9, 14). It is possible that the addition of a beta-lactam antibiotic does not merely provide protection from aminoglycoside nephrotoxicity, but rather that it delays the course of the process or alters only certain features. In addition, lysosomal acid hydrolases, such as NAG, are imperfect markers of aminoglycoside nephrotoxicity (11) and must be interpreted with caution.

The reason for the amelioration of aminoglycoside toxicity by cephalosporins is not entirely clear. Dellinger et al. (3) noted that aminoglycoside concentrations in renal tissue were lower in animals that also received cephalothin. They suggested that this effect might result in decreased nephrotoxicity. In the present study, beta-lactam antibiotics did not consistently affect gentamicin renal concentrations. Since these measurements were made only once, after 14 days of treatment, it is possible that earlier differences might have been missed. However, in a previous study (12), we found no effect of cephalosporins on aminoglycoside renal concentrations in a similar rat model in which measurements were made on days 5, 10, and 15. Roos and Jackson, on the other hand, found that cephalothin in sufficient doses decreased the renal concentration of gentamicin (17). They also observed that cephalothin increased the excretion of gentamicin. The increased excretion of gentamicin correlated with decreased nephrotoxicity to a greater degree than did the final concentration of gentamicin in the kidney. Their study suggested that protection from gentamicin nephrotoxicity was a function of the enhanced renal excretion of gentamicin by cephalothin. Aminoglycoside measurements in renal tissue by means of bioassay are difficult to interpret because of problems with protein binding (10). Resolution of the discrepancies in results reported thus far may require a study utilizing an isotopically tagged aminoglycoside.

Dellinger et al. (4) have also reported the possibility that protection may be related to the presence of a nonresorbable anion in the urine. They found that the protective effect of cephalothin was reproduced by the administration of sodium sulfate. Sugarman et al. (Abstr. Proc. Am. Soc. Nephrol. **9**:80, 1976) found that the administration of sodium aminohippurate or sodium chloride alone was also capable of ameliorating gentamicin nephrotoxicity. Prior saline loading is known to protect from decreases in the glomerular filtration rate associated with various other nephrotoxic insults. Dibona and associates (5) poisoned saline-loaded rats with $HgCl_2$ and noted no decrease in inulin clearance, although frank tubular necrosis occurred. Non-saline-loaded rats exhibited a marked decrease in inulin clearance, but no greater evidence of tubular damage. Their study indicated that functional impairment was not necessarily related to structural damage. In the present study, carbenicillin, which contains twice the sodium

per weight as cephalothin, afforded protection at a lower dose than that of cephalothin. However, it is unlikely that the protection in this case was simply a matter of volume expansion since both the degree of histological injury and the degree of impairment in glomerular filtration were attenuated. This interpretation is in accord with the study of Roos and Jackson, who found that the protective effect of cephalothin was not a function of its sodium content (17).

Although the doses of cephalothin and carbenicillin employed in the present study were extremely large, when compared with humans on a weight basis, the findings nevertheless may have clinical relevance. A more appropriate perspective is obtained when one compares the dose of a drug in humans with that in rats in terms of the glomerular filtration rate. Maximum doses of carbenicillin and cephalothin in humans approach 30 and 16 g/day, respectively. A 30-g/day carbenicillin dose in humans provides 167 mg of carbenicillin per liter of glomerular filtrate per day, assuming a normal glomerular filtration rate of 125 ml/min. A 16-g/day cephalothin dose provides 88 mg of cephalothin per liter of glomerular filtrate. In rats, protection was observed at a dose of 8.3 mg of carbenicillin per liter of filtrate per day and 42 mg of cephalothin per liter of glomerular filtrate per day. It is conceivable that in humans, beta-lactam antibiotics do not contribute to aminoglycoside nephrotoxicity, but rather that they afford various degrees of protection. Such an interpretation in no way detracts from the recent observations in humans (6, 18), but would serve to reconcile these observations with those in rats.

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