Role of Sodium in the Protective Effect of Ticarcillin on Gentamicin Nephrotoxicity in Rats

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Coadministration of sodium ticarcillin with an aminoglycoside is known to reduce the nephrotoxicity of the aminoglycoside. However, it is not known whether the penicillin or the obligatory sodium load confers protection. To investigate this, gentamicin has been administered intraperitoneally in doses of 50, 60, or 80 mg/kg per day for 12 days in groups of rats receiving either a normal or a low sodium intake. Alterations in creatinine clearance have been measured. Salt depletion resulted in an enhanced nephrotoxic response with a shift in the dose-response curve to the left. Administration of sodium ticarcillin to rats with a salt-depleted intake at a dose sufficient to replace sodium intake conferred an equal degree of protection to rats with a normal salt intake. We report that the obligatory salt supplement with ticarcillin is sufficient to account for the renal sparing effect of the combination treatment without having to infer a direct chemical interaction of penicillin with the aminoglycoside.

Aminoglycosides are rarely used alone in contemporary medicine. They are usually administered as a component of broad antibacterial spectrum cover, often being given in combination with extended-spectrum penicillins such as ticarcillin to provide additional antipseudomonal activity. However, prior admixture of aminoglycosides with penicillins will result in the two drugs forming an inactive complex (11, 12, 27-29). The interaction is also thought to occur in vivo (9), but it has been suggested that this has an influence only on an effective available drug for antimicrobial action when the dose of penicillin is very high or the dosage interval of aminoglycoside is prolonged, as in renal failure (4, 9, 14). In these situations, the combination results in a shortening of half-life.

If complex aminoglycoside-penicillin formation occurs in vivo, it is probable that the interaction would occur in the extracellular fluid space. This would result in less aminoglycoside being available for renal uptake. This might be expected to influence the potential of the aminoglycoside to induce nephrotoxicity (1, 16, 25), particularly as aminoglycosides are concentrated in the renal cortex (24) and the half-life of aminoglycosides in the cortex is longer than in plasma (23, 24). Experimental studies in rats with the combination of tobramycin and ticarcillin have confirmed that the change in renal function during chronic but separate administration of these drugs was less than that found by administration of tobramycin alone (13). This protection occurs despite equal or even higher concentrations of tobramycin in renal tissue in the combination therapy group (13). A similar study has also demonstrated a protective effect by the combination of gentamicin and carbenicillin (5). These observations in animal models would be consistent with the clinical findings of a low incidence of nephrotoxicity when combination therapy was used in immunocompromised patients in the European Organization for Research on Cancer Report (15). Similarly, Wade and co-workers showed that the incidence of nephrotoxicity with the aminoglycoside-

ticarcillin combination (3.1%) was lower than the incidence with the aminoglycoside-cephalothin combination (18.3%) (33).

The evidence that coadministration of extended-spectrum penicillins with aminoglycosides ameliorates nephrotoxicity is convincing. However, it is possible that the mechanism conferring this protection is unrelated to drug complex formation. An alternative hypothesis is that sodium loading, which is obligatory with administration of a divalent anion such as ticarcillin, will have an effect independent of the penicillin, particularly since the nephrotoxic potential of aminoglycosides is salt sensitive (3, 23).

This alternative hypothesis has been investigated in this study by firstly determining the dose-response relationship of gentamicin to reduced renal function under normal salt and low salt conditions. A dose of ticarcillin selected to provide the equivalent of salt replacement was subsequently given to salt-depleted animals. It was reasoned that comparison of the response in rats treated with combination chemotherapy to the two series of dose-response curves would permit evaluation of the relative contribution of the sodium cation and ticarcillin anion to protection from nephrotoxicity.

MATERIALS AND METHODS

Experiments were performed on Sprague-Dawley male rats (SASCO Corporation, Omaha, Nebr.) 7 weeks old and weighing approximately 200 g. Rats were randomized into two groups: sodium-depleted rats and rats given a normal sodium diet. Salt-depleted rats received 2 mg of furosemide per kg intraperitoneally 1 week before the study and were maintained on sodium-free drinking water and a low-sodium purified diet (sodium composition, 0.05%; Ralston Purina Co., St. Louis, Mo.). Rats with normal salt intake were maintained on a regular diet (sodium composition, >0.39%; Wayne Lab Blox; Continental Grain, Chicago, Ill.) and tap water. Rats in each group were divided into three groups depending upon the dose of gentamicin injected: 50, 60, and 80 mg/kg per day given as a single daily dose. The dose of gentamicin was adjusted in each rat, by weight, each day. Gentamicin was injected daily for 12 days into the peritoneal cavity via a permanently implanted tygon rubber-ended

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catheter, with the rubber end being brought subcutaneously to the back of the neck and exteriorized. This catheter was implanted in the rat 2 days before the start of the study, under light ether anesthesia.

Another group of sodium-depleted animals was given a combination of gentamicin (60 mg/kg per day intraperitoneally) and ticarcillin (300 mg/kg per day), which was administered subcutaneously. This dose of ticarcillin was selected to provide a sodium equivalent approximating that of a normal salt diet.

Blood and 24-h urine samples were collected on the day before the start of the study and on days 3, 6, 9, and 12 of the study. Blood (0.5 ml) was obtained from the tail vein in each rat for the measurement of plasma creatinine. Urine samples were collected in rat metabolic cages (Nalge Company, Rochester, N.Y.) for measuring urinary creatinine and electrolyte (sodium and potassium) concentrations. To ensure stable conditions, rats were placed in metabolic cages 2 days prior to each collection day. Creatinine was measured in triplicate with a Beckman creatinine autoanalyzer. Sodium and potassium concentrations were analyzed with a flame photometer.

Each group studied consisted of six rats. Results are presented as the mean plus or minus the standard error of the mean. The relationships between the creatinine clearance and time in three groups were evaluated by analysis of variance and then were applied to multiple comparison analysis by using a Newman-Keul multiple comparison test. A regression line was calculated by least squares analysis. The minimum level of statistical significance was considered to be $P < 0.05$.

RESULTS

The 24-h urinary sodium excretion measured in salt-depleted rats and rats on a normal salt diet at the end of the study is shown for each group of rats in Fig. 1. As anticipated, salt-depleted rats had markedly reduced urinary sodium excretions, compared with the rats on a normal salt diet ($P < 0.05$), but had comparable total urine volumes. Ticarcillin administration to salt-depleted rats increased the urinary sodium excretion almost to the level found in rats on a normal salt diet ($706 \pm 70 \mu\text{eq}$ per day versus $887 \pm 318 \mu\text{eq}$ per day, respectively).

The relationship between creatinine clearance and time for rats receiving each dose is presented for rats on a normal salt diet (Fig. 2) and salt-depleted rats (Fig. 3). In rats on a normal salt diet, gentamicin at a daily dose of 50 mg/kg per day was not associated with any change in creatinine clearance over this time period. However, doses of 60 and 80 mg/kg per day produced significant, dose-dependent reductions in creatinine clearance of 36.8 ± 5.4 and $51.7 \pm 7.9\%$ by day 12. In salt-depleted rats, there were marked, dose-dependent reductions of creatinine clearance in all three groups. A dose of 50 mg/kg per day of gentamicin reduced creatinine clearance by $62.0 \pm 4.7\%$ by day 12. All three doses of gentamicin induced reductions in creatinine clearance that were greater in salt-depleted rats than in rats on a normal salt diet at equivalent doses. Three sodium-depleted rats receiving the highest dose of gentamicin (80 mg/kg per day) died during the last 3 days of the study.

The group of salt-depleted rats receiving gentamicin (60 mg/kg per day) with ticarcillin (300 mg/kg per day) have been compared with rats with salt-depleted and normal salt diets receiving the same dosage of gentamicin (Fig. 4). The reduction in creatinine clearance in salt-depleted rats was

FIG. 1. Urinary sodium excretion on day 12 of daily gentamicin treatment (doses indicated in milligrams per kilograms per day) of salt-depleted rats, with or without concomitant ticarcillin treatment, and rats with normal salt levels. Asterisks indicate $P < 0.05$, compared with salt-depleted rats. The urinary sodium excretion is shown in milliequivalents per day.

greater than in rats receiving a normal salt diet ($P < 0.05$) (Fig. 2 and 3). In contrast, ticarcillin coadministration with gentamicin to salt-depleted rats reduced the change in creatinine clearance from the salt-depleted group to a change comparable to that found in rats with a normal salt diet.

The relationship between the dose of gentamicin and the reduction of creatinine clearance, expressed as percent

FIG. 2. Creatinine clearance over time in rats on a normal salt diet given daily intraperitoneal doses of gentamicin at 50, 60, or 80 mg/kg per day. Asterisks indicate $P < 0.05$, compared with rats receiving gentamicin at 50 mg/kg per day. Each group of rats was evaluated on days 0, 3, 6, 9, and 12.

FIG. 3. Creatinine clearance over time in salt-depleted rats given daily intraperitoneal doses of gentamicin at 50, 60, or 80 mg/kg per day. Symbols: *, $P < 0.05$, compared with rats receiving gentamicin at 50 mg/kg per day; ‡, $P < 0.05$, compared with rats receiving gentamicin at 60 mg/kg per day. Each group of rats was evaluated on days 0, 3, 6, 9, and 12.

change from base line by day 6 (the time of maximal differences between groups), illustrates the extent of enhancement of the nephrotoxic response due to sodium depletion (Fig. 5). It can be seen that coadministration of ticarcillin with gentamicin to salt-depleted rats caused a change in the renal response equivalent to that observed in animals receiving gentamicin on a normal salt diet.

DISCUSSION

Gentamicin-induced nephrotoxicity is a well-recognized and anticipated limitation of the therapeutic usefulness of

FIG. 4. Creatinine clearance over time in three groups of rats, each treated with a daily dose of gentamicin at 60 mg/kg per day. Rats received either a normal salt diet or a salt-depleted diet, and one group of salt-depleted rats received ticarcillin (TC) at a dose of 300 mg/kg per day. Asterisks indicate $P < 0.05$, compared with rats on a normal salt diet. Each group of rats was evaluated on days 0, 3, 6, 9, and 12.

FIG. 5. Comparison of the change in renal function, expressed as percent change in creatinine clearances, after 6 days of treatment with various doses of gentamicin in rats with salt-depleted (●) and normal salt (○) diets. One salt-depleted group of rats also received ticarcillin (TC) (300 mg/kg per day).

this drug. However, aminoglycosides, including gentamicin, are still the initial drugs of choice in the management of suspected gram-negative bacterial infections because of their undoubted efficacy. If gentamicin-induced nephrotoxicity could be prevented or minimized, it would represent a major therapeutic advance in the therapy of such infections. This advantage would be further strengthened if the intervention also provided a broader and more effective range of antimicrobial coverage.

The results of this study with the combination of ticarcillin and gentamicin support previous studies in rats with carbenicillin-gentamicin (5) or ticarcillin-tobramycin combinations (13). These studies suggest that coadministration of an extended-spectrum penicillin with an aminoglycoside provides protection against nephrotoxicity. This study now extends these observations to suggest that protection may be conferred by the obligatory sodium load associated with ticarcillin administration rather than by the penicillin itself.

The mechanism by which gentamicin induces changes in renal function is not clearly understood. It is likely that there are at least three major contributing components. Firstly, there is concentration of drug within the kidney; secondly, there appear to be primary effects probably induced by changes at cell membranes (19); and thirdly, there is activation of renal regulatory mechanisms. Thus, interaction with any of these steps would be expected to alter the outcome. Renal uptake of gentamicin is avid, with over 50- to 100-fold concentrations in renal tubule cells compared with that in plasma (19). However, the extent of drug accumulation in renal tissue does not necessarily relate to the extent of change in renal function (2, 7, 20, 24).

The clearest example of a dissociation between accumulation of aminoglycosides in the kidney and extent of renal damage occurs after salt status is changed at the time the aminoglycoside is administered to rats. Salt depletion enhances nephrotoxicity without changing tissue levels (3, 23). Similarly, in studies with dogs, renal tissue levels of aminoglycosides in salt-loaded dogs and in salt-depleted dogs are similar despite widely disparate glomerular filtration rates (36). Furthermore, coadministration of aminoglycosides with osmotic diuretics decreases nephrotoxicity without changing renal tissue uptake of drug, and furosemide provides protection with a trend toward increases in renal tissue

levels (8, 26). In each of these examples, changes in salt status, rather than renal uptake of the aminoglycoside, have been associated with alterations in response.

A variety of primary events have been implicated in the initiation of gentamicin-induced renal damage. The observation that gentamicin enhances generation of hydrogen peroxide in mitochondria isolated from renal cortical cells suggested the hypothesis that hydroxyl radical formation is involved (34). This contention was supported by the demonstration that administration of hydroxyl scavengers to rats prior to administration of gentamicin reduced the extent of change in renal function in the intact rat (35). An alternative, and not mutually exclusive, mechanism is that aminoglycosides bind to membrane-bound phosphoinositides (30). Once bound, several aminoglycosides have been shown to inhibit phosphatidylinositol phospholipase C, the enzyme responsible for cleavage of phosphatidylinositol to phosphoinositol and diacylglycerol, with the same rank order of potency as their ability to produce nephrotoxicity (22). This could explain the ability of aminoglycosides to inhibit renal sodium potassium ATPase activity (21), as the inhibition of this enzyme is prevented by phosphoinositides. These and other events may also induce primary changes in renal cells. These are then likely to act on secondary regulatory mechanisms of renal function. The minimal evidence of structural damage at a time when the glomerular filtration rate is markedly reduced and the ability of interventions like salt loading to modify the response support the concept of the importance of these secondary regulatory mechanisms.

In this experiment, there was evidence of a dose-response relationship between daily doses of aminoglycoside and changes in creatinine clearance in both salt-depleted and normal salt diets. Furthermore, there was a clear-cut difference between gentamicin-induced reductions in creatinine clearance in salt-depleted rats and those reductions found in rats with normal salt diets. These observations confirm previous observations and illustrate the extent of influence of salt status on changes in renal functions induced by gentamicin. It should be noted that the doses of gentamicin required to induce nephrotoxicity in rats are higher than those required in humans when extrapolated on a weight basis. The reason for this interspecies variation is unknown, but it means that caution should be used in extrapolating experimental information obtained in rats to humans. The mechanism by which changes in salt status influence the renal response to aminoglycosides is unknown and could be due to modification of any of the previously discussed factors but is unlikely to relate to a simple dilutional effect in urine, as urine volume was comparable in each group. The impact of salt status on response is, however, shared by other nephrotoxins such as glycerol (31), mercuric chloride (10), and amphotericin B (17), suggesting a common denominator. In the instance of amphotericin B, it has been suggested that direct injury to renal tubular cells results in a secondary activation of a renal vasoconstrictor response, which results in afferent arteriole constriction and reduction in glomerular filtration rate (17). The nature of this vasoconstrictor is as yet unknown, but it is unlikely to be either catecholamine or angiotensin II, and it can be inhibited by calcium channel blockers (32). Such a mechanism could also be responsible for changes in glomerular filtration rate with other nephrotoxins. It is of interest that reductions in renal function after amphotericin B administration to patients were also less in patients who received concomitant administration of ticarcillin, as well as alternative methods of sodium supplementation (6).

Having defined a relationship between gentamicin dose and change in glomerular filtration rate in salt-depleted rats and rats on a normal salt diet, we reasoned that if ticarcillin is administered together with the aminoglycoside to salt-depleted animals in a dose that would replace the normal sodium intake (Fig. 1), then the extent of change in renal function should be similar to that found in rats on a normal salt diet if the protection was solely due to the sodium component. If the penicillin moiety also contributes to protection, then the change in creatinine clearance should be reduced to an even greater extent than can be explained by sodium replacement only. Results of the experiment (Fig. 4 and 5) clearly suggest that the sodium content of the divalent salt ticarcillin is sufficient to explain renal sparing and that it is unnecessary to speculate on an additional mechanism, such as intrarenal complex formation.

The relevance of discriminating between alternative mechanisms is that in vivo complex formation implies that less of the drug administered is available for antimicrobial action; in contrast, the sodium dependence hypothesis does not imply any change in drug availability. At this time, direct measurements of in vivo complexes have not been made and it remains to be determined whether doses of aminoglycosides should be modified when giving the combination. A further implication of the salt supplement hypothesis is that ticarcillin is a divalent salt. It would be anticipated that extended-spectrum penicillins which are monovalent salts would have a lesser protective effect. This may explain the reason that over three times the dose of piperacillin was required, compared with the dose of ticarcillin used in this study, to provide a renal sparing effect in the rat (18). This dose would confer a sufficient salt load to confer protection. It may be prudent, therefore, to select a penicillin with a high salt content rather than a low one when choosing combination chemotherapy with an aminoglycoside.

In summary, this study illustrates the extent of salt sensitivity of aminoglycoside-induced nephrotoxicity and shows that concomitant administration of ticarcillin with gentamicin to salt-depleted rats confers a level of protection against nephrotoxicity that can be attributed to the obligatory sodium supplement.

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