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Comparative Effects of Aminoglycosides on Renal Cortical and Urinary Phospholipids in the Rat (42300)

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Abstract. We examined the relationship between the nephrotoxicity potential of four aminoglycosides and the capacity of the drugs to induce a renal cortical phospholipidosis. Sprague–Dawley rats were injected subcutaneously with neomycin, gentamicin, tobramycin, or netilmicin, 100 mg/kg per day, for 1 to 4 days, and phospholipid accumulation in the renal cortex and phospholipid excretion in the urine were measured. The rank order of the drug-induced renal cortical phospholipidosis was netilmicin > tobramycin > gentamicin > neomycin. This order is the reverse of the previously established nephrotoxicity potentials of these drugs. Conversely, the rank order according to peak urinary excretion of phospholipids was gentamicin > neomycin > tobramycin > netilmicin. The rank order of the total urinary phospholipid excretion during the 4 days of the study was neomycin ≥ gentamicin > tobramycin ≥ netilmicin. Urinary phospholipid excretion may prove to be a sensitive indicator of aminoglycoside nephrotoxicity.

Aminoglycoside antibiotics induce a phospholipidosis in cells grown in culture (1–3) and in the renal cortex of man (4) and experimental animals (5–8). The phospholipidosis which has been shown to be enriched in acidic phospholipids (2, 3, 5, 8) is believed to be a consequence of lysosomal accumulation of aminoglycosides which inhibit lysosomal phospholipases (7, 9, 10). This gives rise to the prominent ultrastructural feature of aminoglycoside toxicity, the myeloid body, which we have recently shown is composed of phospholipids identical to those comprising the renal cortical phospholipidosis (11).

The relevance of the lysosomal phospholipidosis to the pathogenesis of aminoglycoside nephrotoxicity remains uncertain, because other drugs are known to induce a similar lysosomal phospholipidosis without causing cellular necrosis (12). Therefore, it seems likely that some event other than or in addition to the lysosomal phospholipidosis is required to elicit cellular injury. Nevertheless, this does not exclude the possibility that the magnitude of the phospholipidosis may serve as a predictive index of the nephrotoxicity potential of aminoglycoside antibiotics. In a previous study we presented evidence consistent with this hypothesis (5).

The objective of this study was to compare the effects of four aminoglycoside antibiotics on the accumulation of phospholipids in the renal cortex of the rat to determine if the magnitude of the renal cortical phospholipidosis correlated with the known nephrotoxicity potentials of these agents. Because aminoglycosides also promote phospholipiduria derived from the kidney (13), we also compared the effects of these drugs on the renal excretion of phospholipids.

Methods. Male Sprague–Dawley rats weighing 200–240 g were housed in metabolic cages that permitted the collection of daily urine specimens in glycine buffer, pH 8. The rats were injected subcutaneously with neomycin, gentamicin, tobramycin, or netilmicin at a dose of 100 mg of base/kg body wt per day for 1 to 4 days. Control rats were injected with an equivalent volume of 0.9% NaCl. Twenty-four hours after the last injection six rats from each group were anesthetized by intraperitoneal injection of pentobarbital and sacrificed by exsanguination from the aorta. Blood was analyzed for serum creatinine. The kidneys were removed and the cortex was dissected free. Renal cortical phospholipid was extracted in chloroform:methanol (2:1 v/v) containing 10 m*M* tetrabutyl ammonium sul-

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phate and quantitated by measuring phospholipid phosphorus as described by Bartlett (14). The data were expressed as micromoles of phospholipid per gram dry weight of renal cortex. Urine specimens were centrifuged at 15,000 rpm for 10 min and the pellet was extracted and assayed for phospholipids as previously reported from this laboratory (13).

The data in the text and figures are expressed as means ± standard error (SE). The data were subjected to analysis of variance and the new Duncan multiple range test to define statistically significant differences.

Results. Figure 1 summarizes the renal cortical phospholipid data. Rats injected with aminoglycoside for 1 day had an increase of total phospholipid compared to the saline-injected rats. In rats injected with neomycin the total cortical phospholipid did not increase further after two and three injections of the drug. After four injections the total phospholipid declined to control. In rats injected with gentamicin or tobramycin the total phospholipid in renal cortex continued to rise until the fourth injection when a plateau was reached. In rats injected with netilmicin the total renal cortical phospholipid continued to increase after the fourth injection of the drug and attained the highest level of the four experimental groups. The rank order of the drug-induced phospholipidosis was netilmicin > tobramycin > gentamicin > neomycin.

The urinary phospholipid excretion data are illustrated in Fig. 2. Rats injected with neomycin for 1 day excreted increased phospholipid in the urine compared to rats injected with saline. After the second day all rats injected with aminoglycoside excreted progressively more phospholipid in the urine compared to that of saline-injected rats, and only in the case of neomycin-injected rats was a plateau reached by the fourth injection. The rank order according to peak urinary excretion of phospholipid was gentamicin > neomycin > tobramycin > netilmicin. The rank order of the phospholipiduria determined as the sum of the urinary phospholipid excreted during the 4 days of drug injections was neomycin ⩾ gentamicin > tobramycin ⩾ netilmicin.

Figure 3 summarizes the serum creatinine data. Only neomycin-injected rats developed a statistically significant increase of serum creatinine which first became evident after the

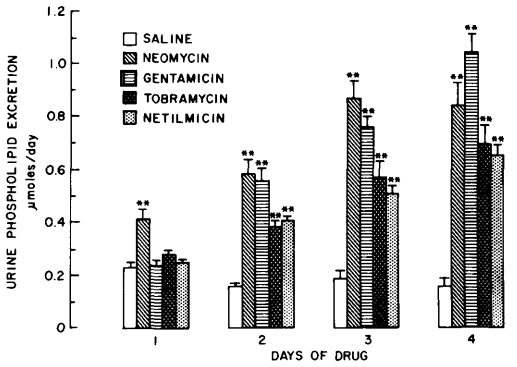

FIG. 2. Effect of drug treatment on the urinary excretion of phospholipid. **Significantly different from saline, $P < 0.01$.

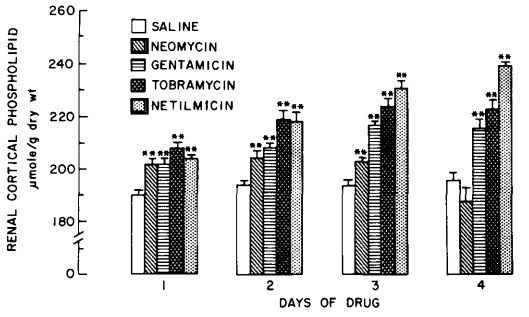

FIG. 1. Effect of drug treatment on the phospholipid content of the renal cortex. **Significantly different from saline, $P < 0.01$.

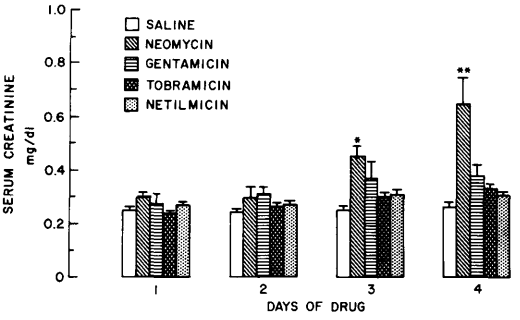

FIG. 3. Effect of drug treatment on serum creatinine concentration. *Significantly different from saline, $P < 0.05$. **Significantly different from saline, $P < 0.01$.

third injection of the drug and rose further after the fourth injection.

Discussion. Aminoglycoside antibiotics have been shown to induce a wide variety of functional and biochemical derangements in the renal cortex of man and experimental animals (15). Prominent among the biochemical defects is an alteration of phospholipid metabolism. With the exception of one study (16) all investigators who have examined the question have found that aminoglycosides induce an accumulation of phospholipids in the renal cortex (4–8) or in cells grown in culture (1–3) and that there is a preferential accumulation of acidic phospholipids (2, 3, 5, 8). We reported previously that the magnitude of the renal cortical phospholipidosis in rats treated with gentamicin was significantly greater than that of rats injected with netilmicin (5). The fact that gentamicin has been shown to be significantly more nephrotoxic than netilmicin in animals (17) and in man (18) raised the possibility that the magnitude of the phospholipidosis might correlate with the nephrotoxic potential of these drugs. To test this hypothesis we compared the effects of four aminoglycosides commonly used in clinical medicine on the accumulation of phospholipid in the renal cortex of Sprague–Dawley rats. We had established previously in our laboratory that the injection of these drugs at the dose used in this study causes a predictable degree of nephrotoxic injury in this animal model, as assessed by depression of renal function and altered morphology, with the following descending order of severity: neomycin > gentamicin > tobramycin > netilmicin (17, 19). In contrast to the expectation generated by our previous study (5), we found that the rank order of these drugs based on the magnitude of the induced renal cortical phospholipidosis (netilmicin > tobramycin > gentamicin > neomycin) correlated inversely with their known nephrotoxicity potentials. Neomycin, the most toxic of the four agents, induced the smallest increment in the phospholipid content of renal cortex whereas netilmicin, the least nephrotoxic, caused the greatest increase of renal cortical phospholipids. The latter observation contradicts our earlier report that 2 days of gentamicin treatment induced a greater renal cortical phospholipidosis than did 2 days of netilmicin (5). The reason for this discrepancy cannot be stated with certainty; however, it may be related to the fact that in our previous study the experiments involving gentamicin and netilmicin were performed consecutively whereas in the present study all experiments were conducted concurrently.

The observation that the magnitude of the renal cortical phospholipidosis correlated inversely with the established nephrotoxicity potentials of these drugs may be explained in large part by the pattern of phospholipiduria. Neomycin caused a significant increase in urinary phospholipid excretion after the first dose of the drug with further increases evident after the second and third dose of the drug. We did not attempt to ascertain the origin of the phospholipiduria in this study. In a previous study we reported evidence which supports the conclusion that the urinary phospholipids are derived in part from the excretion of lysosomal myeloid bodies as well as the loss of proximal tubular cell brush border membrane (13). We could not distinguish between exocytosis of lysosomal contents into the tubular lumen and sloughing of cell components consequent to toxic injury. In either case if the urinary loss of phospholipid were pronounced, it would limit the accumulation of phospholipid in the renal cortex. Moreover, to the extent that phospholipid synthesis was depressed as a consequence of toxic injury, this would magnify any urinary losses. Taking into consideration that neomycin rats manifested an increase of serum creatinine which we have shown correlates with proximal tubular cell necrosis in this animal model (17), a reasonable synthesis of these data is that neomycin caused a significant degree of proximal tubular cell injury evident functionally by Day 3 but probably detectable morphologically by Day 1. The increased urinary excretion of phospholipid first evident on Day 1 is probably derived from the injured cells. By Day 4 we infer that the degree of injury was sufficiently diffuse that the phospholipid content of the renal cortex declined significantly reflecting continued urinary losses and in all probability decreased phospholipid synthesis. In rats injected with gentamicin the renal cortical phospholipidosis

achieved a plateau by Day 3 which probably reflects in large part the progressively increasing losses of phospholipid in the urine. We do not exclude a component of depressed phospholipid synthesis as well, given the fact that serum creatinine tended to rise in this group of rats. The phospholipiduria was significantly less pronounced in rats injected with tobramycin and netilmicin and this probably contributed to the greater accumulation of phospholipid in the renal cortex of these rats.

Our analysis of these data leads us to conclude that the magnitude of the renal cortical phospholipidosis induced by aminoglycosides is a multifactorial process involving three major factors. The first is the rate of phospholipid accumulation within lysosomes which is thought to reflect impaired degradation of phospholipid consequent to aminoglycoside-induced inhibition of lysosomal phospholipase activity (7, 9). It should be noted that aminoglycoside antibiotics have been shown to be concentrated within the renal cortex, presumably within proximal tubular cell lysosomes, at differing rates (17, 19, 20) and have differing potentials for inhibiting lysosomal phospholipases as assessed *in vitro* (7, 9, 10). It is the interaction between these two variables which will determine the net effect of a given aminoglycoside antibiotic on the degradation of lysosomal phospholipid. The second major factor is the rate of urinary excretion of phospholipid derived from proximal tubular cells either by exocytosis of lysosomal contents or more likely by sloughing of components of injured cells. The third factor is the capacity of proximal tubular cells to sustain synthesis of phospholipid and to effect cell repair or regeneration. Of the three factors only the urinary excretion of phospholipids lends itself to continuous monitoring. The results of our studies raise the possibility that measuring the daily urinary excretion of phospholipids may prove to be a sensitive indicator of aminoglycoside nephrotoxicity.

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