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Role of Sodium in Protection by Extended-Spectrum Penicillins against Tobramycin-Induced Nephrotoxicity

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Salt depletion is known to potentiate aminoglycoside nephrotoxicity, while salt replacement attenuates it. Recent studies have shown that ticarcillin protects against tobramycin and gentamicin nephrotoxicity. It has been suggested that this protection is due to an interaction between ticarcillin and the aminoglycoside. However, it can also be explained by the salt load associated with ticarcillin administration. This study was conducted to examine this question. Tobramycin was administered to eight groups of rats at 100 mg/kg per day intraperitoneally for 10 days. Group 1 rats were salt depleted, while group 2 rats were on a normal salt diet. Rats in groups 3 through 8 were also salt depleted but received, in addition, the following interventions intraperitoneally: group 3, ticarcillin, 300 mg/kg per day (0.37 to 0.39 meq of Na supplement per day); group 4, ticarcillin, 300 mg per day (1.56 meq of Na supplement per day); group 5, ticarcillin, 300 mg/kg per day, and NaCl supplement (1.17 to 1.19 meq/day), resulting in a total load of 1.56 meq/day; group 6, piperacillin, 400 mg/day (0.76 meq of Na supplement per day and equimolar to the ticarcillin dose [300 mg/day] in group 4 rats); group 7, piperacillin, 400 mg/day, and NaCl supplement (0.8 meq/day) for a total Na load of 1.56 meq/day; and group 8, 1.56 meq of Na per day as NaCl. Rats in groups 2, 4, 5, 7, and 8, which received a normal salt diet or its equivalent Na supplement, had no significant change in creatinine clearance (CL_{CR}) over the 10-day period. The remaining groups sustained significant reductions in CL_{CR}, as follows: group 1, −53.0% ($P < 0.05$); group 3, −66.2% ($P < 0.05$); group 6, −79.8% ($P < 0.05$). A positive correlation was found between the concentration of tobramycin in the kidneys and the percent change in CL_{CR} at the end of the study. Concentrations of drug in plasma were highest in group 1 rats, lowest in the rats in groups in which protection was observed, and moderately elevated in the remaining groups of rats. The results of this study suggest the following: (i) that the protective effect of ticarcillin against tobramycin nephrotoxicity is secondary to the obligatory sodium load associated with it, (ii) pharmacokinetic and pharmacodynamic interactions between salt and tobramycin are proposed to explain this effect, (iii) the nephrotoxicity of tobramycin is probably related to the degree of accumulation of the drug in the kidney, and (iv) an in vivo interaction between tobramycin and ticarcillin does not contribute to the protective effect of the penicillin but may influence concentrations in plasma, especially under conditions of severe renal impairment.

Aminoglycosides (AGSs) are frequently used for the treatment of gram-negative bacterial infections, usually in combination with extended-spectrum penicillins, to provide synergistic antipseudomonal activity. One of the major limiting factors in their use is the development of nephrotoxicity, which remains a problem, despite close monitoring of plasma drug levels in patients. Several factors and maneuvers have been reported to modify this effect. In experimental models of AGS nephrotoxicity, a low-sodium diet or dehydration potentiate the fall in the glomerular filtration rate with AGS administration, while NaCl loading or rehydration tend to minimize this fall and decrease renal cortical accumulation of the drug (3, 8, 16).

Concomitant administration of certain antipseudomonal penicillins has also been reported to protect against AGS nephrotoxicity. In vitro studies show that combinations of AGSs and penicillins, such as ticarcillin or carbenicillin, result in a more rapid loss of the AGS than that which occurs in the absence of the penicillins (26). Other studies confirm an in vitro interaction that suggests the formation of an inactive complex (9, 10, 18, 22). If this complex formation occurs in vivo, it may account for the observed decrease in the incidence of nephrotoxicity when combination therapy is used (13, 25). An in vivo interaction is also suggested by the observation that patients with end-stage renal failure have reduced half-lives of gentamicin in serum and increased clearance when gentamicin is coadministered with either carbenicillin or ticarcillin (4, 12). However, an increase in gentamicin clearance is only seen in patients with severe renal impairment, while patients with normal renal function are not expected to be affected since the contribution of this mechanism to the total clearance of the AGS is negligible (7). English et al. (11) have demonstrated in vivo attenuation of functional and structural evidence of tobramycin nephrotoxicity in rats that received ticarcillin concomitantly. Their results are not consistent with drug inactivation in plasma since tobramycin decay curves were not altered significantly. Instead, they suggested that the drug-drug complex is formed in the proximal tubular cell to modify the renal effects of tobramycin. An alternative explanation for the renal-sparing effect is that ticarcillin administration is associated with an obligatory sodium load, since it is available as a divalent sodium salt. This sodium load itself may provide protection against nephrotoxicity.

A recent study from our laboratory examined the effect of ticarcillin on gentamicin-induced nephrotoxicity in salt-depleted rats (20). The results suggest that the salt load associated with the administration of ticarcillin is sufficient to explain its protective effect, the magnitude of which was similar to that of a rat on a normal salt diet. In view of the demonstration of English et al. (11) of a protective effect of ticarcillin against tobramycin-induced nephrotoxicity and

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TABLE 1. CL_{CR}, sodium excretion, urine flow, and potassium excretion in all groups of rats before and after treatment with tobramycin[a]

Group	CL_{CR}		Na excretion (meq/day)		Urine flow (ml/day)		K excretion (meq/day)	
	Before	After	Before	After	Before	After	Before	After
1	1.27 ± 0.11	0.56 ± 0.11[b,c]	0.01 ± 0.00	0.02 ± 0.00[c]	11.3 ± 1.7	18.4 ± 4.0	1.56 ± 0.16[c]	1.40 ± 0.25[c]
2	1.08 ± 0.07	1.16 ± 0.19	1.47 ± 0.11	1.41 ± 0.22	15.7 ± 2.0	27.3 ± 2.6[b]	4.35 ± 0.49	4.12 ± 0.34
3	0.70 ± 0.04	0.23 ± 0.03[b,c]	0.03 ± 0.01	0.38 ± 0.07[b,c]	13.6 ± 3.4	33.0 ± 10.3	1.86 ± 0.12[c]	1.60 ± 0.28[c]
4	1.20 ± 0.11	1.11 ± 0.06	0.01 ± 0.01	1.28 ± 0.13[b]	13.0 ± 2.3	17.9 ± 1.8	1.61 ± 0.19[c]	1.72 ± 0.22[c]
5	1.17 ± 0.13	1.00 ± 0.07	0.02 ± 0.00	1.10 ± 0.12[b]	9.3 ± 0.9	17.3 ± 3.6	1.25 ± 0.16[c]	2.26 ± 0.18[c]
6	1.39 ± 0.17	0.28 ± 0.06[b,c]	0.01 ± 0.01	0.36 ± 0.06[b,c]	11.8 ± 1.4	30.2 ± 7.5[b]	2.05 ± 0.20[c]	1.58 ± 0.15[c]
7	1.39 ± 0.17	0.96 ± 0.30	0.02 ± 0.00	1.08 ± 0.03[b]	17.0 ± 3.5	34.4 ± 2.4[b]	1.62 ± 0.28[c]	2.83 ± 0.26[c]
8	1.04 ± 0.08	1.35 ± 0.09	0.03 ± 0.01	1.26 ± 0.14[b]	13.1 ± 2.0	22.3 ± 3.0[b]	1.77 ± 0.16[c]	2.65 ± 0.22[b,c]

[a] Tobramycin was administered at 100 mg/kg per day for 10 days. Values are means ± standard error of the mean. The interventions for each group are given in the text and the legend to Fig. 1.
[b] $P < 0.05$ compared with the value before drug administration.
[c] $P < 0.05$ compared with group 2.

their suggestion that this may be due to the formation of a complex between the two drugs in the renal tubule, we conducted the present study with the hope of extending our previous observations with gentamicin to tobramycin. To this end we compared the effects of various doses of high salt content (ticarcillin) and low salt content (piperacillin) extended-spectrum penicillins. Our results are consistent with the hypothesis that the protection observed is accounted for by the salt load administered rather than the nature of the penicillin.

MATERIALS AND METHODS

Male Sprague-Dawley rats (weight, approximately 200 g) were used in this study. Rats were prepared for intraperitoneal administration of drugs by implantation of a silastic tube extending from the peritoneum subcutaneously to the nape of the neck and exteriorized. This was implanted 2 days before tobramycin was administered. Tobramycin was administered to all rats for 10 days at a dose of 100 mg/kg intraperitoneally. This dose of tobramycin was chosen based on preliminary experiments in salt-depleted rats, in which it resulted in a 55 to 60% reduction in creatinine clearance (CL_{CR}) 10 days later.

In the first series of experiments, the influence of the salt status of the animals on development of tobramycin-induced nephrotoxicity was assessed. Rats were randomized into two groups. Group 1 ($n = 7$) consisted of salt-depleted rats that were maintained on a low-Na purified diet (Na composition, <0.05%; Ralston Purina Co., St. Louis, Mo.) and tap water for at least 1 week prior to the start of treatment. Group 2 rats ($n = 6$) were fed a regular diet (Na composition, >0.39%; Wayne Lab Blox) and tap water.

In a second series of experiments, the effects of coadministration of tobramycin with ticarcillin or piperacillin on tobramycin nephrotoxicity were assessed in six groups of sodium-depleted rats. Na depletion was achieved in these groups as described above for group 1 rats. Daily intraperitoneal injections of ticarcillin at 300 mg/kg per day (group 3; $n = 5$), ticarcillin at 300 mg/day (group 4; $n = 7$), ticarcillin at a dose equal to that given to group 3 rats (300 mg/kg per day) and supplemented with NaCl to provide a total load of 1.56 meq of sodium per day (group 5; $n = 4$), piperacillin at 400 mg/day (group 6; $n = 6$), piperacillin at 400 mg/day and supplemented with NaCl to provide a total load of 1.56 meq of sodium per day (group 7; $n = 4$), or NaCl at 1.56 meq/day (group 8; $n = 6$) were administered. Rats in groups 4, 5, 7, and 8 received the same amount of Na daily, which was equivalent to that in a normal daily diet (group 2). Rats in groups 4, 6, and 7 received equimolar amounts of ticarcillin and piperacillin but different quantities of salt: 1.56, 0.76, and 1.56 meq/day, respectively. For rats in groups 3 through 8, at least 5 h was allowed between injections of tobramycin and the intervention to minimize intraperitoneal interactions between the drugs.

Blood samples and 24-h urine collections were taken on days 1 and 10 of the study. Rats were placed in metabolic cages at least 2 days prior to the collection day to ensure stable conditions. Measurement of the creatinine concentrations in plasma and urine was done with creatinine autoanalyzer (Beckman Instruments, Inc., Fullerton, Calif.). Sodium and potassium concentrations in urine were measured by flame photometry. At 24 h after the last dose of tobramycin was administered, blood was collected for measurement of tobramycin concentrations in plasma, and the kidneys were excised and frozen until subsequent measurement of tobramycin concentrations in tissue. It was assumed that following a 10-day period of tobramycin administration, these concentrations would represent trough steady-state levels. On the day of measurement, the kidneys were weighed and homogenized by using a Tekmar tissue homogenizer in 0.1 M phosphate-buffered saline (pH 7.4). The homogenate was centrifuged at 3,000 × g for 20 min, and the supernatant was measured for tobramycin concentration by radioimmunoassay. The assay had a sensitivity of 0.18 μg/ml, which was defined as the concentration that could be distinguished from zero with 95% confidence. The coefficient of variation within and between runs ranged between 2.9 and 3.6% for levels of 1 and 8 μg/ml, respectively. The standard curve for the concentrations in tissue was linear between 0.5 and 10 μg/ml, with a correlation coefficient of 0.998 and $P < 0.0001$.

Results are presented as means ± standard errors of the mean. Statistical comparison of intragroup values was done by using the paired Student's t test. Intergroup comparisons were done by using one-way analysis of variance followed by multiple comparisons by the Neuman-Keuls test. The minimum level of statistically significant difference was considered as $P < 0.05$.

RESULTS

Urinary sodium excretion rate. Table 1 shows the mean 24-h urinary Na and K excretion rates in all groups of rats. As expected, Na depletion (group 1) resulted in markedly reduced urinary Na excretion compared with that in group 2

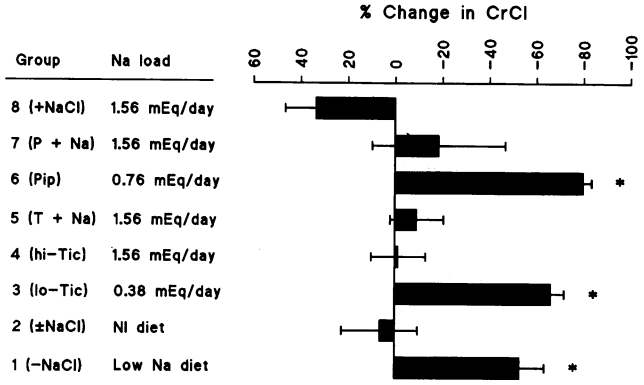

FIG. 1. Percent change in CL_{CR} (CrCl) in eight groups of rats receiving tobramycin at 100 mg/kg per day intraperitoneally and various interventions over 10 days. Abbreviations: −NaCl, salt depleted (group 1); ±NaCl, normal salt diet (group 2); lo-Tic, ticarcillin at 300 mg/kg per day (group 3); hi-Tic, ticarcillin at 300 mg/day (group 4); T + Na, ticarcillin at 300 mg/kg per day plus NaCl (group 5); Pip, piperacillin at 400 mg/day (group 6); P + Na, piperacillin at 400 mg/day plus NaCl (group 7); +NaCl, 1.56 meq of Na per day as NaCl (group 8). Values are means ± standard errors of the mean. Asterisks indicate $P < 0.05$.

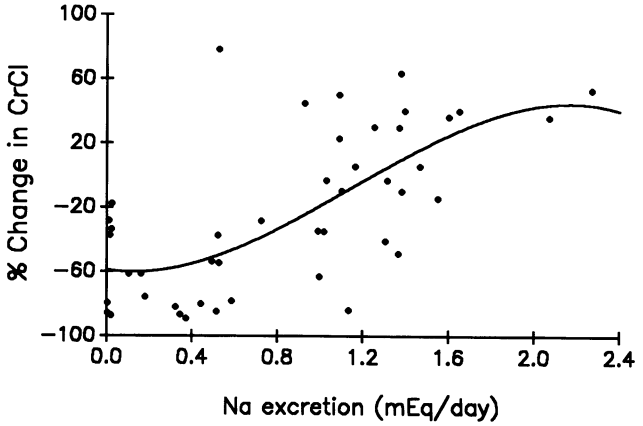

FIG. 2. Relationship between urinary sodium excretion and percent change in CL_{CR} (CrCl) 10 days after daily administration of tobramycin at 100 mg/kg intraperitoneally in eight groups of rats with various interventions (see text) ($r = 0.67$; $P < 0.001$).

rats ($P < 0.005$). Rats in groups 3 through 8, which were all salt depleted to start with, had significantly increased urinary Na excretion rates when they were compared with the starting rates. However, when these rates were compared between groups at the end of the study, rats in groups 4, 5, 7, and 8, which received 1.56 meq of Na per day as ticarcillin, piperacillin, and NaCl, alone or in combination, had Na excretion rates that were not significantly different from those in rats in the normal salt group (group 2). Rats in the remaining groups (3 and 6) had lower excretion rates than rats in group 2.

When urinary flow rates were compared in each group before and after treatment with tobramycin, there was a trend for the rates to increase; however, this did not always reach a level of significance (Table 1). When urine volumes between groups of rats were compared at the end of the study, no significant differences were observed.

Effect of salt status on tobramycin-induced nephrotoxicity. Treatment with tobramycin for 10 days resulted in changes in renal function, with a variation in response between groups. Mean values for CL_{CR} before and after treatment are given in Table 1, and percent changes are depicted in Fig. 1. Group 1 rats had a 53.0 ± 10.5% reduction in CL_{CR} ($P < 0.05$), while group 2 rats had no significant change (1.08 ± 0.07 versus 1.16 ± 0.19 ml/min).

Effect of ticarcillin and piperacillin on tobramycin-induced nephrotoxicity. Rats in groups 4, 5, 7, and 8, which received an Na load of 1.56 meq/day as ticarcillin, piperacillin, or NaCl, alone or in combination, had no change in mean CL_{CR} over the 10-day period. Rats that received the low dose of ticarcillin (group 3), which contained an average of 0.38 meq of Na per day, sustained a significant decrease in CL_{CR}. Rats that received the same molar amount of piperacillin as the protective dose of ticarcillin given alone (group 6) also sustained a significant decrease in CL_{CR}. When enough Na supplement was added to these nonprotective doses of both drugs to raise the daily Na intake to 1.56 meq/day, no significant change in CL_{CR} was observed (groups 5 and 7). Finally, group 3 rats, which received the low dose of ticarcillin containing 0.37 to 0.39 meq of Na per day, started out with a low CL_{CR} (0.70 ± 0.04 ml/min; $P < 0.05$ compared with group 1 rats). The reason for this reduced base-line CL_{CR} is unknown, but tobramycin still induced a 66.2 ± 5.5% reduction in CL_{CR} ($P < 0.005$). Furthermore, when CL_{CR} between groups of rats was compared at the end of the study, there was no difference between values in the groups of rats in which the CL_{CR} fell.

The weights of rats in all groups increased over the 10-day study period. When CL_{CR} was expressed as a function of body weight, results similar to those presented above were obtained.

Figure 2 presents the relationship between Na excretion and CL_{CR} at the end of the study for all groups of rats. A positive correlation existed between these two parameters, with $r = 0.67$ and $P < 0.001$. This relationship could be fitted with a linear, parabolic, or sigmoid model with similar values of $r = 0.66$ to 0.67 and $P < 0.001$ in each case. The sigmoid model is presented in Fig. 2.

Levels of tobramycin in plasma and kidneys. Trough steady-state concentrations of tobramycin in plasma and kidneys, which were collected 24 h after administration of the last dose of drug, exhibited marked variations (Table 2).

Rats on normal salt diet (group 2), which had no change in renal function, had a 600-fold kidney:plasma ratio, indicating extensive renal uptake. In salt-depleted rats (group 1), in

TABLE 2. Tobramycin concentrations in plasma and kidneys 24 h after the last dose of tobramycin

Group[a]	Concn (mean ± SEM) in:	
	Plasma (μg/ml)	Kidney (μg/g)
1	4.86 ± 1.28[b]	429 ± 43
2	0.66 ± 0.18[c]	391 ± 45
3	0.61 ± 0.08[c]	514 ± 67
4	0.35 ± 0.03[c]	382 ± 23
5	0.38 ± 0.05[c]	539 ± 105
6	1.97 ± 0.97[c]	590 ± 11[b,c]
7	1.39 ± 0.76[c]	466 ± 58
8	0.29 ± 0.03[c]	146 ± 32[b,c]

[a] Interventions for each group are explained in text and the legend to Fig. 1.
[b] $P < 0.05$ compared with group 2.
[c] $P < 0.05$ compared with group 1.

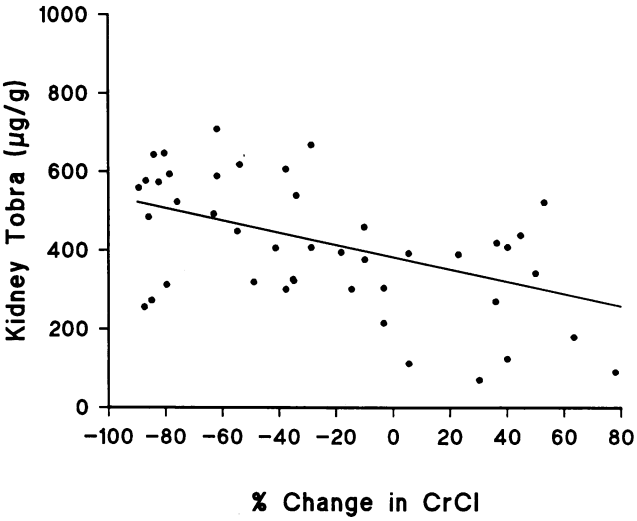

FIG. 3. Relationship between the concentration of tobramycin in the kidneys and the percent change in CL_{CR} (CrCl) in eight groups of rats 10 days after the daily administration of tobramycin at 100 mg/kg per day intraperitoneally and various interventions (see text). Values are means ± standard errors of the mean ($r = -0.44$; $P < 0.01$).

which a decrease in CL_{CR} occurred, there was a significant, sevenfold increase in the concentration of tobramycin in plasma, but there was no change in the concentration in renal tissue. Analysis of tobramycin concentrations in serum and renal tissue in individual rats from groups 1 and 2 did not show any correlation between these two variables.

Rats in the remaining groups had concentrations of tobramycin in plasma that were not significantly different from those in group 2 (normal salt) rats, but all had significantly lower levels than those in the salt-depleted group (group 1). Interestingly, rats in groups 3 and 6, both of which sustained marked decreases in CL_{CR}, had tobramycin concentrations in plasma that were not different from those in group 2 rats, although they tended to be higher in group 6 rats.

Finally, comparison of tobramycin levels in the kidneys among rats in all groups by one-way analysis of variance showed a significant difference. Group 8 rats had values that were significantly lower than those in rats in each of the other groups. The only other difference was between group 6 and each of groups 2 and 4. When correlation analysis was applied to CL_{CR} and tobramycin concentrations in plasma and tissue, no correlation was found between levels in plasma and CL_{CR}, but a negative correlation existed for CL_{CR} (expressed as percent change in CL_{CR}) and concentrations of drug in kidneys, with $r = -0.44$ and $P < 0.01$ (Fig. 3).

DISCUSSION

Our results are consistent with those previous studies showing that salt depletion potentiates the nephrotoxicity of aminoglycosides, while a normal salt diet protects against it (2, 8). Whereas in the salt-depleted group of rats (group 1) there was a 53 ± 10% decrease in CL_{CR}, no significant change was seen in rats in the normal salt group (group 2), which received the same dose of tobramycin for the same period of time as the salt-depleted rats did.

The data from the second series of experiments which showed protection by ticarcillin against tobramycin nephrotoxicity are also consistent with those obtained in other studies (11). The lower dose of ticarcillin, which provided an average of 0.38 meq of Na per day, was ineffective. It is not clear why this group of rats started out with a low CL_{CR}, and it is not certain whether this contributed to the fall in CL_{CR} after tobramycin administration. However, in rats given a dose containing the same milliequivalents of Na as those given to rats (group 2) on a normal salt diet, the deterioration in renal function was prevented (group 4). To examine whether this effect was due to the Na content or the interaction of the penicillin molecule with tobramycin, we readministered the low dose of ticarcillin and supplemented it with enough NaCl to raise the daily Na load to be similar to that given to rats in the high-dose ticarcillin group (group 4). Rats in this group did not sustain any significant fall in CL_{CR}, suggesting that the increase in Na intake rather than in the amount of ticarcillin administered is what explains the protective effect in group 4 rats. To investigate this protective effect further, we made use of a closely related compound, piperacillin. This compound is available as a monovalent Na salt and contains approximately one-third of the milliequivalents of Na per gram as does ticarcillin. Hence, a dose was chosen that provided the same amount of piperacillin as the protective dose of ticarcillin (piperacillin at 400 mg/day). This, however, afforded no protection to the kidneys. Piperacillin, like ticarcillin, has been shown to interact with tobramycin and gentamicin in vitro (23). If this interaction provides an explanation for the protective effect of ticarcillin in vivo, then piperacillin should produce a similar effect. In this study, there was no demonstrable protection by piperacillin against tobramycin nephrotoxicity, which further argues against an in vivo interaction, similar to the in vitro one, explaining the protective effect. An attempt to raise the amount of Na supplement by increasing the dose of piperacillin was a failure because of the intrinsic toxicity of the huge dose of piperacillin required, causing a mortality rate of approximately 50% (data not shown). However, an increase in Na intake by supplementing the piperacillin with NaCl produced the desired protective effect (group 7). It should be kept in mind, however, that ticarcillin and piperacillin are structurally distinct compounds and that if an interaction does occur between them and tobramycin, the stoichiometry of the interaction may be different for the two penicillins.

In the last group of rats (group 8), we administered what, effectively, is the vehicle for the protective dose of ticarcillin, namely, 1.56 meq of Na per day as NaCl. This proved to be as effective as the high dose of ticarcillin (given to group 4 rats) in preventing the fall in CL_{CR} induced by tobramycin. These results strongly suggest that the protection of kidney function observed when ticarcillin is coadministered with tobramycin is explained by the obligatory Na load associated with it. The results obtained with group 8 rats further support our conclusion that the difference in renal function observed between rats in groups 1 and 2 is explained by the difference in the Na content of their diets rather than some other change in diet, since parenteral NaCl replacement while the rats were on an Na-deficient diet was as protective as a normal salt diet was. The positive relationship between the salt status and the percent change in CL_{CR} (Fig. 2) suggests that the main determinant of renal function at the end of the treatment period is the sodium status of the animal, independent of whether or not a penicillin was administered and independent of the nature of the penicillin. The salt status of the animals is reflected by their daily

sodium excretion, since the homeostatic control of sodium is preserved until the end stages of renal failure.

Our results with piperacillin are in contrast to those of Hayashi et al. (15). They found that piperacillin at a dose of 1,000 mg/kg given intravenously to rats and followed by the intramuscular injection of gentamicin (100 mg/kg) daily for 5 days resulted in lower elevations of blood urea nitrogen, serum creatinine, and urinary *N*-acetyl-β-D-glucosaminidase than those that occurred when gentamicin was given either alone or in combination with 3.6% NaCl containing the moles of NaCl equivalent to 1,000 mg of piperacillin per kg. This latter regimen had a protective effect, although to a lower extent. However, there was no mention of the statistical significance of the difference between the two groups, i.e., piperacillin versus NaCl. The dose of piperacillin used in those experiments (15) would, on average, be slightly less than the lower dose used in our experiments. At present, we have no clear explanation for this discrepancy. However, the fact that the rats used in their experiments were not salt depleted but were maintained on a regular diet might have minimized the importance of salt replacement as a protective mechanism. The different methodology and routes of administration may have contributed to this difference as well.

The major determinant of trough steady-state concentrations in plasma at the end of the experiment was renal function. The concentrations of tobramycin in plasma were significantly lower in all groups of rats in which protection was demonstrated compared with those in the Na-depleted group of rats ($P < 0.02$). This is consistent with the maintained kidney function and the adequate excretion of AGS in rats in these groups rather than the result of a postulated interaction with ticarcillin in group 4 rats. However, despite a reduction in renal function to a similar extent in groups 1, 3, and 6, rats in the latter two groups had concentrations of tobramycin in plasma that were lower than those in rats in the salt-depleted group (group 1). It is tempting to speculate that under these conditions of marked impairment in renal clearance, both AGS and penicillin accumulate in the plasma, making an interaction more favorable. This could account for the lower concentrations of tobramycin not bound to the penicillin in these groups of rats compared with those in group 1 rats, to which no penicillin was administered. This is in agreement with the observed increase in clearance and decrease in half-life of gentamicin by ticarcillin, which is only observed in patients with end-stage renal failure (12). Another explanation for these lower concentrations of tobramycin in plasma is that salt loading led to expansion of the extracellular volume, which resulted in dilution of the drug levels. However, since Na homeostasis is preserved despite marked reductions in the glomerular filtration rate, the volume changes, and, hence, the dilutional effect, are probably modest.

In comparison with wide intergroup variations in the concentrations of tobramycin in plasma, there were smaller variations in the steady-state levels of tobramycin in the kidneys. A consistent pattern could be discerned, however. As depicted in Fig. 3, a negative correlation existed between the percent change in CL_{CR} and concentrations of tobramycin in the kidneys at the end of the study. Alternative explanations for this correlation are possible. On the one hand, it suggests that the renal accumulation of tobramycin determines the extent of nephrotoxicity. The extent of drug uptake into the kidney would then be a function of the salt status of the animal. This hypothesis, therefore, postulates a pharmacokinetic interaction between tobramycin and salt that modifies the renal uptake of the drug. An alternative hypothesis is that the salt status determines the extent of decrease in renal function induced by tobramycin. Consequent to this, the drug accumulates in the body, as reflected by its higher concentration in the kidneys. This suggests that a pharmacodynamic interaction takes place between the drug and the salt to minimize the nephrotoxicity of the drug. A corollary of this hypothesis is that the levels of tobramycin in plasma should have a profile similar to those in the kidneys. However, this was not the case in this study. Furthermore, no correlation was observed between CL_{CR} and concentrations of tobramycin in plasma, which further argues against a pharmacodynamic interaction. This assumes that no interaction occurs between the drugs that would account for the relatively lower concentrations of tobramycin in plasma in the groups of rats which sustained marked reductions in CL_{CR}.

The relationship between the accumulation of AGS by the kidney and nephrotoxicity has been addressed in previous studies. Aronoff et al. (1) and Bennett et al. (3) suggested that the deterioration in renal function is related to the degree of drug accumulation in the kidney. Others provided evidence contrary to this (6, 11, 17, 19). It is evident that this question has yet to be answered, but our results support a relationship between renal uptake of drugs and change in renal function.

The mechanism by which Na depletion potentiates AGS nephrotoxicity remains obscure. It has been suggested that a state of salt depletion stimulates the renin-angiotensin system, leading to renal vasoconstriction, which may contribute to nephrotoxicity (24). In contrast, Luft et al. (17) showed that captopril had no protective effect on functional or morphological changes induced by gentamicin in rats.

Another possible explanation for the effect of salt status on AGS nephrotoxicity may involve activation of tubuloglomerular feedback. Amphotericin B is another nephrotoxic agent, the toxicity of which is modified by salt intake in a manner similar to that of AGS (14). In addition, ticarcillin protects against amphotericin B nephrotoxicity (5). Gerkens and Branch (14) suggested that the tubular injury induced by amphotericin B would result in activation of tubuloglomerular feedback, leading to renal vasoconstriction mediated by adenosine (21). A similar process may be involved in AGS nephrotoxicity, although more specific experiments are required either to confirm or refute this hypothesis.

In conclusion, our study demonstrates the protective effect of ticarcillin against tobramycin nephrotoxicity. This protection can be explained by the Na load in the ticarcillin preparation. The evidence for a chemical interaction in vivo, under conditions of normal renal function, is not convincing, especially in the absence of any demonstration of complex formation. The validity of either one of these theories has an important bearing on clinical practice. The Na theory implies that the availability of both drugs is unchanged, while the interaction theory indicates that levels of the drugs in plasma and tissues are reduced, which entails the modification of dosages. Results of this study support the contention that adjustment of AGS doses when penicillins are coadministered is not warranted. In addition, and in the absence of prospective clinical trials, it is reasonable to suggest that when an extended-spectrum penicillin is to be administered together with an AGS, a drug with a high sodium content is preferable, if the hemodynamic status of the patient permits.

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