Retinal Toxicity of Intravitreal Gentamicin

An Electron Microscopic Study

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Retinal ultrastructure was examined at various intervals following a single intravitreal injection of 100-4,000 µg of gentamicin in rabbit eyes. Three days after injections of 100-500 µg, numerous abnormal lamellar lysosomal inclusions were observed in the retinal pigment epithelium (RPE) and in macrophages in the subretinal space. These changes were typical of drug-induced lipid storage and were comparable to inclusions reported in kidney and other tissues as manifestations of gentamicin toxicity. One week after similar injections, focal areas of RPE necrosis and hyperplasia with disruption of outer segments appeared, but the inner segments and inner retina were intact. Doses of 800-4,000 µg produced a combined picture of RPE/macrophage lipidosis within the first 3 days, with increasing, superimposed, inner, retinal necrosis. This study provides the first evidence of lysosomal alterations in ocular tissues following the intravitreal injection of gentamicin and implicates the RPE as the primary site of observed toxicity. Invest Ophthalmol Vis Sci 25:564-572, 1984

Aminoglycosides are used frequently in ophthalmology to treat or to prevent bacterial infections. These antibiotics are known to be ototoxic and nephrotoxic. They also may be toxic for retinal structures if high concentrations are administered in the vitreous, as demonstrated by light microscopic and electrophysiologic studies.1–3

A selective accumulation of these drugs within lysosomes has been demonstrated in cultured fibroblasts4–6 and in the proximal tubular cells of the kidney.7,8 It results from trapping by protonation of the molecules in the low pH lysosomal content.9,10 This accumulation produces important perturbations of the phospholipid catabolism, possibly through a lowered activity of sphingomyelinase and phospholipases4,6,10 together with important storage of these substrates within lysosomes, as shown by electron microscopic and biochemical studies.6,11–15

We know by previous experiments that such lysosomal storage disorders induced by aminoglycosides may be observed in cultured cells obtained from various ocular structures16 and in ocular fibroblasts in vivo after subconjunctival injections.17 The present study demonstrates that a similar mechanism is involved in the toxic reactions of the retina following intravitreal injections of gentamicin.

Materials and Methods

Twin studies have been performed in two different laboratories (Massachusetts Eye and Ear Infirmary; Boston, MA and Universite Libre de Bruxelles; Brussels, Belgium) following similar protocols. The results were compared after completion of the experimental phase in order to avoid bias.

Dutch Belted rabbits weighing 2–3 kg were used for both studies. They were housed in separate cages with a 12-hr light/dark cycle. All animal procedures were performed following guidelines of the ARVO Resolution on the Use of Animals in Research. Animals were anesthetized with 60-100 mg intramuscular ketamine and received intravitreal injections of gentamicin or saline through a 30-gauge needle inserted approximately 4 mm posterior to the limbus. A paracentesis was performed in Belgium but not in USA experiments. The volume for injection was always 0.1 ml, and the doses of gentamicin, ranging from 200-4,000 µg, were made by appropriate saline dilutions of commercially available gentamicin. Several experiments were replicated using preservative-free intrathecal preparation of gentamicin to control for possible preservative effects. Preservative-free saline was employed for all dilutions and for control injections.

The animals were periodically examined and photographed after tropicamide 1% and phenylephrine 10% dilation in the USA series. They were killed at intervals ranging from 1 day to 1 month after injection.
The eyes were enucleated immediately, sectioned, and placed in 2.5% glutaraldehyde fixative for 24-48 hr. After a buffer wash, the retina was dissected into 2-mm fragments, postfixed in 1% osmium, dehydrated in graded alcohols, and embedded in Epon. Sections were examined by phase-contrast and transmission electron microscopy.

In some cases, the vitreous was carefully dissected, frozen, and sent for gentamicin determination by an immunofluorometric method.

Results

Ophthalmoscopic studies of the retina after intravitreal injection of high doses of gentamicin (4,000 μg) demonstrated a severe retinal necrosis after 24 hr, with marked retinal edema, hemorrhage, optic disc swelling, and vitreous inflammation. Following injections of 1,000-2,000 μg, diffuse mottling of the previous uniform pigmentation of the RPE was seen as early as 2 days after drug administration. Lower doses did not seem to induce ophthalmoscopic alterations during the first week.

Some rabbits had an estimation of the gentamicin levels 3 days after the injection. Although the series is not significant for quantitative evaluation, it is obvious that the antibiotic has not been eliminated totally at that time, and that a significant concentration of the drug was still present (Table 1).

<table>
<thead>
<tr>
<th>Dose injected (mg)</th>
<th>Concentration* (mg/ml)</th>
<th>Concentration day 3 (mg/ml)</th>
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<tbody>
<tr>
<td>1.600</td>
<td>1.150</td>
<td>0.076</td>
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<tr>
<td>1.000</td>
<td>0.720</td>
<td>0.062</td>
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<tr>
<td>0.500</td>
<td>0.360</td>
<td>0.040</td>
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<tr>
<td>0.250</td>
<td>0.180</td>
<td>0.020</td>
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* Column 2 represents an estimate of immediate postinjection concentration based on an assumed rabbit vitreous volume of 1.4 ml.

Table 1. Intravitreal levels of gentamicin

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Fig. 1. Three days after injection of 200 μg of gentamicin, extensive absorption of photoreceptor outer segments (arrowheads) with sparing of inner segments (IS). A macrophage (asterisk) is visible on the pigment epithelial surface and contains numerous lamellar cytoplasmic inclusions. The basal infoldings (bracketed) of the RPE are maintained. Bruch's membrane and choriocapillaris appear unremarkable. (X4,500).

Inset: higher magnification of macrophage (asterisk) reveals whorled lysosomal inclusion of complex lipid (X25,000).
Both experiments conducted in Brussels and Boston showed identical histopathologic lesions, related to the concentration of injected gentamicin. These alterations mainly involved the RPE-photoreceptor complex when low or moderate doses of the drug were administered; whereas a severe disintegration of all retinal structures was evident with doses ranging from 800–4,000 μg.

Only minimal changes could be seen after intravitreal injection of 200 and 250 μg of gentamicin. After 3 days (Fig. 1), the basal portion of the RPE cells displayed round storage lysosomes, which contained lamellar material arranged in a parallel or concentric manner. Numerous transitional images between normal outer segment phagosomes and storage lysosomes were evident, but the amount of lamellar lipid-containing organelles was definitely higher than in controls. Most specifically, abnormal cells appeared either on the surface of the RPE or free in the interphotoreceptor matrix. These cells had a macrophage-like configuration, contained few melanosomes, and displayed an important accumulation of lamellar lysosomal lipids. In some areas, outer segments seemed to be disorganized; whereas no abnormality could be discovered elsewhere. The inner retina was totally normal as was the choroid. These changes were still evident after 7 days.

Three days after the intravitreal injection of 400 or 500 μg, a more important lysosomal storage of complex lipid was evident within the RPE cells and the macrophage-like cells infiltrating the interphotoreceptor spaces (Fig. 2). In addition, cysts were occasionally noted between the RPE cells. After 1 week (Fig. 3), a loss of polarity of the RPE cells appeared with dissemination of melanosomes and storage lysosomes throughout the cytoplasm, disappearance of the infoldings of the basal portion of the cytoplasmic mem-

Fig. 2. Three days after injection of 500 μg of gentamicin. A macrophage is infiltrating the interphotoreceptor matrix and contains numerous polycyclic lysosomal inclusions of complex lipids. Homogeneous intracytoplasmic inclusions of probable neutral lipids are also evident. The photoreceptor inner segment mitochondria appear unremarkable (×14,000).
Intravitreal injection of 800–1,000 μg of the drug produced an important storage of lamellar lipids within the RPE and macrophage-like cells. This process clearly increased from the first to the seventh day in cells that brane, and alteration of the apical villi. Disintegration of the photoreceptor outer segments was more prominent, while the other retinal and choroidal structures remained unaffected.

Fig. 3. One week after injection of 500 μg of gentamicin: an RPE cell displays loss of basal infoldings, loss of cellular polarity with dissemination of melanosomes, and storage lysosomes (asterisks) throughout the cytoplasm. Disorganized outer segment material (OS) is visible at the superior border of the cell (×8,900).
Fig. 4. One week after injection of 1,000 μg of gentamicin. Inset: Outer segment destruction is evidenced by direct apposition of inner segments to hyperplastic RPE (paraphenylenediamine, ×250). Main figure: there is total loss of outer segment discs with necrotic debris (asterisks) in the subretinal space. Inner segments (IS) contain numerous mitochondria with normal appearance (×8,900). (Arrowheads delineate outer limiting membrane in both figures.)

kept their differentiation. After the third day, areas of focal necrosis of the RPE appeared, with loss of cellular polarity, cytoplasmic edema, swelling of mitochondria and endoplasmic reticulum, rupture of the cell membrane, and dispersion of necrotic material and free melanin granules in the interphotoreceptor spaces.
Fig. 5. Inset, phase contrast micrograph: 24 hr after injection of 2,000 µg of gentamicin. A macrophage (M) is visible in the subretinal space with focal obliteration of the RPE (paraphenylenediamine, ×250). Main figure: 3 days after injection of 2,000 µg of gentamicin, there is focal disappearance of the RPE, with direct apposition of the outer segments to intact RPE basal lamina (arrowhead). Additionally, there is apparent diapedesis of a macrophage-like cell (arrow) within the inner collagenous layer of Bruch's membrane (×14,000).

Curiously, no storage material could be evidenced in these dying cells. Photoreceptors also were degenerating in these areas, with disintegration of the regular lamellar pattern of their outer segment discs, whereas the inner segment seemed to remain normal (Fig. 4). At the margins of these necrotic areas, foci of cell proliferation often appeared with two or three layered arrangement of pigmented, nonpolarized cells that were free of storage material. Animals that received 1,000 µg often displayed focal necrosis in the inner plexiform and in the inner nuclear layer, with appearance of dense, irregular, whorled, lamellar structures in the extracellular spaces and in axons, as well as nuclear pyknosis.

Necrotic images were prominent following injection of 1,600–2,000 µg of gentamicin; whereas lysosomal storage process was found only in rare macrophage-like cells, located between Bruch's membrane and the retina (Fig. 5). From the first day, the RPE cells were profoundly altered and most of them totally disappeared, leaving photoreceptor outer segments in direct contact with bare Bruch's membrane. The retinal architecture was very disorganized after three days, with increasing foci of RPE cell necrosis, disappearance of outer segments and profound cell loss in all retinal layers, together with a marked proliferation of Müller cells. After 7–14 days (Fig. 6), these latter cells occupied most of the retinal sections, from Bruch's membrane to the inner limiting membrane. They were infiltrated in places by pigment-containing cells. Only a few bipolar cells and atrophic photoreceptors were in their
Fig. 6. One week after injection of 2,000 μg of gentamicin. There is total loss of photoreceptor outer segments with direct contact of inner segments to disorganized RPE. Other areas, not depicted, show direct inner segment contact to Bruch's membrane (x8,900).
original location; whereas ganglion cell loss was apparently total. The choroid remained normal, but some macrophage-like cells were seen to proceed through the elastic network of Bruch’s membrane in the direction of the retina. Sections were not obtained, however, which documented further progression of these cells through the remaining basal membrane of the necrotic RPE.

With higher doses of gentamicin (2,000–4,000 μg), an end-stage of retinal necrosis was evident from the first day. This was followed by rapid atrophy and gliosis. The process was so severe that it totally masked the nature of the pathogenetic mechanism.

Discussion

The present study provides the first evidence of a lysosomal dysfunction in RPE cells after intravitreal injection of gentamicin. The lysosomal inclusions—observed by electron microscopy in duplicate experiments performed in both laboratories—present constant and typical structural characteristics, closely resembling those described in numerous studies performed in cultured cells, kidney or conjunctival tissue, both of animal and human origin.6,11,13,14,16,17

The preponderance of the lamellar components within lysosomes strongly suggests the accumulation of complex lipids. Biochemical analysis on cultured cells has demonstrated that these materials consist mainly of phospholipids.6 Although the basic mechanism responsible for aminoglycoside toxicity remains speculative, the accumulation of these molecules together with the concomitant perturbations of acid hydrolase activities suggest that drug-induced enzyme deficiencies might be a reasonable explanation.6,8,10 although other physiopathologic mechanisms involving stabilization of the lysosomal membranes also have been suggested.18

The storage process seems to be related to the concentration of antibiotic in our experiments, well demonstrated by studies in which doses ranging from 200–1,000 μg of gentamicin were injected into the vitreous. This dose-related accumulation had been previously documented in cultured cells,6 and in the proximal tubules of the kidney.12,14

When doses ranging from 500–1,000 μg of gentamicin were injected into the vitreous, an increasing number of necrotic areas appeared within the RPE-photoreceptor complex, paralleling the increasing severity of the lamellar lipid accumulation. Foci of cellular necrosis involving the inner plexiform and inner nuclear layers were more prominent after injection of 1,000–2,000 μg of gentamicin. A very severe necrotic reaction of all retinal structures masked the lysosomal accumulation if doses higher than 2,000 μg were employed.

A similar necrotic phenomenon has been described in the tubular cells of the kidney,11,13,14 and a possible explanation has been suggested of a lysosomal disruption resulting in autolysis and necrosis.8,15,18 However, biologic arguments suggest that this lysosomal theory for tubular necrosis might be incomplete and that other mechanisms such as mitochondrial dysfunction might be involved.8,10,12,13

The appearance of necrotic areas within the inner plexiform and nuclear layers of the retina without pre-existing lysosomal storage, as well as the absence of massive lysosomal overloading within the necrotic RPE following injection of high doses, also suggest that gentamicin is capable of producing cell necrosis by alternate mechanisms in addition to lysosomal dysfunction.

The enormous clinical importance of aminoglycosides in the prevention and therapy of severe infections demands their continued use despite a variety of toxic side effects. The recent report of an accidental injection of tobramycin, another aminoglycoside with presumably similar toxicity, into a patient’s eye with disastrous consequences19 is better understood in the light of the present study. It, furthermore, underlies the need to explore more subtle drug effects and suggests that studies should be initiated for comparison of the relative retinal toxicity of other aminoglycosides to provide for safer intraocular administration.

Key words: gentamicin, lysosomes, lipidosis, aminoglycosides, toxicity

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