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Effect of Sodium Intake on Gentamicin Nephrotoxicity in the Rat (39296)

WILLIAM M. BENNETT, MICHAEL N. HARTNETT, DAVID GILBERT, DONALD HOUGHTON, AND GEORGE A. PORTER

Department of Medicine and Pathology, University of Oregon Health Sciences Center and Veterans Administration Hospital, Portland, Oregon 97201

Nephrotoxicity is a serious adverse effect of gentamicin sulfate therapy. In rats with experimental acute renal failure due to glycerol and mercuric chloride, sodium depletion enhances while sodium loading modifies the renal insult (1, 2). In doses which produce proximal tubular necrosis pathologically, gentamicin accumulates in rat renal cortex (3). The present investigation examines the effect of low and high sodium intake on renal function, ultrastructure, and renal cortical gentamicin concentrations in a rat model. In preliminary experiments a 10-day treatment period with 40 mg/kg of gentamicin reliably produced reversible azotemia and extensive ultrastructural damage in animals on normal diets.

Methods. Male Fischer 344 rats weighing 250–300 g were divided into three groups of 10 rats. Nine rats in each group were given either standard Purina rat chow and tap water *ad libitum* (group 1), standard purina rat chow plus 1% saline substituted for drinking water (group 2), or sodium-deficient rat chow (Nutritional Biochemical) plus tap water *ad libitum* (group 3). In each diet group, the tenth rat served as a sham-injected control. After 2 weeks on the diet all experimental rats were given gentamicin sulfate (40 mg/kg body wt) daily sc for 10 days in two divided doses. Weight was recorded before and after gentamicin treatment.

Animals were sacrificed by exsanguination 7 days after the last gentamicin dose. Blood was flushed from the kidneys by cardiac infusion of Krebs-Ringer solution. The kidneys were fixed for electron microscopy in situ by cardiac perfusion of 1% gluteraldehyde in 0.1 *M* phosphate buffer at 100 cm of water pressure. Both kidneys then were removed, postfixed in 2% osmium tetroxide, dehydrated, and embedded in Araldite 502. After staining with uranyl acetate and lead citrate, thin sections were examined at 60 kV with a Phillips EM-200 electron microscope. Araldite embedded tissue (1 μm thick) was examined by light microscopy.

Terminal vena caval blood was analyzed for blood urea nitrogen (BUN), bicarbonate, and creatinine with Technicon Autoanalyzer. Gentamicin was determined in serum, renal cortex, and bladder urine by a modification of the adenylating enzyme method of Benveniste and Davies (4). Weighed renal tissue was homogenized in the buffer used for enzyme assay. The homogenate was centrifuged and the supernatant was assayed. The pellet was resuspended in fresh buffer, agitated, and recentrifuged. The gentamicin concentrations of both supernatants were summed. Results were expressed as micrograms of gentamicin per gram of renal tissue. All determinations were done in triplicate. The coefficient of variation for individual tissue samples was $\pm 15\%$ and for individual serum or urine samples it was $\pm 10\%$. Recovery of [^{14}C]gentamicin added to supernatants was 97% of that found in crude homogenate. After injection of [^{14}C]gentamicin iv, radioenzymatic assay was compared to isotopically determined gentamicin concentration. The range of isotopically determined gentamicin was 30 to 89% of that found in the adenylating enzyme assay.

Results. Light and electron microscopic appearances of tissue from control rats in all diet groups were normal and there was no biochemical evidence of renal failure. In group 1 animals, light microscopy showed proximal tubular cells regaining normal appearance and height from the toxic insult. In scattered areas cells had earlier stages of regeneration with lower height, few organelles, and rudimentary microvilli. Debris was present in the tubular lumens consisting primarily of unicentric myeloid bodies. Group 2 animals showed a similar appear-

ance to group 1 but clearly different from controls. Group 3 animals had extensive proximal tubular necrosis and desquamation by light microscopy. Electron microscopy showed severe tubular disruption with many cells containing only remnants of severely damaged organelles (Fig. 1). Four rats died in group 3 prior to scheduled sacrifice.

BUN, creatinine, bicarbonate, and renal cortical gentamicin as a function of the type of salt intake are summarized in Table I. BUN, creatinine, and cortical gentamicin were greater in group 3 animals than the two other groups ($P < .001$). Although cortical gentamicin was further reduced in the group 2 rats compared to group 1 ($P < .001$), renal function was similar. On the day of sacrifice urine gentamicin was 9.5 ± 2 μg/ml in group 1, 8.8 ± 1 μg/ml in group 2, and 12.6 ± 2 μg/ml in group 3. These differences were not significant. Serum gentamicin was 10.2 ± 3 μg/ml in group 3 but undetectable in the other two groups. Animals in groups 1 and 2 lost 10 ± 2% body weight and animals in group 3 lost 15 ± 4% ($P < .01$).

Discussion. In rats, ultrastructural evidence of gentamicin nephrotoxicity has been well described with doses comparable to those used in patients (3, 5). To produce renal failure in rats, however, takes many times the therapeutic dosage (5). It is known that acute renal failure in experimental animlas may be enhanced by extracellular volume depletion (1, 2). The present study provides evidence that sodium restriction by dietary means markedly potentiates renal failure in a standard rat model when compared to animals on regular or high sodium intakes. The mortality of 44% in sodium depleted animals correlates well with the severe pathological changes observed. The mechanism of enhancement of the renal insult by volume depletion remains speculative; however, activation of the renal pressor system within the kidney is an attractive

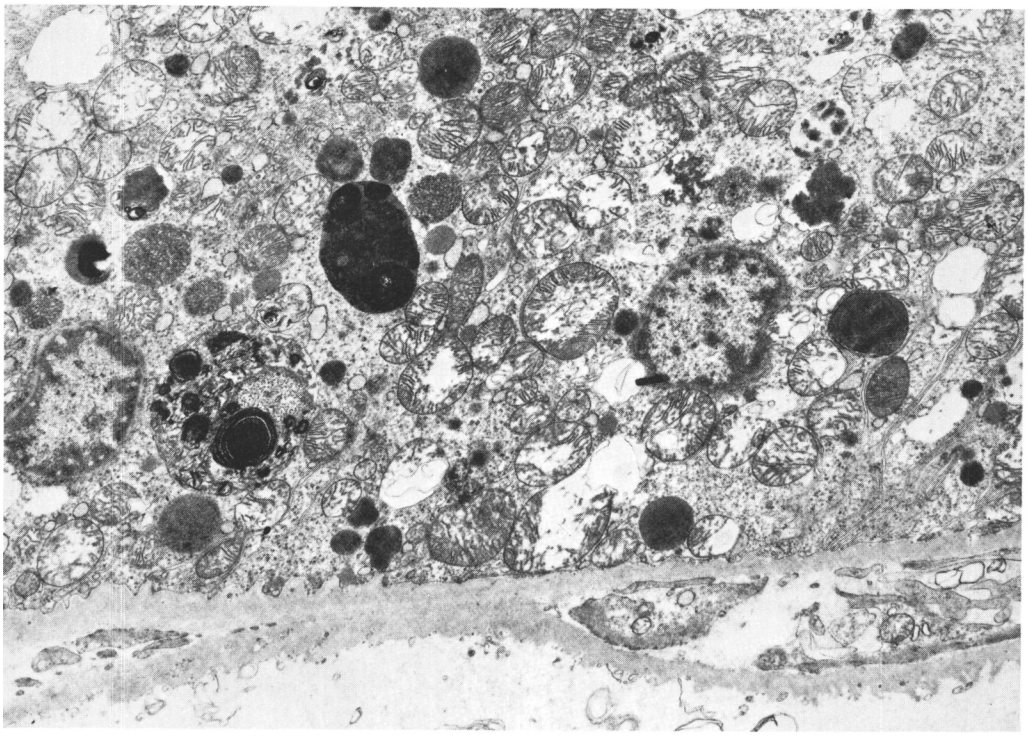

FIG. 1. Proximal tubule from an animal treated for 10 days with gentamicin. Mitochondria are in varying stages of degeneration and myeloid bodies are present within several cytosomes. Visible in the lower portion of the figure is an adjacent tubular basement membrane which has lost its epithelial cell layer. Uranyl acetate and lead citrate, × 6600.

TABLE I. SERUM CHEMISTRIES AND RENAL CORTICAL GENTAMICIN IN RATS WITH VARYING SODIUM INTAKE.[a]

	Group 1 n = 9	Group 2 n = 8[b]	Group 3 n = 5[c]
BUN (mg%)	31 ± 3	28 ± 2	276 ± 19 ($P < .001$)[d]
Creatinine (mg%)	1.2 ± .1	1.2 ± .1	9.8 ± 3 ($P < .001$)[d]
Bicarbonate (mEq/L)	21 ± 2	22 ± 3	19 ± 2
Renal cortical gentamicin (μg/g tissue)	381 ± 22	278 ± 21 (P < .001)[e]	900 ± 123 ($P < .001$)[d]

[a] Gentamicin 40 mg/kg body wt daily for 10 days in two divided doses. Animals sacrificed 1 week after therapy.
[b] One rat died during therapy of obstructive uropathy due to a bladder tumor.
[c] Four rats died of renal failure prior to scheduled sacrifice.
[d] Statistical significance using Student's t test comparing group 3 to both groups 1 and group 2.
[e] Statistical significance using Student's t test comparing group 2 to group 1.

possibility that would be consistent with our data. Recently, however, Oken *et al.* have demonstrated resistance to mercury induced nephropathy in rat with high renal renin content due to previous acute renal failure (6). It is equally possible that a decrease in excellular fluid volume reduces the volume of distribution of the drug thus increasing the serum concentration when the dose is calculated on the basis of body weight. The converse might be true in animals on high sodium intakes.

The kidney has been assumed to excrete gentamicin almost entirely by glomerular filtration (7). Luft *et al.* have recently described a prolonged half life of aminoglycoside antibiotics in rat renal cortex associated with ultrastructural evidence of proximal tubular cell autophagy (3). Although the exact mechanism of nephrotoxicity is unknown, drug transported into the cell may damage organelles resulting in the appearance of organelle fragments within lysosomes and ultimately cell rupture. Since concentrations of gentamicin in cortical tissue are increased by sodium depletion, it is tempting to speculate that the nephrotoxicity is related to enhanced intracellular concentration of gentamicin. Hsu *et al.* have recently reported that metabolic acidosis increases nephrotoxicity of gentamicin in rats given 20 mg/kg body wt (8). Bicarbonate values in our rats were not decreased in the salt depleted state. Although determinants and mechanisms of gentamicin transport into the cell are unknown, it is clear from the prolonged urinary excretion after the drug is discontinued, when serum levels are undetectable, than renal handling of gentamicin is more complex than has been assumed.

Summary. Gentamicin nephrotoxicity was examined in rats on normal, high, and low sodium diets. Low sodium diet markedly potentiated nephrotoxic effects of the drug as evidenced by animal mortality, renal failure, pathological changes, and increased renal cortical concentration of the drug. High sodium intake reduced the cortical concentration of gentamicin but renal function and ultrastructure were similar to normally fed rats given in the same dose.

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