Recovery from Aminoglycoside Nephrotoxicity with Continued Drug Administration

FRIEDRICH C. LUFT,[1]* LAURA I. RANKIN,[1] REBECCA S. SLOAN,[1] AND MOO NAHM YUM[2]

Departments of Medicine[1] and Pathology,[2] Indiana University Medical Center, Indianapolis, Indiana 46202

To examine the nephrotoxicity of prolonged gentamicin administration compared to the effect obtained when a less toxic aminoglycoside is substituted during the course of treatment, we gave gentamicin (67.5 mg/kg per day) to rats for 21 days, gentamicin for 14 days followed by either netilmicin or tobramycin for 7 days, or gentamicin for 14 days followed by saline diluent. After initial tubular, necrosis, the animals recovered from renal injury whether the drug was continued or discontinued or another drug was substituted. These data are consistent with the observation that regenerating renal epithelium is resistant to continued or additional nephrotoxic insults. These findings suggest that improvement in renal function during aminoglycoside therapy cannot necessarily be attributed to the substitution of another aminoglycoside or other therapeutic interventions.

Aminoglycoside antibiotics differ in their propensity to cause renal damage in experimental animals. Previous studies have shown that tobramycin and netilmicin are less nephrotoxic than gentamicin in rats (1, 3, 7). Should nephrotoxicity occur during gentamicin therapy in humans, clinicians have the option of changing their therapy to an aminoglycoside purported to be less nephrotoxic. In all likelihood any improvement in renal function would be attributed to the drug substitution. We studied the course of prolonged gentamicin administration in rats with or without substitution of another aminoglycoside late in the course of treatment. After 2 weeks, renal function and structure recovered whether the gentamicin was continued or another drug was substituted. These studies indicate that, in rats, recovery from aminoglycoside nephrotoxicity may occur despite continued administration of the same, or a different, aminoglycoside.

MATERIALS AND METHODS

Adult, male Sprague-Dawley rats (Cox Laboratories, Indianapolis, Ind.), weighing 225 to 250 g, were housed and fed as described elsewhere (4). Ten groups of 12 rats each were studied. Four groups were given gentamicin, 67.5 mg/kg per day, as a daily subcutaneous injection in 1 ml of saline diluent for 14 days. Thereafter, group 1 received saline diluent, group 2 received netilmicin (90 mg/kg per day), group 3 received tobramycin (67.5 mg/kg per day), and group 4 received gentamicin (67.5 mg/kg per day) for an additional 7 days, after which the animals were sacrificed. Groups 5 and 6 received netilmicin, 90 mg/kg, and tobramycin, 67.5 mg/kg, daily for 21 days, respectively, whereas group 7 received only saline diluent. Groups

8, 9, and 10 received gentamicin, netilmicin, or tobramycin respectively, at the above doses for 14 days prior to sacrifice on day 15. The experiments were performed in two phases. Rats studied for 3 weeks (groups 1 through 7) were studied simultaneously, as were rats studied for 2 weeks (groups 8, 9, and 10). The dose of gentamicin was chosen because it regularly produces a relatively homogeneous nephrotoxic injury in rats after 2 weeks of administration (6). The dose of netilmicin was adjusted upward since that drug is given at a dose higher than gentamicin in humans (F. C. Luft, J. Intern. Med. Res., in press).

On the day prior to the first injection (day 0), day 7, day 14, and, for groups 1 through 7, day 21, 24-h urine specimens were collected, and volume, osmolality, protein, and *n*-acetyl-glucosaminidase (NAG) were measured. Blood was obtained from the tail for blood urea nitrogen (BUN) determinations. On the day of sacrifice, creatinine was measured in serum and urine for the calculation of creatinine clearance. The kidneys were prepared for light microscopy by standard techniques. Histological changes were graded as follows by a pathologist unaware of the regimens: grade 0, normal; grade 1, cloudy swelling of proximal tubular epithelium without necrosis; grade 2, necrosis of <25% of the cortical area; grade 3, necrosis ≥25% and <50% of the cortical area; grade 4 necrosis ≥50% and <75% of the cortical area; and grade 5, necrosis ≥75% of the cortical area.

NAG in urine was measured by the technique of Patel et al. (12), BUN was measured by the method of March et al. (10), and creatinine was measured by the method of Martinez and Doolan (11). The urine osmolality was determined by freezing-point depression (Advanced Instruments, Newton Highlands, Mass.). Protein in urine was measured by the Coomassie dye reaction (2). Statistical comparisons were performed by one-way analysis of variance and by Student's *t* test as appropriate.

RESULTS

Data from rats studied for 3 weeks (groups 1 through 7) are outlined in four figures. Urine protein excretion (Fig. 1), urine NAG excretion (Fig. 2), urine osmolality (Fig. 3), and BUN (Fig. 4) all indicated that in groups receiving gentamicin (1 through 4) renal injury occurred earlier and to a greater extent than with netilmicin or tobramycin. For the excretion of protein and NAG, these differences were significant by day 7 ($P < 0.05$). In groups receiving gentamicin, these two indicators increased abruptly, early in the course of renal injury, and then decreased. Specific details on these two variables on day 7 for all 10 groups are provided in Table 1. By day 15, proteinuria and enzymuria decreased. Groups receiving netilmicin or tobramycin displayed progressive increases in protein and NAG excretion over the entire treatment period.

Urine osmolality decreased progressively in all treatment groups but most markedly in those

given gentamicin (Fig. 3). By day 15, a significant ($P < 0.05$) separation between groups receiving gentamicin and those receiving other regimens was apparent. The changes in BUN by day 15 provided a similar ($P < 0.05$) discrimination (Table 1). However, those groups (1 through 4) receiving gentamicin or some other regimen for an additional 7 days all had decreases ($P < 0.05$) in BUN toward normal values.

Table 1 outlines the creatinine clearance values at sacrifice and the pathological scores. Animals receiving gentamicin for 2 weeks had a mean creatinine clearance of 0.59 ± 0.37 (standard deviation) ml/min compared to 1.29 ± 0.33 (standard deviation) ml/min in animals receiving gentamicin for 3 weeks ($P < 0.05$). Similarly, the group receiving gentamicin for 2 weeks had the most severe pathological score ($P < 0.05$). That group showed 75 to 100% necrosis of the outer cortical tubular epithelium. Though not quantitated by means of a graded scale, early regenerative changes were evident. The group receiving gentamicin for 3 weeks, on the other hand, showed progressive, advancing regeneration of the cortical epithelium. The regenerating
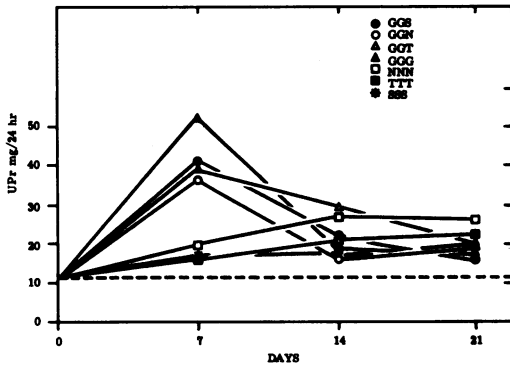

FIG. 1. *Effect of the regimens on mean urine protein excretion; broken line represents mean day 0 value for all groups. G, Gentamicin; S, saline; N, netilmicin; T, tobramycin.*
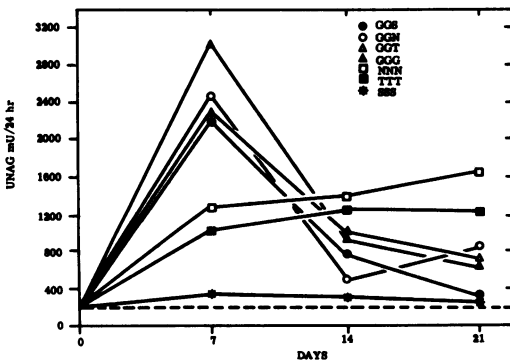

FIG. 2. *Effect of the regimens on the mean excretion of NAG. Abbreviations as in legend to Fig. 1.*
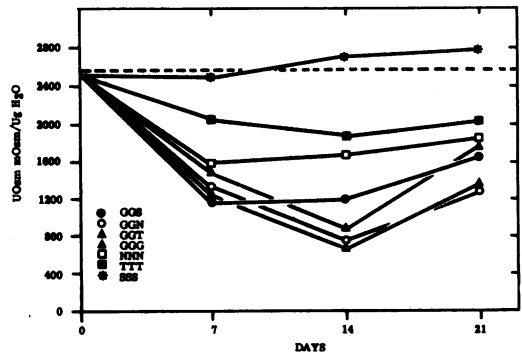

FIG. 3. *Effect of the regimens on mean urine osmolality. Abbreviations as in legend to Fig. 1.*
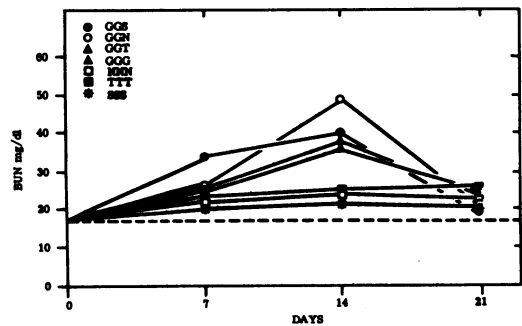

FIG. 4. *Effect of the regimens on mean BUN. Abbreviations as in legend to Fig. 1.*

TABLE 1. *Effects of regimens on discriminating variables (mean ± standard deviation)*

		Day 7		Day 15		Sacrifice	
Group	Regimens[a]	UPr[b] (mg/24 h)	NAG (mU/24 h)	Uosm[c] (mosmol/kg per H_2O)	BUN (mg/dl)	Ccr[d] (ml/min)	Path score (0–5)
1	GGS	42 ± 23	2,430 ± 844	1,188 ± 489	40 ± 22	1.50 ± 0.27	2.20 ± 0.42
2	GGN	36 ± 15	2,697 ± 950	724 ± 353	47 ± 24	1.62 ± 0.37	2.43 ± 0.79
3	GGT	38 ± 15	2,458 ± 1,103	874 ± 376	36 ± 12	1.57 ± 0.36	2.75 ± 0.97
4	GGG	53 ± 17	3,247 ± 1,291	688 ± 477	38 ± 14	1.29 ± 0.33	3.17 ± 1.27
5	NNN	20 ± 6	1,387 ± 404	1,738 ± 395	25 ± 4	1.67 ± 0.34	2.30 ± 0.48
6	TTT	16 ± 5	1,168 ± 397	1,868 ± 291	25 ± 3	1.62 ± 0.31	2.58 ± 0.51
7	SSS	18 ± 5	338 ± 49	2,891 ± 362	21 ± 3	1.61 ± 0.28	0.00
8	GG	69 ± 27	2,933 ± 1,335	773 ± 244	50 ± 17	0.59 ± 0.37	4.90 ± 0.35
9	NN	23 ± 9	1,525 ± 336	1,602 ± 297	19 ± 6	1.20 ± 0.34	2.20 ± 0.36
10	TT	24 ± 4	762 ± 210	1,852 ± 273	27 ± 8	1.42 ± 0.28	2.40 ± 0.35

[a] G, Gentamicin; N, netilmicin; T, tobramycin; S, saline; 7 days of treatment with each drug.
[b] UPr, Urine protein excretion.
[c] Uosm, Urine osmolality.
[d] CCr, Creatinine clearance.

cells were more numerous and more mature in appearance and had hyperchromatic nuclei. Specimens from animals in which another drug had been substituted or in which gentamicin had been discontinued were similar in appearance to those receiving gentamicin for 3 weeks.

DISCUSSION

The data indicate that rats receiving gentamicin, 67.5 mg/kg, for more than 2 weeks did not die of progressive uremia, but rather proceeded to recover essentially normal renal function. The renal architecture was indistinguishable from that of rats switched to other aminoglycosides or rats switched to saline diluent. Similar findings have very recently been reported by Bennett et al. (W. M. Bennett, K. Reger, C. Plamp, D. Houghton, G. A. Porter, and D. N. Gilbert, Clin. Res. **26**:390A, 1978). These investigators also reported that renal cortical concentrations of gentamicin decreased progressively during treatment. These findings are consistent with much earlier observations that animals recovering from acute tubular necrosis are resistant to a second nephrotoxic insult from the same, or a different, nephrotoxin (9). We recently observed that rats recovering from acute tubular necrosis induced by mercuric chloride were resistant to the toxic effects of gentamicin (8). It is apparent that recently regenerated renal tubular epithelium is relatively resistant to nephrotoxic insults.

Houghton et al. (5) observed that the necrosis following gentamicin administration was patchy in nature and was accompanied by active regeneration. The susceptibility of the epithelium appears to be considerably more heterogeneous than that observed with mercuric chloride (8).

The organism, therefore, has the opportunity to regenerate cortical epithelium during the course of injury since active necrosis and regeneration occur simultaneously. As the regeneration proceeds, the susceptible epithelium is eventually replaced by resistant epithelium. The reason for the recovered epithelium's apparent resistance to injury from gentamicin and other nephrotoxins is not known. The results of Bennett et al. (Clin. Res. **26**:390A, 1978) suggest that the lower gentamicin accumulation observed in the recovering epithelium may be important.

In humans, aminoglycoside nephrotoxicity is diagnosed by increasing BUN and plasma creatinine values, or by a decrease in creatinine clearance. Recovery from aminoglycoside nephrotoxicity, during continued administration, has not been reported in humans, since clinical detection of renal injury prompts the discontinuation of the drug. Conceivably, subclinical renal injury and concomitant repair occur in humans, but are not detected by presently used techniques. Patients receiving aminoglycosides for 6 weeks, such as those with endocarditis or malignant otitis externa, may provide a future opportunity to observe such phenomena if more sensitive tests of renal function are used. Our results suggest that improvement in renal function during aminoglycoside therapy may occur as a natural course of events and that improvement in renal function after changes in therapy or other interventions must be interpreted with caution.

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