Effect of Cefamandole Nafate on the Toxicity of Tobramycin

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Because of the potential for an interaction between cephalosporins and aminoglycosides leading to renal injury, an evaluation of the effect of a new cephalosporin, cefamandole nafate, on the toxicity of the aminoglycoside tobramycin was performed in laboratory animals. High doses of cefamandole nafate did not increase the acute toxicity (lethality) of tobramycin in rats or mice. In a subacute experiment in rats, dose-related tobramycin nephrotoxicity, as evidenced by blood urea nitrogen changes, increased kidney weights, and histologically determined tubular necrosis, was observed. Concomitant treatment with cefamandole nafate, 500 mg/kg, did not increase tobramycin nephrotoxicity, but protected against the aminoglycoside-induced renal injury. Determination of tissue radioactivity after administration of [14C]tobramycin indicated that cefamandole nafate treatment resulted in uniformly lower tobramycin tissue concentrations compared with the control, suggesting that the protective effect was produced by enhanced excretion of tobramycin after cefamandole nafate treatment.

The concomitant use of an aminoglycoside and a cephalosporin represents a frequently used approach to the initial therapy of life-threatening infections.

There are a number of case reports attributing renal injury to the simultaneous use of the aminoglycoside gentamicin (Garamycin, Schering Laboratories, Bloomfield, N.J.) and the cephalosporin cephalothin (Keflin, Eli Lilly & Co., Indianapolis, Ind.) in seriously ill patients (3, 4, 8, 12, 15, 16).

However, in an examination of data on 1,073 patients collected by the Boston Collaborative Drug Surveillance Program, Fanning et al. (7) have concluded that no appreciable interaction leading to impaired renal function exists between cephalothin and gentamicin.

Tobramycin (Nebcin, Eli Lilly & Co.) is a recently introduced aminoglycoside with an antibiotic spectrum and profile of activity in laboratory animals that compares favorably with those of gentamicin (17, 20). Cefamandole nafate (Mandol, Eli Lilly & Co.) is a new cephalosporin antibiotic with an expanded antibacterial spectrum compared with cephalothin (21).

The combined use of cefamandole nafate and tobramycin represents a potentially useful therapy for serious infections. Because of the controversy surrounding the potential for nephrotoxicity resulting from the concomitant use of aminoglycosides and cephalosporins, an evaluation of the interaction between cefamandole nafate and tobramycin in laboratory animals was undertaken as a part of the toxicological evaluation of this new cephalosporin antibiotic.

MATERIALS AND METHODS

Animals. In acute toxicity experiments, female Wistar rats weighing 110 to 135 g or female ICR mice weighing 16 to 18 g (Harlan Industries, Cumberland, Ind.) were used. In the subacute experiments, male and female Wistar rats, specific pathogen-free and cesarean derived, weighing 104 to 146 g (Harlan Industries) were caged individually with free access to water and food.

Antibiotic administration. Cefamandole nafate was dissolved in 1.67% aqueous sodium carbonate (3.3 ml/g) to make a 25% solution comparable to that obtained after reconstitution of the formulated product (11). Tobramycin was administered as tobramycin sulfate in the commercially available formulation containing 4% tobramycin. This solution was diluted with saline for administration of lower doses, and a 10% solution was prepared for the administration of high doses in the acute toxicity experiments.

In acute toxicity experiments, mice (10 per dose) received intravenous doses of saline, 8 ml/kg, or cefamandole nafate, 2,000 mg/kg, followed after 1 min by intravenous doses of tobramycin, 40 to 80 mg/kg. Injections were made via the tail vein. Rats (10 per dose) received similar intravenous treatments, with the tobramycin doses ranging from 80 to 125 mg/kg. Similar subcutaneous doses of saline or cefamandole nafate were followed within 1 min by a second subcutaneous injection of tobramycin (300 to 900 mg/kg) at a separate injection site. Animals were observed for signs of toxicity occurring shortly after antibiotic administration, and mortality was
determined at 7 days. The LD₅₀ (median lethal dose) was calculated by the method of Bliss (2).

The effect of cefamandole nafate on the subacute toxicity of tobramycin was evaluated in a 15-day study in which 16 groups of 10 rats per sex per treatment received daily subcutaneous injections of: saline; cefamandole nafate, 500 mg/kg; tobramycin, 30, 120, or 160 mg/kg; or cefamandole nafate, 500 mg/kg, plus tobramycin at 30, 120, or 160 mg/kg. In groups that received both antibiotics, the second injection was made at a separate site within 5 min of the first injection.

Rats were weighed at intervals throughout the 15-day period. At the termination of the study, surviving rats were bled, and serum was prepared for determination of urea nitrogen (14). The kidneys were removed, weighed, and examined microscopically after conventional preparation and staining with hematoxylin-eosine.

Tissue distribution. The effect of cefamandole nafate on tissue concentrations of tobramycin was studied with [¹⁴C]tobramycin. [¹⁴C]Tobramycin was obtained from M. Stark, Lilly Research Laboratories, Indianapolis, Ind., and was prepared by fermentation in a medium containing [U-¹⁴C]glucose. The [¹⁴C]tobramycin was identified by thin-layer chromatography and was shown to have radiochemical purity of greater than 99.7% by thin-layer chromatography and liquid scintillation counting. The [¹⁴C]tobramycin was diluted with non-radioabeled tobramycin to provide a final dose of [¹⁴C]tobramycin with a specific activity of 3.2 μCi/g.

Rats were given a subcutaneous dose of [¹⁴C]tobramycin, 160 mg/kg, and either cefamandole nafate, 500 mg/kg, or saline, 2.0 ml/kg. Four hours after antibiotic administration the rats were sacrificed, and serum and various tissues were collected for determination of radioactivity. Tissue samples (200 mg) were digested in 2.4 ml of NCS tissue solubilizer (Amersham/Searle, Arlington Heights, Ill.) at 50°C for 16 h and counted in PCS scintillation cocktail (Amersham/Searle) with a Packard model 3330 liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.). Quench correction was accomplished by the channels ratio method.

RESULTS

Acute toxicity. The LD₅₀ values for rats and mice receiving cefamandole nafate and tobramycin or tobramycin alone are presented in Table 1. Observations of signs of toxicity resulting from the administration of tobramycin or tobramycin plus cefamandole were similar to those reported previously for tobramycin (18) and included clonic convulsions and loss of righting reflex after intravenous administration and leg weakness and loss of righting reflex after subcutaneous injection. Deaths occurred from 1 to 60 min after intravenous administration and 10 to 160 min after subcutaneous administration. The premortem responses and times of death suggested that the acute toxicity was mediated through the central nervous system and was not related to nephrotoxic properties of tobramycin.

The tobramycin LD₅₀ values (Table 1) with the concomitant administration of saline or cefamandole nafate clearly indicate no exacerbation of tobramycin toxicity by cefamandole nafate. In cases where differences occurred (e.g., subcutaneous administration to mice or rats), the tobramycin LD₅₀ for concomitant cefamandole nafate treatment was higher than that for saline plus tobramycin, suggesting a slight protective effect of the cephalosporin on tobramycin acute toxicity.

Subacute toxicity. Data from the 15-day subacute study are presented in Table 2. All male rats survived the 15-day study. There was 90% and 60% survival in groups of females receiving 120 and 160 mg/kg, respectively, of tobramycin alone. However, all rats receiving the same tobramycin doses in combination with cefamandole nafate survived the study.

There were no significant effects on terminal body weights in male rats as a result of treatment with tobramycin alone or in combination with cefamandole nafate (Table 2). A dose-related depression in body weights of female rats produced by tobramycin was reversed by concomitant cefamandole nafate administration. The body weights of the female rats receiving cefamandole nafate in addition to tobramycin at 120 or 160 mg/kg were significantly higher than those receiving corresponding doses of tobramycin alone.

Terminal mean blood urea nitrogen values in surviving rats were slightly elevated in both males and females receiving tobramycin alone (Table 2). The elevation was statistically sig-
significant only in males receiving 120 or 160 mg of tobramycin per kg. Concomitant cefamandole nafate administration resulted in no further increase in blood urea nitrogen, and, for five of the six groups receiving both antibiotics, the mean blood urea nitrogen values for rats receiving concomitant cefamandole nafate were slightly lower than for those receiving tobramycin alone.

There was a dose-related increase in relative kidney weights in both male and female rats receiving tobramycin alone (Table 2). The relative kidney weights in rats receiving concomitant cefamandole nafate were lower, and in most instances significantly lower, than relative kidney weights for rats receiving tobramycin alone.

The renal pathology of rats receiving tobramycin alone or in combination with cefamandole nafate was similar to the effects of tobramycin reported previously (20). Histopathological evaluation of kidney tissue indicated a dose-related increase in severity of renal tubular reparative nephrosis and necrosis in rats receiving tobramycin (Table 2). The mean evaluation of renal injury in rats receiving concomitant cefamandole nafate treatment was significantly lower than that for groups receiving the corresponding dose of tobramycin alone at both 120 and 160 mg of tobramycin per kg per day.

Tissue distribution. The effect of cefamandole nafate on the concentration of radioactivity in rat tissues after the administration of [14C]tobramycin is shown in Table 3. Concomitant cefamandole nafate treatment resulted in significantly lower tobramycin concentrations 4 h after subcutaneous tobramycin administration, compared with the saline control. The decrease was consistent in each of the tissues studied. The [14C]tobramycin concentration in the cefamandole nafate group ranged from 58 to 78% of the radiocarbon concentration in the control group.

Determination of urinary radioactivity at the same 4-h time point indicated that 72 ± 7% of the dose of tobramycin had been excreted into the urine in animals receiving saline, whereas 80 ± 5% of the dose was excreted by rats receiving concomitant cefamandole nafate treatment.

**DISCUSSION**

The data presented in this report indicate that high, but nontoxic, doses of cefamandole nafate do not enhance but appear to decrease the acute, as well as subacute, toxicity of tobramycin. The doses of cefamandole nafate (2,000

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**Table 2. Effect of cefamandole nafate on the subacute toxicity of tobramycin**

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Sex</th>
<th>Survivors (%)</th>
<th>Body wt (g)</th>
<th>BUN (mg/100 ml)</th>
<th>Relative kidney wt (g/100 g body wt)</th>
<th>Histopathological evaluation, mean rank (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobramycin</td>
<td>Male</td>
<td>0</td>
<td>100</td>
<td>12.9 ± 0.7</td>
<td>0.75 ± 0.02</td>
<td>0</td>
</tr>
<tr>
<td>Cefamandole nafate</td>
<td>0</td>
<td>500</td>
<td>100</td>
<td>12.7 ± 0.5</td>
<td>0.73 ± 0.02</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>100</td>
<td>107</td>
<td>14.3 ± 0.6</td>
<td>0.86 ± 0.02</td>
<td>0.8 (0-1)</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>500</td>
<td>100</td>
<td>13.5 ± 1.8</td>
<td>0.79 ± 0.02</td>
<td>0.8 (0-1)</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>100</td>
<td>105</td>
<td>17.1 ± 1.1c</td>
<td>0.95 ± 0.02</td>
<td>2.5 (1-4)</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>500</td>
<td>100</td>
<td>17.4 ± 0.5c</td>
<td>0.83 ± 0.02e</td>
<td>1.3 (0-3)c</td>
</tr>
<tr>
<td></td>
<td>160</td>
<td>0</td>
<td>96</td>
<td>19.6 ± 1.3c</td>
<td>0.96 ± 0.03c</td>
<td>2.8 (1-4)c</td>
</tr>
<tr>
<td></td>
<td>160</td>
<td>500</td>
<td>100</td>
<td>15.7 ± 1.3d</td>
<td>0.91 ± 0.04c</td>
<td>1.5 (1-3)c</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0</td>
<td>100</td>
<td>16.2 ± 0.6</td>
<td>0.71 ± 0.02</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>500</td>
<td>100</td>
<td>13.5 ± 0.6</td>
<td>0.72 ± 0.02</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>100</td>
<td>99</td>
<td>15.0 ± 0.7</td>
<td>0.83 ± 0.02e</td>
<td>0.9 (0-1)</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>500</td>
<td>100</td>
<td>14.2 ± 0.7</td>
<td>0.75 ± 0.02e</td>
<td>0.5 (0-1)</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>90</td>
<td>92</td>
<td>23.0 ± 5.2</td>
<td>0.99 ± 0.06c</td>
<td>3.2 (3-4)</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>500</td>
<td>100</td>
<td>22.7 ± 7.0</td>
<td>0.88 ± 0.04c</td>
<td>2.1 (1-5)c</td>
</tr>
<tr>
<td></td>
<td>160</td>
<td>0</td>
<td>91</td>
<td>19.7 ± 2.1</td>
<td>1.06 ± 0.11c</td>
<td>4.1 (3-5)</td>
</tr>
<tr>
<td></td>
<td>160</td>
<td>500</td>
<td>100</td>
<td>15.9 ± 0.9</td>
<td>0.88 ± 0.01c</td>
<td>2.7 (2-4)c</td>
</tr>
</tbody>
</table>

*Survivors only; BUN, blood urea nitrogen; SE, standard error.

0, Normal; 1, very mild reparative nephrosis; 2, mild reparative nephrosis; 3, moderate reparative nephrosis; 4, marked reparative nephrosis; 5, severe necrosis.

*Significantly different from control, analysis of variance and Duncan's new multiple range test (18), P ≤ 0.05.

*Significantly different from corresponding dose of tobramycin alone, analysis of variance and Duncan's new multiple range test (18) P ≤ 0.05.

*Significantly different from corresponding dose of tobramycin alone by Mann-Whitney U test (9), P ≤ 0.05.
mg/kg in acute experiments and 500 mg/kg per day in subacute experiments) produced no significant toxicity when given alone in these experiments or in more extensive studies (J. S. Wold, J. S. Welles, N. V. Owen, W. R. Gibson, and D. M. Morton, J. Infect. Dis., in press).

These cefamandole nafate doses did, however, decrease the acute lethality of tobramycin and nonspecific aspects of subacute tobramycin toxicity evidenced by the effects on body weight and survival in the 15-day study. Additionally, the parameters related to nephrotoxicity, including blood urea nitrogen, kidney weight, and renal histopathology, indicated both a dose-related nephrotoxic response to tobramycin alone and a decrease in tobramycin nephrotoxicity when cefamandole nafate was given concomitantly.

The protective effect of cefamandole nafate on tobramycin nephrotoxicity in rats correlates with the observations of other laboratories on other cephalosporin-aminoglycoside combinations. The combination of cephalothin and gentamicin, an aminoglycoside that is slightly more nephrotoxic in laboratory animals (N. E. Reiner, D. D. Bloxham, and W. L. Thompson, Fed. Proc., p. 953, 1977) has been studied in a number of laboratories. Luft et al. (13) found that cephalothin (100 mg/kg, four times a day) did not augment gentamicin (5 mg/kg, four times a day) nephrotoxicity and that high doses of cefazolin (20 to 50 mg/kg, four times a day) and cephaloridine (50 mg/kg, four times a day) protected against gentamicin nephrotoxicity in 15-day experiments in rats. Dellinger et al. (6) have demonstrated that cephalothin at doses up to 800 mg/kg per day protected against nephrotoxicity produced by gentamicin (6 to 50 mg/kg per day) in a 10-day experiment. Similar results have also been reported by Sugarman et al. (19). Harrison et al. (10) observed that the concomitant administration of cephaloridine, cephalothin, or cefazolin, 500 mg/kg per day, and gentamicin, 20 mg/kg per day, to rats for 4 weeks produced no more renal injury than the same dose of gentamicin alone.

In contrast, Wilhelm and Sack (22) have reported that cephalothin, 1,000 mg/kg, plus gentamicin or tobramycin at 2.5 mg/kg per day resulted in increased renal cell excretion and enzymuria in rats, compared with either aminoglycoside alone. Alterations in renal function or histological evidence of renal injury were not different, however.

The experiment in which the concentration of radioactivity in the renal cortex was determined after the administration of [14C]tobramycin was conducted to gain insight into the mechanism by which cefamandole nafate reduced tobramycin nephrotoxicity. Although the radioactivity in tissue, urine, and serum was not characterized in these studies, the assumption that the radioactivity represents predominantly unchanged tobramycin is supported by the work of Yamada et al. (23). These workers found that 93.4% of administered [14C]tobramycin was recovered in rat urine in 24 h and that urinary radioactivity was comprised solely of tobramycin, with no evidence for the presence of any metabolites.

The uniform decrease in radiocarbon concentrations in serum, liver, lung, and renal medulla as well as the renal cortex suggests that the effect of cefamandole nafate on tobramycin nephrotoxicity is not mediated by a specific effect limited to the mechanisms responsible for concentration of tobramycin in renal cortical tissue. The generalized decrease in tissue concentrations plus the effects of cefamandole nafate on the acute toxicity of tobramycin both suggest a nonspecific effect of cefamandole nafate resulting in slightly more rapid excretion of tobramycin. This hypothesis is also supported by the slightly greater urinary excretion at 4 h in rats receiving concomitant cefamandole nafate. In a comparable experiment, Dellinger et al. (5) have reported significantly lowered gentamicin concentrations in the renal cortex of rats at 4 h and at up to 6 days after the administration of 12 mg of gentamicin per kg in combination with cephalothin (400 mg/kg), compared with saline controls.

The apparently increased renal excretion of tobramycin resulting from concomitant cefamandole nafate was not examined in detail in the experiments reported here. Work on gentamicin nephrotoxicity, however, suggests that increased sodium intake alone is not responsible for decreased aminoglycoside nephrotoxicity (11). Dellinger et al. (6) observed that sodium sulfate protected against gentamicin nephro-
toxicity in the rat in a manner similar to that observed for cephalothin and suggested that cephalothin was acting as a non-reabsorbable anion and influencing cortical accumulation of the aminoglycoside.

Elucidation of the mechanism of the protective effects of cephalosporins on aminoglycoside toxicity in laboratory animals will require further experimentation. The protective effect, however, is of secondary importance to the observation that the concomitant administration of cefamandole nafate does not enhance the toxicity of tobramycin in laboratory animals.

LITERATURE CITED