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Preclinical and Clinical Studies of Unrelieved Aural Fullness following Intratympanic Gentamicin Injection in Patients with Intractable Ménière's Disease

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Abstract

Objective—To clarify whether gentamicin affects vestibular dark cells in guinea pigs and relieves patients of aural fullness with intractable Ménière's disease following intratympanic administration.

Materials and Methods—Purified gentamicin-Texas Red (GTTR) was injected intratympanically in guinea pigs that were sacrificed at 1, 3, 7, 14 and 28 days. GTTR uptake was examined in hair cells, and transitional cells and dark cells in vestibular end-organs were examined. Specific attention was paid to its distribution in dark cells under confocal microscopy, and the ultrastructure of dark cells using electron microscopy, following intratympanic injection.

Results—Dark cells in the semicircular canals showed weak GTTR uptake at 1, 3, 7, 14 and 28 days after intratympanic injection, with no significant differences at various time points after injection. However, the adjacent transitional cells demonstrated intense GTTR uptake that was retained for at least 28 days. Ultrastructural studies demonstrated negligible characteristics associated with apoptosis or necrosis in these dark cells. The tight junctions between dark cells showed no signs of disruption at 7 or 28 days after injection.

Conclusion—Intratympanic gentamicin has little direct impact on vestibular dark cells.

Clinical Application—A modified low-dose titration intratympanic approach was used in 29 patients with intractable vertigo and the clinical outcomes were followed. Aural fullness following intratympanic gentamicin injection was not relieved based on our subjective scales, demonstrated by no statistically significant difference between preinjection (4.16 ± 3.08) and postinjection (3.58 ± 2.93 ; $p > 0.05$) aural fullness scores. Vertigo control was achieved in 88% of patients, with hearing deterioration identified in 16% of patients. Intratympanic gentamicin administration might not lead to relief of aural fullness in patients with intractable vertigo, although it can achieve a high vertigo control rate with some cochleotoxicity.

Keywords

Gentamicin; Intratympanic injection; Dark cells; Ménière's disease; Aural fullness

Introduction

Ménière's disease (MD) is characterized by episodic vertigo, fluctuating hearing loss, tinnitus and aural fullness. The symptoms of vertigo in 5–10% of MD patients cannot be controlled through medical treatment. For these patients with intractable MD, surgical interventions such as labyrinthectomy and vestibular neurectomy were previously frequently recommended [Gacek and Gacek, 1996]. In recent years, chemical labyrinthectomy with gentamicin have become more popular due to its effective vertigo control while minimizing surgical complications and preserving residual hearing function [Zhai et al., 2010]. Cohen-Kerem et al. [2004] concluded in a meta-analysis that complete vertigo control was achieved in 74.7% of patients, with complete and substantial vertigo control obtained in 92.7% of patients.

Despite its apparent clinical efficacy in vertigo control, it remains uncertain whether intratympanic gentamicin administration could reduce endolymphatic hydrops, relieving aural fullness in patients with MD. Endolymphatic hydrops causes distortion or even eruption of the membranous labyrinth, and is thought to be the basis of the four fundamental characteristic complaints of MD: aural fullness, hearing loss, tinnitus and vertigo [Minor et al., 2004]. Yen et al. [1995] demonstrated that symptoms of fullness were completely controlled in 22 out of 24 patients (92%) after endolymphatic sac surgery, suggesting that aural fullness is correlated with endolymphatic hydrops.

Dark cells are specialized nonsensory epithelial cells involved in the regulation of vestibular endolymph composition [Pitovski and Kerr, 2002; Wangemann, 2002]. It has been suggested that destruction of vestibular dark cells could improve clinical outcomes in patients with MD [Harner et al., 1998]. Previous studies on the effects of gentamicin on dark cells remain unclear. Roehm et al. [2007], using autoradiography of tritiated gentamicin, reported that utricular dark cells rapidly took up gentamicin following intratympanic injection and retained it within the cell body while staining levels fell to background levels in the rest of the injected ear over 14 days [Roehm et al., 2007]. Pender [1985] observed dark cell death in the vestibular end-organs following gentamicin tympanolysis. On the contrary, Cureoglu et al. [2003] reviewed histopathologically human temporal bones of patients who had received aminoglycoside treatment of various durations, and did not observe significant changes in the number of vestibular dark cells, possibly because drug treatment duration was too short. Furthermore, a recent study revealed no evidence of reduced endolymphatic hydrops following intratympanic gentamicin treatment while using a 3-dimensional fluid-attenuated inversion recovery (3D-FLAIR) sequence in 3-tesla (3 T) MRI units to evaluate endolymphatic hydrops [Fiorino et al., 2012]. In addition, another study measured summing potential/action potential (SP/AP) ratios by noninvasive electrocochleography, which is associated with the degree of endolymphatic hydrops [Yamamoto et al., 2010], and reported that endolymphatic hydrops did not improve statistically even as vertigo was well-controlled following intratympanic gentamicin administration [Büki et al., 2011].

Wang and Steyger [2009] demonstrated that the distribution of gentamicin-Texas Red (GTTR) is closely related to the distribution of immunolabeled or tritiated gentamicin and that the cochlear distribution and serum pharmacokinetics of systemic GTTR uptake is similar, albeit slower due to its larger molecular size, thus making GTTR a valid probe to

assess the cellular distribution of gentamicin with great sensitivity using confocal microscopy.

The goal of this study was to clarify whether intratympanic gentamicin has direct impact on vestibular dark cells, and alleviates endolymphatic hydrops and the associated aural fullness in patients with intractable vertigo. We applied GTTR intratympanically in guinea pigs and assessed the distribution of GTTR in ampullar dark cells. In addition, we examined the ultrastructure of ampullar dark cells following intratympanic gentamicin injection in guinea pigs using electron microscopy. Meanwhile, patients with intractable vertigo were treated with a modified low-dose titration gentamicin dosing technique [Zhai et al., 2010]. Patients were also asked to report the severity of aural fullness using a visual analog scale before the first intratympanic gentamicin injection and within 6 months after the last intratympanic gentamicin injection.

Materials and Methods

Guinea Pig Studies

Agent Preparation—GTTR was prepared and purified as described previously [Li et al., 2011]. The stock GTTR solution was diluted for administration with sterile phosphate-buffered saline (PBS; define, pH 7.4).

Animals—Adult male and female albino guinea pigs weighing 250–300 g with normal Preyer's reflex were divided into 3 groups. Group 1: guinea pigs received 50 μ l of GTTR (0.1 mg/ml) via intratympanic injection of the left ear, and inner ear tissues were collected on days 1, 3, 7, 14 and 28, with 5 animals for each time point. Group 2: guinea pigs were injected intratympanically in the left ear with 50 μ l of gentamicin (30 mg/ml), and inner ear tissues were collected 7 and 28 days later, with 3 animals for each time point. Group 3 (control): 3 guinea pigs received intratympanic injections of 50 μ l of Texas Red (0.065 mg/ml), and 3 additional guinea pigs received 50 μ l of PBS, and inner ear tissues were collected 7 days later. All animals were provided by the Animal Care Centre of the Eye, Ear, Nose and Throat Hospital, Fudan University, and this study was approved by the Committee on Care and Use of Animals of Fudan University. All procedures were performed using accepted veterinary standards.

Intratympanic Injections and Tissue Collection—Guinea pigs were anesthetized using intramuscular ketamine (40 mg/kg) and xylazine (8 mg/kg), and the external auditory canal was sterilized with 75% ethanol solution. Animals were placed on their right side with their left ears turned toward the operating microscope. Fifty microliters of solution (GTTR, Texas Red, gentamicin or saline) was slowly injected into the tympanic cavity through a hole in the posterior part of the tympanic membrane after puncturing the anterior superior tympanic membrane using a 100- μ l micro-syringe. The animal's head remained stationary with the treated ear and nose turned towards the ceiling so as to bathe the round window niche in solution for at least 30 min.

At each time point for tissue collection, guinea pigs were again deeply anesthetized as above, the temporal bones excised and bullae opened. In groups 1 and 3, inner ears were perfused with 4% paraformaldehyde through small holes at the apex of cochlea and common crus, and then immersed in the same fixative solution overnight at 4°C. The tiny blood vessel was removed with Biemer scissors and vestibular end organs were exposed, excised and washed in PBS, and then dehydrated in graded sucrose solutions. Specimens were embedded in OCT (Leica RC-LDB-004) compound by cooling to approximately -70°C with dry ice, and transversely cut into 10- μ m thick sections using a cryostat (Leica CM3050S). In group 2, inner ears were perfused with 4% paraformaldehyde plus 0.25%

glutaraldehyde, and then immersion-fixed in 2.5% glutaraldehyde overnight before vestibular end organs were excised and further preserved in 0.1 M PBS.

Immunohistochemistry and Confocal Microscopy—In groups 1 and 3, sections were washed in 0.01 M PBS (pH = 7.2–7.4; 3×10 min). Sections were blocked for nonspecific immunoreactivity (10% goat serum in 0.2% Triton X-100) for 1 h at room temperature (20–25°C), then were incubated in mouse anti-calretinin monoclonal antibodies (1:250; Millipore) diluted in blocking solution for 24 h at 4°C, and secondarily labeled with Alexa Fluor-488-conjugated goat anti-mouse antibodies (1:200 in PBS containing 5% goat serum and 0.1% Triton X-100; Invitrogen, Calif., USA) for 2 h at room temperature. Subsequently, sections were incubated with 4',6-diamidino-2-phenylindole (DAPI, 1:500 in PBS; Invitrogen, Carlsbad, Calif., USA) for 20 min at room temperature to label nuclei. Between all labeling procedures, 3×10 min rinses in PBS were performed. Specimens were then mounted under coverslips on glass slides in a 1:1 mixture of 0.1 M PBS and glycerol. Control tissues were processed with the same protocol except using PBS to replace the primary antibody.

Specimens from the mid-portion of the cristae (4–7 sections per end organ) were scanned using a confocal laser scanning microscopy (TCS SP5, Leica, Wetzlar, Germany). Serial optical image slices (1.0 μ m) through the 8- μ m sections were obtained with identical parameters (laser power, gain and pinhole). The GTTR intensity was measured with a grayscale value (0–255 arbitrary fluorescent units). All images were prepared for publication using Photo-shop.

Transmission Electron Microscopy—After washing in 0.1 M PBS, vestibular tissues in group 2 were treated with 1% osmium tetroxide in 0.1 M PBS, pH of 7.4, for 1 h, and then were rinsed in PBS and dehydrated through a graded alcohol series (from 70° proof to absolute alcohol) at 4°C. The tissues were embedded with resin (No. 618 Shanghai Resin Factory). Ultrathin sections (60 nm) were prepared using a LKB-1 ultramicrotome (LKB Company), contrasted with uranyl acetate and lead citrate, and examined under a Philips CM120 electron microscope at 60 kV.

Statistics—General linear model analyses of variance were performed on these outcomes to determine the significance of the input variables of treatment, animal, canal and cell type. Variables identified as significant were further analyzed through post hoc ANOVA and t tests.

Clinical Studies

Patient Selection—This study was carried out in the Otolaryngology department of the Eye, Ear, Nose and Throat Hospital of Fudan University. Twenty-nine patients diagnosed with definite unilateral intractable MD were enrolled from January 2008 to November 2010. The diagnosis of MD was made according to the American Academy of Otolaryngology Head and Neck Surgery Diagnostic Criteria [Committee on Hearing and Equilibrium, 1995]. The details of vertigo attacks and concomitant symptoms (tinnitus, hearing loss and aural fullness) of all patients were recorded. Physical examination revealed normal ear canal and ear drum. Pure tone audiogram (PTA) and videonystagmography were detected in all patients. Hearing threshold was detected according to 0.5, 1, 2 and 3 kHz frequency average. Aural fullness was assessed through a 1–10 visual analog scale regarding the presence/absence and annoyance level of aural fullness (fig. 1). The criteria for patient inclusion into the present study were as follows: (1) each patient had already received medical treatment such as a low-salt diet, β -histine or diuretics for at least 1 year without any improvement of

vertigo; (2) the hearing and vestibular function (caloric test) of the contralateral unaffected ear in all patients were normal, and (3) no central nervous system abnormality was detected.

Clinical Intratympanic Gentamicin Injection—This gentamicin solution and dose schedule was applied routinely in our institution as described previously [Zhai et al., 2010]. Briefly, gentamicin sulfate (40 mg/ml) was buffered with 8.4% sodium bicarbonate to pH 6.4 to reach a final concentration of 30 mg/ml. Under the microscope, the patients were kept in a supine position with their heads turned 45 degrees away from the affected ear. Topical anesthesia with 1% Dicaine was administered in the external auditory canal. Approximately 0.5 ml of solution was delivered slowly to the middle ear cavity with a 26-gauge syringe through the inferior posterior tympanic membrane, while the air bubble in the middle ear cavity was driven out via a tiny hole pierced through the anterior superior tympanic membrane. After the injection, the patient remained in position for at least 30 min to allow for pooling of the solution over the round window. The patient was instructed to avoid swallowing or speaking to prevent solution drainage through the Eustachian tube. Patients were followed up at 3 weeks postinjection to determine whether additional injections were needed based on the assessment of ototoxicity to the vestibule and cochlea. Additional injections were judged to be unnecessary if signs of vestibular hypofunction appeared (i.e. spontaneous nystagmus and positive head shake test or head thrust test) or obvious symptom relief was obtained. In addition, a second injection was not given if the hearing threshold (by PTA) increased by 10 dB.

Follow-Up—All the 29 patients were followed up according to physician's instructions. Patients were generally assessed at 3 weeks, 3 months, 6 months and 2 years after injection. At every follow-up visit, evaluation included a detailed assessment of patient symptoms as well as a complete head and neck examination, PTA results, nystagmus (spontaneous nystagmus, head shake and head thrust tests), and the assessment of aural fullness (within 6 months; the measurement was the same as at preinjection).

Statistics—A number of statistical procedures were used for data analysis. Statistical study was performed using the Stata 10.0 software program. Paired statistical analysis was performed with paired t tests. When analyzing the statistics of male patients, we used Wilcoxon matched-pairs signed-rank test as a result of abnormal distribution. A p value <0.05 was considered statistically significant.

Results

Guinea Pig Studies

Distribution of GTTR in Vestibular End-Organs after a Single Intratympanic Injection—The regions of dark cells in semicircular canals were identified by melanin granules generated by the melanocytes underlying the dark cells (fig. 2a). Central zone type I hair cells were indicated by the epithelial region predominately immunolabeled with calretinin (fig. 2c). The dark cell area was negative for calretinin immunolabeling. After a single intratympanic GTTR (0.1 mg/ml) injection, the most intense GTTR fluorescence was present in transitional cells, with obvious GTTR fluorescence identified in the sensory epithelium (including hair cells) of the cristae. However, comparatively little GTTR fluorescence was observed in the dark cell area (fig. 2d).

In the semicircular canals, GTTR fluorescence in dark cells or nonsensory epithelial cells was weak on all days examined (1, 3, 7, 14 and 28 days; fig. 3) following a single intratympanic injection. Statistical analysis showed no significant difference between the various time points. However, more intense fluorescence was observed in the transitional

cells, particularly in their apical cytoplasm close to the luminal surface of these cells (fig. 3). In addition, GTTR fluorescence was obvious in type I hair cells, and type II hair cells in the central zone of the semicircular canal. A distinct time course of GTTR fluorescence was present in ampullar hair cells: GTTR intensity was weak at 24 h, increasing in intensity to peak at 3–7 days before declining in fluorescence over the remaining 3 weeks (fig. 3).

In addition, 7 days after intratympanic injection of gentamicin (30 mg/ml) in guinea pigs, negligible characteristic apoptotic or necrotic morphologies were observed in the dark cells of the semicircular canals. The ultrastructure of mitochondria, cytoplasm and nuclei all appeared intact. Similar results were also observed after 28 days. The tight junctions between dark cells were normal with no signs of cellular degeneration at these two time points (fig. 4).

Clinical Studies

A total of 29 patients with intractable vertigo were treated with intratympanic gentamicin administration (30 mg/ml). Twenty-five patients completed the 2-year follow-up, while 4 patients were followed for only 6 months, and thus excluded from statistical analysis. Among these 25 patients, 23 cases were diagnosed with MD, while 2 were diagnosed with delayed endolymphatic hydrops. There were 13 women and 12 men, with an age range of 18–67 years (mean 47.3 years). Fourteen patients were affected in the right ear, and 11 had left ear involvement. The mean duration of disease was 11 years with a range of 0.5–30 years. Twenty patients (80%) received 1 injection, 3 patients (12%) had 2 injections and 2 patients (8%) underwent 3 injections.

Aural fullness was present in 21 patients (84%) before injection and 21 cases after injection. The average score was 4.16 ± 3.08 before injection and 3.58 ± 2.93 after injection (table 1), which presented no statistically significant difference ($p > 0.05$). We also compared aural fullness in the male and female patients, again finding no significant difference ($p > 0.05$; fig. 5). Eleven patients reported relief of aural fullness (44%) while 6 complained of deterioration (24%). The severity of aural fullness did not change in the remaining 8 patients (32%).

Vertigo control was achieved in 22 (88%) of 25 patients (assessed 2 years postinjection). Complete control of vertigo was obtained in 16 patients (64%; class A), good control in 4 patients (16%; class B) and moderate control in 2 patients (8%; class C). Vertigo recurrence was noted in 3 patients (12%); however, vertigo attacks were less frequent and less severe.

The posttreatment PTA threshold was 62.5 dB on average, compared with 60.0 dB before treatment. Only 4 patients (16%) complained of significant increase in hearing thresholds (>10 dB). Hearing function was stable in 19 patients (76%) and apparently improved hearing thresholds were identified in 2 patients (8%; over 10 dB hearing threshold decrease). Tinnitus was resolved in 1 patient (4%) and 10 patients (40%) self-reported relief of tinnitus; however, tinnitus remained in 14 patients (56%).

Discussion

The goal of clinical management of intractable unilateral MD has been to minimize the frequency and severity of vertigo attacks by ablating vestibular hair cells following intratympanic injection of gentamicin. It has been proven effective in clinical practice, and cochleotoxicity is not a major or frequent side effect [Cohen-Kerem et al., 2004]. Intratympanic injection of gentamicin may also be effective in controlling tinnitus and aural fullness to some extent [Sala, 1997; McFeely et al., 1998].

Although a few clinical studies demonstrated that aural fullness can be reduced following intratympanic gentamicin administration, the evidence was based only on subjective evaluation [Sala, 1997; McFeely et al., 1998], and animal studies have yielded incomplete data [Harner et al., 1998; Roehm et al., 2007]. Therefore, further data on clinical outcomes and basic scientific investigation are required to establish the effectiveness of using intratympanic gentamicin to relieve aural fullness.

In this study, we observed GTTR distributed predominantly in the characteristic morphological location of transitional cells adjacent to the dark cells and sensory epithelium of the semicircular canals following intratympanic administration. However, GTTR did not appear to be concentrated in dark cells or other nonsensory epithelial cells in the semicircular canals on days 1, 3, 7, 14 and 28 after intratympanic injection. Further study revealed there was no significant difference in GTTR labeling in the dark cells among different time points following intratympanic injection. In the present study, the GTTR labeling in the ampullar dark cells is consistent with the results of the study of Schmid et al. [2011], which used the acutely isolated inner ear of the rat that had been exposed to GTTR for 10 min, but is not consistent with the results of the study of Roehm et al. [2007], which observed substantial autoradiographic deposits for tritiated gentamicin in dark cells within the utricle.

We suppose the different results of gentamicin distribution in vestibular dark cells lies in these two different detection methods. Our technique cannot rule out that when intratympanic gentamicin is given at the clinically relevant dose (instead of GTTR) there may be greater uptake of gentamicin by dark cells. Roehm et al. [2007] suggest that by retaining gentamicin, dark cells may function to effectively filter vestibular fluids to impede the entry of a potential cationic toxin into hair cells. Whether gentamicin has toxic effects upon vestibular dark cells remains unknown.

To further clarify this, we examined the ultrastructure of dark cells after gentamicin administration intratympanically by electron microscopy. However, we observed normal dark cell densities without any characteristic apoptotic or necrotic morphologies, or other degenerative changes, which was consistent with previous temporal bone pathological outcomes [Cureoglu et al., 2003]. These ultrastructural findings, together with our GTTR distributions by confocal microscopy, suggest that dark cells do not appear to be affected morphologically following intratympanic gentamicin injection. Consequently, dark cell regulation of endolymph may also be unaffected, and thus endolymphatic hydrops are unlikely to be alleviated at the dose of gentamicin used in this study. However, we found the strongest GTTR fluorescence in transitional cells, which might change endolymph homeostasis. Transitional cells appear to provide a simple barrier to sustain the high concentration differences of K^+ and Na^+ between endolymph and perilymph, and actively absorb cations by cellular mechanisms homologous to co-chlear outer sulcus cells [Oudar et al., 1988; Lee et al., 2001]. Transitional cells may act as a pathway for GTTR trafficking from vestibular perilymph into endolymph or, alternatively, sequester GTTR from vestibular endolymph. However, little is known about the effect of gentamicin upon the function of transitional cells, which merits further study. This hypothesis is supported by the recent MRI study by Fiorino [2012] and electrocochleography results [Büki et al., 2011].

In the past few years, alternative measurements of endolymphatic hydrops have been tried in clinical practice, such as MRI scanning and electrocochleography [Büki et al., 2011; Fiorino et al., 2012]. A 3D-FLAIR sequence in 3 T MRI units, after intratympanic gadolinium administration had been used to visualize endolymphatic hydrops indirectly, relying on the imaging of the enlarged space occupied by nongadolinium-enhanced fluid space, reduced the area/volume of the gadolinium-enhanced perilymphatic compartments [Nakashima et al.,

2007; Naganawa et al., 2011]. Fiorino et al. [2011] observed that reduced or absence of enhancement of the vestibule occurred at an early stage in all subjects with MD, after which the cochlea was involved. The progression in the prevalence of enhancement defect in all co-chlear and vestibular sites was directly proportional to MD duration. The author found no evidence of reduced endolymphatic hydrops through a 3D-FLAIR MRI following intratympanic gentamicin treatment for 8 patients with definite MD, therefore coming to the conclusion that the therapeutic effect of intratympanic gentamicin is mediated by vestibular deafferentation, rather than relief of endolymphatic hydrops [Fiorino et al., 2012]. Although some aspects of MRI imaging of endolymphatic hydrops agree with the clinical manifestations of MD, such as the behavior of auditory threshold, it is not clear whether there is a direct cause-effect relationship, or if both are a consequence of more subtle biochemical and ultrastructural modifications. Further studies are necessary to clarify the sensitivity and specificity of this technique.

Electrocochleography has been clinically applied in the diagnosis of MD in some vestibular laboratories [Iseli and Gibson, 2010]. The most frequently used indicator for the detection of endolymphatic hydrops is the SP/AP amplitude ratio [Kumagami et al., 1982; Campbell et al., 1992; Sass, 1998; Shea and Ge, 1998; Ge and Shea, 2002]. Yamamoto et al. [2010] concluded that elevation of the SP/AP ratio is related to not only the degree of endolymphatic hydrops but also to the persistence of hydrops. Ferraro et al. [1999] and Devaiah et al. [2003] reported that the SP/AP area curve ratio in extratympanic electrocochleography significantly improved the diagnostic sensitivity for endolymphatic hydrops in comparison with conventional SP/AP amplitude ratio in MD [Ferraro and Tibbils, 1999; Devaiah et al., 2003]. Buki et al. [2011] proved that SP/AP ratios did not improve statistically even after patients had become symptom free, thus leading to the assumption that the beneficial effect of gentamicin seems more likely to depend on a reduction in the sensitivity of the vestibular periphery, rather than the improvement of endolymphatic hydrops. However, further studies evaluating the diagnostic sensitivity and specificity of the SP/AP area ratio together with the SP/AP amplitude area and other methods, such as vestibular-evoked myogenic potentials, the caloric test or the glycerol test, should still be conducted to increase the sensitivity of early diagnosis of MD and endolymphatic hydrops.

In our retrospective clinical analysis, intratympanic injection of gentamicin was effective in controlling vertigo (88%), with hearing deterioration identified in only 16% of patients. However, no statistical differences were noted in aural fullness postinjection compared to preinjection, although some subjects (44%) reported partial relief of aural fullness, in contrast to previous studies by McFeely et al. [1998] and Sala [1997] who found that aural fullness was significantly improved after gentamicin injections. Our clinical outcomes, together with these animal studies, suggest that intratympanic gentamicin administration is insufficient to relieve the symptoms of aural fullness.

Although not the main complaint of patients with MD, aural fullness can often be extremely distressing. One patient with intractable MD in the present study achieved complete vertigo control following 2 intratympanic gentamicin injections. However, this patient continued to complain of intolerable aural fullness. Only after endolymphatic sac surgery was the aural fullness resolved [Zhai et al., 2010]. Endolymphatic sac decompression appears to be effective in relieving aural fullness, but the rate of vertigo control is not as effective as intratympanic gentamicin administration [Brinson et al., 2007], and may have other complications due to general anesthesia [Sajjadi et al., 1998]. Well-designed clinical studies regarding the effectiveness of different treatments for relieving vertigo are still needed. In addition, dark cells may also be a potential target for the reduction of endolymphatic hydrops and thereby aural fullness, if these cells can be selectively targeted.

Conclusion

Vestibular dark cells had only weak uptake of fluorescent gentamicin over 28 days following intratympanic GTTR injection (compared to transitional cells), and no obvious morphological changes were observed in vestibular dark cells using electron microscopy. In addition, no statistically significant differences in aural fullness were demonstrated clinically between the postinjection group and the preinjection group, although some subjects reported partial relief of aural fullness. The animal study, combined with clinical follow-ups, suggests that intra-tympanic gentamicin administration does not directly affect dark cells and does not relieve aural fullness in patients with unilateral intractable MD.

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Fig. 1.
Aural fullness and the degree of annoyance were rated on a 10-point scale.

Fig. 2.

Identification of dark cells in the ampulla 3 days after a single intratympanic GTTR administration (0.1 mg GTTR). **a** The horizontal ampulla of a guinea pig. Arrows indicate the region of dark cells overlying the melanin granules visible in the melanocytes underlying the dark cells. **b** The same section as in **a** labeled with DAPI (blue; colors online only). Arrows indicate the region of dark cell epithelia. **c** The horizontal ampulla immunolabeled with calretinin; the epithelial region containing central zone type I hair cells is immunolabeled with calretinin (small arrows). The dark cell region had negligible calretinin immunolabeling (arrows). **d** The distribution of GTTR (red) in the horizontal ampulla. Intense GTTR fluorescence was present in transitional cells, with obvious GTTR fluorescence in the sensory epithelium (including hair cells). However, comparatively little GTTR was observed in the dark cell area (arrows). Scale bar = 40 μ m.

Fig. 3.

Time course of GTTR fluorescence in the horizontal ampulla. GTTR uptake in dark cell regions (arrows) did not differ significantly between the various time points after a single intra-tympanic GTTR injection (0.1 mg/ml). However, GTTR fluorescence (red; colors online only) in the sensory epithelium varied at different time points, with the greatest GTTR intensity observed 7 days following a single intratympanic GTTR injection. **a, a1** One day after GTTR administration. **b, b1** Seven days after GTTR injection. **c, c1** Twenty-eight days after GTTR administration. **d, d1** Control group (Texas Red only). In the lower row of panels calretinin immunolabeling (green) indicates the sensory epithelial region containing central zone type I hair cells (arrows). Scale bar = 20 μ m.

Fig. 4.

b Seven days after intratympanic injection of gentamicin (30 mg/ml) in albino guinea pigs, negligible characteristic apoptotic or necrotic morphologies were observed in the dark cells of horizontal semicircular canals. The ultrastructure of mitochondria (small arrow), and nuclei all appeared intact. **c** Similar results were also observed at 28 days. **b1, c1** The tight junctions (arrows) between dark cells displayed no signs of disruption or degeneration at these two time points. Control group (**a, a1**); 7 days after GTTR injection (**b, b1**); 28 days after GTTR administration (**c, c1**). Scale bar = 2 μ m (**a–c**); scale bar = 0.4 μ m (**a1–c1**).

Fig. 5. Average scores of aural fullness self-evaluated by patients with unilateral intractable MD before and after injection (mean $\pm$ SD). There was no significant difference between the preinjection ($n = 25$) and postinjection ($n = 25$) aural fullness scores ($p > 0.05$). We also compared results of male (M; $n = 12$) and female patients (F; $n = 13$), for which no significant difference was demonstrated either ($p > 0.05$).

Table 1

Subjective results of aural fullness

No.	Sex	Age, years	Diagnosis	Preinjection score	Postinjection score	Injections, n
1	M	58	MD	3	3	1
2	M	30	MD	6	3	2
3	M	59	MD	6	3	1
4	M	40	MD	6	1.5	1
5	M	18	DEH	0	0	1
6	M	53	MD	0	0	1
7	M	41	MD	7	3	1
8	M	41	MD	4	4	1
9	M	33	MD	8	5	1
10	M	45	MD	7	2	1
11	M	48	MD	3	5	3
12	M	57	MD	5	8	1
13	F	57	MD	10	10	1
14	F	48	MD	10	9	1
15	F	26	MD	6	3	2
16	F	62	DEH	2	8	3
17	F	43	MD	6	6	1
18	F	59	MD	0	0	1
19	F	67	MD	0	0.5	1
20	F	56	MD	1	0	1
21	F	41	MD	3	1.5	1
22	F	49	MD	2	3	1
23	F	47	MD	1	1	1
24	F	48	MD	6	4	1
25	F	57	MD	2	6	2
Mean±SD				4.16 ± 3.08	3.58 ± 2.93	1.28 ± 0.61

DEH = Delayed endolymphatic hydrops.