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Cochlear Ototoxicity of Chlorhexidine Gluconate in Cats

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Summary. Chlorhexidine gluconate is a derivative of chlorhexidine and is a popular desinfectant with a strong bactericidal action that is widely used for preoperative sterilization in ear surgery. The purpose of this investigation is to ascertain the potential ototoxity of this agent. After topically applying chlorhexidine gluconate solutions to the middle ear cavities of 12 cats, we observed the excised cochleas using both scanning and transmission electron microscope studies. Either 0.05% or 2% chlorhexidine gluconate solutions were infused into the right ear of the test animal through one of two tubes chronically installed in the tympanic bullae. The left ears were utilized as controls and were infused with sterilized physiological saline. The solutions were administered once every other day for three separate infusions. Nine animals were decapitated 7 days after the third application, while the other three animals were sacrificed at 4 weeks. In the 2% chlorhexidine group, we found that hair cells in the organ of Corti had degenerated and had lost their hair bundles over a wide range. This pathology was more marked in the lower cochlear turns. In the animals sacrificed at 4 weeks, the injuries present seemed to have progressed. Even at a clinical concentration of 0.05%, chlorhexidine caused intracellular degeneration but with little surface damage. Our findings would suggest a cause of hearing loss when chlorhexidine is used clinically in the ear.

Key words: Ototoxity – Chlorhexidine gluconate – Electron microscope

Introduction

Chlorhexidine (1,6-di-[4′chlorophenyldiguanido]hexane) is one of the most popular agents for skin disinfection and is bactericidal to both gram-positive and gram-negative bacteria. Even at such low concentrations as 0.05%, it exerts its action promptly on staphylococcal organisms. In otological surgery, this agent has been used more and more widely as a skin disinfectant prior to

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tympanomastoid surgery. However, when used to disinfect skin at the beginning of surgery, instances of inner ear damage by this agent may occur when it is accidentaly dropped into the tympanic cavity through a perforated eardrum. In 1971, Bicknell [4] reported cases of severe sensorineural hearing loss following successful myringoplastic operations. He pointed out from his clinical observations that chlorhexidine used preoperatively appeared to be the cause of this loss. Subsequent reports by other authors have also led otologists to pay careful attention to the use of preoperative disinfectants.

In spite of the extensive use of chlorhexidine and its potential ototoxity, ultrastructural studies of its actions on the inner ear have not disclosed any pathological changes. Recently, Aursnes [2] reported cochlear damage in the guinea pig following a single intratympanic application of chlorhexidine. Hair cell damage was observed with a surface preparation technique under a light microscope and Aursnes concluded that there was a strong correlation between the pathology seen and the dose of disinfectant applied.

The aim of our present investigation was to clarify any morphological changes of the organ of Corti in cats by electron microscopy following intratympanic applications of chlorhexidine gluconate solutions at a high concentration (2%) and at concentrations used clinically (0.05%).

Clorhexidine gluconate is a derivative of chlorhexidine and may currently be more widely used clinically. The bactericidal property of both compounds is almost the same. Our pilot study revealed that no definite changes were present in the cat's inner ears following a single application of the agent in the above-mentioned concentrations and implied that these results stemmed from normal function of the Eustachian tube, facilitating drainage of the applied solution from the tympanum to the pharynx. Our pilot study also showed that three applications of the disinfectant, given once every other day, induced both middle ear pathology and inner ear injury. Consequently, we repeated intratympanic applications of this agent in this investigation to assess the ototoxic effects on the inner ear.

Materials and Methods

Twelve healthy adult cats, weighing 2.8–3.5 kg, were used. Before starting the experiment, careful observations through the external canals of all animals showed that no animal had either fluid retention or inflammatory changes in the tympanum. All cats were anesthetized with intraperitoneal pentobarbital (35 mg/kg body weight), and bilateral postauricular skin incisions were made to expose the clean bony surfaces of the tympanic bullae. Holes 2 mm in diameter were drilled open on the posterior walls of both bullae. Two epidural tubes (10 cm in length) were inserted approximately 5 mm into each tympanic cavity and were fixed to the surrounding muscle and connective tissues. The shortest distance between the posterior wall of the tympanic bulla and the round window membrane in the adult cats was previously found to be 6–8 mm in our pilot study. Consequently, the tip of each tube inside the tympanic bulla was so placed from the round window membrane that no mechanical injury would be incurred. The two tubes were also positioned so that any increased pressure from an infused solution would easily escape from the tympanic cavity through the drainage tube in order to prevent infusion barotrauma on the round window membranes. All of the left ears were used as controls and were compared with the right ears for any morphological changes occurring. Sterilized physiological saline (SPS), 0.6 ml, was infused each time into the left ears. The

Table 1. Protocol for using topical chlorhexidine gluconate (0.05 or 2%) solutions in the cat ear

Cat no.	Ears		Time of animal sacrifice	
	Right disinfectant	Left control	1 Week	4 Week
1	2% CH	SPS	*	
2	2% CH	SPS	*	
3	2% CH	SPS	*	
4	2% CH	SPS	*	
5	2% CH	SPS	*	
6	0.05% CH	SPS	*	
7	0.05% CH	SPS	*	
8	0.05% CH	SPS	*	
9	0.05% CH	SPS	*	
10	2% CH	SPS		*
11	2% CH	SPS		*
12	2% CH	SPS		*

CH, chlorhexidine gluconate; *SPS,* sterilized physiological saline

chlorhexidine gluconate (CH) solutions were infused into the test ear in the same quantity as the control (0.6 ml per infusion). Solutions were injected into both ears once every other day for a total of three separate infusions.

Our pilot study revealed that acute otitis media occurred in almost all ears following the application of an irritative disinfectant solution. To reduce the effect of this occurrence on the inner ear, benzylpenicillin procaine (100,000 units/kg body weight per day) was administered intramuscularly to each animal for 7 days after the first otic application of the test solution. This effectively prevented acute bacterial infection from occurring.

Seven days after the third application of the test solutions, nine cats were sacrified by decapitation, and the entire whole tympanic bullae were fixed with 2.5% glutaraldehyde in 0.1 *M* phosphate buffer (pH 7.4). The specimens were then processed for electron microscopic observations. The remaining three cats were sacrified 4 weeks later and the six temporal bones thus obtained were also prepared for electron microscopic observations of possible inner ear changes. Surface structures of the cochlear sensory epithelia were observed by scanning electron microscope (SEM, Hitachi HHS-2R) and intracellular pathological changes were examined by transmission electron microscope (TEM, Hitachi HS-9). All procedures are summarized in Table 1.

Results

Behavioral observations on the animals preceding death revealed that the heads of all cats in the 2% CH group tilted down on the side to which the disinfectant was applied and that the animals were squatting. This abnormal behavior was not noted in any of the cats given 0.05% CH. Histological studies of these animals showed that the middle ear mucosa on the SPS side had a clear, nearly normal surface structure. In contrast, the middle ears on the CH side contained thick, inflammatory mucosa and some serous fluid retention was present.

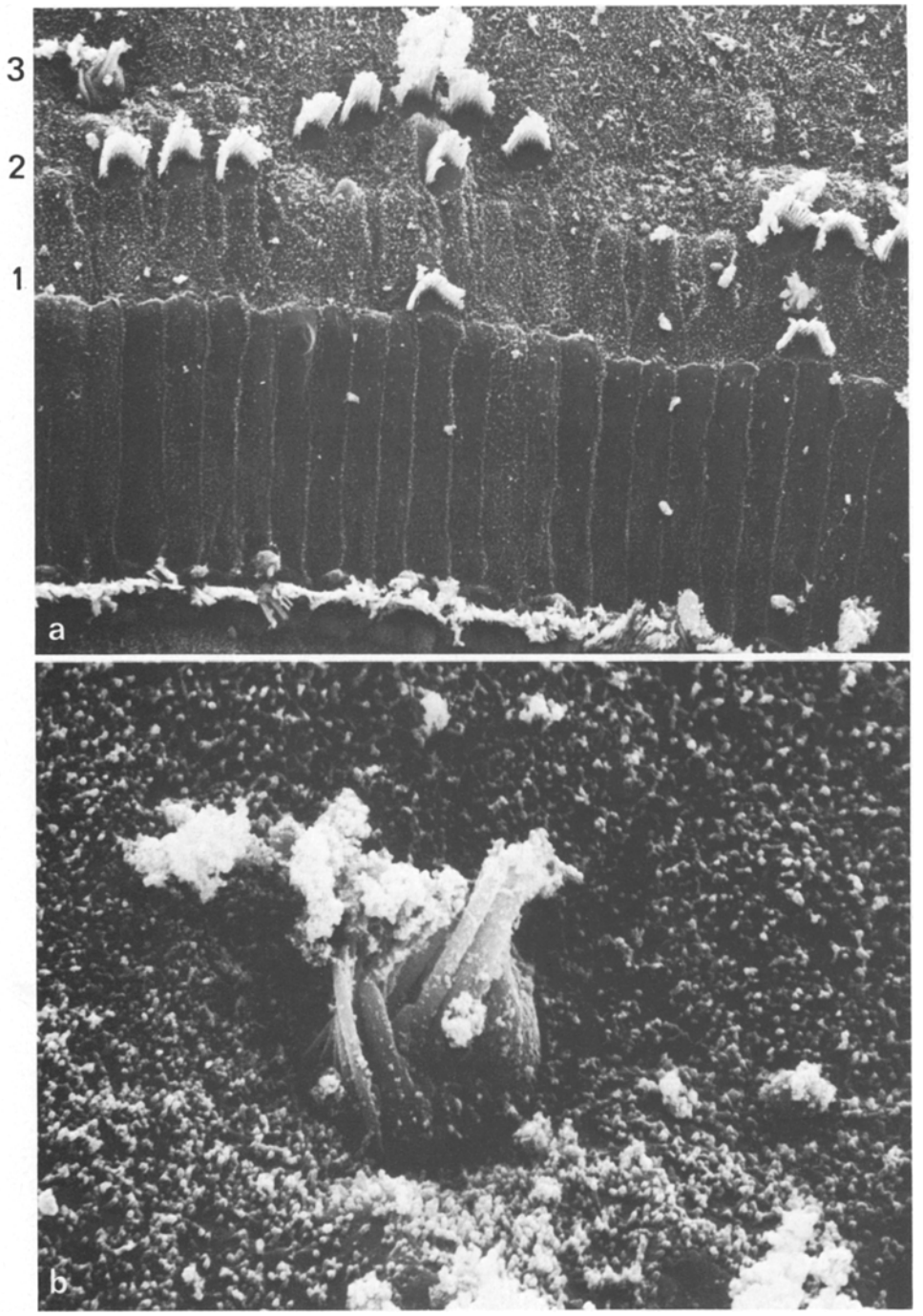

Fig. 1a. Outer hair cell missing at the middle turn of the right ear following exposure to 2% chlorhexidine gluconate; CH: (cat no. 1). Nearly 85% of the outer hair cells (OHCs) were missing and missing sensory hair bundles were found between three OHC rows (1–3). Bundles of sensory cilia of inner hair cells (IHCs) seem distorted. × 2250. **b** Higher magnification (× 9900) of degenerated OHCs in a part of Fig. 1a. Some cilia were twisted and swollen, and cilia of the bundle coalesced with each other

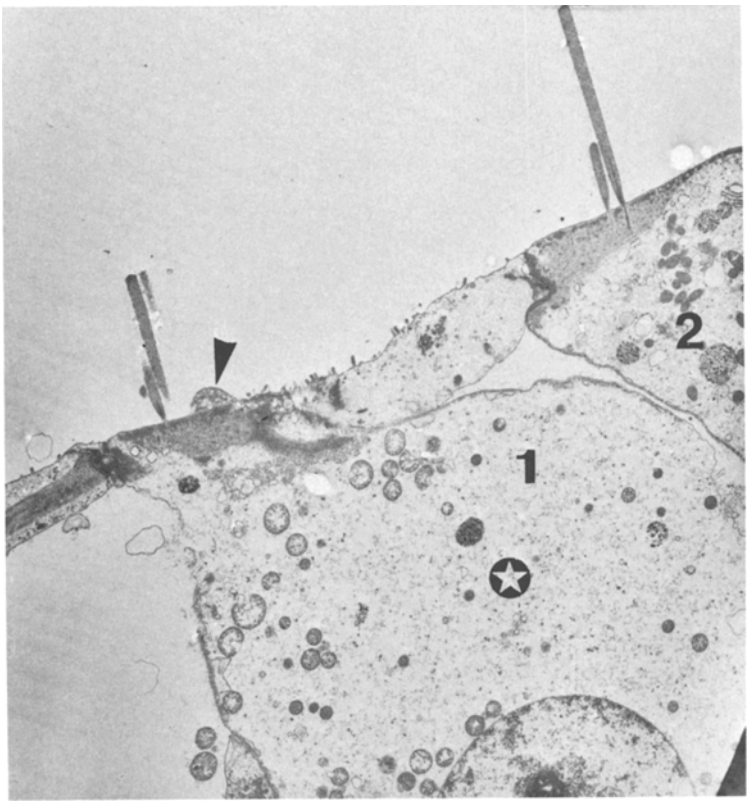

Fig. 2. Intracellular degeneration of OHCs of the middle turn in the 2% CH group. The body of the first row of OHCs is grossly distorted and contains degenerating mitochondria (*star*). The second row of OHCs seem less damaged in this section. A small protrusion (*arrowhead*) is seen from the cell surface of the first row of OHC. 1,2: first and second outer hair cells respectively. × 7020

In all control ears, sensory cells of the organ of Corti were intact and had normal surface structures in all cochlear turns, except in the apical turn, where a normal variation of hair cell loss was observed. Sensory hair cells in the five cochleae (cats 1–5) exposed to 2% chlorhexidine gluconate showed moderate to severe hair cell loss, which was marked in the lower cochlear turns.

The middle cochlear turn on the CH side showed that the bundles of sensory cilia of the outer hair cells (OHCs) had partially disappeared (Fig. 1a). Some of the remaining OHCs showed the normal characteristic shapes and normal arrangement of sensory cilia, while others showed some deformation, twisting or shrinkage (Fig. 1a, b). In the surrounding area of these deformed cilia, some OHCs were missing, indicating more severe injury. The area where hair cells were missing was replaced by supporting cells with microvilli. The inner hair cells (IHCs) showed some loss and some disarrangement of sensory bundles (Fig. 1a). The TEM study revealed changes of the remaining OHCs, such as ballooning of the cell bodies and a varied extent of degeneration of mitochondria and some other intracellular organellae (Fig. 2).

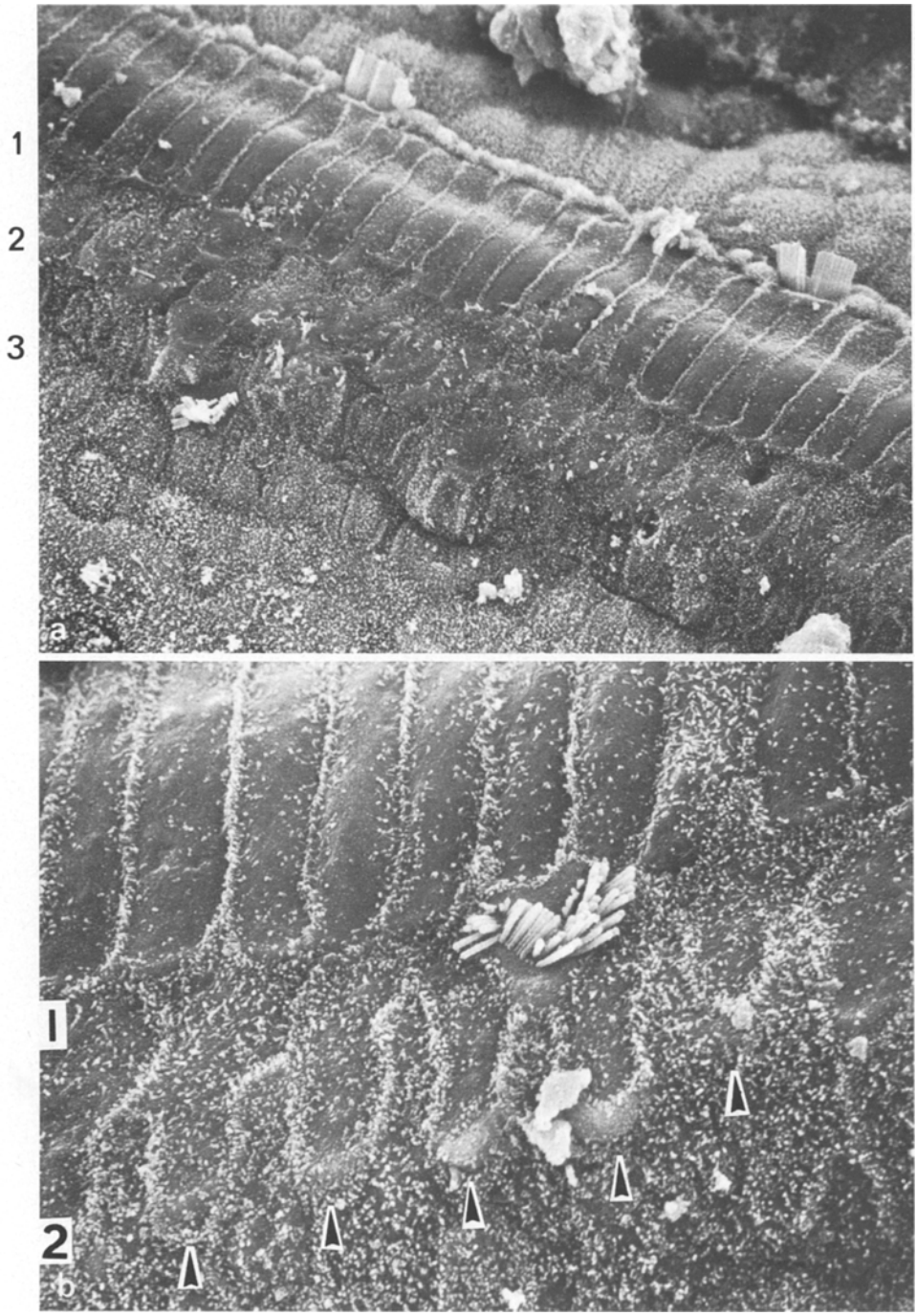

Fig. 3. a Upper basal turn of the 2% CH group (cat no. 3). Almost all OHCs have disappeared and are replaced by the surrounding supporting cells with surface microvilli. A few hair bundles of OHCs remain, but show distorted and decreased number of cilia. Inner hair cells have also lost their sensory hair bundles. × 2250. **b** Higher magnification of the area depicted in Fig. 3a. Area of disappearance of OHCs (*arrowheads*). 1, 2: first and second rows of OHCs. × 4860

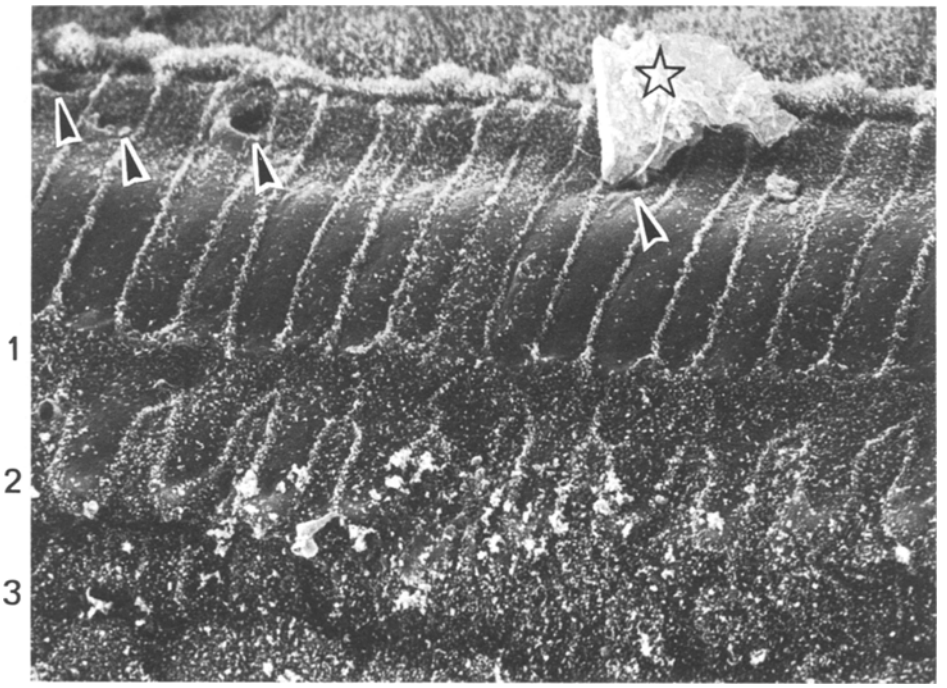

Fig. 4. Lower basal turn of 2% CH group (cat no. 3) showing complete loss of all sensory cilia. On the surface of the pillar cells, several holes (*arrowheads*) can be observed. Substance or probably degenerated intracellular organelle (*star*) protrudes from one of these holes. × 3240

In the upper basal turn of the treated cochleae the changes of the hair cells were more severe than those in the middle turn. Almost all the OHCs completely lost their sensory hairs, with the exception of a few deformed bundles of cilia. In these specimens, 85% of the IHCs showed disappearance or deformity of sensory hairs in this area (Fig. 3a). Further observations of the sites where the hair bundles disappeared revealed various stages of replacement of these cells by the neighboring supporting cells with microvilli on their surfaces (Fig. 3b).

In the lowest cochlear turn, a total loss of sensory hair cells and the presence of small holes or roundish depressions on the supporting cell surface were noted. Intracellular components seemed to come to the surface of the organ of Corti through these holes (Fig. 4). The above observations on all the cochlear turns indicated that sensory cell damage was more intensive in the lower cochlear turns than in the upper ones.

As for the group of animals treated with 0.05% chlorhexidine gluconate, the SEM study showed little loss of sensory hair bundles and no abnormality on the surface of the organ of Corti in any of the cochlear turns (Fig. 5a). The TEM observations, however, indicated some degree of intracellular degeneration, such as the appearance of vacuoles, markedly degenerated mitochondria and disruption of the cell membrane in some of the OHCs (Fig. 5b). Both the surfaces and the intracellular structures of the IHCs were normal.

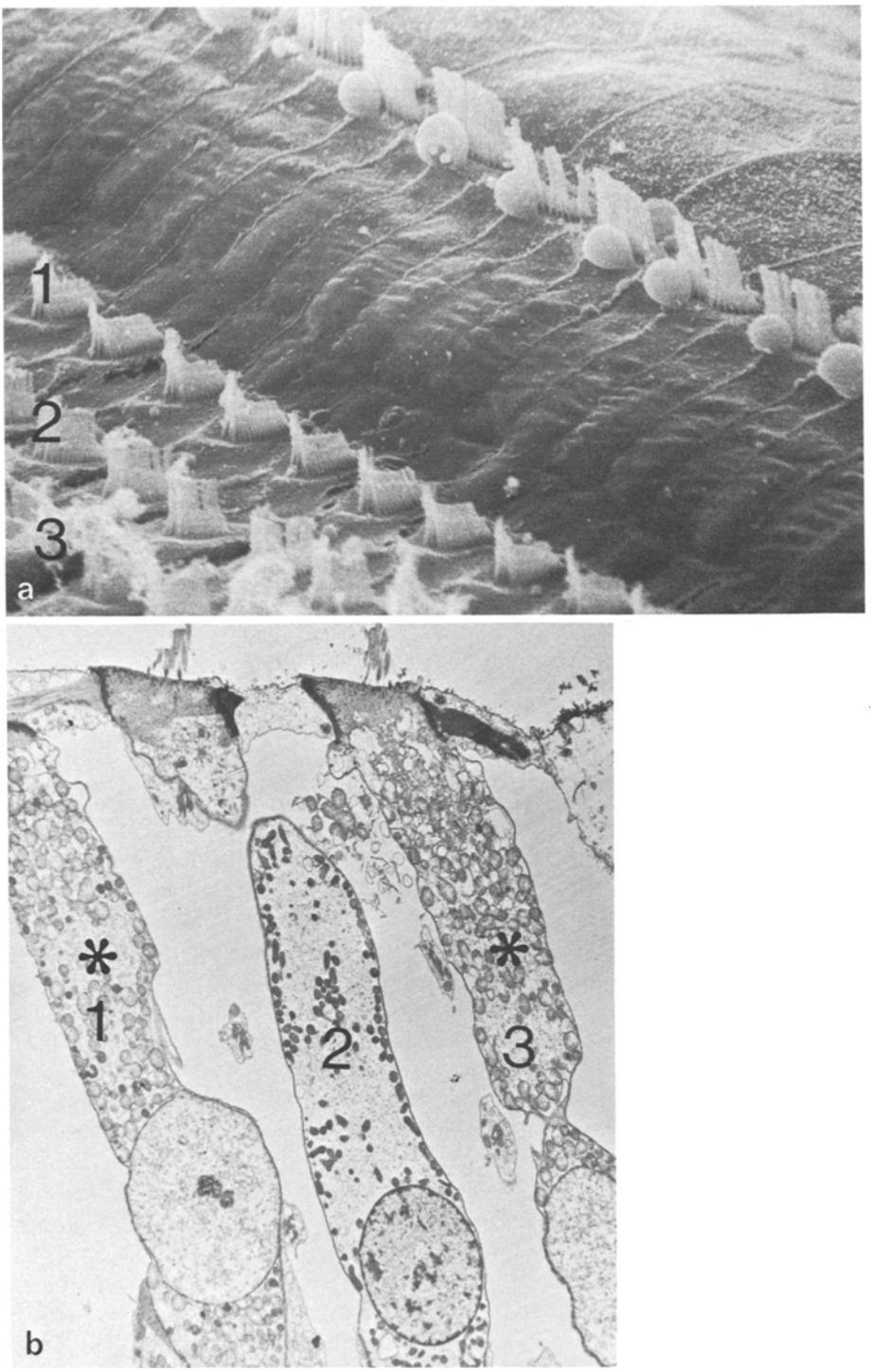

Fig. 5. a Upper basal turn of the 0.05% CH group. Surface structures of outer and inner hair cells were seemingly intact. 1–3: three rows of OHCs. × 2880. **b** Intracellular degeneration of OHCs at the basal turn in the same cat as in Fig. 5a. Degeneration is marked in the first and third OHCs as compared with the second. No protrusion or collapse is noted on the top of the organ of Corti. 1–3: first to third rows of OHCs. × 3150

In the cats sacrificed 4 weeks after the third applications of 2% chlorhexidine gluconate, the observations in all three ears revealed even more extensive degenerations, i.e., the cochlear ducts of almost all cochlear turns were filled with dense fibrous tissue and were completely obliterated. Neither the organ of Corti nor the surrounding supporting structures of the scala media were distinguishable.

Discussion

There have been numerous reports of ototoxity induced by certain antibiotics and other parenteral medications. Such ototoxic drugs have included the aminoglycoside antibiotics (streptomycin, kanamycin, gentamycin, tobramycin, etc.), with ear pathology demonstrated in both clinical and animal studies [1, 3, 5–9]. Some of these drugs have also been reported to have an ototoxic action when used topically as "ear drops" [10, 11]. When the use of these drugs could not be avoided in therapy, otologists have been very careful in their method of application. In 1971, Bicknell [4] reported several cases of severe postoperative sensorineural hearing losses after simple tympanoplastic surgery. He suspected that chlorhexidine, one of the preoperative sterilizing agents, might have been the responsible agent. Since then, few studies have been conducted on the toxic actions of this agent, despite the increased numbers of tympanoplastic surgery and the increased possibility of accidental contact of the disinfectant with the inner ear during such surgery. In 1981, Aursnes [2] reported that chlorhexidine had an obvious cochlear ototoxity. In an experimental study using a guinea pig model, he noted that cellular injuries were most marked in the lower cochlear turn and were worse when higher concentrations of disinfectant were used or when exposures were prolonged.

Our present investigation utilizing electron microscopy supports Aursnes' findings. Moreover, we were able to demonstrate morphorlogical characteristics of the damaged cochlear sensory cells. With a higher concentration of chlorhexidine (2%), hair cell damage was found in test ears and the percentage of OHC loss was 85% in the middle turns. There were small individual differences. The remaining hair bundles of the cochlear sensory cells appeared to have little damage, although the hair cell loss was more extensive toward the lower turns. In the lowest cochlear turn near the round window membrane, all hair bundles had disappeared.

Several routes for ototopical drugs reaching the inner ear have been suggested. These include the round window, the annular ligament of the oval window, and the mucosal lymphatic vessels of the middle ear [10]. Although our study has not involved possible routes for drug transport, some—probably the round window route—seem to be related to the induction of inner ear damage following the topical application of chlorhexidine. It is important to realize that even with the dilute concentration of CH (0.05%) used clinically, our investigation showed that some cellular changes were induced by the antiseptic, i.e., intracellular degeneration of cochlear hair cells was initiated earlier than were changes on surface structures.

At the present time, we recommend the insertion of a cotton ball soaked with physiological saline into the external ear canal of patients at the time of preoperative disinfection for otologic surgery. This will prevent the disinfectant from coming in contact with the middle ear through a perforated eardrum and possibly cause injury to the inner ear.

Our present study had indicated that chlorhexidine gluconate will cause ultrastructural injury to inner ear cells, beginning with the sensory hair cells. This injury can then spread to other structures in the organ of Corti. 2% chlorhexidine gluconate caused hair cell degeneration predominantly in the lower turns of the cochlea, particularly in the outer hair cells. At a clinical concentration of 0.05%, CH caused sensory cell body damage at a time when few gross sensory hair changes were noted. These observations indicate that this disinfectant solution may be an important potential cause of postoperative perceptive hearing loss following successful tympanoplasty.

References

1. Aran J-M, Erre J-P, Guilhaume A, Aurousseau C (1982) The comparative ototoxities of Centamycin, Tobramycin and Dibekacin in the guinea pig. Acta Otolaryngol (Stockh) [Suppl] 390:1–30
2. Aursnes J (1981) Cochlear damage from chlorhexidine in huinea pigs. Acta Otolaryngol (Stockh) 92:259–271
3. Ballantyne J (1970) Iatrogenic deafness. J Laryngol Otol 84:967–1000
4. Bicknell PG (1971) Sensorineural deafness following myringoplasty operations. J Laryngol Otol 85:957–961
5. Engstrom H, Kohonen A (1965) Cochlear damage from ototoxic antibiotics. Acta Otolaryngol (Stockh) 59:171–178
6. Hawkins JE jr (1976) Drug ototoxity. In: Handbook of sensory physiology, vol V-3. Auditory system. Springer, Berlin Heidelberg New York, pp 707–748
7. Hawkins JE jr, Engstrom H (1964) Effect of kanamycin on cochlear cytoarchitecture. Acta Otolaryngol (Stockh) [Suppl] 188:100–106
8. Johnsson L-G, Hawkins JE, Kingsley TC, Black FO, Matz GJ (1981) Aminoglycoside-induced cochlear pathology in Man. Acta Otolaryngol (Stockh) [Suppl] 383:1–19
9. Kohonen A (1965) Effect of some ototoxic drugs upon the pattern and innervation of cochlear sensory cells in the guinea pig. Acta Otolaryngol (Stockh) [Suppl] 208:1–70
10. Kohonen A, Tarkkanen J (1969) Cochlear damage from ototoxic antibiotics. Acta Otolarnygol (Stockh) 68:90–97
11. Proud GO, Mittelman H, Seiden GD (1968) Ototoxity of topically applied chloramphenicol. Arch Otolaryngol 87:580–587

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