Relationship Between Rat Renal Accumulation of Gentamicin, Tobramycin, and Netilmicin and Their Nephrotoxicities

MICHAEL E. BRIER,' PHILIP R. MAYER,1* RICHARD A. BRIER,2 DAVID VISSLCHER,2 FRIEDRICH C. LUFT,2 AND GEORGE R. ARONOFF2

School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 479072 and Nephrology Section, Department of Medicine, Indiana University School of Medicine, Indianapolis, Indiana 462232

Received 13 November 1984/Accepted 18 February 1985

Gentamicin, tobramycin, and netilmicin were given to rats in daily doses of either 5 or 20 mg/kg for 30 days to determine the renal accumulation kinetics of the compounds and to correlate steady-state renal parenchymal concentrations with nephrotoxicity. Four rats from each group were sacrificed daily and renal parenchymal tissue concentrations were determined microbiologically. Nephrotoxicity was assessed by changes in creatinine values in serum, renal creatinine clearances, and pathological scores. There was no indication of aminoglycoside-induced nephrotoxicity in any tests performed. The following steady-state levels resulted: 36, 148, and 176 μg/g after 5 mg/kg per day and 148, 260, and 510 μg/g after 20 mg/kg per day for tobramycin, gentamicin, and netilmicin, respectively. We conclude that aminoglycoside parenchymal accumulation in rats follows this order: tobramycin < gentamicin < netilmicin. Therefore, differences in the relative toxicities of gentamicin, tobramycin, and netilmicin do not correlate with the renal parenchymal accumulation of these agents and may be more dependent on intrinsic toxicity to the renal proximal tubule than to the concentration of the aminoglycoside in the kidney.

Gentamicin, tobramycin, and netilmicin are aminoglycoside antibiotics well known for their gram-negative activity as well as their detrimental effects on the kidney. The degree of nephrotoxicity of these agents differs in a dose-related fashion (2, 8, 11, 16). At equivalent doses, the order from most to least nephrotoxic is gentamicin > tobramycin > netilmicin (10, 21). Studies measuring the degree of toxicity and the concentration of the aminoglycosides within the kidney have not regularly shown a positive correlation between these two variables (11, 21). In a detailed kinetic study of gentamicin and tobramycin at nontoxic doses, it was concluded that the renal parenchymal concentration of the aminoglycosides may be directly related to their toxicity (1). Therefore, if the relative nephrotoxicity of an aminoglycoside is related to the concentration of that aminoglycoside in the renal parenchyma, we would expect to see netilmicin accumulate to a lesser extent than both gentamicin and tobramycin.

We measured the accumulation kinetics of gentamicin, tobramycin, and netilmicin in rat renal parenchyma to test the hypothesis that the renal accumulation of netilmicin is less than that of gentamicin and tobramycin. As in the previous study, we chose doses which would cause neither a decrease in glomerular filtration rate nor tubular necrosis in any of the treatment groups, as viewed by the tests performed. Thus, our model for tissue accumulation should be minimally affected by any alteration in renal function due to renal proximal tubule injury.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 150 to 200 g were randomly assigned to six groups of 120 rats each. They were housed in groups of four rats each, allowed free access to water, and fed a standard Wayne diet ad libitum. Rats received daily subcutaneous injections of aminoglycoside on a milligram-per-kilogram basis, diluted to 1 ml in 0.9% saline

* Corresponding author.
TABLE 1. Pharmacokinetic model parameters for tobramycin, gentamicin, and netilmicin accumulation in rat kidney tissue

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug (dose size, mg/kg per day)</th>
<th>A (g−1)a</th>
<th>k (days−1)a</th>
<th>t1/2 accum (days)b</th>
<th>CₚSS (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tobramycin (5)</td>
<td>1.90 (0.35)</td>
<td>0.233 (0.043)</td>
<td>3.01</td>
<td>36</td>
</tr>
<tr>
<td>2</td>
<td>Gentamicin (5)</td>
<td>3.12 (0.30)</td>
<td>0.100 (0.013)</td>
<td>6.93</td>
<td>148</td>
</tr>
<tr>
<td>3</td>
<td>Netilmicin (5)</td>
<td>5.05 (0.41)</td>
<td>0.134 (0.013)</td>
<td>5.17</td>
<td>176</td>
</tr>
<tr>
<td>4</td>
<td>Tobramycin (20)</td>
<td>0.48 (0.05)</td>
<td>0.063 (0.012)</td>
<td>11.00</td>
<td>148</td>
</tr>
<tr>
<td>5</td>
<td>Gentamicin (20)</td>
<td>1.44 (0.13)</td>
<td>0.105 (0.012)</td>
<td>6.60</td>
<td>260</td>
</tr>
<tr>
<td>6</td>
<td>Netilmicin (20)</td>
<td>1.93 (0.17)</td>
<td>0.073 (0.011)</td>
<td>9.49</td>
<td>510</td>
</tr>
</tbody>
</table>

a The standard deviation of the estimated parameter is given in parentheses. b t1/2, Accumulation half-life.

determined. A nonparametric statistical test was then performed.

Parenchymal aminoglycoside accumulation kinetics were assessed by two independent techniques. We performed a pharmacokinetic analysis of the renal concentration-versus-time profile, using the nonlinear regression program NONLIN (13). An accumulation model was used (7) described by the general form: Cₚ = dose × A e⁻kt × (1 − e⁻nt)/(1 − e⁻kt), where Cₚ is the renal parenchymal aminoglycoside concentration, T is the dosing interval, n is the number of doses, and A and k are constants. A and k were determined simultaneously as the best estimates of the constants during least-squares analysis. The steady-state renal parenchymal concentration (CₚSS) was determined from these parameters by using the following equation: CₚSS = dose × A e⁻kt × 1/(1 − e⁻kt). The accumulation half-life (t1/2 accum) was predicted from the relationship t1/2 accum = (ln 2)/k and indicates the time required to accumulate half of the steady-state renal parenchymal aminoglycoside concentration.

A statistical evaluation of the model was performed by measuring the standard error of the estimate for the model parameters A and k and by plotting the residual difference between the model-predicted renal aminoglycoside concentrations and the measured concentrations, with the time after the first dose as the independent variable.

Independently of the kinetic model, renal parenchymal aminoglycoside concentrations, creatinine clearances, and creatinine values in serum were compared by two-way analysis of variance. Statistical evaluations of parenchymal concentrations from days 1 through 15 and days 16 through 30 were performed separately, and an evaluation of concentrations from days 1 through 30 as a continuum was also performed. A Newman-Keuls range test was performed to detect any differences between groups treated with 5 mg/kg per day and those treated with 20 mg/kg per day. The pathological scores were analyzed nonparametrically by a Kendall coefficient of concordance.

RESULTS

Mean renal parenchymal aminoglycoside concentrations are shown in Fig. 1 (5 mg/kg per day) and Fig. 2 (20 mg/kg per day) along with the concentrations predicted by the kinetic model. The model parameters are listed in Table 1. When given at a dose of 5 mg/kg per day, the aminoglycosides accumulated to significantly different levels (P < 0.0001). Further testing with a Newman-Keuls range test showed that gentamicin accumulated to over 4.1 times the concentration of tobramycin (P < 0.05) and that netilmicin accumulated to a greater extent than did gentamicin (P < 0.05). These differences were true for the comparisons between groups on days 1 through 15, 16 through 30, and 1 through 30 as a whole.

When the dose was increased to 20 mg/kg per day, the plateau concentration of gentamicin was 1.8 times greater than the concentration of tobramycin (P < 0.05). Netilmicin renal parenchymal concentration was observed to be greater than 1.9 times the concentration of gentamicin in the kidney (P < 0.05). Once again, these differences were seen when groups were compared on days 1 through 15, 16 through 30, and 1 through 30 with a Newman-Keuls range test. Renal parenchymal concentrations were seen to follow this pattern: netilmicin > gentamicin > tobramycin at both dose levels.

When the dose was increased fourfold from 5 mg/kg per day to 20 mg/kg per day, an associated increase in steady-state renal parenchymal aminoglycoside concentrations was observed. Tobramycin, gentamicin, and netilmicin concentrations increased 4.1-, 1.8-, and 2.9-fold, respectively.

An analysis of variance on creatinine values in serum for days 1 through 30 for each group showed no change in creatinine level in serum. Creatinine clearances, measured on 5 days during the study, showed a significant increase over the study period rather than a decrease which would have been indicative of kidney toxicity. This increase in creatinine clearance may be due to an approximately two-fold increase in body weight during the study. It is not likely that kidney function, as estimated through creatinine comparisons, was modified, since the creatinine level in serum was measured in every animal and did not significantly change during the study.

The Kendall test showed no significant difference in the kidney pathological scores for the low-dose groups. There was a significant change (P < 0.05) in the scores for the high-dose gentamicin and high-dose netilmicin treatments. Although these tests were statistically significant, they are of limited practical importance, since no kidney had a total pathological score above 3 out of a possible score of 24.

DISCUSSION

The comparative nephrotoxic potential of the aminoglycosides has been extensively evaluated. In the rat, several investigators have shown that gentamicin is more toxic to kidneys than is tobramycin (8, 10, 11). Further, tobramycin is more toxic than netilmicin at equivalent doses (10). Many factors are known to influence the nephrotoxicity of aminoglycosides. The precise mechanisms of this injury are unknown; however, these agents accumulate primarily in the renal cortex, where they have long elimination half-lives (12). Therefore, renal parenchymal kinetics may be of value in predicting nephrotoxic potential.

Any attempt to correlate renal drug concentrations with toxicity should follow the guidelines specified by Whelton (22). These suggestions are that the drug dose, the duration of therapy, and the time relationship of renal tissue removal for drug analysis versus the concomitant serum drug concentration must be precisely defined. Measurements of parenchymal concentrations should be frequent enough to characterize the concentration-time profile, and the study should run for at least four times the tissue accumulation half-life to guarantee that steady-state plateau tissue concentrations have been reached. Finally, a detailed statistical analysis should be performed on the data to compare the regimen used.

In following the suggestions of Whelton, we gave equivalent doses of the three aminoglycosides on a milligram-per-
kilogram basis. Further, rats were sacrificed 24 h after the previous dose so that the parenchymal concentrations measured represent trough concentrations. Concurrent concentrations of the aminoglycosides in serum were also at a trough and were below the sensitivity of the assay. Finally, we conducted the study over a 30-day period so that we could adequately characterize the entire concentration-time profile.

We studied the renal parenchymal accumulation kinetics of gentamicin, tobramycin, and netilmicin in an effort to compare known toxicities to their respective accumulation kinetics. Experiments have been performed to correlate

FIG. 1. Mean renal parenchymal aminoglycoside concentration (± standard deviation) after a daily 5-mg/kg dose of tobramycin (○), gentamicin (●), or netilmicin (□). Lines represent the model-predicted concentrations.

FIG. 2. Mean renal parenchymal aminoglycoside concentration (± standard deviation) after a daily 20-mg/kg dose of tobramycin (○), gentamicin (●), or netilmicin (□). Lines represent the model-predicted concentrations.
toxicity with parenchymal drug concentration. These experiments have not regularly shown an association between drug accumulation and toxicity (11, 21). Since most of these investigations primarily addressed the issue of relative toxicity, large doses were used to induce renal injury. Decreasing glomerular filtration rate, tubular necrosis, and cellular regeneration may have prevented the demonstration of a consistent correlation between renal parenchymal aminoglycoside concentration and resultant toxicity. Thus, any differences in accumulation may have been influenced by a decrease in kidney function and not by the propensity of the aminoglycoside for accumulation. In this study, it has been demonstrated by measurements of creatinine level in serum and creatinine clearance, and by a detailed microscopic examination that no renal tubular injury occurred; therefore, any accumulation is dependent only on the affinity of the aminoglycoside for renal tissue. Aminoglycoside concentrations in the rat kidney mirror those of a previous experiment (1), with the addition of a netilmicin treatment group.

Josepovitz et al. (9) report a method of blocking the cortical uptake of gentamicin through the use of other aminoglycosides and similar polycationic molecules. They suggest that the uptake of gentamicin and the other aminoglycosides may be mediated by a high-capacity, low-affinity system. Since apical transport of the aminoglycosides is thought to be mediated by anionic phospholipids (17), one may expect a correlation between cationic charge on the aminoglycoside and the parenchymal concentration. In most cases, Josepovitz et al. report a correlation between net cationic charge at pH 7.4 as measured in vitro and the ability to block gentamicin uptake (9). The net charges on netilmicin, gentamicin, and tobramycin were +3.48, +3.46, and +3.10, respectively. These values correlate in a rank-order fashion with the results of our study, in which accumulation in the kidney followed this scheme: netilmicin > gentamicin > tobramycin. Our results support the hypothesis that the binding affinity to the acidic phospholipids may be related to the charge on the molecule and could explain the differences in the concentrations of the aminoglycosides in the rat kidney.

In a comparison of the renal cortical uptake of netilmicin in the rat after a 1-h infusion, Pastoriza-Munoz et al. (15) have concluded that netilmicin uptake was mediated by a low-affinity, high-capacity system similar to that observed for gentamicin. Pastoriza-Munoz et al. show that the absorptive flux of gentamicin is twice the absorptive flux of netilmicin. They also state that the secretory flux of netilmicin is significantly greater than the secretory flux of gentamicin. As a result of these differences, they found that gentamicin accumulated to significantly greater renal cortical concentrations than did netilmicin. These findings differ from ours, but the difference may be due to the shorter time of exposure to the aminoglycosides in the study of Pastoriza-Munoz et al. Even though the levels in serum may be at equilibrium after an infusion time of 1 h, our results indicate that the renal tissue will not be at steady state for at least 1 week, and other evidence also estimates that the kidney half-life of these drugs is in excess of 100 h (12). Therefore, the results of Pastoriza-Munoz and colleagues may only be indicative of the initial concentrations of these compounds in the rat kidney and not predictive of the steady-state concentrations after multiple administrations of the aminoglycosides.

Brion et al. (4) have investigated the tubular reabsorption of gentamicin, netilmicin, dibekacin, and amikacin in rabbits after a dose of 4 mg/kg per day for 14 days. Each of the aminoglycosides exhibited tubular reabsorption, but the rate of reabsorption for gentamicin was 10 times the rate for netilmicin. These results are similar to those seen earlier by Pastoriza-Munoz et al. (14, 15). In the study by Brion and colleagues, the concentrations of gentamicin and netilmicin found in the rabbit renal cortex on day 14 do not differ significantly. In a comparison of those results with those obtained in our low-dose administration, netilmicin accumulated to a greater extent than did gentamicin in both studies. Our data resulted in a statistically significant difference since we were able to make comparisons over the entire 30-day experiment, whereas the earlier study was restricted to data collected on day 14.

Collier et al. (5) reported that in experiments with isolated perfused rat kidneys, filtering kidneys had renal gentamicin concentrations four times greater than nonfiltering kidneys. This study shows that some antiluminal uptake may occur. Therefore, any differences in concentration between gentamicin and netilmicin may be due to an increase in antiluminal uptake of netilmicin. Further investigation in the isolated perfused rat kidney may show an important difference in the antiluminal uptake of gentamicin and netilmicin.

The steady-state renal concentrations of the aminoglycosides are the result of many processes occurring simultaneously. Since results of studies with acute exposure of kidneys to the aminoglycosides predict that gentamicin will accumulate to a greater extent than netilmicin (15), there must be another process which partially determines the final disposition of the aminoglycosides in the kidney. Gentamicin and netilmicin may have different rates of removal from the proximal tubule cells during chronic exposure, possibly in the form of myeloid bodies, the electron-dense material found in renal lysosomes. It is possible to speculate that netilmicin interacts with lysosomes to a lesser degree than does gentamicin. This may result in higher renal parenchymal levels of netilmicin due to less netilmicin being excreted in the form of myeloid bodies.

The accumulation of aminoglycosides in humans has been studied by Edwards et al. (6) and by Schentag and co-workers (18–20). They reported mean tissue elimination half-lives of 198, 112 and 146 h for netilmicin, gentamicin, and tobramycin, respectively. These results are many times that of the serum elimination half-life and are similar in magnitude to the values reported for elimination from rat kidneys (12). When the accumulation of tobramycin in tissue was compared with that of gentamicin, Schentag and co-workers demonstrated that tobramycin accumulated to a lesser extent than did gentamicin. Netilmicin had greater estimated tissue accumulation than gentamicin, but the difference was not statistically significant. These results are similar to our study with doses of 5 mg/kg per day, but we found that netilmicin accumulated to a significantly greater concentration than did gentamicin. Since Edwards et al. were unable to sample the kidneys of the patients involved, they used linear pharmacokinetic principles and assumed that the terminal gamma phase in serum mirrored overall tissue elimination (6). This assumption allowed the mathematical calculation of theoretical kidney concentrations and elimination half-lives rather than the actual parameters obtained in our animal model.

In a linear pharmacokinetic system, a fourfold increase in the dose would result in an increase in the drug concentration by a factor of four. When the dose was increased fourfold from 5 to 20 mg/kg per day, we observed increases in concentration of 1.8, 2.9, and 4.1 times for gentamicin, netilmicin, and tobramycin, respectively. These results indi-
cate that aminoglycoside renal parenchymal accumulation kinetics are nonlinear and that saturation of some uptake mechanism may be occurring. The saturation of this mechanism may occur at lower doses for gentamicin than for netilmicin and tobramycin.

The steady-state renal concentrations of the aminoglycosides are ordered at the same dose levels; netilmicin accumulates to a greater extent than does gentamicin, and gentamicin concentrations are greater than those of tobramycin. In a similar study involving only gentamicin and tobramycin, we have concluded that nephrotoxicity may be related to the renal parenchymal concentration of the aminoglycoside (1). The results of the current study indicate that aminoglycoside nephrotoxicity is not solely related to the concentration of drug in renal tissue; rather, aminoglycoside toxicity is dependent on other incompletely understood interactions between the drugs and the cells of the renal proximal tubule. Although the concentration of an aminoglycoside in renal parenchyma may still be a factor, investigation into the role of drug structure and toxicity should continue.

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LITERATURE CITED