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OTOTOXIC EFFECTS OF MIDDLE EAR GENTAMICIN INSTILLATION IN THE CHINCHILLA

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Abstract

Gentamicin sulphate is used clinically to produce vestibular organ damage in patients with intractable vertigo. The drug is given locally by middle ear instillation. In humans, gentamicin appears to have a more toxic effect on the vestibular parts of the inner ear than on the cochlea. However, cochlear effects are not insignificant and to further explore the utility of monitoring vestibular and cochlear function during treatment, we developed an animal model (chinchilla) of this procedure. We present morphological findings, comparing vestibular sensory epithelial damage (crista ampullaris and otolith organs) with cochlear damage, in both ipsilateral and contralateral inner ears. In the chinchilla, we have failed to find a differential effect where the vestibular organs are more damaged than the cochlea. Importantly, we find significant contralateral damage even with very low gentamicin regimes. We conclude that the chinchilla is an unsuitable model for gentamicin induced, selective vestibulectomy in humans.

Key Words: Meniere's disease, ototoxic, gentamicin, chinchilla, treatment

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Introduction

There are a number of possible treatments for disabling vertigo in Meniere's disease. In cases where hearing is unserviceable on the affected side, surgical ablation of the vestibular apparatus by such means as labyrinthectomy or translabyrinthine vestibuloneurectomy will prove effective (Fisch and Mattox, 1988; Kemink *et al*., 1989; Levine *et al*., 1990). In situations where hearing is serviceable, a middle or posterior fossa vestibular neurectomy are effective hearing conservation procedures (Fisch and Mattox, 1988; McElveen *et al*., 1988; Silverstein *et al*., 1990). However, injuries to the facial nerve, cochlear nerve and the cochlea itself can result. Middle ear instillation of vestibulotoxic agents, resulting in the destruction of the vestibular apparatus and relief from vertiginous attacks without causing damage to the cochlea, is a possible option to surgical procedures.

Schuknecht (1957), first proposed the instillation of ototoxic medication (streptomycin) to achieve vestibular ablation. Early work using this procedure was marked by a high prevalence of cochlear damage and resulting deafness. This procedure was revised in the 1970's when several centers began using the instillation of gentamicin, which has been reported to be less cochleotoxic than streptomycin, to relieve the symptoms of unilateral Meniere's disease (Beck and Schmidt, 1978; Lange, 1981). Clinical studies published to date indicated a wide variety of delivery schemes, dosages and established endpoints for treatment with gentamicin (Lange, 1981; Odkvist, 1988; Pender, 1985; Bagger-Sjöbäck *et al*., 1990; Nedzelski *et al*., 1992; 1993). In order to investigate several of the unresolved issues regarding the use of gentamicin in vestibular ablation, we set out to establish an animal model. The chinchilla was chosen as a potential model due to its large, easily accessible bulla which provides ample access to the middle ear cavity without perforating the tympanic membrane. The aim of this study is to compare vestibular and cochlear selectivity from topical application of gentamicin.

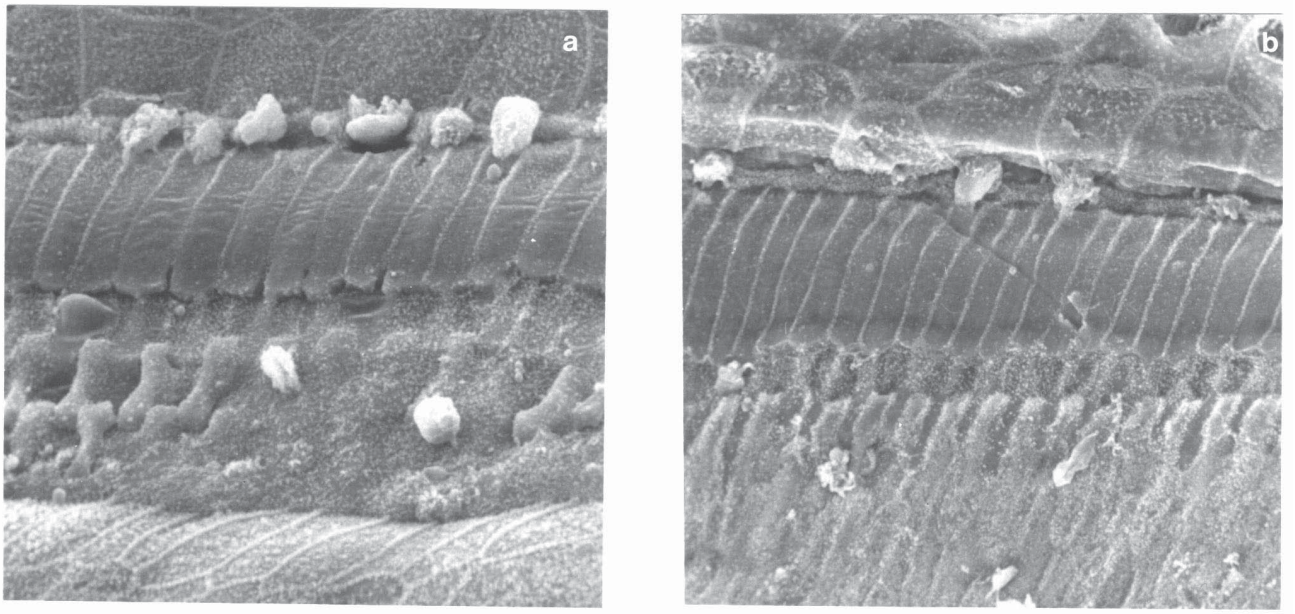


Figure 1. Severe cochleotoxicity resulting from middle ear gentamicin instillation: Hook (**a**) and apical (**b**) regions of cochlea 7 days after instillation of a 1:4 dilution of 26.7 mg/ml gentamicin. No sensory cells remain. Picture width = (**a**) 55 µm, (**b**) 90 µm.

Materials and Methods

Ten adult female chinchillas served the subjects of the study. All were healthy animals with no indication of middle ear infection. Baseline normal data was collected for each animal. This included auditory brainstem response (ABR) audiograms, otoacoustic evoked response measurements and electronystagmograms. Animals were cared for in accordance with national and local guidelines as overseen by the University of Toronto, Animal Care Committee.

Animals were anaesthetized using ketamine 15 mg/kg and xylazine 2.5 mg/kg. An incision approximately 1 cm in length was made over the left superior bulla, a 3 mm square opening was made to provide entry to the middle ear cavity. The middle ear cavity was injected with either gentamicin solution or dextrose (control) (1.5 ml per injection). After injection, the incision was sutured closed. In animals receiving more than one injection, further injections were made 7 days apart through the skin overlying the opening into the bulla.

Gentamicin (40 mg/ml in dextrose) was buffered with sodium bicarbonate (pH 6.4) to a stock concentration of 26.7 mg/ml. This is the concentration currently applied clinically (Nedzelski *et al*., 1993).

Two animals received 1:2 dilution of gentamicin solution: one of these (301) received three weekly injections and was sacrificed two weeks following the third injection: the second animal (309) received two weekly injections and was sacrificed two weeks following the final injection. One animal (311) received a 1:4 dilution of gentamicin stock solution in a single injection and was sacrificed seven days following the injection. The final animal (313) was injected with a 1:10 dilution of stock gentamicin in a single injection and was sacrificed one week after the injection. Four control animals were given 3 weekly injections of dextrose and sacrificed two weeks after the last injection. Electrophysiological monitoring was performed prior to each injection and at one week intervals up to the date of sacrifice.

Histological Processing

Animals were deeply anaesthetized with pentobarbital at 50 mg/kg body weight. The animal was decapitated and the temporal bones removed. The inner ears were rapidly dissected free. Round and oval windows were opened and the vestibule and cochlea perfused with 2.5% phosphate buffered glutaraldehyde at 4°C. Inner ears were fixed for 2 hours, washed in pH 7.4 phosphate buffer overnight, postfixed for 1 hour in buffered 1% OsO_4 the following morning. Specimens were dehydrated to 70% ethanol and dissected. The crista of semicircular canals were removed and the organ Corti exposed. Following dehydration to absolute ethanol specimens were critical point dried from CO_2, mounted on stubs and sputter coated with gold. Specimens were examined on an Hitachi S570 scanning electron microscope.

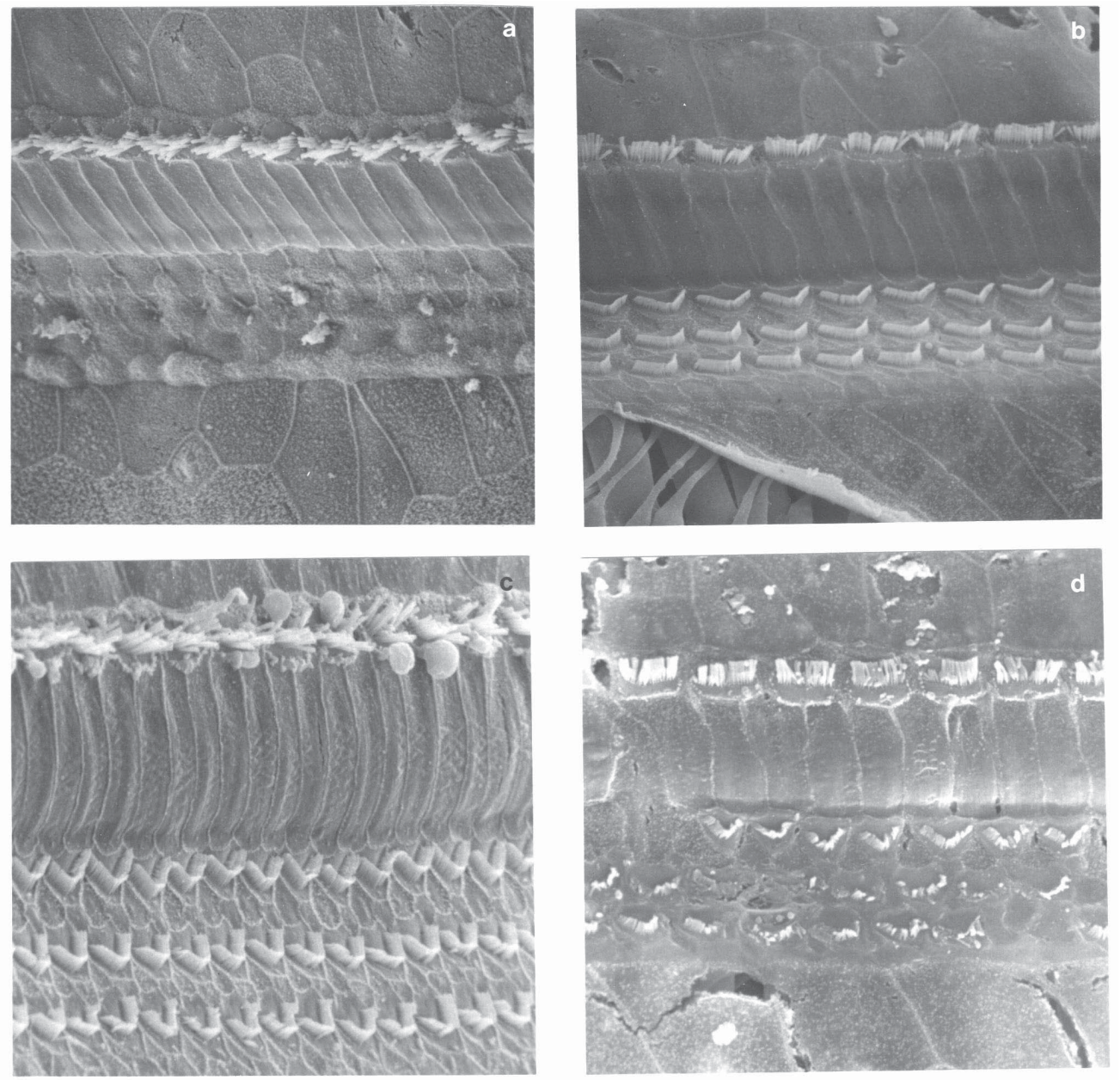


Figure 2. Cochleas contralateral to gentamicin instillation site having various degrees of damage which is unrelated to the dosage. Illustrated are hook regions from contralateral cochleas of animals receiving, (**a**) 3 X 1:2 gentamicin, (**b**) 2 X 1:2 gentamicin, (**c**) 1 X 1:4 gentamicin, (**d**) 1 X 1:10 gentamicin. Picture width = (**a**) 75 µm, (**b**) 60 µm, (**c**) 75 µm, (**d**) 50 µm.

Results

Histologic evaluation by scanning electron microscopy (SEM) showed that the cochlear and vestibular sensory epithelium of both ears were within the normal range of appearance in all control animals. In the experimental group, we found a great variation in damage resulting from gentamicin instillation. General findings will be presented first, followed by descriptions of specific animals.

In all cases gentamicin instillation caused a profound loss of hair cells in the cochlea of the target ear (side of drug instillation); outer hair cells were absent from all but the most apical region and inner hair cells were absent or severely damaged in most instances (as shown in the typical examples of Figure 1). Figure 2 shows examples of damage to cochlear hair cells which was noted in 2 of 4 contralateral ears. Vestibular sensory damage, ranging from mild to severe, was present in all crista of the target ears. In no case was

there a total absence of vestibular hair cells; even in the most severely damaged specimens populations of vestibular hair cells were noted at all peripheral areas of the crista as shown in Figure 3. Contralateral damage was present in the crista of some animals (Fig. 4), but was not necessarily related to the presence of contralateral cochlear damage. Utricular damage was present in the target ear. Even at low dosages (Fig. 5), no contralateral utricular damage was noted. The saccule was not examined. In the ipsilateral ear the degree of damage, both cochlear and vestibular, decreases with decreasing dosage (either concentration or total dose). The degree and/or site of contralateral damage does not appear to be directly related to dose.

In the vestibular organs the central regions of the crista and the striola region of the utricle were damaged more severely than peripheral areas. Where a gradient of damage was observed, the posterior crista was the most damaged, the anterior crista the least. The sensory cells of the utricle were less susceptible to damage than those of the crista. Dark cells of both the crista and utricle appeared normal in all specimens. Specific findings for each animal are given below.

Animal 301 received three weekly injections of 1:2 gentamicin and was sacrificed two weeks after the last injection. Severe damage to sensory epithelium was noted in both the cochlea and vestibular apparatus (Fig. 3) of the left (injected) ear. The contralateral ear suffered mild damage to the vestibular crista (Fig. 4a) and severe damage to the hook region of cochlear (Fig. 2a) with mild to moderate damage in the basal turn. Middle and apical turns appeared normal.

Animal 309 received two injections of 1:2 gentamicin one week apart and was sacrificed two weeks after the last injection. The left (injected) ear showed severe damage to both vestibular and cochlear hair cells. The contralateral ear showed severe damage to the lateral and anterior crista (Fig. 4b) and moderate damage to the posterior crista. The cochlea appeared normal throughout its length (Fig. 2b).

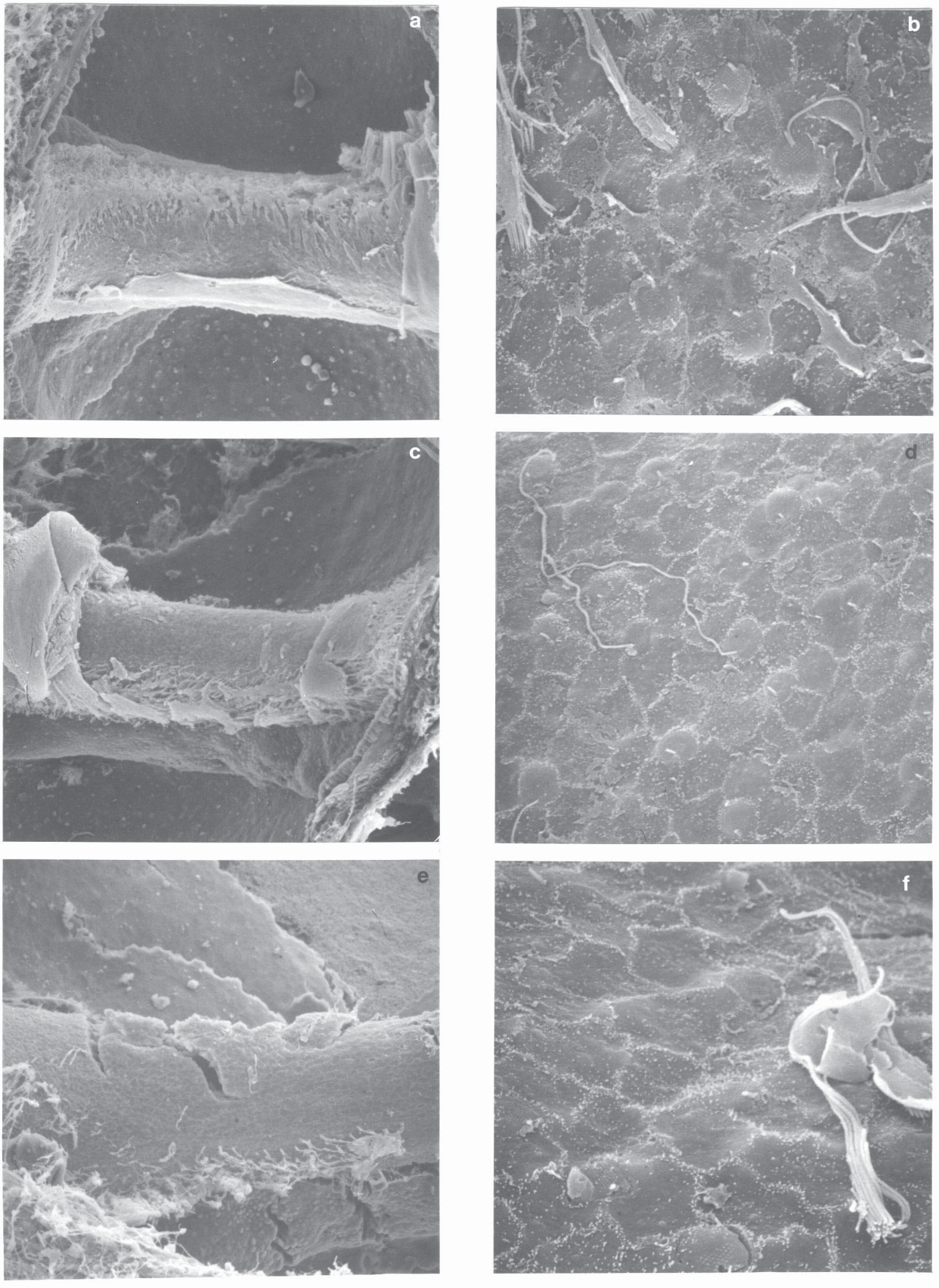
Animal 311 received a single injection of a 1:4 dilution of gentamicin and was sacrificed 7 days post injection. The ipsilateral ear demonstrates a complete absence of cochlear hair cells (Fig. 1). Moderate to severe damage was seen in the vestibular crista with moderate damage in the utricle. The contralateral ear shows mild vestibular damage to the posterior crista. The lateral and anterior crista and the utricle appear normal. The cochlea appeared normal throughout its length (Fig. 2c).

Animal 313 received a single injection a 1:10 dilution of gentamicin and was sacrificed 7 days post injection. The injected ear demonstrated a complete absence of outer hair cells in the basal and hook regions, inner hair cells were present. Some outer hair cells were present in the upper middle turn and approximately 60% of outer hair cells were present in the apical turn. Moderate to severe vestibular damage was noticed. The posterior crista was most severely damaged. The lateral crista exhibited moderate damage and the anterior crista, mild damage. Moderate damage was also noted in the utricle (Fig. 5). The right (contralateral) ear demonstrated moderate to severe damage in the hook region of its cochlea (Fig. 2d). The middle and the apical turns appear normal. The posterior crista suffered mild to moderate damage; other vestibular apparatus appeared normal.

Figure 3 (*on facing page*). Severe vestibulotoxicity resulted from gentamicin instillation, the peripheral areas of the neuroepithelium remained intact in all specimens. Crista ampularis following 2 injections of a 1:2 dilution of 26.7 mg/ml gentamicin; (**a**, **b**) anterior, (**c**, **d**) lateral, and (**e**, **f**) posterior crista. High magnification photomicrographs are from the central region of the crista. Photo width = (**a**, **c, e**) 500 µm, (**b**) 30 µm, (**d**) 40 µm, (**f**) 25 µm.

Discussion

The use of ototoxic antibiotics in the treatment of unilateral Meniere's disease has been driven by a desire to conserve hearing. It is to this end that titrated regimes of gentamicin installation have been proposed (Magnusson and Padoan, 1991; Nedzelski *et al*., 1992; 1993). Clinically, a number of different regimes for gentamicin intratympanic treatment have been suggested. These range from multiple daily injections to weekly application. The clinical success in controlling vertigo resulting from Meniere's disease is approximately 90%. Severe hearing loss resulting directly from gentamicin treatment occurs in about 15% of the treated population (Nedzelski *et al*., 1993). In the present study, severe cochlear damage occurred in our animal model after a single gentamicin treatment despite a reduction to a 1:10 dosage. Whilst this result is at odds with the majority of clinical experience, it is not unheard of. Bagger-Sjöbäck *et al*. (1990), in a clinical report of two patients, indicated gentamicin treatment resulted in the complete destruction of the organ of Corti while vestibular sensory cells (showing signs of degeneration) were retained. In both these cases gentamicin treatment proved ineffective and surgical ablation of the vestibular apparatus was ultimately carried out. In animal studies Norris *et al*. (1990), have reported near total vestibular and cochlear sensory cell damage in two cats treated with topical gentamicin. Kimura *et al*. (1988) report that 15/15 guinea pigs receiving middle ear injections of gentamicin had sensory cell lesions in all turns, while only 12/15 showed lesions in all three crista. After reducing gentamicin uptake by covering the round window with fat, cochlear damage was noted in 22/23 animals while 3/23 had lesions in all three crista (only 6/23 had damage in at least one crista).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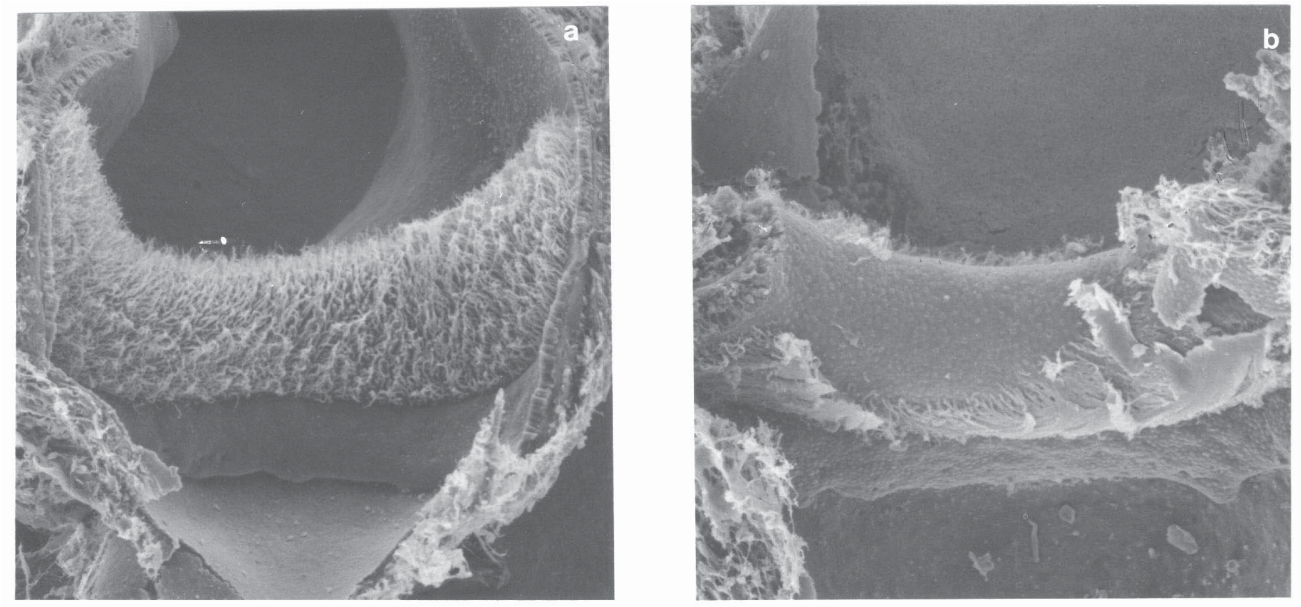

Figure 4. The contralateral vestibular organs suffered varying degrees of damage which was unrelated to the dosage instilled in the opposite ear. Illustrated are anterior crista ampularis from the contralateral ear of animals receiving, (**a**) 3 X 1:2 gentamicin (compare to Fig. 2a) and (**b**) 2 X 1:2 gentamicin (compare to Fig. 2b). Photo width = (**a**, **b**) 500 µm.

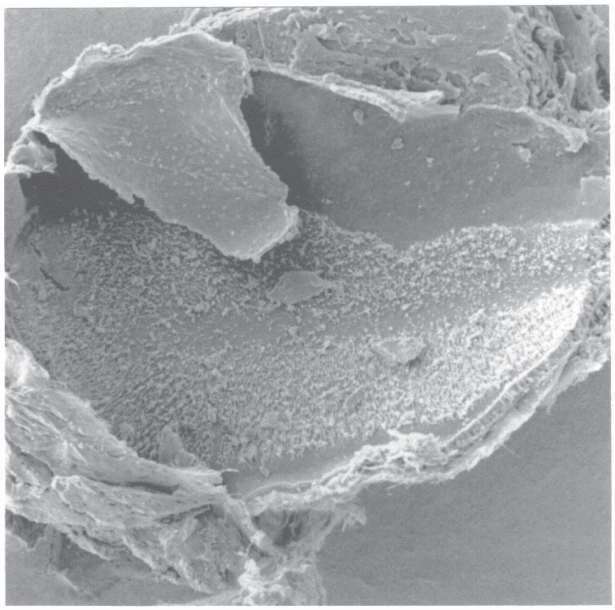

Figure 5. The striola region of the utricle was damaged at even the lowest dosage (1 X 1:10 gentamicin), no contralateral utricular damage was noted at any dosage. Picture width = 1 mm.

Further manipulation of the dosage and delivery regime in chinchillas will be necessary to determine if there is a scheme in which vestibular function is affected without cochlear hair cell destruction. However, our preliminary data suggest that the chinchilla is not a good model for the human gentamicin treatment. Whilst we have explored a range of dosage regimes, we have yet to observe an animal in which there is more vestibular damage than cochlear damage (at least as indicated at the SEM level).

It is largely an assumption (with the lack of post-mortum data) that in humans this therapeutic use of gentamicin is differentially affecting the vestibule and cochlea. There is a scarcity of human ototoxicity data for high frequency (>8 kHz) regions of the cochlea (Fausti *et al*., 1984). If the vestibular selectivity assumption is correct, then the chinchilla differs considerably. Such species variance may relate to differing physical characteristics of the middle ear, cochlear windows and inner ear fluid flow. Species specific biological factors may render one type of hair cell more vulnerable than another. It may be that the therapeutic effects of gentamicin in Meniere's disease does not relate to actual damage to the vestibular epithelium. Odkvist (1988), indicates that the beneficial effects of gentamicin in the treatment of Meniere's disease can be realized without loss of vestibular function. Aminoglycosides are also reported to damage the dark cells, responsible for endolymph production prior to damaging the vestibular and cochlear sensory cells (Beck and Schmidt, 1978; Pender, 1985). In normal animals this phase may pass unnoticed whilst in patients with Meniere's disease it may provide relief from vertigo without sensory cell destruction. Kimura *et al*. (1991) have shown, in guinea pigs, that both gentamicin and streptomycin are more ototoxic in hydropic

ears than normal ears. In the present study pathological changes to the dark cells of the utricle (such as those, for example, recently reported by Ge and Shea, 1995) were not found, neither were changes to the dark cells of the crista.

The most interesting finding in this study is the presence of the contralateral damage resulting from middle ear instillation of gentamicin. To our knowledge there is no other reported instances where middle ear instillation of ototoxic agents causes contralateral damage. Wright and Meyerhoff (1984) instilled Cortisporin into chinchillas and found no contralateral damage. In a supplementary experiment to the present study, two chinchillas were instilled with streptomycin unilaterally; no contralateral damage was found. There is a lack of consistency to the pattern of vestibular and cochlear damage between animals. However, all animals, whether receiving a 1:2 dosage of gentamicin in 3 treatments or a 1:10 dosage of gentamicin in a single treatment showed damage to the contralateral vestibular and/or cochlear epithelium.

There are three possible routes for gentamicin to affect the contralateral ear: absorption into the blood stream, absorption into the cerebral spinal fluid and direct communication between the bullae or endolymphatic ducts. Odkvist (1988) reported in patients receiving up to 11 daily dosages of 40 mg/ml gentamicin instillation that no trace of antibiotic was detected in blood serum. We undertook a quick study to investigate direct communication by instilling a dye solution into the bulla of a normal animal. After 30 hours, the animal was sacrificed and the skull carefully dissected. The ipsilateral cochlear and vestibular passages were dyed, as was the endolymphatic duct. There was no indication of dye in the contralateral ear, nor was there any evidence of dye transport away from the ipsilateral middle and inner ear. Whilst no analysis of cerebrospinal fluid (CSF) was performed, it has previously been reported that gentamicin injected into cranial CSF results in ototoxicity (Parrish *et al*., 1981). That study indicated a lack of relationship between dosage and degree of ototoxicity (0.5 mg - 0% ototoxicity; 2 mg - 50%; 4 mg - 100%; 6 mg - 0%), similar to the contralateral findings in the present study.

Gentamicin uptake into the inner ear is primarily through the round window membrane (Nomura, 1984). Diffusion from the perilymph into cochlear compartments and intracellular accumulation then occurs over the course of several days (Hiel *et al*., 1992). The degree of ototoxicity seen in the present study is significantly higher than that which would be expected based on clinical experience. However, the presence of severe contralateral damage in our animal model indicates the need for bilateral monitoring of hearing and vestibular function in clinical applications. Such monitoring regimes may include behavioural audiograms, the use of otoacoustic emissions and/or electrophysiological measures of cochlear and vestibular function. In any case, attention should be paid to the fact that aminoglycosides are known to accumulate slowly in inner ear tissues, the toxic effects having a delayed onset and continuing for several days after treatment has ceased (Hiel *et al*., 1993).

References

Bagger-Sjöbäck D, Bergenius J, Lundberg AM (1990) Inner ear effects of topical gentamicin treatment in patient with Meniere's disease. Am J Otology **11**: 406-410.

Beck C, Schmidt CL (1978) Ten years of experience with intratympanic applied streptomycin (gentamicin) in the therapy of Mobius Meniere. Arch Otolaryngol **221**: 149-152.

Fausti SA, Rappaport BZ, Schechter MA, Frey RH, Ward TT, Brummett RE (1984) Detection of aminoglygoside ototoxicity by high-frequency auditory evaluation. Am J Otolaryngol **5**: 177-182.

Fisch U, Mattox D (1988) Microsurgery of the Skull Base. Georg Thieme Verlag, Stuttgart, Germany. pp. 460-461.

Ge X, Shea JJ (1995) Scanning electron microscopic observations of dark cells after streptomycin perfusion of the vestibule in guinea pigs. Scanning Microsc **9**: 283-288.

Hiel H, Bennani H, Erre JP, Aurousseau C, Aran JM (1992) Kinetics of gentamicin in cochlear hair cells after chronic treatment. Acta Otolaryngol (Stockh) **112**: 272-277.

Hiel H, Erre JP, Aurousseau C, Bouali R, Dulon D, Aran JM (1993) Gentamicin uptake by cochlear hair cells precedes hearing impairment during chronic treatment. Audiology. **32**: 78-87.

Kemink JL, Telian SA, Grahm MD, Joynt L (1989) Transmastoid labyrinthectomy: reliable surgical management of vertigo. Otolaryngol-H and N Surg **101**: 5-10.

Kimura RS, Iverson NA, Southard RE (1988) Selective lesions of the vestibular labyrinth. Ann Otol Rhinol Laryngol **97**: 577-584.

Kimura RS, Lee K-S, Nye CL, Trehey JA (1991) Effects of systemic and lateral semicircular canal administration of aminoglycosides on normal and hydropic inner ears. Acta Otolaryngol (Stockh) **111**: 1021-1030.

Lange G (1981) Transtympanic treatment for Meniere's disease with gentamicin sulfate. In: Meniere's Disease. Pathogenesis, Diagnosis, and Treatment. Vosteen KH, Schuknecht HFS, Pfaltz CR et al. (eds). Georg Thieme Verlag, Düsseldorf, Germany. pp. 208-215.

Levine SG, Glasscock ME, McKennan KX (1990) Long term results after labyrinthectomy. Laryngoscope **100**: 125-127.

Magnusson M, Padoan S (1991) Delayed onset of ototoxic effects of gentamicin in treatment of Meniere's disease: Rationale for extremely low dose therapy. Acta

Otolaryngol (Stockh) **111**: 671-676.

McElveen JT, Shelton C, Hitzelberger WE, Brackmann DE (1988) Retrolabyrinthine vestibular neurectomy: A reevaluation. Laryngoscope **98**: 502-506.

Nedzelski JM, Bryce GE, Pfleiderer AG (1992) Treatment of Meniere's disease with topical gentamicin: a preliminary report. J Otolaryngol **21**: 95-101.

Nedzelski JM, Chiong C, Fradet G, Schessel DA, Bryce GE, Pfleiderer AG (1993) Intratympanic gentamicin instillation as treatment of unilateral Meniere's disease: Update of an ongoing study. Am J Otol **14**: 278-282.

Nomura Y (1984) Otological significance of the round window. Advances in ORL. S Karger, Basel. **33**: 1-162.

Norris CH, Amedee RG, Risey JA, Shea JJ (1990) Selective chemical vestibulectomy. Am J Otol **11**: 395-400.

Norris CH, Aubert A, Shea JJ (1994) Comparison of cochleotoxicity and vestibulotoxicity of streptomycin and gentamicin. In: Proceedings of the Third International Symposium on Meniere's Disease. Filipo R, Barbara M (eds). Kugler, New York. pp. 485-490.

Odkvist LM (1988) Middle ear ototoxic treatment of inner ear disease. Acta Otolaryngol (Stockh) Suppl **457**: 83-86.

Parrish KL, Tachibana M, Domer FR, Norris CH, Guth PS (1981) Ototoxicity of intracisternal gentamicin in cats. Ann Otol Rhinol Otolaryngol. **90**: 255-260.

Pender DJ (1985) Gentamicin tympanoclysis: Effects on the vestibular secretory cells. Am J Otolaryngol **6**: 359-367.

Schuknecht HF (1957) Ablation therapy in the management of Meniere's disease. Acta Otolaryngol (Stockh) Suppl **132**: 1-42.

Silverstein H, Norrel H, Rosenberg S (1990) The resurrection of vestibular neurectomy: A ten year experience with 115 cases. J Neurosurg **72**: 533-539.

Wright CG, Meyerhoff WL (1984) Ototoxicity of otic drops applied to the middle ear in the chinchilla. Am J Otolaryngol **5**: 166-176.

Discussion with Reviewers

X. Ge: Usually ototoxicity has a positive correlation with the dosage. The authors mentioned that the degree of damage did not directly relate to a dosage of gentamicin received. Would the authors give an explanation?
Authors: While it is true that there is usually a direct correlation between dosage and ototoxicity, this is not always the case in middle ear instillation of ototoxic agents. For example, in the paper by Wright and Meyerhoff (1984), Cortisporin was instilled into the bulla of chinchilla and it was found that the severity of damage in inner ear did not depend on the dosage. Additionally, Kimura *et al*. (1988), found that in guinea pigs receiving 5 to 8 equal injections of gentamicin into the middle ear, there was only a slight difference in the extent of damage among vary dosages. In the present study, we found a positive correlation in the ipsilateral ear whilst the damage in the contralateral ear show no correlation to dosage at all.

C.H. Norris: I understand the need for using gentamicin in these experiments since this is most often used in clinical therapy. However, the first used aminoglycoside was streptomycin and there are definite differences in the relative effects of these two substances. Do you intend to extend this study to include other members of aminoglycoside family?
Authors: We are well aware of the controversy. Whilst gentamicin is favored, some groups report no difference between gentamicin and streptomycin (Kimura *et al*., 1991) and others reported streptomycin is more beneficial (Norris *et al*., 1994). We have no plans to continue the study further.

C.H. Norris: There are reports in the literature that vestibular hair cells regenerate after aminoglycoside toxicity. Do you have any plans for using longer survival times prior to sacrificing the animals?
Authors: We did not note any evidence of vestibular hair cell regeneration. It has been reported in chinchillas with hair cell loss (but no supporting cell damage) at 4-8 week post-treatment period and beyond. However, our longest surviving animal was 5 weeks post-treatment. At present, we have no plans for longer survival times.

C.H. Norris: Since you have observed such extensive damage in the chinchilla, maybe this is an incorrect animal model of the human situation. Do you have plans for another animal model?
Authors: We agree that the results seen in this study indicate that the chinchilla is an incorrect animal model of the human situation. We have no plans to investigate a further animal model.

A. Campos: Under normal conditions, there is some degree of background damage in the inner ear sensory cell observed with SEM. How do you interpret your findings in light of this?
Authors: In the normal inner ear, there may occasionally be missing or damaged sensory cells. Within the cochlea, the apical region often shows a degree of disarrangement of stereocillia in the normal animal. In more basal regions, one will occasionally find individual missing or damaged hair cells. Likewise, in the vestibular organ occasionally there are damaged or missing hair cells evident in normal preparations. The degree of damage indicated in this report far exceeds anything which is seen in normal inner ear structure.

A. Campos: Ethanol can lead to the appearance of precipitate in the specimen. What has your experience been?
Authors: We have compared results obtained drying cochlear specimens from ethanol, acetone and amyl acetate and found no noticeable differences. Our only experience in finding participate on critical point dried specimen resulted from the presence of powdered molecular sieve in the final ethanol.

J.-M. Aran: The authors should also consider participation the efferent system which has been demonstrated to be transiently affected after a single injection of gentamicin. Then the contralateral ear could be more sensitive to noise during some time after the ipsilateral application of gentamicin. Since the chinchilla is very susceptible to acoustic damage, that could be a possibility.
Authors: The reviewer raises a very interesting point which is important to keep in mind where assessing inner ear pathology resulting from gentamicin application. However, to the best of our knowledge, the animals in this study were not exposed to any acoustic insult after treatment. The contralateral damage observed in the present study exists both within the cochlea and within the vestibular organs. It is highly unlikely that efferent impairment would lead to contralateral vestibular damage as there is very limited possibility of vestibular trauma.