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Potentiation of Gentamicin Nephrotoxicity by Metabolic Acidosis[1] (38213)

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One of the major adverse reactions to gentamicin sulfate administration is nephrotoxicity. Factors potentiating or modifying this toxicity have not been studied. The purpose of this investigation is to examine through use of an experimental animal model the effect on gentamicin nephrotoxicity of metabolic acidosis, a common physiological alteration frequently observed in clinical practice.

Methods. Male Sprague–Dawley rats weighing 220–240 g were fed Purina Rat Chow and weighed daily. Animals were divided into 2 groups. In group one, 23 rats were given tap water *ad libitum,* 18 of which were injected im with gentamicin sulfate 20 mg/kg body wt daily for 16 days. The remaining 5 animals were given 40 mg/kg body wt daily for 12 days. In group 2, metabolic acidosis was induced in 27 rats by substituting 1% NH_4Cl solution for drinking water throughout the experiment. Gentamicin sulfate 20 mg/kg body wt was administered im daily for 18 of these rats beginning 24 hr after acid loading; 9 rats were given an equivalent volume of normal saline for 16 days.

Two milliliters of blood were sampled from the tail vein on days 6, 12 and 16. Serum urea nitrogen (SUN) was determined by a Technicon Autoanalyzer. Serum CO_2 content was measured by a Natelson Microgasameter Model 600.

The animals in each series were sacrificed following completion of the experiment. Kidneys were excised and submitted for macroscopic and microscopic examination. Tissues were stained with hematoxylin-eosin for histological examinations.

Renal cortical uptakes of paraaminohippurate (PAH) and tetraethylammonium bromide (TEA) were also evaluated *in vitro* as measures of renal function (1) in the following groups of male Sprague–Dawley rats, weighing 160–170 g at the beginning of the experiment. Group I: Twelve rats were given tap water *ad libitum,* 6 of which were injected im with gentamicin sulfate 20 mg/kg body wt daily for 16 days. The other 6 rats were injected with an equivalent volume of saline for the same duration of time. Group II: Twelve rats were made acidotic as previously described. Gentamicin sulfate 20 mg/kg body wt was given im to 6 rats for 16 days; another 6 rats were given saline im as controls. All animals were sacrificed on the 16th day, kidneys removed and immediately immersed in ice cold saline for PAH and TEA uptake studies.

Renal cortical slices 0.3–0.4 mm thick and weighing approximately 70–90 mg were prepared with a Stadie-Riggs microtome. Incubation of slices at pH 7.40 in 3.0 ml Cross and Taggert's solution (2), containing 2×10^{-5} *M* each of ^{3}H-paraaminohippurate and ^{14}C-tetraethylammonium bromide was performed on a Dubnoff metabolic shaker at 25°, shaking at 100 cycles/min with 100% oxygen as the gas phase for 90 min.

After incubation slices were suspended in 10% trichloroacetic acid, homogenized, and centrifuged. One ml supernatant was placed

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in a glass vial containing 20 ml scintillation liquid (Aquasol-New England Nuclear Corp.). One ml incubation medium was treated similarly after deproteinization with 10% trichloroacetic acid. Counting was performed using a Packard Tri-Carb 3003 scintillation spectrometer. Slice to medium ratios (S/M) of PAH and TEA were calculated from the isotopic counts in 1 g tissue to those in 1 ml medium.

Results. SUN of 26 normal rats ranged from 18.5 to 24.5 mg% with a mean of 22.3 mg% ± 2.1 SD (Standard Deviation). Metabolic acidosis was evident 24 hr after feeding rats with 1% NH_4Cl solution. The serum CO_2 content decreased in 24 hr from the normal mean of 24.7 mM ± 0.87 SD, (N = 10) to 16.6 mM ± 1.62 and to 19.4 mM ± 2.54 on the 3rd day. By the 6th day the serum CO_2 content returned to normal (24.7 mM ± 1.69) indicating that renal compensation corrected the altered metabolic state.

As shown in Fig. 1 by the 6th day there was no significant difference between the SUN of the acidotic rats injected with gentamicin and that of normal rats injected with gentamicin. However, on the 12th day the average SUN of the acid-loaded rats injected with gentamicin, as compared to normal rats similarly injected, was significantly elevated (31.9 mg% ± 5.1 compared to 23.2 mg% ± 1.9; $P < 0.001$); the average SUN of acidotic rats injected with normal saline was 24.2 mg% ± 1.3, not being significantly different from normal rats injected with gentamicin. By the 16th day acid-loaded rats injected with gentamicin had even higher SUNs with an average of 38.6 mg% ± 11.5 ($P < 0.001$) compared to the SUNs of normal rats injected with gentamicin (26.8 mg% ± 1.9). Acidotic rats injected with saline had SUNs of 24.2 mg% ± 1.7.

Although acidosis induced by NH_4Cl administration may decrease glomerular filtration rate in the human because of depletion of the volume of body fluid (3), the weight increase of the acidotic rats (Fig. 2) was similar to that of the normal rats and SUNs of control acidotic rats were normal indicating that dehydration did not occur as a result of 1% NH_4Cl loading.

All 5 normal rats injected with gentamicin 40 mg/kg body weight were azotemic by the 12th day with average SUN of 41.5 mg% ± 14.6, ranging from 29.5 mg% to 65.5 mg%, whereas the mean SUN of normal rats given 20 mg/kg was 23.2 mg% ± 1.9.

Macroscopic examination disclosed pale, swollen, edematous kidneys in all animals receiving 40 mg/kg body wt of gentamicin. Histological examination revealed the presence of edema of the interstitium especially in the area of the proximal convoluted tubules. Focal tubular necrosis and cellular infiltration were remarkable in the cortical area. Some proximal tubules were widely dilated with flattened epithelial cells. Glomeruli were generally not affected.

The kidneys of animals receiving gentamicin 20 mg/kg body wt and of acidotic rats injected with saline appeared to be nor-

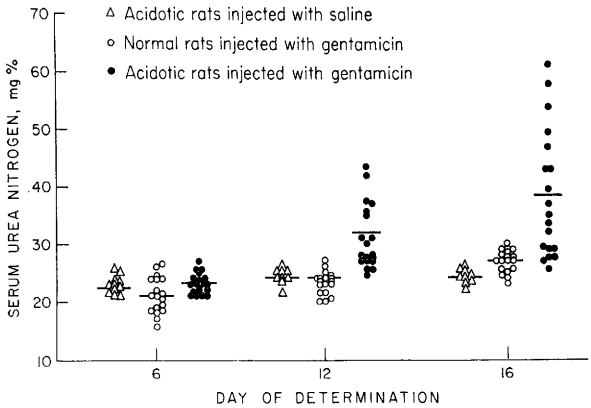

FIG. 1. SUNs of normal and acidotic rats both injected with gentamicin sulfate 20 mg/kg body wt and acidotic rats injected with saline.

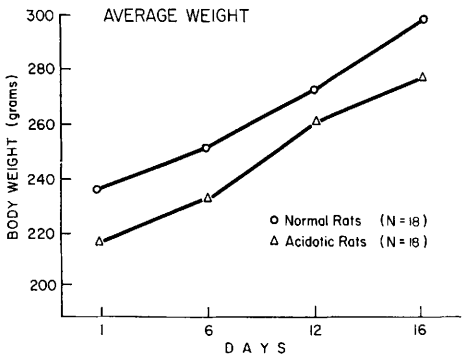

FIG. 2. Average weight increase of 18 normal and acidotic rats both injected with gentamicin sulfate 20 mg/kg body wt.

mal grossly and histologically were normal. However, kidneys of animals rendered acidotic by feeding 1% NH_4Cl solution and injected with gentamicin 20 mg/kg body wt showed pathological findings similar to those of rats receiving higher doses (40 mg/kg) of gentamicin. Grossly they were edematous and pale; proximal tubular necrosis was focal in distribution and cellular debris was desquamated into the tubular lumens. Some proximal tubules were abnormally dilated. Multifocal cellular infiltration and interstitial edema were present between tubules.

The uptake of PAH and TEA by renal cortical slices is summarized in Table I. PAH and TEA uptakes, expressed as S/M ratio, of acidotic rats injected with gentamicin for 16 days were significantly lower than those of normal rats injected with gentamicin indicating that moderately severe functional impairment of cortical renal tubular cells occurred in the acidotic rats. There were no significant differences of PAH and TEA uptake between control normal rats, acidotic rats and normal rats injected with gentamicin. However, TEA uptake of acidotic rats injected with saline was significantly higher than any other group, thus the depression of TEA uptake in acidotic rats injected with gentamicin is even more significant.

Discussion. Nephrotoxicity of gentamicin has been well established in animal experiments (4). In this study, gentamicin, 20 mg/kg, given daily to normal rats only raised the SUN slightly from an average of 21.2 mg% on the 6th day to 26.8 mg% on the 16th day. Pathological examination of these animals' kidneys failed to reveal renal tubular necrosis. Furthermore, the uptakes of PAH and TEA by renal cortical slices in normal rats treated with gentamicin for 16 days revealed no significant differences from those of control animals injected with saline. However, chronic metabolic acidosis significantly exacerbated gentamicin nephrotoxicity as shown in Fig. 1. The pathological lesions mainly involved the proximal portion of the convoluted tubules similar to those reported by Flandre *et al.* (4). Renal tubular uptake of PAH and TEA were also significantly decreased in these animals (Table I). Since PAH is transported across the proximal tubules (5) and depression of organic acid (PAH) and base (TEA) uptake in tissue slices is the earliest and most consistent proximal tubular dysfunc-

TABLE I. PAH and TEA Uptake in Normal and Acidotic Rats Given Gentamicin and Normal and Acidotic Rats Injected with Saline.[a]

	Normal rats + gentamicin	Acidotic rats + gentamicin	Normal rats + saline	Acidotic rats + saline
PAH S/M	13.87 ± 1.64	6.53 ± 1.64 $P < 0.001$*	13.05 ± 1.08	12.75 ± 1.15
TEA S/M	13.45 ± 2.17	9.47 ± 1.03 $P < 0.05$*	13.73 ± 1.73	20.32 ± 1.13

[a] S/M Ratio = Mean uptake of slice to medium ratio ± SEM. Number of rats = 6 in each experimental group.

* Statistical significance using Student *t* test comparing normal rats with acidotic rats both injected with gentamicin 20 mg/kg body wt.

tion (1), this further indicates that nephrotoxicity of gentamicin probably occurs mainly in the proximal tubule.

The exact mechanism by which gentamicin causes nephrotoxicity, as well as how chronic acid loading potentiates the renal tubular damage, is unknown. It is possible that nephrotoxicity in the rat arises as a result of a relatively higher intracellular concentration of the antibiotic. Cephaloridine is known to cause necrosis of the proximal convoluted tubule (6, 7). It is actively transported by renal tubular cells and its transport and toxicity can be inhibited and protected by probenecid administration (6, 7), suggesting that tubular transport indeed plays an important role in the development of antibiotic nephrotoxicity. The data on gentamicin clearance indicates that net renal tubular secretion of gentamicin occurs and accounts for approximately 25–35% of the gentamicin excretion when corrected for protein binding (8). It is, therefore, likely that nephrotoxicity occurs during proximal tubular secretion of the antibiotic. How metabolic acidosis potentiates gentamicin toxicity is not clear. Whether chronic acid loading would stimulate and increase renal tubular transport of gentamicin and potentiate toxicity through this mechanism remains to be investigated.

Summary. Gentamicin nephrotoxicity was examined in normal rats and in rats chronically ingesting 1% NH_4Cl solution. Metabolic acidosis significantly exacerbated gentamicin nephrotoxicity as manifested by elevation of SUN and depression of tissue uptake of PAH and TEA by renal cortical slices. In contrast normal rats given the same dose of gentamicin did not develop nephrotoxicity. Pathological examination confirmed that acute tubular necrosis occurred only in the acid-loaded rats injected with gentamicin.

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