Biochemical Pharmacology, Vol. 40, No. 11, pp. 2383–2392, 1990.
Printed in Great Britain.

0006-2952/90 $3·00 + 0.00

COMMENTARY

AMINOGLYCOSIDE-INDUCED RENAL PHOSPHOLIPIDOSIS AND NEPHROTOXICITY

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Since the isolation of streptomycin by Waksman in 1944, aminoglycoside antibiotics have become one of the major groups of agents used in modern chemotherapy to combat bacterial infections. The main aminoglycosides administered today in clinical practice are gentamicin and tobramycin, two naturally-occurring compounds, and netilmicin and amikacin, which are semi-synthetic derivatives resistant to many of the bacterial enzymes that inactivate gentamicin and tobramycin. Comprehensive surveys of available aminoglycosides and of those in current development appear in papers published previously [1, 2]. Aminoglycosides exhibit a broad spectrum of activity, especially against Gram-negative bacteria, and are effective against organisms potentially resistant to beta-lactam antibiotics. Thus, their clinical indications cover a variety of infectious diseases of bacterial origin, including nosocomial infections [3, 4], and today they account for approximately one-fifth of all antimicrobial drug use in hospitalized patients.

Because of their toxicity, aminoglycosides are delivered almost exclusively in clinical settings—at least when they are given parentally. Aminoglycoside-induced untoward effects consist of nephrotoxicity, ototoxicity and neurotoxicity. The two former side-effects can be encountered under normal conditions of administration. Neurotoxicity occurs less frequently, and is mostly associated with overdosing or the presence of specific risk factors, such as *myasthenia gravis* or the concomitant administration of neuromuscular blocking agents. The present commentary will only focus on nephrotoxicity.

The clinical manifestations of renal toxicity associated with aminoglycoside therapy mostly consist of a rise in blood urea nitrogen (BUN) and serum creatinine, indicative of a fall in glomerular filtration rate (GFR). Aminoglycoside nephrotoxicity is also characterized by other signs of functional impairment, such as polyuria and urine hypoosmolality, which have been reviewed and discussed in previous papers [3, 5]. If not palliated, drug-induced renal dysfunction can lead eventually to non-oliguric renal failure. The reported incidence of functional impairment related to aminoglycoside administration fluctuates between 5 and 26%, depending on such parameters as the criteria applied to define nephrotoxicity, the aminoglycoside derivative and the possible presence of predisposing factors [3, 6–8]. Although it is usually reversible and seldom leads *per se* to a fatal outcome, kidney dysfunction due to aminoglycoside therapy complicates the management of hospitalized patients and represents a heavy economical burden [9].

Although it is typically distinguished by a decrease in GFR, the renal toxicity of aminoglycosides does not appear to involve structural alterations of the glomeruli. In contrast, a number of studies on animal models indicate that these drugs primarily affect renal proximal tubules, causing necrosis in the convoluted (S1-S2) portion [10, 11]. This histopathological pattern of tissue injury is consistent with the pharmacokinetics and disposition of aminoglycosides.

After parenteral administration, aminoglycoside antibiotics distribute in a compartment equivalent to the volume of extracellular body fluids. No metabolism has been reported for these compounds which are excreted almost exclusively by the kidneys. While aminoglycosides are eliminated primarily by glomerular filtration and appear unchanged in urine (see Refs. 3 and 5 and references therein), a small but sizeable fraction of the injected dose (approx. 5%) is retained by the renal cortex where the drug reaches concentrations several-fold higher than in serum [12, 13]. Most aminoglycoside accumulated in renal tissue is found in proximal tubular cells [14, 15], but patchy accumulation in more distal parts of the nephron also has been reported [16, 17]. Thus, one finds a striking relationship between the tissue distribution of aminoglycosides and the location of damage that they cause in the kidney. As shown by autoradiography studies, the reabsorption of these antibiotics by proximal tubular cells does not result from diffusion—they are polyaminated compounds and much too hydrophilic to freely diffuse across cell membranes (Fig. 1)—but is due to a process of adsorptive endocytosis occurring at the luminal pole (brush border membrane) of proximal tubular cells [18]. The original finding that aminoglycosides are predominantly taken up by proximal tubules has prompted several groups of investigators to examine further the fate of these drugs after reabsorption. Several studies based on tissue fractionation techniques have given

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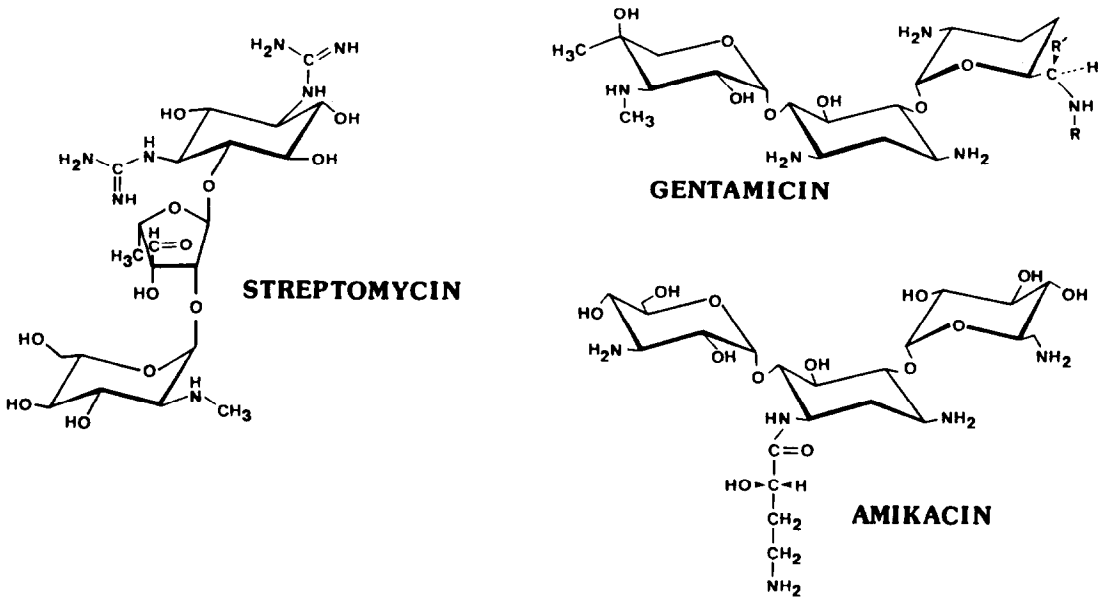

Fig. 1. Structural formulas of aminoglycoside antibiotics. Streptomycin, the first aminoglycoside to be used in clinics, was isolated by Waksman in 1944. After considerable success, it has been progressively replaced by other compounds such as gentamicin (isolated in 1963, and presently utilized as a mixture of three major components, i.e. C1: R = R′ = CH_3; C1a: R = R′ = H; C2: R = H, R′ = CH_3), and the more recent aminoglycoside amikacin (a semisynthetic derivative of kanamycin A, created in the beginning of the 1970s). Aminoglycoside molecules possess an aminocyclitol (streptidine in the case of streptomycin, 2-deoxystreptamine for all other compounds of clinical interest) connected through glycosidic bonds to two or more amino sugars. The polycationic character of aminoglycosides accounts for their peculiar pharmacokinetics, and probably for their renal toxicity.

consistent results showing that, within kidney cortex, aminoglycosides are associated almost exclusively with lysosomes, at least before the onset of overt tubular necrosis (which may lead to drug redistribution) [19, 20].

Aminoglycoside-induced lysosomal phospholipidosis

Concomitantly with the drug accumulation in kidney tissue, the epithelial cells of proximal tubules (and to a much lesser extent, that of distal tubules and collecting ducts) show typical ultrastructural alterations [10, 11, 21, 22] which precede the onset of tubular necrosis. These sublethal morphological abnormalities consist of a progressive swelling of the lysosomes, whose content becomes more osmiophilic and assumes a lamellar appearance (hence the term of "myeloid bodies" which is commonly used to describe that type of abnormal entities). Myeloid bodies have been observed both in rats and humans exposed to low, clinically-relevant doses of aminoglycosides [10, 23], and their accumulation has been shown to be time- and dose-dependent as long as aminoglycoside administration does not lead to acute tubular necrosis.

The original interpretation of Kosek *et al* [10] that gentamicin-induced myeloid bodies corresponded to an overloading of the lysosomes with undegraded polar lipids was particularly perspicacious. Indeed, in both animal and human, the development of that lysosomal alteration occurs in parallel with a rise of phospholipid content in renal cortex [13, 21, 23–25] and an increased phospholipiduria [26, 27]. Furthermore, Ramsammy *et al.* [28] have demonstrated recently that the rate of phospholipid turnover is decreased in proximal tubular cells grown in the presence of gentamicin, a finding which points to an impairment of phospholipid catabolism. Similarly, aminoglycoside administration causes a marked reduction of the activity of lysosomal hydrolases involved in phospholipid catabolism. The activities of acid sphingomyelinase and phospholipase A_1 are depressed in the renal cortex of experimental animals, or even in that of patients, after administration of low doses of aminoglycosides [21, 23, 24]. A decrease of sphingomyelinase activity has also been reported in cultured cells exposed to gentamicin or other aminoglycoside antibiotics [29–31].

It is not known whether the catabolism of other polar lipids than phospholipids is also affected by aminoglycoside antibiotics. We have observed lately that gentamicin treatment causes a significant increase of the renal tissue content in lipid- and protein-bound sialic acid [32]. Yet, examination of the activity of sialidases and several other enzymes responsible for the lysosomal catabolism of glycolipids has failed to reveal sizeable changes, either in treated animals or in cells exposed to gentamicin. However, as will be pointed out below for the phospholipases, the conditions of enzyme assay, and particularly the choice of substrate, may be critical in this respect.

Mechanism of aminoglycoside-induced phospholipid accumulation

The use of *in vitro* cell-free models has proven to

be particularly valuable to get a better insight of the mechanism underlying the aminoglycoside-induced inhibition of lysosomal phospholipase activity.

The amino groups displayed by aminoglycoside molecules confer to these compounds a marked polycationic character. Thus, one may expect aminoglycosides to interact with cell membranes which are negatively-charged because of the presence of acid phospholipids amidst their components. Indeed, aminoglycoside binding to brush-border membranes isolated from renal cortex has been demonstrated experimentally [33–35], and accounts for the endocytosis of the antibiotic by proximal tubular cells. Moreover, once transported into lysosomes, aminoglycosides are exposed to a fairly acid environment (pH around 5.4) and must also be bound to negatively-charged membranes which are physiologically brought into lysosomes by autophagy (see Ref. 36 for review). Thus far, the latter point has not been established unequivocally on isolated lysosomes, but studies using model membranes of defined compositions have clearly indicated that such binding may indeed occur.

Two types of experimental models have been utilized to examine aminoglycoside interactions with membranes: planar lipid monolayers and lipid vesicles (liposomes). Alexander *et al.* [37] have shown by microelectrophoresis that the drug causes a charge reversal of negatively-charged liposomes prepared from phosphatidylinositol or phosphatidic acid. Using gel permeation [24] or equilibrium dialysis [38], we have extended this original finding and measured the binding of various aminoglycosides to unilamellar vesicles reconstituted from major lipid constituents of cell membranes. As expected, drug binding is enhanced by an increase in the content of vesicles in acid phospholipids (such as phosphatidylinositol), and is maximized when the pH and ionic strength of the incubation medium are diminished, all these facts indicating a major involvement of electrostatic interactions between aminoglycoside molecule and negative phospholipids [24, 39, 40]. In accordance with the observations of Alexander *et al.* [37], a comparative study run on several aminoglycoside antibiotics of clinical interest has revealed differences between compounds, with regard to their propensity to associate with negatively-charged lipid bilayers (e.g. gentamicin = dibekacin = tobramycin > kanamycin A > amikacin > streptomycin) [39]. The existence of interactions between aminoglycosides and acid phospholipids has also been confirmed by independent studies performed on molecular lipid films, where these drugs have been reported to cause calcium displacement from the lipid monolayers [41] and increase their surface pressure [42].

The association of aminoglycosides with phosphatidylinositol has been analyzed in more detail, thanks to the development by Brasseur *et al.* [43] of computer-aided procedures for the conformational analysis of molecules at the lipid–water interface. All aminoglycosides that have been examined by this approach appear to form supramolecular complexes with several (usually four) molecules of phosphatidylinositol [39, 43–45]. Interestingly, the energy of interaction characterizing the stability of each complex varies according to the compound examined, showing a striking parallelism with the extent of aminoglycoside binding to lipid vesicles, measured experimentally by gel permeation. Computer-generated models of the most probable conformers show, however, that different aminoglycoside derivatives may adopt quite distinct shapes and orientations relative to the surrounding phospholipids.

The experimental demonstration and molecular characterization of aminoglycoside binding to lipid bilayers happened to be a major step towards the unraveling of the mechanism responsible for the drug-induced inhibition of phospholipid catabolism. Indeed, the use of liposomes as a membrane model has offered the opportunity to reproduce this inhibition in a defined cell-free system. The activity of lysosomal enzymes upon phospholipids was visualized by including ^{14}C-labeled phospholipids in liposomes and incubating the resulting preparation in the presence of disrupted purified lysosomes (isolated by tissue fractionation) [46]. Amazingly, we found in this system that the degradation of phosphatidylcholine, mediated by phospholipases A_1 and A_2, and lysophospholipase, was noticeably stimulated by the presence of negative phospholipids (e.g. phosphatidylinositol) along with the zwitterionic substrate [47]. The addition of gentamicin to the assay mixture reduced in a concentration-dependent fashion the hydrolysis of ^{14}C-labeled phosphatidylcholine by lysosomal enzymes [24]. Dose–effect relationships obtained with a number of aminoglycoside derivatives have revealed that different compounds are not characterized by the same inhibitory potency, gentamicin, for example, being a stronger inhibitor than amikacin, and particularly streptomycin. For many derivatives, the ability to interfere with phospholipid hydrolysis appears closely related to their propensity to interact with negatively-charged lipid bilayers [39, 43, 46], although the relationship is not absolute (see Ref. 43 for discussion). Apparently, other factors, such as the conformation and the orientation of the aminoglycoside molecule in the drug–phospholipids complexes, also contribute along with the extent of binding to determine the inhibitory potency [45]. The inhibition by aminoglycosides of lysosomal phospholipase activity has been demonstrated independently by other studies also performed on cell-free systems [48], though these studies do not arrive at exactly the same conclusions concerning the specificity of the enzymes which are affected and the degree of inhibition exerted by various derivatives. Such discrepancies, however, can be attributed to differences in experimental conditions (see the discussion in Ref. 46).

The realization that aminoglycoside-induced inhibition of phospholipase activity toward phosphatidylcholine is clearly associated with drug binding to lipid bilayers allows one to speculate on the mechanism of inhibition. Steric hindrance resulting in a lesser accessibility to the substrate (namely phosphatidylcholine) comes to mind as the most obvious explanation, but more detailed studies have proven this view to be naive. Indeed, a decrease of the

phosphatidylinositol content of liposomes, if it diminishes aminoglycoside binding to the vesicles [39], also results in an increased drug-induced inhibition of phosphatidylcholine degradation [47]. Consequently, the inhibitory effect exerted by aminoglycosides on the activity of lysosomal phospholipases A is due to another mechanism. As an alternative hypothesis, one may propose that these antibiotics decrease the availability of acid phospholipids that some phospholipases [47] and lipases [49] require to function properly. Thus, aminoglycosides would impair the lysosomal catabolism of phosphatidylcholine (and possibly of other zwitterionic phospholipids as well) by sequestering acid phospholipids and creating less favorable conditions for the hydrolysis of neutral phospholipids.

As pointed out above, aminoglycoside inhibition of lysosomal phospholipase A_1 activity upon phosphatidylcholine has been evidenced in renal cortex tissue *in vivo* [23, 24]. Although that remains to be established experimentally, the inhibition of phospholipase A_1 activity within the cell probably occurs through a mechanism similar to that derived from *in vitro* studies on cell-free systems. Because lysosomal phospholipase A_2 is inhibited by detergents commonly used to disperse cell homogenates, the influence of aminoglycoside treatment on its activity has not been reliably established. Lysosomal phospholipase C activity towards phosphatidylinositol is inhibited by aminoglycosides *in vitro* or in the kidney cortex of animals treated with these antibiotics [50]. The mechanism of this inhibition has not been established, but this observation may be of special significance, since the kidney cortex of animals, as well as the urine of animals and patients exposed to aminoglycosides, are enriched markedly in phosphatidylinositol [26, 27].

In vivo and cell culture studies have also evidenced an impairment of the activity of lysosomal sphingomyelinase upon treatment with or exposure to aminoglycosides [24, 29–31], but thus far this phenomenon has not been reproduced *in vitro* with model membranes. However, sphingomyelinase has been found to lose its activity *in vitro* when exposed to an excess of phospholipids other than sphingomyelin (especially acid phospholipids) [24, 51] and that may explain the loss of activity associated with aminoglycoside-induced phospholipidosis.

Studies with model membranes have made possible the comparison of different aminoglycosides on the basis of their binding to lipid bilayers and of the resulting inhibition of phospholipid degradation. Comparative studies have also been conducted *in vivo* to determine whether similar variations could be found in the degree of phospholipidosis induced by various derivatives, under experimental conditions relevant to the therapeutic use of aminoglycosides (4- to 10-day treatments with daily dosages equivalent to those administered in clinical practice). Initial research focused on five compounds of current use in clinics, which could be ranked as follows with respect to the extent of phospholipidosis that they caused in rat renal cortex: gentamicin $\simeq$ dibekacin $\simeq$ netilmicin $>$ tobramycin $>$ amikacin [21, 52, 53]. Except for tobramycin, this ranking roughly corresponds to the inhibitory potency of the respective compounds previously determined in cell-free systems [46]. With regard to tobramycin, which exhibits *in vivo* a milder effect than could be expected from its behaviour *in vitro*, it is known that this compound accumulates less in kidney tissue than other aminoglycoside derivatives [54–56]. More recently, the comparison has been extended to other compounds undergoing preclinical testing for renal safety, namely isepamicin [57] and micronomicin [58]. For the latter two compounds, an excellent agreement has also been found between the phospholipidosis induced *in vivo* and the inhibitory potency evaluated *in vitro* (tobramycin $>$ amikacin $\geqslant$ isepamicin; gentamicin $>$ tobramycin $>$ micronomicin).

Relationship between aminoglycoside-induced phospholipidosis and tubular necrosis

Since it actually represents the key to the comprehension of aminoglycoside nephrotoxicity, the cause of tubular necrosis has been the topic of many investigations. However, it still remains to some extent a matter of speculation despite the data that have accumulated. Depending on the experimental approach, the cytotoxicity of aminoglycoside has been attributed to drug interactions with plasma membrane enzymes (such as Na^+,K^+-ATPase [59], and phosphatidylinositol-specific phospholipase C [60]), to mitochondrial dysfunction [61, 62], to a perturbation of neoglucogenesis [63] or to an impairment of protein synthesis [64]. Although some of these alterations are potentially harmful and may compromise cell viability, they have been observed mostly during *in vitro* experiments or in studies on animals exposed to high doses of aminoglycosides, non-relevant to the clinical use of these antibiotics. Therefore, it remains difficult to determine whether they are a cause or merely a result of tubular necrosis. In contrast, the lysosomal phospholipidosis is the earliest abnormality associated with aminoglycoside accumulation within proximal tubular cells, and actually precedes overt epithelium injury. Thus, it may reasonably be assumed to play a crucial role in the onset of tubular damage. That point has proven difficult to test experimentally since there is no direct measurement for tubular necrosis. On the one hand, serum creatinine and BUN levels mostly reflect GFR, which itself depends on tubular epithelium integrity in an indirect fashion. Moreover, increase in serum creatinine, usually taken as a criterion of nephrotoxicity, only occurs in animals where a large proportion (more than 30%) of proximal tubules are necrotic [65]. On the other hand, urinalysis, if it can estimate the amount of material lost during tubular injury, is subject to such a variability that it only allows for a crude evaluation. It also does not unambiguously differentiate between cell necrosis and shedding or release of cellular material without cell death. Alternative approaches, therefore, need to be used to estimate the extent of nephrotoxin-induced tubular necrosis.

Loss of tubular epithelium due to ischemia or nephrotoxic injury is normally followed by a tissue repair reaction characterized by an increased proliferation of epithelial cells (frequently, this is also accompanied by a mild interstitial hyperplasia) (see review in Ref. 66). This process, often referred to as

"tubular regeneration," is distinct from renal compensatory growth since it does not entail any sizeable increase of renal mass. It has been reported to occur in experimental animals after exposure to various nephrotoxins (including mercuric chloride, uranyl nitrate and unleaded gasoline) that cause tubular necrosis. Usually, tubular regeneration leads to a restoration of renal tissue architecture [67]. Aminoglycosides induce a dose-related increase of cell proliferation (estimated by measuring the rate of [^{3}H]thymidine incorporation into DNA and by evaluating the frequency of S-phase cells) in the renal cortex tissue of treated animals [66, 68, 69]. Because aminoglycosides are unlikely to interact directly with DNA synthesis and mitosis in eucaryotic cells, the most plausible interpretation for this proliferative response is that it is elicited by focal tubular necrosis. Close examination of the renal tissue indeed reveals that administration of aminoglycosides at low doses causes the appearance of pycnotic nuclei and of apoptosis ("shrinkage necrosis") in tubular epithelium [68]. When different aminoglycosides administered at low, equitherapeutic doses are ranked with respect to their effect on renal cell proliferation, the following sequence is observed: gentamicin = dibekacin > netilmicin > tobramycin > micronomicin ≥ amikacin ≥ isepamicin. Such a ranking is in good agreement with that obtained for the degree of renal phospholipidosis [57, 58, 70]. Thus, the extent of tubular damage, as assessed by tubular regeneration, appears related to the extent of inhibition exerted by aminoglycosides on phospholipid catabolism.

Further experimental evidence of a close relationship between aminoglycoside-induced phospholipidosis and tubular necrosis stems from the protective effect of anionic polypeptides against the renal toxicity of these antibiotics. The co-administration of poly-L-aspartic acid with gentamicin or amikacin largely suppresses the histopathological signs and the renal dysfunction associated with aminoglycoside nephrotoxicity [71, 72]. In parallel, it has also been shown that poly-L-aspartic acid almost completely prevents the lysosomal phospholipidosis and phospholipiduria, as well as the focal tubular necrosis induced by gentamicin [73, 74]. Thus, as for the studies comparing various aminoglycosides, a correspondence is found between phospholipidosis and aminoglycoside nephrotoxicity. Originally, it was proposed that poly-L-aspartic acid exerts a protective effect by decreasing aminoglycoside binding to membranes of renal tubular cells [34]. However, poly-L-aspartic acid does not decrease but actually increases gentamicin accumulation within renal cortex issue of experimental animals [71, 75]. Recent *in vitro* investigations based on equilibrium dialysis have revealed that poly-L-aspartic acid binds gentamicin and displaces it from negatively-charged lipid bilayers. Consequently, poly-L-aspartic acid relieves the gentamicin-induced inhibition of phosphatidylcholine hydrolysis by lysosomal phospholipases [75]. Since *in vivo* polyaspartic acid and gentamicin are both accumulated in lysosomes of proximal tubular cells [76], the lesser inhibition of phospholipid catabolism can reasonably be explained by the complexation of the drug within the lysosomes, which is favoured by the low pH of these organelles [77, 78].

Even though there is now convincing experimental evidence for a causal relationship between the kidney phospholipidosis induced by aminoglycosides and their nephrotoxicity, one must still figure out how phospholipid accumulation within lysosomes eventually leads to cell death, and thus to tubular necrosis. It is conceivable that the phospholipid overload in lysosomes may alter the stability of these organelles, eventually making them burst and release within the cell a variety of potentially harmful hydrolases and the drug itself. Indeed, in other situations, lysosomal alterations have been reported to cause tissue damage. In particular, this is the case for experimental liver ischaemia where the necrosis of hepatic parenchyma has been attributed to the leakage of lysosomal enzymes in the cytosol [79]. Besides, prolonged exposure of cultured proximal tubular cells to gentamicin results in an increased fragility of the lysosomes [80]. Although the intracellular release of lysosomal hydrolases and of entrapped aminoglycosides may well result in cell destruction, such an event still remains speculative since its occurrence has not been established experimentally in the case of aminoglycoside-induced tubular injury. One must stress, however, that the self-destruction of cells following the leakage of lysosomal content could happen quickly and even elude a careful study of the cytotoxic process at the ultrastructural level.

There is also evidence that a critical threshold in phospholipid accumulation needs to be reached before it leads to cell death, whereas keeping below this critical value will allow the cell to survive, and even return to its normal state if the drug is withdrawn ([13] see also discussion in Refs. 21 and 53).

Alternatively, the lysosomal phospholipidosis resulting from exposure to aminoglycosides may lead to more subtle—albeit not less deleterious—modifications in cell structure and physiology. The proximal tubule reabsorbs various biological substances, including small molecular weight proteins and, therefore, the epithelium lining that section of the nephron is the site of extensive endocytic activity. Gentamicin interferes with the process of lysosomes-endocytic vacuoles fusion [20] and has been shown to inhibit the enzymatic breakdown of reabsorbed proteins within proximal tubules [81]. Since endocytosis, with the associated process of membrane recycling, is prominent in the same cells where aminoglycosides accumulate, the inhibitory effect of these antibiotics on lysosomes-endocytic vacuoles fusion may provide a plausible explanation for their cytotoxicity at the tubular level.

It has often been argued that agents other than aminoglycosides (namely the so-called cationic amphiphilic drugs) can also induce a lysosomal phospholipidosis without apparent signs of tissue injury [82]. Yet, both types of compounds bind to negatively-charged lipid layers and inhibit lysosomal phospholipases [47]. Development of focal necrosis following exposure to cationic amphiphiles, however, must not be ruled out since limited tissue damage can easily be overlooked in the kidney (and in the liver as well) examined by conventional histological approach (see discussion in Ref. 66). Moreover, because of their pharmacological properties

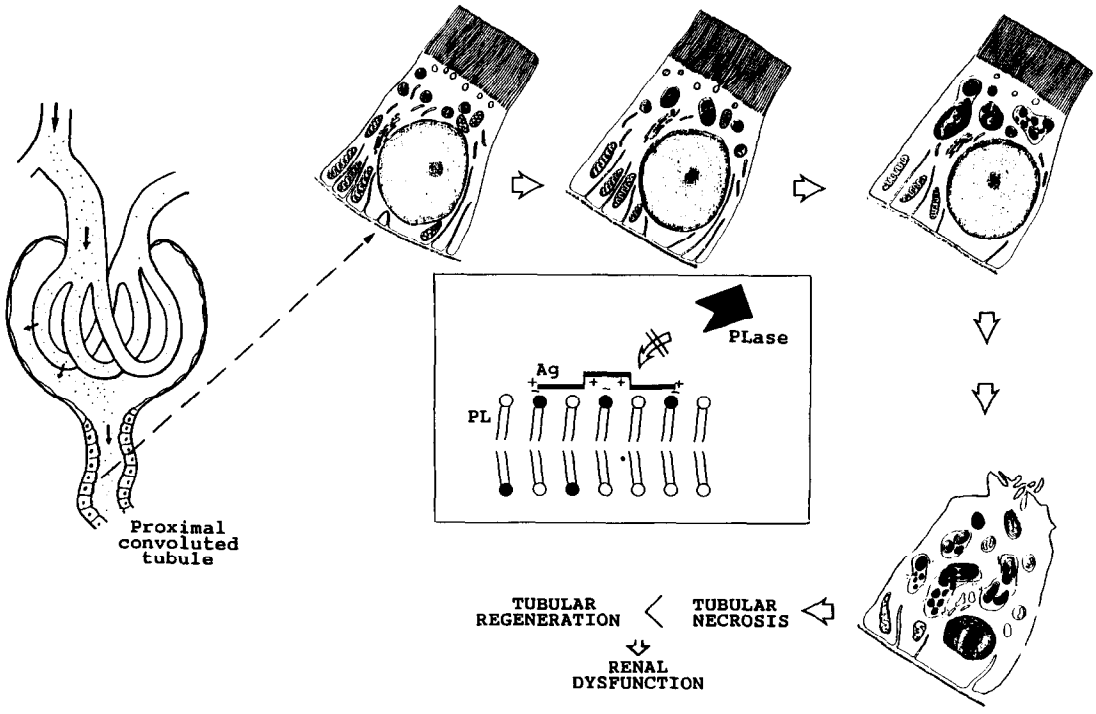

Fig. 2. Putative sequence of events leading to aminoglycoside nephrotoxicity. Aminoglycosides are primarily eliminated by glomerular filtration (left). A small proportion of the antibiotic is however reabsorbed at the proximal tubular level (dash-arrow), this reabsorption resulting from adsorptive endocytosis. Intracellular aminoglycoside (Ag) accumulates within the lysosomes where it is bound to negatively-charged phospholipids (filled circles, center of the figure) present within the lipid bilayer (PL) of membranes. The existence of aminoglycoside-negative phospholipid complexes interferes with the catabolism of phospholipids by phospholipases (PLase). The ensuing lysosomal phospholipidosis (open arrows) may lead eventually to rupture of the lysosomes and/or to lysosomal dysfunction, resulting in the necrosis of proximal tubular cells. It must be stressed, however, that renal functional impairment only develops when tubular necrosis is not compensated for by regeneration. The figure only gives a symbolic representation and is not meant to represent the actual structure or the relative proportions of the different elements.

and their wide tissue distribution, cationic amphiphilic drugs may affect various physiological functions before causing alteration in kidney histology.

Conclusions and perspectives

Many studies devoted to the renal toxicity of aminoglycosides have overlooked the early metabolic abnormalities that these antibiotics cause in renal proximal tubules. In our discussion on the pathogenesis of aminoglycoside-induced tubular necrosis, we have put emphasis on the drug–phospholipid interaction underlying the lysosomal phospholipidosis which precedes cell death. We do not pretend by any means to provide an ultimate explanation for the tubular injury and renal dysfunction caused by aminoglycosides, and other consequences of aminoglycoside–phospholipid interaction, such as the impairment of prostaglandin synthesis [83] or the disturbance of phosphoinositide metabolism [84], have not been commented on in this article. Yet, there is an abundance of experimental data suggesting that aminoglycoside nephrotoxicity could develop through the sequence of events depicted in Fig. 2. The existence of such a sequence obviously calls for the use or the development of approaches capable of decreasing the renal toxicity of aminoglycosides, a perspective which should be present in the mind of any student of the involved mechanisms. Some of these approaches also appear in Table 1.

Decreasing aminoglycoside accumulation by competing with the drug at the level of its binding sites on the brush-border membrane has been attempted in animals [85], but seems difficult to achieve under conditions of clinical administration, in view of the large number of these sites and their relatively low affinity [35]. The critical observation that aminoglycoside uptake by renal cortex is saturable at clinically-relevant serum concentrations [86] has, however, led to the interesting concept that appropriate changes in the dosing regimen can modify markedly the nephrotoxicity of these drugs. Hence, animal studies [68, 90] have demonstrated that drug administration at long intervals (viz. once-a-day) reduces aminoglycoside tissue accumulation, and decreases the severity of tubular necrosis and renal functional impairment, as compared to equivalent daily doses delivered at short intervals (viz. every

Table 1. Possible approaches leading to a reduction of aminoglycoside-induced nephrotoxicity

Event	Mechanism	Potential mean of prevention
Drug uptake ↓	Drug binding to brush borders [18, 35]	Saturate or compete with drug binding sites [85–87]
Lysosomal phospholipidosis ↓	Drug binding to phospholipids [24, 37, 40, 46]	Use aminoglycosides with minimal intrinsic binding or displace phospholipid-bound drug [39, 45, 75]
Tubular necrosis ↓	Numerous possibilities	Keep sublethal lesions below critical threshold
Kidney dysfunction	Necrosis/regeneration [66, 70, 88]	Enhance tissue repair. Avoid or correct for risk factors [3, 89]

8 hr). These observations take their full meaning when one considers that aminoglycosides are endowed with a substantial "post-antibiotic" effect and should not normally require frequent administrations to display full therapeutic activity [91]. Accordingly, in a rat model of subcutaneous abscess the same daily dose of tobramycin has been shown to be as effective and less nephrotoxic when given once a day instead of every 4 hr [92]. In addition, recent controlled studies on non-neutropenic patients treated with netilmicin or with amikacin have led to similar conclusions concerning the benefits of less frequent aminoglycoside administration [87, 93].

As explained above, polyanions may displace aminoglycosides from phospholipids, and thereby protect against nephrotoxicity. Clinical applications of this concept, however, have not been made. Conversely, although the screening of new aminoglycosides has not been guided primarily by toxicological considerations, it now appears that some compounds, such as amikacin or isepamicin, have a structure that results in lesser binding to phospholipids (see review in Ref. 45). Accordingly, their renal tolerance is greater [6, 23, 57, 94, 95]. Yet, more progress in the rational design of intrinsically less nephrotoxic aminoglycosides is warranted.

Finally, it must be stressed that the tubular damage caused by aminoglycosides only results in kidney dysfunction when it reaches a level such that it is no longer counterbalanced by renal tissue repair. Thus, asynchronous tubular necrosis, as observed upon chronic gentamicin administration, may be associated with a preserved glomerular function in animals [88]. It is conceivable that an enhancement of the renal tissue repair could prevent aminoglycoside-induced tubular necrosis from leading to functional impairment.

Animal and clinical studies have also identified a number of conditions that can potentially increase the nephrotoxic risk associated with aminoglycoside administration [3, 89, 93]. Some of these risk factors probably relate to an increased extent of drug-induced primary lesions (high drug tissue levels, prolonged treatment), whereas others result from an impaired tissue repair reaction (age, concomitant administration of other tubulotoxins), or from a greater susceptibility to nephrotoxic insult (preexisting alteration of renal function due to shock or hypovolemia). Hopefully, the correction of recognized risk factors, along with the design of protective approaches, will lead to the avoidance of aminoglycoside toxicity.

Acknowledgements—P. Tulkens was *"Maître de Recherches"* and G. Laurent is *"Chercheur Qualifié"* of the Belgian "Fonds National de la Recherche Scientifique" (FNRS). B. K. Kishore is the recipient of Fellowships from the International Institute of Cellular and Molecular Pathology and from the Fonds de Développement Scientifique of the Université Catholique de Louvain. The experimental work of the authors was supported by the Belgian FNRS (Grant 1.5213.87), the "Fonds de la Recherche Scientifique Médicale" (Grants 3.4516.79 and 3.4553.88) and the "Service de la Politique et de la Programmation Scientifique" (Grants 82-87/39 *Actions Concertées* and 7bis *PAI*). The expert secretarial assistance of Ms. Amat-Peiro is gratefully acknowledged.

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