Scanning Electron Microscopic Observation of Dark Cells After Streptomycin Perfusion of the Vestibule in Guinea Pigs

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SCANNING ELECTRON MICROSCOPIC OBSERVATION OF DARK CELLS AFTER STREPTOMYCIN PERFUSION OF THE VESTIBULE IN GUINEA PIGS

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Abstract

Hearing has been stabilized in the majority of patients studied in the treatment of Meniere's disease with streptomycin. This observation suggests that effects of streptomycin may ameliorate endolymphatic hydrops, possibly by attenuating the activity of secretory tissue. The purpose of this study is to observe the dark cells of the utricle in guinea pigs after streptomycin perfusion of the vestibule. Twelve pigmented guinea pigs weighing 250-350 grams were used in this study. The vestibules in five guinea pigs were perfused monolaterally with 150 μg of streptomycin in artificial perilymph and, in seven, the vestibules were perfused only with artificial perilymph as a control group. Specimens were processed for observation with a scanning electron microscope.

After streptomycin perfusion, the margin of the dark cells became indistinct. The luminal surface of the cells bulged out like a dome. The microvilli decreased or were absent, and some debris was deposited on the surface. In four of the five animals, the luminal membrane of the dark cell ruptured. The cytoplasm and organelle extruded into the endolymphatic space. After the cellular debris moved out into the endolymph, either a vanished cell or a nucleus in an empty nest was observed. These cells appeared damaged and destroyed.

The results indicate that the dark cells in the membranous wall of the utricle were affected by streptomycin. The results lead to the assumption that streptomycin may reduce the volume of endolymph by damaging the dark cells of the utricle.

Key Words: Scanning electron microscopy, dark cells, utricle, Meniere's disease, streptomycin perfusion.

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Introduction

Streptomycin has been used in the treatment of Meniere's disease for almost fifty years. Fowler [5] first reported four patients using streptomycin in the treatment of Meniere's disease with good results. Schuknecht and his associates [7, 19, 20, 21, 24] published five papers which included results on fifteen patients who received intramuscular streptomycin sulfate therapy. Wilson and Schuknecht [29] presented 20 cases, with a follow-up period of from 1 to 16 years, in which no one experienced loss of hearing. Schuknecht [22], in his recent paper, stated that if the effect of streptomycin is limited to ablation of vestibular function, one would expect the hearing losses to progress in accordance with that known to occur in the normal course of Meniere's disease. However, the data indicate that this is not the case and that hearing has been stabilized in the majority of patients studied. This observation suggests that the toxic effects of streptomycin may ameliorate endolymphatic hydrops, possibly by attenuating the activity of secretory tissue which is the stria vascularis in the cochlea and the dark cell areas in the vestibular system. A large number of histopathologic studies [3, 13, 14, 27, 28] have demonstrated that the vestibular sensory epithelium is able to be destroyed by streptomycin ototoxicity. Norris et al. [16] reported that streptomycin has a double effect on the hair cells of the vestibular receptors, an immediate effect by blocking the calcium channels which causes interruption of neural discharges, and a permanent effect which includes destruction of the glycocalyx, injury of the stereocilia, and damage of the hair cells. Streptomycin effects on the vestibular sensory epithelium may eliminate vertigo attacks. The purpose of this study is to observe the effect of streptomycin on the dark cells of the utricle with the scanning electron microscope after streptomycin perfusion of the vestibule, which may have major implications in the fluid balance of the inner ear.

Materials and Methods

Twelve pigmented guinea pigs weighing 250-350

Table 1. Composition of an artificial perilymph.

Chemical	Dosage (mM)
NaCl	125
KCl	4
$MgCl_2$	1.14
$CaCl_2$	1.3
$NaHCO_3$	25
NaH_2PO_4	0.51
Glucose	3
Urea	2

*pH: 7.4

grams were used in this study. The vestibules in five guinea pigs were perfused with streptomycin in artificial perilymph. Only artificial perilymph was perfused in seven of the guinea pigs as a control group.

The animals were anesthetized with 50 mg/kg of sodium pentobarbital intraperitoneally. The head of the guinea pig was fixed in a head holder, and a tracheostomy was performed. Ventilation was maintained with an artificial respirator after muscle relaxation. The auditory bulla was opened through a submandibular approach in the supine position and the cochlea was exposed extensively. Under the surgical microscope, the stapes was removed, and the vestibular window was opened to allow the perilymph to release. After most of the perilymph escaped from the vestibule, a glass micropipette with a tip diameter of 3 to 5 micrometers filled with the perfusate, mounted on a micromanipulator, was inserted into the vestibule through the oval window without touching the membranous labyrinth. The pipette was connected to a tube attached to a syringe on the micro perfusion-pump (Sage Instruments, Model 355). In the streptomycin group, streptomycin sulfate dissolved in artificial perilymph was perfused into the vestibule. The concentration of streptomycin was 2 mg in 1 ml of artificial perilymph. The rate of perfusion was 12 μl/min. The duration of perfusion was 6 minutes and 20 seconds. The total dosage of streptomycin into the vestibule was 150 μg. In the control group, the artificial perilymph was perfused at the same rate and over the same period of time. The composition of the artificial perilymph is shown in Table 1. Ten minutes after perfusion, the animals were sacrificed for histopathological study.

The guinea pigs were fixed by heart perfusion with 2.5% glutaraldehyde buffered with 0.1 M phosphate buffer (pH 7.4). The temporal bone was immediately removed and the fixative was introduced into the inner ear
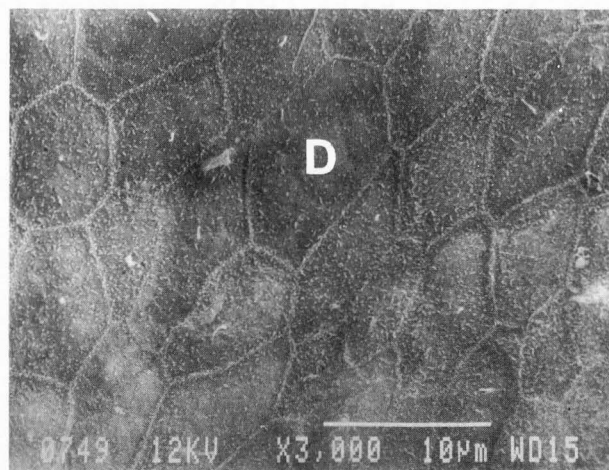

Figure 1. In the control group, the shapes of the dark cells were varied. Most of them were hexagonal, some were pentagonal and a few were quadrilateral. The luminal surface was flat and smooth with microvilli. No otoconia fragments and debris were seen. D = dark cell.

via the round window while the oval window was opened. The specimens were dissected after fixation, dehydrated with graded ethanol, placed in isoamyl acetate and processed for critical point-drying (CO_2). The specimens were then coated with gold-palladium and observed with a Hitachi 2000 scanning electron microscope. The observation was focused on the dark cells of the utricle. The ampulla and the hair cells were not observed in this study.

Results

The control group

The shape of the dark cells varied (Fig. 1). Most of them were hexagonal, some of them were pentagonal and a few were quadrilateral. The luminal surface was flat and smooth with microvilli. Meshwork structures were occasionally observed on the luminal surface of the cells. No otoconia fragments or debris were seen. No mechanical damage was observed.

The streptomycin group

The margin of the cells became indistinct. The luminal surface of the cell bulged out like a dome (Fig. 2). The microvilli had decreased or were absent, and some debris was deposited on the surface.

In four of the five animals, the luminal membrane of the dark cells ruptured (Fig. 3). The cytoplasm and organelle extruded into the endolymphatic space. Either a vanished cell (Fig. 4) or a nucleus in an empty nest
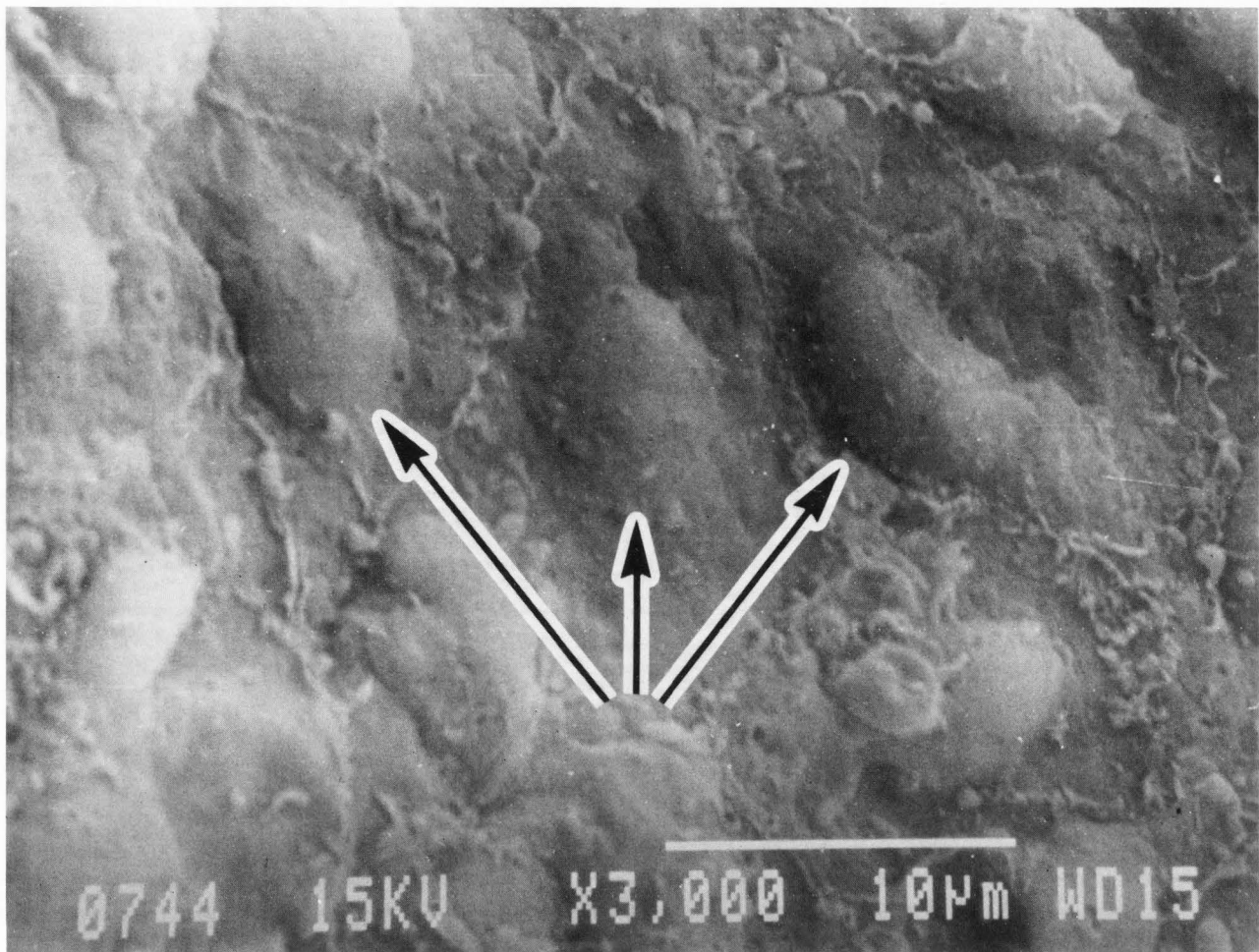
Figure 2. In the streptomycin group, the margin of the dark cells became indistinct. The luminal surface of the cell bulged out like a dome (arrows). The microvilli decreased or were absent and some debris was deposited on the surface.

(Fig. 5) were observed. After the cellular debris moved out into the endolymph, these cells were damaged and destroyed. The pictures of the dark cells observed using scanning electron microscopy in this study correlated well with the observations under the transmission electron microscope in our previous report [6].

Discussion

Hearing has been stabilized in the majority of patients studied in the treatment of Meniere's disease with streptomycin. This observation suggests that the effects of streptomycin may ameliorate endolymphatic hydrops, possibly by attenuating the activity of secretory tissue in the inner ear. The ultrastructure of the dark cells in the utricle was first described by Smith [25]. The morphologic characteristics, particularly, the numerous plasma membrane infoldings, were similar to those

seen in the ion-transporting epithelia in renal tubules. It is suggested that these epithelia play an important part in the production of endolymph. The dark cells in the vestibular system are similar to the marginal cells in the stria vascularis which are known to produce endolymph in the cochlea. The dark cells in the vestibular system are located at the slope of the ampullar crista, the wall of the utricle, and the planum semilunatum. Kimura [12] found that the granules in the transitional cells and the cells of the planum semilunatum of the pigeon were secretory cells. Watanuki *et al.* [26] reported that the dark cells have secretory and perhaps absorptive functions. Kimura [11] suggested that the dark cells were engaged in fluid and electrolyte transport. Nakai and Hilding [15] demonstrated adenosine triphosphatase (ATPase) activity on the surface of the basal infolding and postulated that the dark cells produce endolymph in the vestibule.
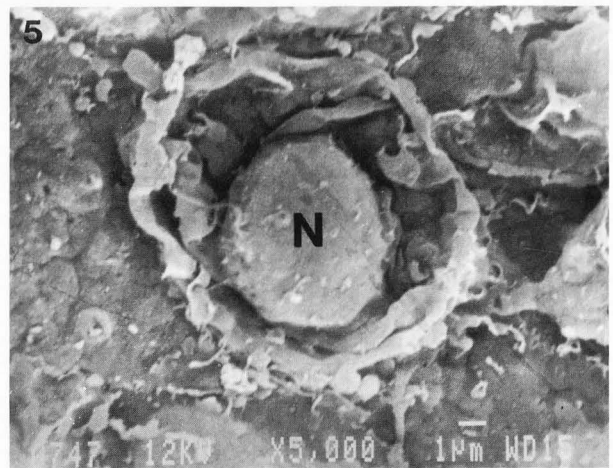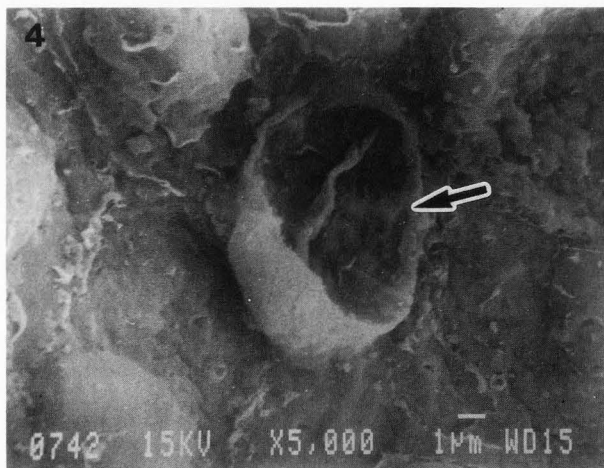
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Figure 3. After streptomycin perfusion, the luminal membrane of the dark cells ruptured (arrow). The cytoplasm and organelle extruded into the endolymphatic space.

Figure 4. At high magnification, a vanished cell was observed (arrow) after streptomycin perfusion.

Figure 5. A nucleus in an empty nest was seen after streptomycin perfusion. Obviously, these cells were damaged and destroyed. N = nucleus.

It is believed that the early damage to the vestibular system by streptomycin is in the sensory cells. Hawkins and Preston [8] stated that the hair cells are the primary target of streptomycin ototoxicity, especially type I hair cells in the vestibular neuroepithelia. Duvall [4] reported that sensory cell damage started with degeneration of the mitochondria, and later swelling appeared in the sensory hairs with deformation of the cell surface. Finally, disappearance of the sensory hairs with rupture and ejection of cell debris into the endolymph occurred. Injury to the vestibular system by streptomycin is not confined to the sensory cells. Streptomycin affects the secretory areas, that is, the stria vascularis in the cochlea and the dark cells in the vestibule, which are responsible for maintaining the microhomeostasis of the inner ear. Sparwald *et al.*, cited by Hawkins [9], described degenerative changes in the dark cells of the crista in the guinea pig after streptomycin treatment. Hawkins and Preston [8] stated that similar effects have been demonstrated in the squirrel monkey. Furthermore, the authors hypothesized that the vestibular toxicity is exerted primarily on the secretory tissues, and that the loss of hair cells is secondary to a disturbance of the microhomeostasis of the vestibular system. Park and Cohen observed the vestibular ototoxicity in the chick. Their experiments showed that streptomycin damaged the dark cells before other cell types [17]. The authors found that the cells damaged by streptomycin were in a specific order, beginning with the dark cells, followed by planal cells, nerve terminals, hair cells, and supporting cells [18]. In our study, following intoxication with streptomycin, an increasing amount of cell fragments were found on the luminal surface of the dark cells. The microvilli decreased or were absent. The margin of the cells became indistinct. The luminal surface of the cell bulged out in a dome shape. In most of the cases, the luminal membranae of the dark cells ruptured and the cytoplasm and organelle extruded into the endolymphatic space. These cells were observed to be damaged. The degenerative changes in the dark cell areas of the vestibular labyrinth, as well as the stria vascularis, might explain why some patients with Meniere's disease experience improved hearing in association with streptomycin treatment. It appears that streptomycin is in some way improving the fluid physiology of the inner ear. Probably the degeneration of secretory epithelia, including the stria vascularis in the cochlea and dark cells in the vestibular system, results in less production of endolymph.

It is well documented by immunomorphological studies that the onset of aminoglycoside antibiotic ototoxicity is a two-step mechanism [10]. At first, the drug attacks on the cell surface, and thereafter the internal parts of the hair cells, primarily, lysosomes in the cytoplasm. The aminoglycoside antibiotics are irreversibly bound to the sensory cells of the vestibular receptors in as little as 10 minutes [23]. Anniko *et al.* [1] reported on the uptake of aminoglycosides; radioactively labelled gentamicin bound irreversibly within 10 minutes, completely saturating all receptor binding sites in the isolated crista ampullaris of the guinea pig. Also, the same authors reported that a rapid, and probably, irreversible binding of tobramycin occurs within 10 minutes [2]. In our study, the luminal surface of utricular dark cells revealed degenerative changes 10 minutes after streptomycin perfusion of the vestibule. Some were severely damaged. The results correlate with the observation by Anniko.

Streptomycin ototoxicity produces an immediate effect and a delayed effect. This study observed only the immediate effects. The delayed effects need to be considered in a separate study.

Conclusions

(1) The dark cells in the membranous wall of the utricle were affected by streptomycin.

(2) The results lead to the assumption that streptomycin may reduce the volume of endolymph by damaging the dark cells of the utricle.

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Discussion with Reviewers

C.H. Norris: Was there any mechanical damage seen in control animals due to the perfusion?
Authors: No, there was no mechanical damage observed.

C.H. Norris: Are these permanent changes or are they reversible?
Authors: Some of the damage was mild and some very severe. According to Anniko's report on the uptake of aminoglycosides, radioactively labelled gentamicin bound irreversibly within 10 minutes, as did tobramycin. Therefore, we assume some of these changes are irreversible.