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Lysosomal Toxicity of Gentamicin in Rat Kidney and Comparisons with Amikacin and Tobramycin

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In earlier publications (1, 3, 4), we have shown that gentamicin induces in cultured cells a lysosomal phospholipidosis characterized by (i) the uptake of the drug in the lysosomes; (ii) the specific loss of activity of lysosomal sphingomyelinase and phospholipase A1 (unpublished data); and (iii) the accumulation of phospholipids, both glycerophosphatides and sphingomyelin, in lysosomes. We report here on similar findings with kidney cortexes of rats. Data obtained with humans will be reported elsewhere (2).

Sprague-Dawley rats were treated with gentamicin for various times (0 to 14 days) at 10 mg/kg or at increasing doses (0 to 60 mg/kg, 9 days). Samples of cortex were analyzed for the following enzyme activities: (i) the lysosomal phospholipases sphingomyelinase (assayed with *N-methyl*-^{14}C-labeled bovine sphingomyelin) and phospholipase A1 (assayed with dipalmitoyl phosphatidyl choline, labeled with either *N-methyl*-^{14}C or 1-[1-^{14}C]palmitoyl, and 1-palmitoyl 2-[1-^{14}C]oleoyl phosphatidylcholine); (ii) lysosomal *N*-acetyl-β-D-hexosaminidase, sulfatase B, α-D-mannosidase, α-L-fucosidase, α-D-galactosidase, cathepsin B (these enzymes are important in the catabolism of glycolipids and glycoproteins) and acid phosphatase (this enzyme hydrolyzes water-soluble phosphoesters); (iii) glucose 6-phosphatase (endoplasmic reticulum), phosphorylase (cell sap), alanyl aminopeptidase, and γ-glutamyl transpeptidase (brush border) (these enzymes are typical of proximal tubular cells); and (iv) cytochrome oxidase (mitochondria).

Tissue was also processed for histopathology (Formalin fixation, periodic acid-Schiff, or hematoxylin-eosin staining) and electron microscopy. Kidney function was monitored by assay of serum creatinine and urea. Urine was collected from animals housed in metabolic cages.

Serum urea and creatinine were elevated only in those animals treated 8 to 9 days at 60 mg/kg. Tubular necroses were then very apparent. The osmolarity of the urine was decreased; aminoaciduria and glucosuria (the latter up to 100 mg/day) were detected, in the absence of significant variation of serum amino acid or glucose concentration.

Mild histopathological alterations were detected at 27 mg/kg for 9 days or 10 mg/kg for 14 days (hyaline globules in cytoplasm, vacuolar degeneration, loss of brush border, focal necroses). Animals that received a lower dose or a shorter treatment could not be distinguished from controls by histopathological evaluation.

Under electron microscopy, typical lesions of lysosomes were, however, seen as early as day 4 at 10 mg/kg (Table 1). These lesions consisted in the deposition of osmiophilic, dense material displaying, on high magnification, a regular, lamellar aspect (packed "membranes" with a periodicity of about 4.5 nm), consistent with a phospholipidic nature. The cell fractional volume occupied by lysosomes increased very significantly, half of this volume being contributed by lamellar material after 7 days of treatment at 10 mg/kg. No other intracellular lesions were observed as long as cell did not display signs of necroses.

TABLE 1. *Morphometrical analysis of the cytoplasm of proximal tubular cells*[a]

Component	Control	% of cell vol occupied							
		Gentamicin, 10 mg/kg				Amikacin, 30 mg/kg			
		1[b]	4	7	14[c]	1	4	7	14
A. Lysosomes	3.9	4.0	6.2	10.2	16.2	3.8	4.2	6.1	6.7
B. Myeloid bodies (dark lamellar) in lysosomes	0.12	0.21[d]	1.18	5.27	7.82	0.22	0.41	0.86	2.1
C. B/A × 100	3.1	5.2	19.0	51.7	48.3	5.8	9.8	14.1	31.3

[a] Pictures, each covering about 200 μm^2, were taken at random, at ×5,900 magnification (minimum of 48 pictures from 2 different animals per point) and enlarged 2.5-fold for evaluation of lysosomal volume or 6-fold for evaluation of the lysosome content. The morphometrical technique used is that of Weibel (Int. Rev. Cytol. **26**:235, 1969) and Baudhuin (Methods Enzymol. **32B**:3, 1974).

[b] Days of treatment.

[c] Necrotic cells were excluded from the score. Other intracellular structures did not show significant alterations, except at day 14 with gentamicin, when extralysosomal lamellar material was observed and necrotic cells became frequent.

[d] With large variations from profile to profile.

At low doses (<27 mg/kg) or after 9 days or less at 10 mg/kg, the only enzymatic alteration observed was a decrease of the activities of both phospholipase A1 and sphingomyelinase (Fig. 1). Kinetic studies showed a reduction of the maximal velocity (V_{max}), without change in apparent affinity (K_M) of the enzymes for their substrate. Total phospholipids were increased to 109.1 ± 2.3% (SD, $n = 3$) after 7 days at 10 mg/kg. Significant decrease in the activity of other enzymes occurred only at day 14 or above 27 mg/kg, except for alanyl aminopeptidase; the variations of activity of alanyl aminopeptidase were, however, not dose related.

Homogenates of kidney cortexes from rats treated with gentamicin at 10 mg/kg for 1, 4, 9, or 14 days were fractionated by isopycnic centrifugation in sucrose gradients. Up to day 9, gentamicin (we used ^{3}H-labeled gentamicin) showed a buoyant density (about 1.20 g/cm^3) and a distribution pattern similar to that of *N*-acetyl-β-D-hexosaminidase, suggesting an association with the lysosomes. At day 14, the lysosomal enzymes were found partly soluble, and gentamicin was partly dissociated from them. This could result either from an in vivo labilization of lysosomes or from an increased fragility of the enlarged lysosomes (containing lamellar material) upon homogenization.

Comparative studies were undertaken with amikacin and tobramycin (at three times and the same dose as gentamicin, respectively). Despite a higher dosage, accumulation of amikacin was similar to that of gentamicin. By all criteria shown above, amikacin induced less severe effects (Table 1 and Fig. 1). Tobramycin accumulated less than gentamicin. Its influence on lysosomal phospholipases and ultrastructure was intermediate between that of gentamicin and amikacin.

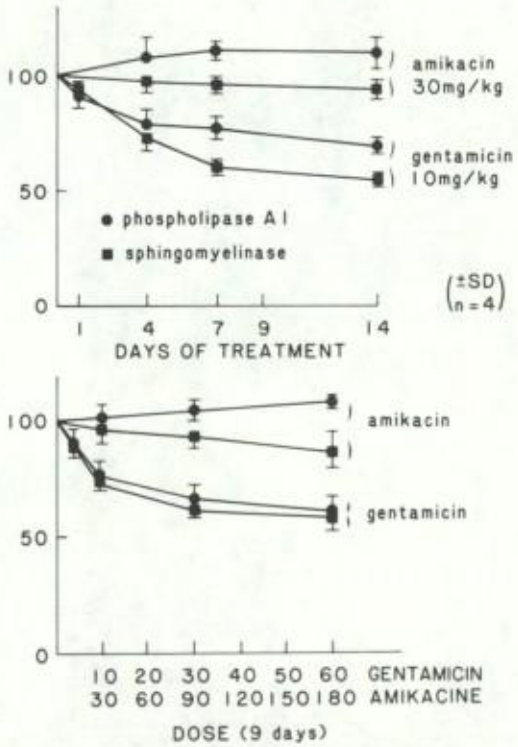

FIG. 1. *Activity of the lysosomal sphingomyelinase and acid phospholipase A1 of kidney cortexes from aminoglycoside-treated rats. The animals received the antibiotic through intraperitoneal injection once a day. Values of control (set at 100 [SD ± 5 for sphingomyelinase, ± 6 for phospholipase A1]) were obtained from a total of 10 animals. Each value is the mean (± SD) of five experimental animals.*

In conclusion, our studies show that aminoglycosides induce a lysosomal phospholipidosis in kidney tubular cells, even at low doses and after

short-term administration. Differences among aminoglycosides can be evidenced in animals (gentamicin > tobramycin > amikacin), in accordance with our previous observations on cultured cells (4). The level of accumulation of the drug is, however, an important parameter to take into account. This may be all the more essential if animal studies are to be extended to humans.

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