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Reversibility of Gentamicin Nephrotoxicity in Rats: Recovery during Continuous Drug Administration[1] (40397)

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Reports from this laboratory of earlier studies have described acute tubular necrosis and azotemia in male Fischer rats given gentamicin over a 14 day period (1, 2). In the course of these studies, morphologic evidence of renal tubular regeneration was observed during the last 4 days of drug administration. The tubular regeneration was considered important in light of the results of Cuppage *et al.*, who used the same rat model and an identical dosage regimen of gentamicin (3). They sacrificed the animals after 28 days of drug administration and found only minimal signs of toxicity as evidenced by scattered areas of necrotic tubular cells, some tubular regeneration, and *in vitro* impairment of renal oxidative metabolism (3). These results, viewed in light of our previous studies, led us to hypothesize that gentamicin-induced renal proximal tubular necrosis may be reversible despite continuous exposure to the drug for longer than one month. The experiments described below support this hypothesis.

Materials and methods. Adult male Fischer 344 rats weighing 175–275 g were housed in metabolic cages, allowed free access to water, and fed a standard Purina diet, *ad libitum.* Screens were placed below the animal living spaces to avoid fecal or debris contamination of urine specimens.

Rats were given either subcutaneous antibiotic-free saline or gentamicin in equal volumes (gentamicin, 40 mg/ml, was supplied by the Schering Corporation, Bloomfield, NJ). Rats were given 40 mg/kg per day of gentamicin in two equal divided doses at 8:00 AM and 4:00 PM.

Student's two-tailed t test was used for statistical analysis of variance.

Rats received gentamicin or saline for as long as 42 days. Drug-treated rats and control rats were sacrificed approximately 16–18 hr after their last doses on days 3, 7, 10, 14, 17, 21, 28, 34, and 42. At sacrifice, blood was obtained to determine blood urea nitrogen (BUN) and serum creatinine concentrations. The kidneys were then perfused with 0.1 *M*, 300 milliosmolar phosphate buffer, pH 7.1 until the kidneys blanched and renal vein effluents were clear. One kidney was processed for electron microscopy; tissue was obtained from the other kidney for determination of the tissue gentamicin concentration and for light microscopy. For histologic studies, kidney fixation was accomplished by perfusion for 5–10 min with 1% gluteraldehyde in 0.1 *M* phosphate buffer (2).

Renal tissue was weighed, homogenized in 0.2 *M* Tris–buffer and centrifuged at 2000 g for 10 min; the eluate was stored in duplicate by radioimmunoassay (radioimmunoassay materials were obtained from Monitor Science Co., Los Angeles, CA). Results are expressed as μg of gentamicin per g wet weight of kidney.

BUN and serum creatinine concentrations were determined by standard methodologies. Osmolality was determined by freezing-point depression, using a Fisk osmometer.

Tissue specimens were coded and examined by a microscopist who had no prior knowledge of the animal's treatment group. The proportions of outer cortical tubules demonstrating light microscopic changes of tubular necrosis and of regeneration were estimated for each treatment and recovery group. Subtler changes of cell injury and

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recovery, including the progression and regression of characteristic aminoglycoside-induced cytosomal changes, were evaluated by electron microscopy.

Results. All rats survived until the scheduled time of sacrifice.

The renal gentamicin concentration varied dramatically over the 42-day experimental period, Fig. 1. Two distinct cycles of increasing and then decreasing renal drug concentration occurred. The duration of each cycle was roughly 17 days. The peak renal gentamicin concentration of 1179 ± 63 (mean ± SEM) μg/g on day 10 was statistically much greater than the renal concentration on day 17, 300 ± 42 μg/g ($P < 0.001$), and statistically much less than the second peak concentration of 1588 ± 78 μg/g ($P < 0.001$) on day 28. Even though the number of animals is small, the decrease in renal concentration between day 28 and day 34 was statistically significant, $P < 0.001$. The increase in renal concentration from 613 ± 63 μg/g on day 34 to 851 ± 45 μg/g on day 42 was barely statistically significant ($P < 0.05$) but may indicate the start of a third cycle.

The serum creatinine concentration reached a maximum mean concentration of 3.5 mg/100 ml on day 14, Fig. 1. Thereafter, the serum creatinine concentration decreased and was within the normal range for these animals by day 21. No second cyclical increase in the serum creatinine concentration occurred. The blood urea nitrogen results followed a curve virtually identical to the serum creatinine. The difference between the peak and the recovery values of BUN and serum creatinine were statistically significant ($P < 0.001$).

The urine osmolality decreased in all rats given gentamicin at the 40 mg/kg per day dose, Fig. 2. The urine osmolality in experimental animals was significantly lower ($P < 0.001$) than the urine osmolality in control animals on days 7, 11, 13, and 17. Thereafter, the urine osmolality in the gentamicin rats gradually increased. The difference between the two groups is not significant on day 35 or day 42 ($P < 0.05$).

Histologically, the most extensive tubular injury was observed after 10 days of treatment, when 75–90% of the outer cortical tubules were necrotic, Table I. With continued treatment, resolution of cell injury and reconstitution of tubular epithelium occurred and after 28 days of drug administration, the renal cortex had regained nearly normal light microscopic appearances. The cycle of cell injury and recovery is exemplified in Fig. 3.

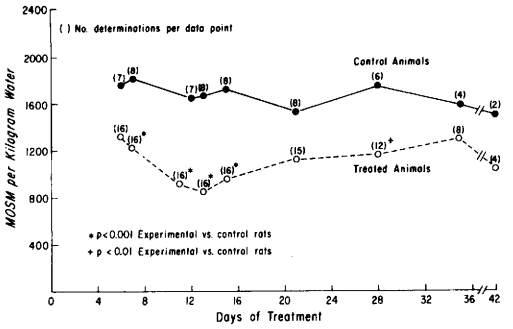

FIG. 2. Urine osmolality of rats administered either gentamicin, 40 mg/kg per day in two divided doses, or saline for 42 days. Only mean values are given.

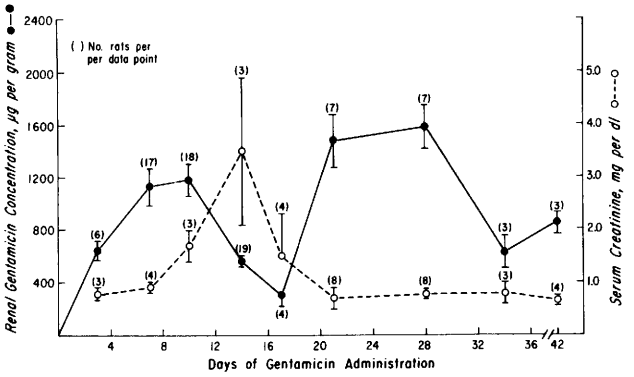

FIG. 1. Gentamicin renal concentrations and serum creatinine concentrations in rats given 40 mg/kg per day in two divided doses for 42 days. Results expressed as mean ± 2 SE.

TABLE I. EXTENT OF RENAL PROXIMAL TUBULAR NECROSIS AND REGENERATION IN RATS GIVEN GENTAMICIN, 40 MG/KG PER DAY, IN TWO DIVIDED DOSES.

Treatment duration (days)	% Acute necrosis[a]	% Regeneration[a]
3	0	0
7	10–25	0
10	75–90	10–25
17	10	25–50
21	10	10–25
28	1–5	0
34	1–5	0
42	1–5	0

[a] Expressed as % of number of cortical tubules.

Ultrastructural changes also reflected discontinuation of cell injury with tubular clearing and progressive regeneration of the epithelium. Regenerating cells were numerous after 17 days of treatment and less conspicuous after 21 and 28 days. Cytosomal changes and myeloid bodies were prominent throughout all treatment periods.

Discussion. These results demonstrate the ability of the rat kidney to recover from gentamicin toxic nephropathy despite the continued presence of the toxin. Recovery is evident by the return of the renal histology, serum creatinine concentrations, and the defect in urine concentrating ability to their pretreatment status. This model of toxicity is distinct from previous models of oliguric drug-induced acute renal failure. As a result, we were able to observe the development and recovery of renal failure over several days.

Other than Cuppage *et al.* (3), studies of gentamicin toxicity in rats have administered either a low subtoxic dose (less than 40 mg/kg per day) for up to 28 days or a toxic dose (40+ mg/kg per day) for 14–16 days, after which the animals were deliberately sacrificed (4–10). In addition, dosage schedules and species of rat vary from study to study. When doses of 150 mg/kg or more are given, especially as a single injection, many animals succumb in a few days (10). It is not clear from the literature whether death is due to neuromuscular blockage and respiratory failure or to uremia. Thus, the previous literature has not addressed the issue of reversible gentamicin nephrotoxicity in the rat.

There are probably other variables in the severity of the aminoglycoside-induced renal injury. We have observed deaths associated with severe uremia in rats which were volume-depleted, either by dietary salt restriction or frequent phlebotomy (11). Similarly, Kosek *et al.* observed uremic deaths after 14–21 days of gentamicin, 40 mg/kg per day, in Fischer 344 rats which were bled frequently during drug exposure (12). We speculate that 40 mg/kg per day of gentamicin in two divided doses rarely results in uremic deaths in rats unless intravascular volume is depleted. Additional studies are required to determine whether a higher gentamicin dosage will result in more severe uremia. It may be that all aminoglycoside-susceptible cells are killed at a dose of 40 mg/kg per day; if this is the case, a higher drug dose might not cause additional renal damage.

The return of serum creatinine concentration to the normal range, despite continued gentamicin exposure, is believed to reflect a restoration of glomerular filtration. Other investigators have measured an impairment of the glomerular ultrafiltration coefficient in the Munich-Wistar rat during exposure to gentamicin (13). Despite impaired filtration, we find no light or electron microscopic changes in the glomerulus. Thus, as in other models of acute renal failure, the mechanism of impaired glomerular filtration is uncertain (14).

The cyclical pattern of renal gentamicin concentrations is difficult to explain. Although the turnover rate of normal tubular epithelial cells is slow, tubular cell regeneration time following a single injection of mercuric chloride in Sprague-Dawley rats was only 4–5 days (15, 16). Perhaps, the 17 day cycle we observed represents tubule cell regeneration in the Fischer rat exposed continuously to gentamicin.

During the second 17 day cycle, there is a dissociation between renal function and renal concentrations of gentamicin. The absence of tubular necrosis during the second cycle may relate to the poorly-understood resistance to toxins of regenerating epithelium (17–19). Alternatively, the cell surface may interact with gentamicin, but the recently regenerated cells may not be able to transport the aminoglycoside intracellularly. Also, it is possible that the changes in renal drug concentration are an indirect reflection of changes in renal blood flow and glomerular filtration. It is difficult to rationalize the second cycle of renal gentamicin concentration with changes

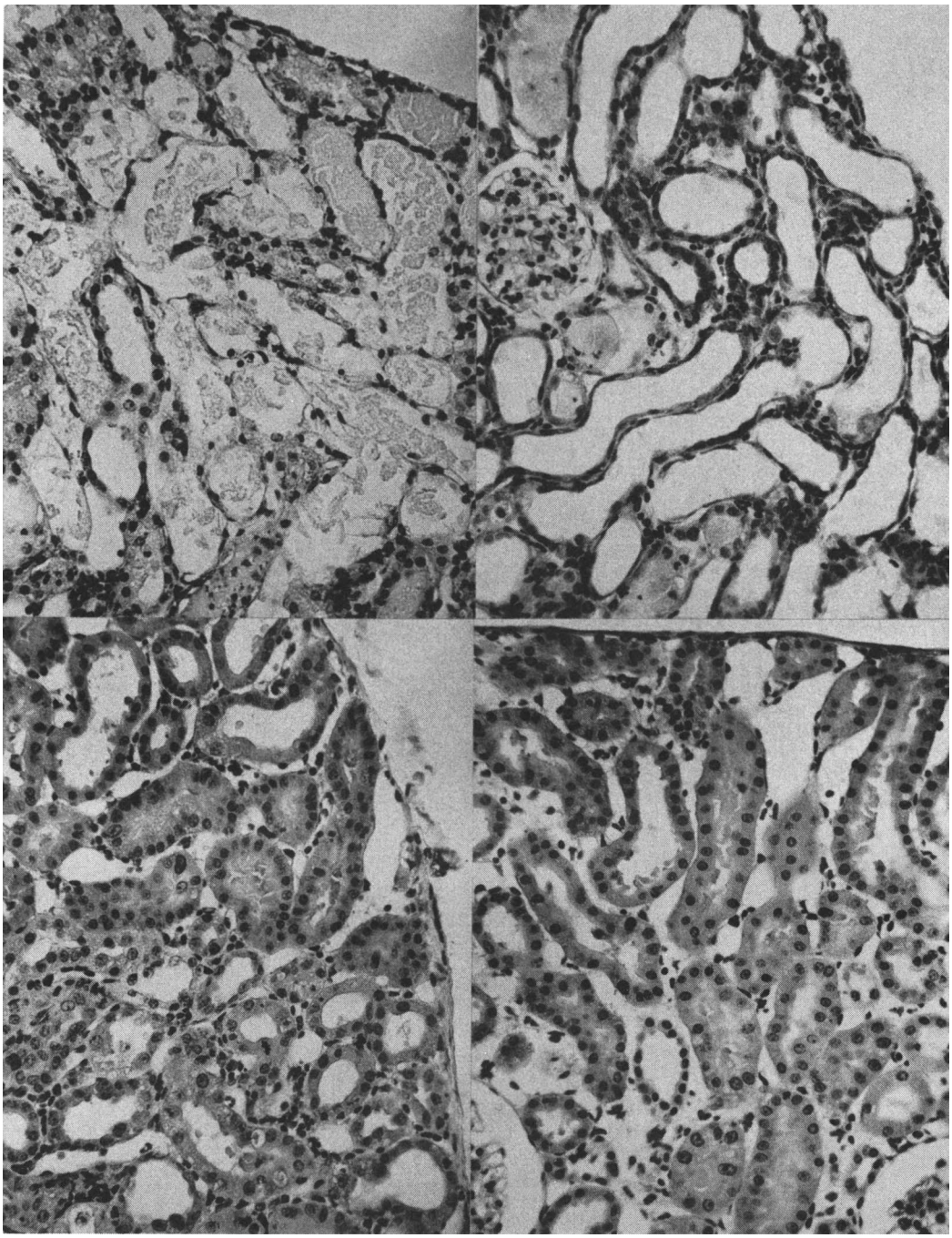

FIG. 3. From upper left, clockwise, renal cortices from animals after 10, 14, 17, and 28 days of gentamicin, 40 mg/kg per day. At 10 days proximal tubular necrosis is extensive. Lumina contain amorphous necrotic cell debris. At 14 days lumina are clear and tubules are lined by squamoid regenerating cells. Mononuclear inflammatory cells are present in the interstitium. At 17 days tubules are repopulated by columnar cells with amphiphilic, sometimes vacuolated cytoplasm and large nuclei. A single mitotic figure is visible. Brush borders are generally absent. After 28 days the cortex is comparable to that of a control animal except for the presence of occasional small scars and collections of inflammatory cells (not shown). All photos × 420, hematoxylin and eosin.

in glomerular filtration rate since the serum creatinine remained normal. In short, the pathogenesis of the cyclical kidney drug concentration remains speculative until additional studies are performed.

Summary. The administration of gentamicin, 40 mg/kg per day in two divided doses for 14 days, to male Fischer 344 rats, predictably resulted in acute tubular necrosis and azotemia. Gentamicin administration, in the same dosage, was continued for a total of 42 days because morphologic evidence of regeneration was observed after only 10 days. Despite the continued drug exposure, the blood urea nitrogen and serum creatinine concentrations returned to, and remained in, the normal range by day 21. The ability of the treated rats to produce concentrated urine improved more gradually and was within the normal range by day 34. Renal morphology progressed from acute tubular necrosis on day 10 to near normal morphology on and after day 21. The renal concentration of gentamicin went through two cycles of large increases and decreases. Since the cyclic renal concentration did not correlate with toxicity, we postulated a relationship to the turnover time of injured regenerating rat proximal tubular cells. The mechanism of gentamicin intracellular toxicity remains speculative.

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