DISTRIBUTION OF GENTAMICIN AMONG SUBCELLULAR FRACTIONS FROM RAT RENAL CORTEX

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Abstract—A substantial amount of data is available to suggest that lysosomal sequestration of aminoglycoside antibiotics plays a role in the pathogenesis of aminoglycoside-induced renal tubule cell injury; however, relatively little information is available on the subcellular distribution of aminoglycosides in the kidney during treatment protocols of the type that ultimately go on to produce extensive lethal renal tubule cell injury and acute renal failure in experimental animals. This study assessed the distribution of gentamicin and subcellular membranes on a discontinuous sucrose density gradient after in vivo exposure of rats to four daily 100 mg/kg doses of gentamicin as compared to in vitro exposure of normal rat renal cortex to gentamicin during tissue homogenization at drug levels comparable to those seen after in vivo treatment. After both in vivo and in vitro exposure, major localization of gentamicin, the lysosomal marker enzyme N-acetyl-β-D-glucosaminidase (NAG), and the endoplasmic reticulum marker enzyme NADPH-cytochrome c reductase, occurred in a very light membrane fraction. Within this membrane fraction, gentamicin was more closely associated with the NAG than with the NADPH-cytochrome c reductase. The results could not be explained by complete lysosomal disruption during subcellular fractionation after in vivo gentamicin. These data provide additional insights into both the possibilities for subcellular interactions of aminoglycosides in the kidney, and into the methodology required to optimally assess such interactions.

The recognition that a large fraction of the high levels of aminoglycoside antibiotics which accumulate in the renal cortex in treated patients and in animal models of aminoglycoside nephrotoxicity is sequestered within lysosomes has been a major advance in the understanding of the pathogenesis of aminoglycoside-induced renal tubular cell injury. However, the most unequivocal data on subcellular distribution of aminoglycosides during prolonged courses of exposure has been obtained not in kidney or in a kidney-derived tissue model, but in cultured fibroblasts [1, 2]. Subcellular fractionation and autoradiographic studies have indeed provided definitive evidence that lysosomal sequestration does occur in the kidney in vivo acutely after tracer doses of gentamicin [3–7], but little is known about the subcellular distribution of aminoglycosides during prolonged courses of exposure of the type associated with significant nephrotoxicity [3, 8–10]. Such information is of substantial importance in assessing the relevance of extralysosomal versus lysosomal events in the pathogenesis of aminoglycoside-induced renal tubular cell injury and nephrotoxicity. In this regard, recent data have indicated that a number of prominent extralysosomal events are demonstrable in the early development of gentamicin-induced renal tubular cell injury: (1) functional and structural defects are present in isolated mitochondria and brush border membranes [3, 9–13], (2) acidic phospholipids are specific binding sites for the aminoglycoside on der membranes [3, 9–13], (2) acidic phospholipids are present in isolated mitochondria and brush border membranes [11], and (3) aminoglycoside treatment appears to produce early alterations in the acidic phospholipid content of non-lysosomal cellular membranes [13]. Furthermore, in vitro gentamicin binding to a variety of subcellular membranes has been documented [3, 11, 14–16]. The present study was designed to obtain more information on the possible subcellular distribution of gentamicin in the kidney after an in vivo course of treatment of sufficient magnitude to result in substantial nephrotoxicity in the rat but at a time prior to the occurrence of advanced renal tubular cell injury and necrosis. Cellular fractionation techniques previously reported to be effective in assessing the subcellular distribution of gentamicin in isolated fibroblasts [1, 2] and the subcellular distributions of cationic amphiphilic drugs which are concentrated in hepatic lysosomes were utilized [17–20].

MATERIALS AND METHODS

Male Sprague–Dawley rats (Harlan) weighing 275–300 g and maintained on a standard lab diet (1% calcium) were used for all studies. Gentamicin treatment was with a single daily subcutaneous dose of 100 mg/kg gentamicin as gentamicin sulfate (Sigma, St. Louis, MO) for 4 consecutive days. Rats were killed 24 hr after the last dose.

Kidneys were rapidly removed and placed in ice-cold 0.27 M sucrose. Then the cortices were dissected, minced and homogenized. For control (C) and in vivo gentamicin-treated (G) groups, the homogenizing solution consisted of 0.27 M sucrose,
In the first series of experiments, gradients were sampled at several discrete points which preliminary studies had suggested would provide the best definition of various subcellular membranes as identified by enzyme markers. Table 1 summarizes the results of these studies as regards enzyme composition of each fraction, predominant membranes present as deduced from the enzyme composition, and gentamicin level factored for mg protein. The G group had a mean ± S.E. homogenate gentamicin level of 8.8 ± 0.3 µg/mg protein while the homogenate gentamicin level of the C + G group was 10.2 ± 0.1 µg/mg protein. As can be seen in Table 1, the major enrichment of gentamicin activity occurred in fraction 1 which was also enriched in the lysosomal marker, NAG, and in the endoplasmic reticulum marker, NADPH-cytochrome c reductase. Furthermore, both group G (in vivo gentamicin exposure) and group C + G (in vivo gentamicin exposure) exhibited generally similar patterns of gentamicin distribution on the gradient.

The sampling methodology employed in the first series of gradient studies did not allow for assessment of extent of recovery of enzymes and gentamicin off the gradient and could have missed differences between closely adjacent areas, or in non-sampled areas. For this reason, another series of experiments was done using the same type of sucrose gradient and the same groups, C, G, and C + G but sampling the entire gradient in 1-ml aliquots and analyzing each for protein, enzyme activity, and gentamicin level. Recoveries for all parameters measured were 85–110% of the amount layered on the gradients. The G group had a mean ± S.E. homogenate gentamicin level of 7.6 ± 0.6 µg/mg protein while the homogenate gentamicin level of the C + G group was 8.4 ± 0.3 µg/mg protein. Representative gradients from each group are illustrated in Fig. 2. As in the initial series of gradients, there were prominent localizations of NAG, NADPH-cytochrome c reductase and gentamicin to light membranes coming off in gradient fractions 10–19. This occurred similarly in the in vivo group (G) and in the in vitro group (C + G).

To better quantify the average behaviors of membrane enzymes and gentamicin in this series of gradients, the data were analyzed to determine the mean percent of total enzyme and gentamicin on the gradient found in each gradient region. Based on enzyme characteristics, values for the 1-ml aliquots were pooled to give results for gradient regions approximately corresponding to the areas sampled in the first group of gradients (Table 1). However, in this series of gradients, no area remained unsampled, the identified regions are contiguous and cover the entire gradient, and a fraction rich in mitochondria (No. 5 in Fig. 1) which was not sampled in the first series of gradients is reported. These data are summarized in Table 2.

Somewhat more cytochrome oxidase activity was present in the area defined as fraction 1 in the second series of gradients (Table 2), but otherwise the overall behavior of marker enzyme activity was similar to that in the initial series of studies (Table 1). It is
<table>
<thead>
<tr>
<th>Fraction</th>
<th>$N$-Acetyl-$\beta$-glucosaminidase</th>
<th>NADPH-cytochrome c reductase</th>
<th>Alkaline phosphatase</th>
<th>Na$^+$-K$^+$-ATPase</th>
<th>Cytochrome $c$ oxidase</th>
<th>Membranes§</th>
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<tr>
<td></td>
<td>$C$</td>
<td>$G$</td>
<td>$C+G$</td>
<td>$C$</td>
<td>$G$</td>
<td>$C+G$</td>
</tr>
<tr>
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<td>6.61</td>
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<td>±0.22</td>
<td>±0.21</td>
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</table>

* All values are means ± S.E. for $N = 3-5$. Abbreviations: ND = activity below accurately quantifiable limits, NM = not measured, C = control, $G = \text{in vivo}$ gentamicin, and $C + G = \text{in vitro}$ gentamicin.

† Relative specific activity is the ratio of enzyme activity in fraction divided by the enzyme activity in renal cortex homogenate prior to centrifugation.

‡ Location of fraction on gradient indicated by symbol in this column corresponds to indicated area in Fig. 1.

§ Predominant membranes present in fraction as determined by enzyme activity profile.

|| Overall significant differences within group by ANOVA: $P < 0.01$. In both $G$ and $C + G$ groups, levels of gentamicin were significantly greater in fraction 1 than in every other fraction.
Fig. 2. Profiles of the distributions of protein, gentamicin and subcellular membrane associated enzymes on representative gradients from a control rat (C), a rat treated with gentamicin in vivo (G), and a control rat whose renal cortex was homogenized in vitro with gentamicin present (C + G). Each bar indicates a single 1-ml aliquot of the gradient with fraction number 1 corresponding to the top of the gradient (S in Fig. 1) and fraction 40 being the bottom of the gradient. For each aliquot, the percent of total activity of the measured parameter found in it is graphed. Abbreviations: protein (Prot), N-acetyl-β-D-glucosaminidase (NAG), alkaline phosphatase (AP), NADPH-cytochrome c reductase (Red), and gentamicin (Gent).

**Fig. 2 Diagram Description:**

- **CONTROL (C)**: Profiles show distribution of proteins, gentamicin, NAG, AP, Red, and Gent across gradient fractions.
- **IN VITRO (C + G)**: Similar profiles with slight variations.
- **IN VIVO (G)**: Profiles demonstrate gentamicin migration in fraction 1.

**Analysis:**

- From Table 2, gentamicin preferentially migrates in fraction 1, which also contains the most NAG and NADPH-cytochrome c reductase activities. Gentamicin behaves similarly in G (in vivo) and C + G (in vitro) groups.
- The analysis in Table 2 does not optimally quantitate the enzyme activity and gentamicin in the unsedimentable cytosolic protein fraction because particulate areas intermix variably with the lower several milliliters of this area of the gradient. To better assess the amount of unsedimentable activity without this confounding factor and, thereby, to gain some estimate of whether changes in fragility of subcellular organelles after in vivo or in vitro gentamicin substantially affected the results, the amounts of protein, enzyme activity, and

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**Evident from Table 2:**

- Gentamicin preferentially migrates in fraction 1, also containing the most NAG and NADPH-cytochrome c reductase activities.
- Gentamicin behaves similarly in G (in vivo) and C + G (in vitro) groups.
- Analysis in Table 2 optimally quantitates enzyme activity and gentamicin in the unsedimentable cytosolic protein fraction because particulate areas intermix variably with the lower several milliliters of this area of the gradient. To better assess the amount of unsedimentable activity without this confounding factor and, thereby, to gain some estimate of whether changes in fragility of subcellular organelles after in vivo or in vitro gentamicin substantially affected the results, the amounts of protein, enzyme activity, and
Table 2. Percent of total gradient content of protein, enzymes and gentamicin in individual fractions*

<table>
<thead>
<tr>
<th>Fraction†</th>
<th>Protein</th>
<th>NADPH-cytochrome c reductase</th>
<th>Alkaline phosphatase</th>
<th>Cytochrome c oxidase</th>
<th>Gentamicin</th>
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<tbody>
<tr>
<td></td>
<td>C</td>
<td>G</td>
<td>C + G</td>
<td>C</td>
<td>G</td>
</tr>
<tr>
<td>S</td>
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<tr>
<td></td>
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</tr>
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<td>48.6</td>
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<td>± 0.8</td>
<td>± 3.6</td>
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</tr>
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<td>7.7</td>
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<td></td>
<td>± 0.2</td>
<td>± 4.1</td>
<td>± 1.5</td>
<td>± 1.0</td>
<td>± 1.8</td>
</tr>
<tr>
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<td>14.2</td>
<td>10.0</td>
<td>18.0</td>
</tr>
<tr>
<td></td>
<td>± 0.9</td>
<td>± 4.0</td>
<td>± 1.5</td>
<td>± 1.1</td>
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<td>± 0.7</td>
<td>± 3.1</td>
<td>± 0.7</td>
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<td>1.5</td>
</tr>
<tr>
<td></td>
<td>± 0.1</td>
<td>± 0.2</td>
<td>± 1.4</td>
<td>± 3.9</td>
<td>± 0.6</td>
</tr>
</tbody>
</table>

* All values are mean percent ± S.E. C = control, N = 3; G = in vivo gentamicin, N = 4; C + G = in vitro gentamicin, N = 3.
† Location of fractions on gradient indicated by symbol in this column corresponds to area indicated in Fig. 1.
‡ Overall significant differences within group by ANOVA: P < 0.01. In both G and C + G groups, the percent of gentamicin found in fraction 1 was significantly greater than in every other fraction. G and C + G groups did not differ significantly from each other in the distribution of gentamicin.
¶ Predominant membranes present in fraction as determined by enzyme profile.

Cytosolic proteins
“Light” lysosomes
Endoplasmic reticulum
Endoplasmic reticulum + Plasma membranes
Mitochondria
Mitochondria + Plasma membranes
“Heavy” lysosomes

Subcellular distribution of gentamicin in kidney

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Gentamicin in the first 5 ml of the gradient were multiplied by 2 in order to extrapolate to the 10 ml volume of 600 g supernatant fraction originally layered on each gradient so as to assess what fraction of the Results. G = in vivo gentamicin. C + G = in vivo gentamicin. Statistically significant (P < 0.05 or better) differences by paired t-test are indicated as follows: (*) G vs control; (#) G vs C + G; and (+) control vs C + G.

gentamicin in the first 5 ml of the gradient were multiplied by 2 in order to extrapolate to the 10 ml volume of 600 g supernatant fraction originally layered on each gradient so as to assess what fraction of each enzyme component of the original postnuclear (600 g) supernatant fraction was nonsedimentable. These data were available for both types of gradient studies done and were pooled. The results are summarized in Fig. 3.

The percent nonsedimentable activity found in the supernatant fraction was similar to that reported in other density gradient studies to the extent that comparable data are available [1, 2, 17, 18]. Group G had slightly but significantly more nonsedimentable protein than group C and groups G and C + G had more nonsedimentable NAG than controls, suggesting the occurrence of some increased organellar fragility with gentamicin, but the differences were small. Unsedimentable gentamicin was significantly higher in group C + G than in group G indicating that some constraints on redistribution of gentamicin after in vivo treatment, probably related to in vivo sequestration, were retained during the separatory procedures.

The second group of gradient studies in which all fractions were analyzed (Table 2) provided the opportunity to correlate levels of gentamicin with levels of NAG and NADPH-cytochrome c reductase within the 7–9 fractions comprising region 1 of the gradient, the area of most prominent gentamicin localization, to ascertain with which enzyme the gentamicin was most closely associated. These data are summarized in Table 3. They indicate that, after in vivo gentamicin, an excellent correlation between gentamicin and NAG activity was uniformly present while gentamicin correlated poorly with NADPH-cytochrome c reductase activity. However, an identical pattern of gentamicin distribution was seen after in vivo gentamicin in two of three experiments.

DISCUSSION

The past several years have witnessed a substantial increase in the understanding of the cellular pathophysiology of aminoglycoside nephrotoxicity. Prominent among the advances in this area has been recognition of major lysosomal effects of aminoglycosides in renal proximal tubule cells. Multiple observations indicate the importance of lysosomes in the effects of aminoglycosides on renal tubular cells. Morphologic changes characterized by increases in lysosomal size and development of myeloid bodies have been well documented and, more recently, carefully quantitated in both animal models and humans [22–24]. Autoradiographic studies have provided evidence for lysosomal sequestration of labeled gentamicin [5–7]. Cell fractionation studies have demonstrated lysosomal localization of gentamicin after in vivo treatment with low doses in animal models [4] and across a wide dose range in cultured fibroblasts [1, 2]. Inhibitory effects of aminoglycosides on lysosomal phospholipases have been documented [25, 26] and increases in lysosomal phospholipid levels have been shown to contribute to the tissue phospholipidosis seen in renal cortex after aminoglycoside treatment [27]. Aminoglycoside-induced increases and decreases in stability of lysosomal membranes have been reported [15, 28, 29].

However, the available data on lysosomal effects of aminoglycosides do not yet provide a full explanation of the pathogenesis of aminoglycoside-induced renal tubule cell injury for several reasons: (1) the mechanisms by which lysosomal dysfunction results in acute lethal cell injury, despite much study in many models of injury, remain incompletely delineated and controversial [30, 31], (2) increases in size of lysosomes associated with increased uptake of slowly metabolized materials do not necessarily lead to either lysosomal dysfunction or to cell injury as illustrated by the benign nature of the so-called osmotic nephropathies resulting from administration of agents such as dextran [32], and (3) induction of a lysosomal phospholipidosis in the kidney as a result

Table 3. Associations between gentamicin and membrane marker enzymes in gradient fraction 1*

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>In vitro</th>
<th>In vivo</th>
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<tr>
<td></td>
<td>NAG</td>
<td>RED</td>
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<tr>
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<td>0.201</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
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<td>0.240</td>
</tr>
<tr>
<td>S.E</td>
<td>0.179</td>
<td>0.033</td>
</tr>
</tbody>
</table>

* Correlation coefficients between levels of gentamicin and NAG and gentamicin and NADPH-cytochrome c reductase (RED) in the seven to nine 1-ml fractions comprising “Fraction 1” of the sucrose density gradient after in vitro and in vivo gentamicin.
of lysosomal sequestration of a cationic amphiphilic compound does not necessarily lead to acute lethal cell injury as illustrated by the effects of treatment with chlorphentermine. This cationic amphiphilic drug produces more widespread morphologic changes of lysosomal phospholipidosis in the kidney than do aminoglycosides but, to the extent that data are available, renal functional impairment is mild and acute renal tubular cell injury is not prominent [33]. While certain properties of aminoglycosides may make them uniquely effective lysosomal toxins, substantial data are available to support the importance of cellular loci in addition to lysosomes as sites of aminoglycoside-induced renal tubular cell injury. Interactions of aminoglycosides with brush border membranes and mitochondria have been detailed with in vitro model systems [3]. Evidence for the in vitro occurrence of such interactions prior to the development of lethal cell injury has been reported [9, 10, 12]. Aminoglycoside inhibition of nonlysosomal phospholipases occurs [34, 35]. The phospholipidosis produced by aminoglycosides in the kidney is not necessarily limited to lysosomes [13]. A recent detailed reassessment of aminoglycoside uptake by radioautography has suggested the occurrence of an early cytoplasmic phase of aminoglycoside intracellular distribution [7].

To better understand the relationship between lysosomal and extralysosomal events in the cellular pathophysiology of aminoglycoside nephrotoxicity, it would be helpful to know more about the potential for gentamicin distribution intracellularly. The present study was designed to better ascertain the utility of subcellular fractionation procedures for obtaining this information under conditions of substantial but unequivocally, prelethal aminoglycoside-induced renal tubular cell injury. It has been stated that cellular fractionation studies after high or prolonged doses of aminoglycosides are fraught with difficulty [4], but no specific data have been reported to provide basis for further investigation or improvement of methodology. The present study provides such information.

Prior subcellular fractionation studies of liver after treatment with cationic amphiphilic drugs which induced the formation of large numbers of myeloid bodies in hepatocytes have shown that these myeloid bodies can be isolated in enriched form, they have the enzymatic characteristics of lysosomes, they tend to equilibrate at lower gradient densities than do normal lysosomes, and they are the major intracellular sites of drug concentration [17-20]. Detailed studies of fibroblasts exposed to a range of concentrations of gentamicin and other aminoglycoside antibiotics have demonstrated similar dose-dependent effects of the aminoglycosides on lysosomes [1, 2].

One important consideration in cell fractionation studies after in vivo treatment is the possibility of redistribution of drug during the fractionation procedure. This is of particular importance for the aminoglycosides in view of their propensity to bind to various subcellular membranes [11, 14-16]. Most of the available studies with the cationic amphiphilic drugs and aminoglycosides have not provided data on distribution of drug after in vivo exposure during subcellular fractionation. In the single study which carefully addressed this issue it was found that streptomycin exposure in vitro did not produce a preferential lysosomal drug distribution such as was seen after in vivo exposure to several aminoglycosides [1]. However, streptomycin is the least cationic of the commonly utilized aminoglycosides and, probably as a result of this, has the lowest in vivo uptake rate, the fewest documented subcellular effects in the kidney, and the least in vivo nephrotoxicity [3]. In contrast to the observations with streptomycin, appreciable in vitro binding of gentamicin to a light microsomal fraction from renal cortex has been described but enzymatic characterization of this fraction was not provided and concomitant studies of the distribution of gentamicin after in vivo administration were not reported [14].

The present study compared the distribution of gentamicin on a discontinuous sucrose density gradient 24 hr after four daily 100 mg/kg doses with the distribution of gentamicin added to the homogenizing solution after normal renal cortex so as to produce levels similar to those found in the treated animals. The four dose protocol has been used extensively in our laboratory in a number of studies of in vivo gentamicin nephrotoxicity. It reproducibly results in widespread but uniformly prelethal proximal renal tubule morphological and functional changes [9, 10].

The gradient procedures utilized for the present studies were chosen, after evaluation of a variety of methods, to reproducibly separate subcellular organelles with the minimum number of steps, the least disruptive handling and without selectively losing and, thereby, failing to assess the contribution of any major subcellular component. As a result, individual subcellular elements are not nearly as purified as would result from procedures dedicated to isolating them uniquely.

The main findings of the present study were that: (a) In both control and in vivo and in vitro gentamicin preparations a major localization of the activity of the lysosomal enzyme NAG occurred in a very light membrane fraction also enriched in the endoplasmic reticulum enzyme NADPH-cytochrome c reductase. (b) After in vivo gentamicin treatment the distribution of gentamicin along the gradient also showed a major localization in the same light membrane fraction as NAG did and, within this gradient fraction, the distribution of gentamicin correlated very closely with the distribution of NAG. This observation, thus, is apparently consistent with previous reports on the behavior of lysosomes after treatment with cationic amphiphilic drugs or aminoglycosides in that lysosomes tended to migrate at lower densities and contained the drug [1, 2, 17-20]. (c) However, when gentamicin was added in vitro to the homogenizing solution used for renal cortices from control rats to levels approximating the gentamicin levels seen after in vivo treatment, the distribution of gentamicin on the gradient was very similar to that seen after in vivo treatment with respect to a major localization of gentamicin in the same light membrane fraction as NAG and a relatively close correlation of gentamicin with NAG within that fraction. In this regard, the results of
these studies of in vitro gentamicin addition are similar to those of the one previously reported study of exposure of subcellular membrane fragments [14] to gentamicin; however, the present study substantially extends that observation by enzymatically defining the nature of the light membrane fraction showing preferential gentamicin localization.

The data obtained in the present study are compatible with several interpretations:

(a) The similarity of gentamicin distribution after in vivo and in vitro exposure may be due to total disruption of lysosomes during homogenization of the renal cortex after in vivo gentamicin. Several observations argue against this as the only explanation for the findings: (1) the major features of enzyme distribution on the gradient in the present study were similar in control and gentamicin-treated rats, suggesting that substantial differences in organelle integrity were not produced by the in vivo gentamicin treatment as utilized. (2) The methodology for tissue homogenization used in this study was the same as that routinely described by other investigators [1, 2, 4, 17–20] and has, in our hands, produced highly functional mitochondria and highly enriched mitochondrial as well as heavy lysosome fractions by differential centrifugation. (3) Unsedimentable enzyme activity left in the supernatant layer after ultracentrifugation of the gradient (Fig. 3) was as low as has been reported in the literature [1, 2, 4, 17–20]. Furthermore, the protein content of the supernatant fraction was no higher than that in any of the other subcellular fractionation studies referenced in this paper which provided evaluable data for this parameter. We did not measure "free" as opposed to latent enzyme activities in our gradient fractions. Although "free" enzyme activity is a valuable parameter for assessing lysosomal integrity, it may arise from unusually permeable as well as from severely damaged lysosomes and is, thus, a more equivocal measure of major lysosomal disruption than is unsedimentable activity. Furthermore, lysosomal membrane changes contributing to increases in "free" enzyme activity may occur in vivo during gentamicin treatment as well as during homogenization so that increased free levels do not necessarily mean that such changes are in vivo artifacts. (4) Unsedimentable gentamicin was significantly higher with in vitro addition than after in vivo treatment suggesting that gentamicin which was sequestered in vivo was not being totally released during subcellular fractionation.

(b) The high levels of gentamicin in the light membrane fraction in association with the lysosomal enzyme NAG may indicate that concentrative uptake of gentamicin by lysosomes can occur as a result of direct gentamicin-lysosomal interactions without the requirement for adsorptive pinocytosis to the plasma membrane, the process which has been felt to account for most in vivo renal tubule cell uptake of gentamicin [3]. Such lysosomal sequestration of gentamicin upon direct exposure would be compatible with results of recent autoradiographic studies suggesting the occurrence of a cytoplasmic phase of gentamicin distribution acutely after renal tubule cell gentamicin uptake and prior to lysosomal sequestration in vivo [7].

(c) The similarity between the distribution of gentamicin on the sucrose gradients after in vivo and in vitro exposure may be due to a coincidence of two events. In vivo gentamicin may indeed be sequestered in light lysosomes while in vitro gentamicin becomes bound to membrane surfaces rich in acidic phospholipids. We have shown previously that the light membrane fraction which contained the most gentamicin after in vivo and in vitro exposure in the present study is particularly rich in one of the major phospholipid binding sites for aminoglycosides, phosphatidylinositol [13]. Binding to phosphatidylinositol does not, however, explain why, after in vivo exposure, the association of gentamicin with a lysosomal enzyme, NAG, is closer than with the other enriched membrane bound enzyme in that fraction, NADPH-cytochrome c reductase. Furthermore, fractions slightly heavier than the membrane fraction which is most enriched in gentamicin also contain relatively high acidic phospholipid levels [13] without showing similar degrees of enrichment of gentamicin.

The data in this study do not allow definitive evaluation of each of these hypotheses; however, with the phenomenon identified and reproducibly characterized it will be possible to design experiments to distinguish between the major mechanisms outlined. The available data do suggest that some caution must remain in attributing gentamicin renal tubule cell uptake exclusively to adsorptive pinocytosis followed by sequestration within lysosomes without opportunity to interact with intracellular structures. Though difficult and requiring careful interpretation, further studies of gentamicin distribution during prolonged and high dose models of the type that ultimately lead to significant renal functional impairment are likely to yield useful information for understanding the cellular pathophysiology of gentamicin-induced renal tubular cell injury.

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