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Aminoglycoside nephrotoxicity

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Nephrotoxicity has been recognized as a major complication of aminoglycoside antibiotics for many years. During the past 6 to 8 years, this problem has attracted the attention and interest of a number of investigators, resulting in the generation of a large body of experimental data that has greatly expanded our understanding of the pathogenesis of this disorder. The purpose of this paper is to review recent advances in this field.

Animal models

Our knowledge of the pathogenesis of aminoglycoside nephrotoxicity has been generated in large measure by studies performed in animal models [1–9]. The major criticism leveled at such studies is that the animal models may not accurately mimic the disease observed in humans because the threshold for aminoglycoside nephrotoxicity in most animals appears to be 13- to 60-fold greater than that deduced for humans [10]. Nevertheless, some animal models, such as the Fischer 344 rat strain [2], do exhibit sensitivity to the nephrotoxic effect of aminoglycosides similar to that of humans. Moreover, the renal functional and histopathologic lesions demonstrated by these "sensitive" animals are similar to those observed in animal models with a high injury threshold. Once the injury threshold is exceeded, all animal models, whether sensitive or resistant, exhibit similar functional and histopathologic lesions.

Functional correlates of aminoglycoside nephrotoxicity

Urine concentrating capacity. A decrease in urine concentrating capacity is one of the earliest abnormalities of renal function detected in animal models of aminoglycoside nephrotoxicity [2, 4, 5]. This abnormality may explain the observation that aminoglycoside antibiotics are a common cause of nonoliguric acute renal failure in human subjects [11]. The urine concentrating defect appears before whole kidney GFR is measurably reduced; it is not accompanied by increased urinary solute excretion or a fall in the tubular reabsorption of solute-free water; and administration of exogenous vasopressin does not correct the abnormality [4]. Cohen, Lapkin, and Kaloyanides [4] proposed that impaired urine concentrating capacity was due to a decreased number of functioning nephrons accompanied by a compensatory increase in filtration rate and a mild solute diuresis per residual nephron. Other investigators have presented preliminary evidence in support of the hypothesis that aminoglycosides interfere with the action of vasopressin on the distal nephron [11, 12]. It should be noted, however, that histopathologic lesions are confined to the proximal tubule [2, 3, 7, 8].

Proteinuria. Increased urinary protein excretion is another early manifestation of aminoglycoside nephrotoxicity [3, 5, 14]. The proteinuria is mild and usually appears before there is frank depression of GFR. Evidence of proximal tubular cell injury by light and electron microscopy [2, 3, 7, 8], together with increased urinary excretion of B_2-microglobulin [15], supports the conclusion that the proteinuria is of tubular origin.

Enzymuria. Aminoglycoside antibiotics promote increased urinary excretion of lysosomal enzymes [3, 5, 16, 17], as well as membrane-bound enzymes derived from proximal tubular cells [18, 19]. Lysosomal enzymuria may be derived from exocytosis of phagolysosomes with release of lysosomal enzymes into the tubular lumen or from frank proximal tubular cell necrosis. The increased urinary excretion of γ-glutamyl transferase [18] and alanine aminopeptidase [19] may reflect accelerated turn-

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over of brush border membrane. Although enzymuria is an early sign of aminoglycoside nephrotoxicity and typically appears before GFR becomes depressed, the level of enzymuria has not been shown to be a reliable predictor of the severity of nephrotoxicity [20].

Alterations of proximal tubular cell transport processes. Because aminoglycoside antibiotics cause proximal tubular cell injury and necrosis, investigators have sought to identify alterations of proximal tubular cell transport processes as an early clue of aminoglycoside-induced toxicity. Cohen et al [4] examined the effect of gentamicin treatment on para-aminohippurate (PAH) transport by rat kidney in vivo and in vitro. Although a depression of PAH transport was anticipated, unexpectedly increased PAH transport in vivo and in vitro was found within 48 hours of starting gentamicin treatment. Moreover, stimulation of this transport system was detected prior to alterations of other proximal tubular cell transport processes, including organic base and amino acid transport. In subsequent studies, it was shown that stimulation of organic acid transport reflected an increased active transport of PAH, as well as decreased passive back-leak of PAH across the basolateral membrane [21]. Kinetic analysis supported the conclusion that gentamicin promoted an increase in carrier protein postulated to mediate the transmembrane transport of PAH [21]. In additional studies, it was shown that other aminoglycosides share with gentamicin the capacity to stimulate PAH transport; however, no correlation between the capacity of aminoglycosides to stimulate PAH transport and their nephrotoxicity was discerned [22]. It should be emphasized that stimulation of organic acid transport was observed early in the course of aminoglycoside administration; with continued drug treatment, depression of organic acid and organic base transport occurred. Other investigators have reported similar results [23].

The significance of organic acid transport stimulation by aminoglycosides remains speculative. It is noteworthy, however, that other proximal tubular cell toxins have been reported to augment organic acid transport early in the course of proximal tubular cell injury [24]. These observations raise the possibility that stimulation of the organic acid transport system may represent a compensatory response to proximal tubular cell injury induced by a variety of toxic agents.

Kluwe and Hook [25] have examined the effect of gentamicin on other parameters of proximal tubular cell function. These investigators found that administration of gentamicin, 100 mg/kg/day, depressed ammoniagenesis in renal cortical slices after 2 days of therapy and depressed gluconeogenesis after 4 days of therapy. Glucose uptake by rat renal cortical slices was not impaired, although glucosuria has been observed in vivo [26]. The appearance of other abnormalities of proximal tubular cell transport appears to parallel the extent of proximal tubular cell necrosis [27].

Glomerular filtration rate. Depression of GFR is a relatively late manifestation of aminoglycoside nephrotoxicity. As noted above, decreased urine concentrating capacity, proteinuria, enzymuria, and alterations of proximal tubule organic acid transport are characteristically seen before there is any significant depression of GFR. In addition, histopathologic lesions of focal proximal tubular cell necrosis may be present in the absence of any depression of whole kidney GFR [14]. When GFR is depressed, however, it usually parallels the severity and extent of proximal tubular cell necrosis. Baylis, Rennke, and Brenner [28] have shown that the depression of single nephron GFR in rats injected with gentamicin is associated with a decreased glomerular capillary ultrafiltration coefficient. These investigators found no abnormalities of the glomerular capillaries by transmission electron microscopy. Recently, other workers, using scanning electron microscopy, have reported a decreased number of glomerular capillary endothelial cell fenestrations in rats injected with gentamicin [29]. This finding may provide an ultrastructural basis for the decline in SNGFR associated with gentamicin nephrotoxicity.

Histopathology

Aminoglycosides induce tubular cell necrosis, which is confined almost exclusively to the pars convoluta and pars recta of proximal tubules [2, 3, 7, 8, 30]. The earliest identifiable lesion seen by electron microscopy is an increase in the number and size of secondary lysosomes, also known as phagolysosomes or cytosegresomes, which contain myeloid bodies (Fig. 1). The myeloid body, an electron-dense lamellar structure suggestive of concentrically arranged and densely packed membranes, has been detected in proximal tubular cells within 48 hours of gentamicin administration [2]. Subsequently, other changes become evident, including a decrease in the number and height of microvillae of brush border membrane, swelling of mitochondria, cytoplasmic vacuolization, and dilatation of the cisternae of rough endoplasmic reticu-

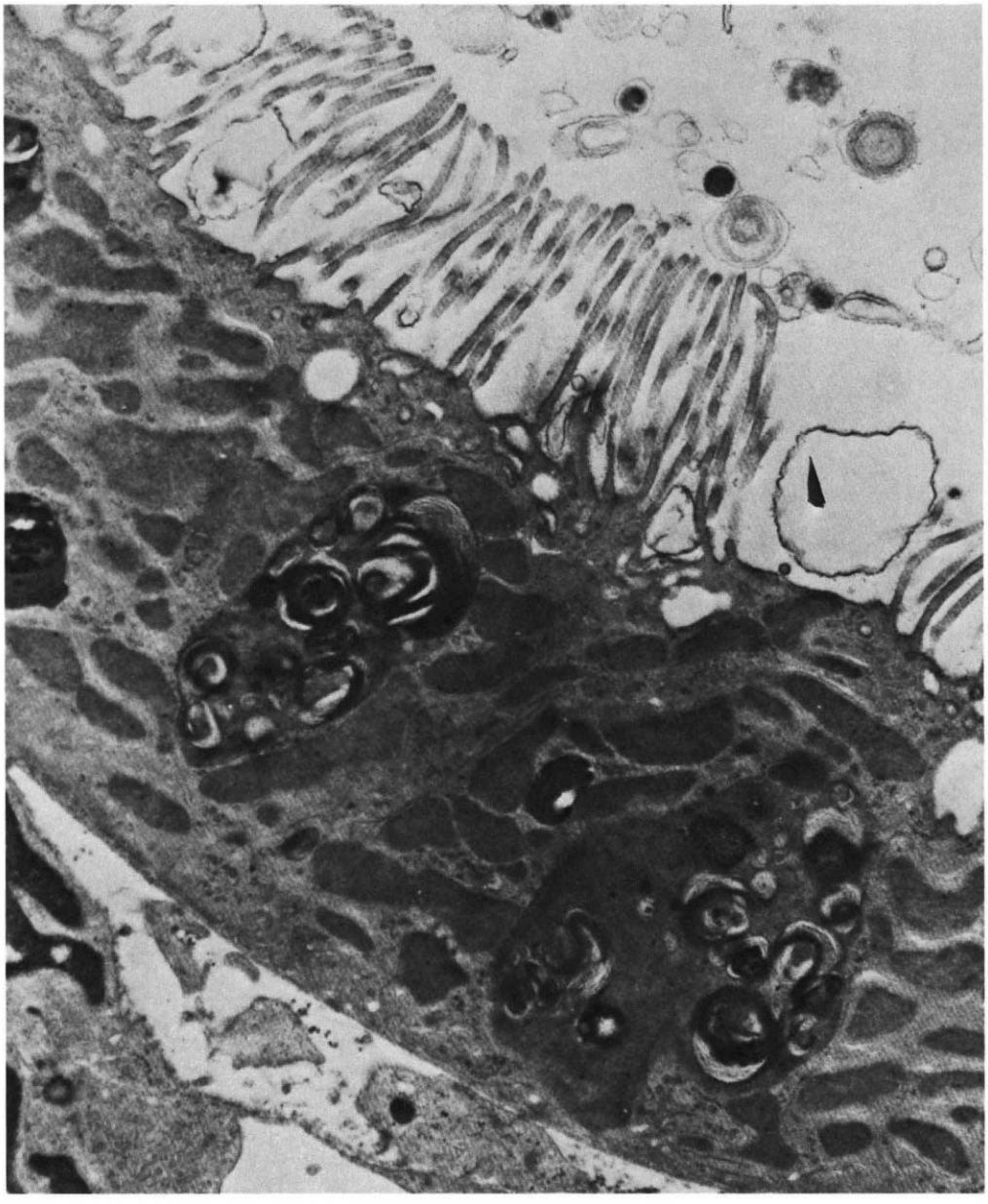

Fig. 1. *Electron micrograph of renal proximal tubule cells of a rat injected with gentamicin, 100 mg/kg for 4 days.* Note multiple myeloid bodies within lysosomes, myeloid bodies in lumen, and loss of microvillae. Cellular organelles otherwise are intact. (×17,500)

lum. These changes progress to total disorganization and disruption of cellular organelles with frank cellular necrosis (Fig. 2).

Determinants of aminoglycoside nephrotoxicity

Depending on the drug and dose schedule, the degree of proximal tubular cell necrosis induced by an aminoglycoside may range from a focal to a diffuse lesion. Thus, it is possible to define a dose schedule of an aminoglycoside that may not depress whole kidney GFR but still causes focal tubular cell necrosis. For example, we have found that netilmicin and tobramycin when administered to rats at a dose of 100 mg/kg/day for 8 days or 75 mg/kg/day for up to 28 days did not depress the creatinine clearance; however, at every time interval of tissue sampling we found evidence of focal tubular cell necrosis [14]. Moreover, we also observed signs of tubular cell regeneration in tissue sections demonstrating tubular cell necrosis. The phenomenon of tubular

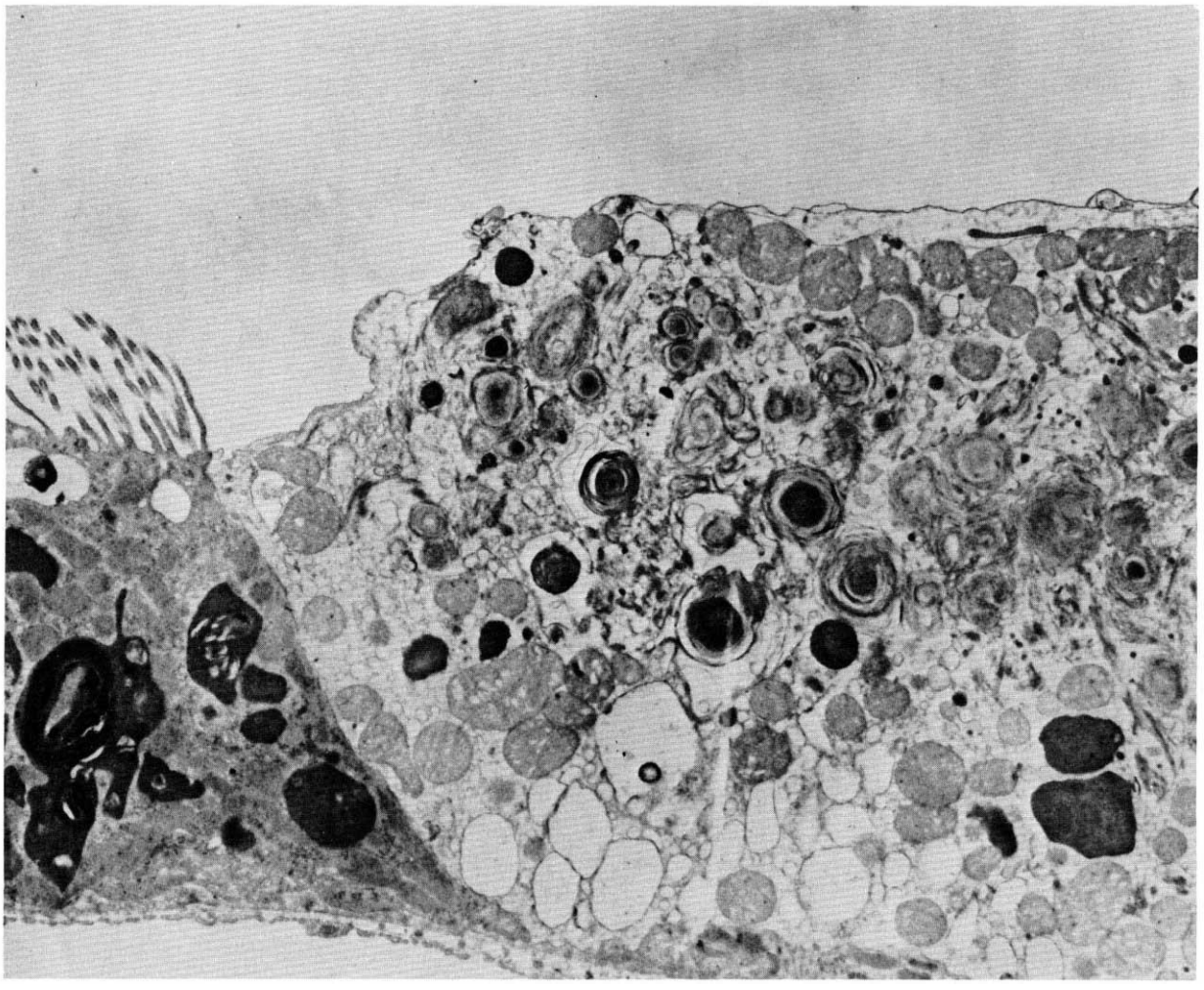

Fig. 2. *Electron micrograph of renal proximal tubule cells of a rat injected with gentamicin, 100 mg/kg for 8 days.* Cell architecture on right is totally disorganized and replaced with numerous myeloid bodies, vacuoles, and degenerating organelles. Cell architecture on left is intact with myeloid bodies within lysosomes. Microvillae of brush border membrane can be seen. (×11,250).

cell regeneration coexisting with tubular cell necrosis has been observed by other investigators [7, 8, 30]. In several studies, recovery from aminoglycoside nephrotoxicity was observed despite continuation of aminoglycosides [31, 32]. It would appear that early in the course of aminoglycoside administration the most susceptible cells experience tubular cell injury and necrosis. With continued drug administration, another population of proximal tubular cells experience injury and necrosis. If the rate of tubular cell regeneration keeps pace with the rate of tubular cell necrosis, however, progressive deterioration of renal function can be obviated. The determinants of the apparent variable susceptibility of proximal tubular cells to the toxic effects of aminoglycosides have not been identified. Age, however, may be an important factor in that young animals appear to be less susceptible to aminoglycoside nephrotoxicity than are older animals [1]. In addition, regenerating cells appear to be more resistant to the toxic effects of aminoglycosides [31–33], although the mechanism remains unknown.

The clinical threshold of aminoglycoside nephrotoxicity, therefore, is determined by the rate of necrosis and the rate of regeneration of proximal tubular cells. An increased rate of tubular cell necrosis, possibly as a consequence of a decreased injury threshold, or a decreased rate of regeneration could explain a reduced clinical threshold of aminoglycoside toxicity. As noted above, increasing age has been postulated to be an important risk factor for aminoglycoside nephrotoxicity [34]. A reduced injury threshold, as well as a reduced capacity for cellular regeneration, could contribute to the height-

ened risk of aminoglycoside toxicity associated with age [9]. Studies in animals suggest frequency of drug administration also may be a risk factor. Plamp et al [35] have reported that administration of the same amount of gentamicin in three divided doses per day was more nephrotoxic than was the same quantity of drug injected as a single dose. The increased nephrotoxicity was associated with increased renal tissue concentration of gentamicin, which is a major determinant of aminoglycoside toxicity (vida infra). Duration of aminoglycoside therapy is another obvious risk factor for nephrotoxicity [9] and probably reflects the progressive rise in renal cortical drug levels observed as a function of duration of therapy [36]. Nevertheless, recovery from aminoglycoside nephrotoxicity may occur despite continuation of therapy. In the study of Gilbert et al [32], recovery of renal function was observed in the face of reaccumulation of drug in renal cortex to levels that earlier were associated with tubular cell necrosis. These observations imply a rise in the injury threshold of regenerating epithelium.

Other risk factors include reduced renal mass [9, 34], sodium depletion [37], metabolic acidosis [38], or the concomitant administration of other nephrotoxic agents. Prior aminoglycoside treatment has been shown to augment the severity of proximal tubular cell injury following exposure to another nephrotoxin even when the dose of aminoglycoside or nephrotoxin by itself causes only mild or no significant injury. In rats, synergism has been demonstrated when aminoglycosides were given in combination with mercuric chloride [33], glycerol [39], or methoxyflurane [40]. The interaction of aminoglycosides with other nephrotoxic agents may underlie the clinical expression of nephrotoxicity even when the dose of aminoglycoside and plasma concentration of drug are well within the recommended ranges [41, 42].

Administration of an aminoglycoside in combination with a cephalosporin has been reported to increase the incidence and severity of nephrotoxicity in human subjects [43–46]. In contrast, no synergism between aminoglycosides and cephalosporins has been demonstrated in rats [47, 48], and in several studies the concomitant administration of a cephalosporin has been shown to protect against aminoglycoside nephrotoxicity presumably by reducing the accumulation of aminoglycoside within the renal cortex [49–51]. The reason(s) for the discrepancy between human studies and animal studies is not known. It should be emphasized, however, that the animal models do not accurately mimic the human model in at least one important respect: the animal models do not have an associated disease process necessitating the administration of antibiotics. It is conceivable that the underlying disease process in humans may be the critically important factor necessary for the interaction between aminoglycosides and cephalosporins.

Relationship between renal accumulation of drug and nephrotoxicity potential

It is well established that aminoglycoside antibiotics are concentrated within the renal cortex of man [36, 41, 42, 52–54] and experimental animals [5, 14, 27, 32, 33, 55, 56], and it has generally been assumed that the renal cortical accumulation of drug is intimately related to the pathogenesis of aminoglycoside nephrotoxicity. For a given aminoglycoside, the risk of nephrotoxicity increases as the renal cortical concentration of drug increases [35, 36]. The concentration of drug in renal cortex increases as a function of dose, frequency of drug administration, and duration of drug treatment until eventually a plateau is reached that reflects saturation of the transport process or the achievement of a balance between uptake and efflux of drug from renal cortex [36]. The aminoglycosides exhibit differing nephrotoxicity potentials, but as summarized in Table 1, the nephrotoxicity potentials of aminoglycosides do not correlate with the degree to which these drugs are concentrated in renal cortex. In this study from our laboratory, rats received a daily s.c. injection of drug for 2 to 8 days. Rats were sacrificed after 2 and 4 days of injection, and the renal cortical concentration of drug was determined by microbiological assay [22]. The remaining rats were sacrificed after 8 days of drug injection, and the rise in serum creatinine was determined and used as an indicator of nephrotoxicity. Neomycin was concentrated in renal cortex at a relatively low rate, yet this drug had the highest nephrotoxicity potential of the aminoglycosides tested. Netilmicin was accumulated in renal cortex to approximately the same extent as gentamicin, yet netilmicin's nephrotoxicity potential has been shown by us [14, 22] and other investigators [27] to be significantly less than that of gentamicin. These observations suggest that the nephrotoxicity of an aminoglycoside is determined by the interaction of at least two factors. The first determinant is the extent to which the drug is accumulated within the renal cortex. Streptomycin exhibits a very low rate of accumulation in renal cortex, and this contributes to the low nephrotoxicity

Table 1. Relation between renal cortical concentration of aminoglycosides and nephrotoxicity potentials[a]

	Cortical drug concentration *μg/g wet tissue wt*		Serum creatinine *mg/dl*
	× 2 days	× 4 days	× 8 days
Saline	—	—	0.34 ± 0.02 (20)
Streptomycin, 100 mg/kg/day	89 ± 8 (4)	76 ± 10 (6)	0.33 ± 0.02 (4)
Neomycin, 100 mg/kg/day	413 ± 61 (6)	940 ± 171 (6)	7.19 ± 0.58[b] (6)
Tobramycin, 100 mg/kg/day	519 ± 73 (8)	833 ± 61 (7)	0.32 ± 0.02 (8)
Kanamycin, 500 mg/kg/day	739 ± 81 (6)	1156 ± 53 (6)	2.18 ± 0.24[b] (6)
Amikacin, 500 mg/kg/day	1067 ± 116 (6)	1759 ± 111 (6)	0.96 ± 0.15[b] (6)
Netilmicin, 100 mg/kg/day	1067 ± 124 (12)	2142 ± 222 (7)	0.34 ± 0.01 (12)
Gentamicin, 100 mg/kg/day	1029 ± 114 (8)	2230 ± 275 (7)	4.73 ± 0.60[b] (8)

[a] Data are expressed as the means ± SEM. Numbers in parentheses denote numbers of rats injected.
[b] Significantly different from control, $P < 0.01$.

potential of this agent. The second factor is the propensity of the drug to cause toxic injury to intracellular organelles. Because netilmicin is accumulated within renal cortex to approximately the same extent as gentamicin is, it implies that netilmicin is significantly less toxic than is gentamicin to intracellular organelles. According to this scheme, neomycin is more toxic than is gentamicin to intracellular organelles.

Renal transport of aminoglycosides

The kidney is the major route by which aminoglycosides are eliminated from the body. Following a single injection, 60 to 80% of the drug is recovered in the urine unchanged over the subsequent 24-hour period; with repeated dosing, the daily urinary recovery of drug approaches 100% of the injected dose [9]. After discontinuation of treatment, however, drug can be detected in the urine for many days, as it is gradually released from tissue stores. Fabre et al have estimated the half-life of gentamicin in rat renal cortex to be between 98 and 166 hours [56]. Similar data have been reported in human studies [36]. The aminoglycosides have differing half-lives in renal cortex [55, 56], which may contribute to differences in nephrotoxicity potentials.

The first step in the renal elimination of aminoglycosides involves glomerular filtration. Clearance studies support the conclusion that the drug is excreted primarily by glomerular filtration. In some clearance studies, tubular absorption of drug has been inferred [36, 57, 58], whereas in other studies correction of clearance data for binding of drug to plasma protein supports the conclusion that aminoglycosides are also secreted [59]. Because the degree of protein binding has been reported to range from 0 to 30% [60, 61], caution should be exercised in drawing inferences based on these protein-binding data.

We have examined the renal handling of gentamicin in the Sprague-Dawley rat [62]. The steady-state renal clearance of ^{14}C-gentamicin was 92.5% of the simultaneously determined inulin clearance, a finding consistent with tubular absorption of gentamicin. But correction of the clearance data for protein binding of gentamicin, determined in vitro by equilibrium dialysis and ultrafiltration techniques, revealed no significant difference between the steady state clearance of gentamicin and the inulin clearance.

Using the microinjection technique, we were able to demonstrate an absorptive flux of ^{3}H-gentamicin along the proximal convoluted tubule and loop of Henle of superficial nephrons. We postulated that the absorptive flux along the loop of Henle (defined as the segment between the last accessible convolution of the proximal tubule and the first accessible convolution of the distal tubule of superficial nephrons) reflects transport of gentamicin along the pars recta [62]. No absorption of gentamicin was detected beyond the earliest accessible segment of the distal convoluted tubule. Although the absolute

absorptive flux of gentamicin increased as the load of gentamicin increased, the fractional absorptive flux decreased with increasing loads and suggested that the mechanism mediating the absorption of gentamicin can be saturated. No evidence of transtubular secretion of gentamicin was found by the precession technique [62].

Recently, Senekjian, Knight, and Weinman [63] reported in abstract form their micropuncture studies of gentamicin transport in which they also found evidence for net tubular absorption of drug along the proximal convoluted tubule. In addition, however, these investigators obtained data consistent with transtubular secretion of gentamicin.

The possibility of basolateral membrane transport of aminoglycosides has been assessed in vitro with the renal cortical slice technique. We [62], as well as other investigators [64, 65], have shown that gentamicin is accumulated in rat renal cortical slices by a process independent of the organic acid and organic base transport systems. That the uptake of aminoglycosides by rat renal cortical slices is mediated by a specific mechanism is suggested by the observation that the cortical uptake of an aminoglycoside can be competitively inhibited in vitro by other aminoglycosides [62]. We have subsequently found that tobramycin is accumulated to a greater degree by rat renal cortical slices in vitro than is gentamicin or netilmicin (unpublished observations) whereas, in vivo, tobramycin is accumulated in the renal cortex to a lesser extent than is gentamicin or netilmicin [14]. If the renal cortical slice technique indeed measures the transport of aminoglycosides across the basolateral membrane of proximal tubular epithelium, then it follows that basolateral membrane transport is quantitatively less important than apical membrane transport in the cellular accumulation of aminoglycosides. This conclusion is supported by the study of Collier et al [66], who found that the accumulation of gentamicin in the filtering isolated rat kidney was four-fold greater than the accumulation of gentamicin in the nonfiltering isolated kidney, and by the study of Chiu and Long [67].

Autoradiographic tracer studies performed by several groups of investigators support the conclusion that gentamicin and presumably other aminoglycosides are transported across the apical membrane of proximal tubular cells by pinocytosis [68, 69]. The first step in this process is the binding of the aminoglycoside to receptors located on the apical membrane of proximal tubular cells. Just and Habermann [70] have characterized the relative binding affinities of several aminoglycosides for receptors on isolated brush border membranes and have demonstrated competitive inhibition of receptor binding of macromolecules by aminoglycosides. The second step is the uptake of drug into apical vesicles. Within 10 min after a pulse injection of ^{3}H-gentamicin, the radioactive tracer can be demonstrated within apical vesicles of proximal tubular cells. One hour later the radioactive tracer can be demonstrated within lysosomes presumably due to the fusion of an apical vesicle with a primary lysosome [68, 69]. The lysosomal accumulation of aminoglycosides has also been demonstrated by ultrastructural and biochemical techniques in fibroblasts grown in tissue culture medium containing aminoglycosides [71, 72] and more recently in cells of rat renal cortex [73].

Although the available evidence unequivocally supports the intralysosomal accumulation of aminoglycosides, it remains unclear whether pinocytosis is the sole or even the major route by which these drugs gain entry into proximal tubular cells. The evidence for basolateral membrane transport of drug has been cited above. Other investigators have suggested that aminoglycosides may be transported by an amino acid transport system based on the observation that infusing large doses of an amino acid mixture decreased the renal cortical accumulation of gentamicin in dogs [74]. In view of the large dose of amino acids required to elicit an effect, it seems likely that the depression of gentamicin transport reflected the inhibitory influence of basic amino acids on binding of gentamicin to apical membrane receptors mediating pinocytosis rather than competitive inhibition of transport by the basic amino acid transport system itself.

Passive diffusion appears to be an unimportant mechanism for cellular accumulation of aminoglycosides. Auslander, Felmeister, and Sciarrone [75] have shown that gentamicin does not exhibit any significant interaction with a series of biologically important lipid membranes when examined over a wide range of pH. This observation supports the conclusion that gentamicin has a low lipid solubility. This conclusion is supported further by the study of Chiu et al [76], who found that under conditions of an alkaline urine, which would increase the fraction of gentamicin existing in the unionized form, renal cortical concentration of gentamicin decreased. This observation suggests that by shifting gentamicin from the ionized to the unionized form the extent of drug binding to apical membrane receptors was reduced, and this resulted in decreased transport of drug into the cell.

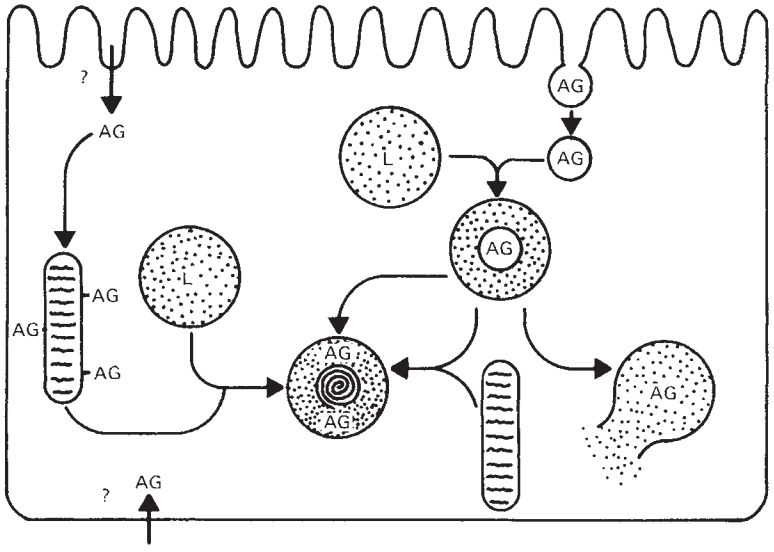

Fig. 3. *Pathways of aminoglycoside-induced cellular injury.* On the right the aminoglycoside (AG) is shown entering the cell by pinocytosis and subsequently fusing with a primary lysosome (L). The aminoglycoside may interfere with normal lysosomal digestion giving rise to myeloid body formation, and the aminoglycosides may labilize lysosomes leading to the release of potent acid hydrolases into the cytosol. If aminoglycosides gained entry into the cell by other pathways as depicted on the left, then the aminoglycosides could cause direct injury to intracellular organelles.

Figure 3 schematically illustrates the possible routes of entry of gentamicin into renal proximal tubular cells. These routes include *(1)* apical membrane transport by pinocytosis with subsequent sequestration of drug within lysosomes, *(2)* apical membrane transport by some mechanism in addition to pinocytosis, and *(3)* basolateral membrane transport of aminoglycosides. It should be apparent that the pathway(s) by which aminoglycosides gain entry into proximal tubular cells holds important implications for the pathogenesis of aminoglycoside-induced nephrotoxicity as well as possible strategies for preventing aminoglycoside nephrotoxicity. If the aminoglycosides are transported into proximal tubular cells primarily by pinocytosis, then it would strengthen the hypothesis that aminoglycoside nephrotoxicity reflects lysosomal dysfunction developing as a direct consequence of aminoglycoside sequestration within lysosomes. Moreover, it might be possible to devise strategies for reducing the uptake of aminoglycosides by pinocytosis, either by competitive inhibition or by modification of molecular structure. Alternatively, if aminoglycosides gain access into proximal tubular cells by some process in addition to pinocytosis, then the possible pathways of aminoglycoside-induced injury are greatly multiplied.

Theories of pathogenesis of aminoglycoside nephrotoxicity

Lysosomal dysfunction hypothesis. The fact that aminoglycosides induce a dramatic increase in the number and size of secondary lysosomes containing myeloid bodies together with the recent evidence that aminoglycosides are accumulated within lysosomes immediately focuses attention on the possibility that aminoglycosides may inhibit one or more lysosomal enzymes leading to impairment of the lysosomal digestive process and giving rise to the myeloid body. It should be emphasized that the myeloid body is seen in conditions other than aminoglycoside nephrotoxicity. It has been found in the lysosomal storage diseases that are characterized by genetically determined deficiency of one or more lysosomal enzymes resulting in the accumulation of incompletely degraded material within lysosomes [77]. Myeloid body formation can be induced by a large number of drugs of differing chemical structure and chemical action, but all share the characteristic of being cationic amphiphilic compounds [78–80]. These amphiphilic drugs have been shown to be concentrated within lysosomes and to induce a form of lipid storage disease characterized by the formation of myeloid bodies, presumably by inhibiting one or more lysosomal enzymes. Recently, Aubert-Tulkens, Van Hoof, and Tulkens [72] reported that gentamicin accumulates within lysosomes and induces a phospholipidosis in fibroblasts grown in culture medium containing gentamicin. The phospholipidosis reflected a generalized increase in all phospholipids and developed in parallel with the formation of myeloid bodies within lysosomes. Lysosomal sphingomyelinase activity was

significantly depressed, whereas the activities of 19 other lysosomal enzymes were unaltered. Morin et al [73] have found that treatment of Wistar rats with gentamicin, 50 mg/kg/day for 8 days, is associated with a depression of lysosomal sphingomyelinase activity obtained from renal cortex. These workers were unable to detect, however, a significant accumulation of phospholipids in rat renal cortex.

We have recently examined the effect of gentamicin on the phospholipid content of renal cortex of Sprague-Dawley rats injected with gentamicin, 100 mg/kg/day for 1 and 2 days. The data are summarized in Table 2. In contrast to Morin et al [73], we detected a significant rise in phospholipid content of rat renal cortex after 2 days of treatment. We have shown previously that after 2 days of treatment at this dose schedule, myeloid bodies are present; however, there is no other histologic evidence of nephrotoxicity, and except for stimulation of organic transport, no other functional abnormalities are detectable. Thus, a phospholipidosis of renal cortex appears to be another early metabolic alteration induced by gentamicin. We have as yet not characterized the nature of the phospholipidosis; nor have we determined whether the phospholipidosis is associated with depression of one or more lysosomal enzymes. Nevertheless, in view of the studies cited above, it is reasonable to postulate that gentamicin and presumably other aminoglycosides inhibit the activity of one or more lysosomal enzymes resulting in the accumulation of phospholipids in the form of myeloid bodies, which probably represent undegraded remnants of cytomembranes.

If it is assumed that aminoglycosides inhibit one or more lysosomal enzymes, then the question arises as to how inhibition of lysosomal enzymes and the resultant phospholipidosis results in cell injury and death. It is possible the impairment of the normal lysosomal degradation process may deprive the cell of critically important substrate. Alternatively, the accumulation of aminoglycosides within lysosomes may, due to mechanical or chemical factors, promote labilization of lysosomes with the release of potent acid hydrolases intracellularly that could attack other intracellular organelles leading to cell injury and necrosis [81].

Other investigators have suggested that aminoglycoside toxicity is a manifestation of altered mitochondrial function [6, 82]. From the available data, it remains uncertain whether the reported changes in mitochondrial function precede, follow, or occur independently of impaired lysosomal function.

Table 2. Phospholipid content of rat renal cortex[a]

	Total phospholipid *mg/g wet tissue wt*
Control	27.0 ± 0.5 (10)
Gentamicin, 100 mg/kg for 1 day	28.4 ± 0.9 (10)
Gentamicin, 100 mg/kg for 2 days	30.5 ± 0.8[b] (10)

[a] Data are expressed as the means ± SEM. Numbers in parentheses denote numbers of rats injected.

[b] Significantly different from control, $P < 0.01$.

Summary. Aminoglycoside nephrotoxicity is manifested functionally by decreased urine concentrating capacity, tubular proteinuria, lysosomal enzymuria, mild glucosuria, decreased ammonium excretion, and depression of GFR. Histopathologic lesions are confined primarily to the proximal tubule and consist of an increase in the number and size of secondary lysosomes and cytosegresomes containing myeloid bodies, disruption of brush border membranes, mitochondrial swelling, and frank tubular cell necrosis. The first step in the pathogenesis of aminoglycoside toxicity involves the accumulation of these drugs within renal proximal tubular epithelium. The renal handling of aminoglycosides includes glomerular filtration and absorption of a variable fraction of filtered drug along the pars convoluta and pars recta mediated in part by pinocytosis, which results in the segregation of aminoglycoside within lysosomes. Basolateral membrane transport of aminoglycosides has been inferred from in vitro studies of drug accumulation by renal cortical slices. The latter mechanism is independent of the organic acid or base transport systems. Unequivocal evidence of basolateral membrane transport and transtubular secretion, however, is lacking. Transport of aminoglycosides across the apical membrane of distal tubular epithelium has not been demonstrated. All aminoglycosides are concentrated in renal cortex, but there is no correlation between cortical concentration of drug and nephrotoxic potential. The second step in the pathogenesis of aminoglycoside nephrotoxicity involves the propensity of these drugs to interact with one or more intracellular metabolic pathways. Recent studies have shown that aminoglycosides induce a phospholipidosis in cells grown in tissue culture. These findings, together with the observation that aminoglycosides accumulate within lysosomes and induce myeloid body formation, suggest that aminoglycoside nephrotoxicity may be a manifestation of drug-induced lysosomal dysfunction involving phospholipid metabolism.

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