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Intracochlear Drug Delivery through the Oval Window in Fresh Cadaveric Human Temporal Bones

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Abstract

Hypothesis—Drug delivered to the oval window can diffuse to the apex of the human cochlea.

Background—We have previously demonstrated that zoledronate, a nitrogen-containing bisphosphonate, can arrest the sensorineural hearing loss in cochlear otosclerosis. We have also shown that, in animals, delivery of bisphosphonate into the cochlea can dramatically increase delivery efficiency. Intracochlear drug delivery has the potential to increase local concentration of drug while decreasing the risk of systemic toxicity. In the present study, a fluorescently labeled bisphosphonate compound (6-FAM-ZOL) was introduced into the human cochlea through the oval window and its distribution within the temporal bone was quantified.

Methods—In three fresh human temporal bones, we introduced 6-FAM-ZOL via the oval window. We compared these specimens to control specimens treated with artificial perilymph alone. Specimens were then processed, embedded into methyl methacrylate, and ground to the mid-modiolar axis. We quantified the fluorescence in confocal images.

Results—We found 6-FAM-ZOL to be distributed up to the apical cochlear turn. In specimens treated with 6-FAM-ZOL, we identified a strong baso-apical gradient of fluorescent signal along the lateral cochlear wall and the modiolus both in the scala vestibuli and in the scala tympani.

Conclusions—Bisphosphonate introduced via the oval window in the human cochlea can be delivered up to the apical cochlear turn. Inter-scalar communication is likely to play an important role in determining patterns of drug delivery in the inner ear.

Keywords

Bisphosphonates; Fluorescence imaging; Human inner ear; Inner ear drug delivery; Otosclerosis

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INTRODUCTION

Otosclerosis is a bone remodeling disorder that affects the otic capsule, inhibiting movement of the stapes, and typically presents as a conductive hearing loss (1). Larger otosclerotic lesions can involve the cochlea and may result in an overlying sensorineural hearing loss due to involvement of the cochlear endosteum and spiral ligament (2). The conductive hearing loss seen in otosclerosis can be addressed via amplification or stapedectomy surgery. Cochlear implantation can be considered for patients with far-advanced otosclerosis (3–5).

Bisphosphonates bind to bone minerals and inhibit osteoclast formation, migration, and activity (6,7) to treat a variety of metabolic bone diseases, most prominently osteoporosis. We have previously reported that zoledronate, a potent third-generation nitrogen-containing bisphosphonate, can arrest the progressive sensorineural hearing loss seen in cochlear otosclerosis when administered to patients (8). However, while rare, complications including osteonecrosis of the jaw, atypical femur fracture, atrial fibrillation, erosive esophagitis, and renal failure have been reported following the systemic administration of nitrogen-containing bisphosphonates at high dose (9). Although none of these complications were seen in our small study, a local delivery mechanism could avoid these systemic side effects while delivering a high local concentration of bisphosphonate to the inner ear. Thus, we recently compared the relative efficacy of systemic vs. local cochlear delivery of bisphosphonate, using a fluorescently labeled derivative of zoledronate (6-FAM-ZOL). When comparing these systemic, round window, and intracochlear delivery methods, we found intracochlear delivery to be the most efficient method, with 50-fold increased potency relative to systemic delivery. Critically, the systemic and the local delivery methods we studied delivered 6-FAM-ZOL without incurring drug-specific ototoxicity (10).

Our *in vivo* guinea pig experiments provided crucial functional information regarding our ability to deliver 6-FAM-ZOL locally to the cochlea in a non-ototoxic manner, and we therefore sought to better understand patterns of drug distribution following delivery to the human cochlea. 6-FAM-ZOL is an ideal probe in this regard, as its strong affinity for bone allows for the straightforward evaluation of the cumulative amount of drug delivered to various cochlear compartments. Cadaveric human temporal bones have previously been used in studies of drug delivery to the human inner ear (11–13), although none of these studies assessed the distribution of drug from base to apex following administration. With respect to cochlear otosclerosis, bisphosphonate administration in patients might be considered either via an intra-fenestral injection or through a drug-eluting stapes prosthesis. Importantly, such delivery methods might be used to improve the efficacy of inner ear delivery of other drugs, including steroids for sudden hearing loss or, in the future, regeneration factors for spiral ganglion neurons and hair cells. To better understand the extent of diffusion of substances placed within the oval window and the scala vestibuli, we studied the distribution of 6-FAM-ZOL in fresh cadaveric human temporal bones after introducing 6-FAM-ZOL through the oval window.

MATERIALS AND METHODS

1. Preparation of a fluorescently labeled bisphosphonate compound (6-FAM-ZOL)

6-FAM-ZOL was synthesized as described previously (14,15). 6-FAM-ZOL was dissolved in artificial perilymph (AP) at a concentration of 10 µg/µL. The content of AP was 120 mM NaCl, 3.5 mM KCl, 1.5 mM CaCl₂, 5.5 mM glucose, and 20 mM HEPES. The pH was adjusted to 7.5 using NaOH.

2. Delivery of 6-FAM-ZOL through the oval window

Six freshly harvested cadaveric human temporal bones, which were obtained within 24 hours after death by the human temporal bone histopathology lab at Massachusetts Eye and Ear Infirmary, were used in this study. The Pathology Quality Assurance Committee at Massachusetts General Hospital approved procurement of the specimens. The squamous and mastoid portions were drilled away, leaving the labyrinth. In three temporal bones, we injected 1 µL of 10 µg/µL 6-FAM-ZOL through the oval window using a micropipette after lifting the stapes footplate. The injection was performed very slowly and we did not pipette “up and down”. The footplate was carefully laid back down. The other three temporal bones were treated with AP alone as controls. All specimens were placed in a sealed, humidified plastic container and left in the dark at room temperature for 4 days.

In our previous study, we infused 1.25 µg of 6-FAM-ZOL over 40 minutes into the cochlea of guinea pigs without causing ototoxicity and damaging hearing (10). Since the inner ear fluid space is approximately 10 times larger in the human than in the guinea pig (204.5 µL Vs. 20.9 µL) (16,17), we decided to test ten times the amount of 6-FAM-ZOL (10 µg) in the present study.

3. Tissue processing

All subsequent steps were performed in covered vials to limit light exposure and prevent 6-FAM-ZOL bleaching. Specimens were fixed in an excess volume of neutral buffered formalin (10%, Fisher Scientific, Pittsburgh PA) at room temperature for one week, with one change of fixative at mid-week. They were then dehydrated in an ascending series of ethanol (Fisher Scientific, 2 days at 70%, 1 hour at 70%, 1 hour at 85%, 40 min at 95%, 80 min at 100%) followed by a wash in xylene (Fisher Scientific) and a 30 minute immersion in xylene. They were cleared of xylene by a one hour infiltration in methyl methacrylate (MMA, Acrylosin soft, Dorn and Hart, Villa Park, IL) under vacuum. The MMA solution was changed and specimens were infiltrated for 24 hours under vacuum. The infiltration solution was changed and the process was repeated for another 2 days. Specimens were embedded in a solution of MMA containing 0.25% w/v (2.5 g/L) of perkadox-16 (Dorn and Hart). Specimens were embedded for several hours under vacuum. After several hours, specimens were sealed in 50 ml polypropylene tubes and maintained at room temperature in a heat sink until fully cured (approximately 5 days). The hardened block was ground to a mid-modiolar section of the cochlea using a 12-inch table mounted rotary wheel.

4. Image collection and analysis

Images (1024 by 1024; 8 bit) were collected using a Leica TCS-SP2 confocal microscope to generate maximum fluorescence images. To identify the surface of each specimen, the point of maximum fluorescence in the z-axis was first identified. We then collected a stack of images to a depth of 500 μm from this point of maximum fluorescence. Images were obtained from control and experimental specimens in parallel at the same sitting under identical microscope settings. ImageJ (18) was used to quantify the amount of fluorescence associated with the labeled bisphosphonate within the lateral cochlear wall and the modiolus of the scala vestibuli and scala tympani at each half-turn (Fig. 1).

Mixed model analysis of variance was performed using the SPSS software package to analyze the effect of dose on fluorescent response in the cochlea. Null hypothesis were rejected at $p < 0.05$.

RESULTS

After application of 6-FAM-ZOL through the oval window, we observed strong fluorescent signal in the bony walls of the vestibular capsule (Fig. 2B) and the cochlea (Fig. 2D). In the cochlea, signal intensity was highest in the basal turn and decreased toward the apex. There was very low autofluorescence in specimens treated with artificial perilymph alone (Fig. 2A and 2C). We quantified the fluorescent signal associated with 6-FAM-ZOL within the lateral and modiolar walls of the scala vestibuli and the scala tympani in both treated and control specimens (see Fig. 1 for locale of analyzed regions). The bony walls of both the scala vestibuli and the scala tympani in specimens treated with 6-FAM-ZOL exhibited significant gradients of signal from base to apex of the cochlea, while those in the control specimens showed minimal fluorescence signal with no gradient (Fig. 3). For both the lateral cochlear wall and the modiolus, a significant effect of dose (6-FAM-ZOL vs artificial perilymph) was observed both in the scala vestibuli and the scala tympani (Fig. 3; all $p < 0.05$). There was no significant effect of scalae location (scala vestibuli vs scala tympani) when evaluating signal either in the lateral cochlear wall or in the modiolus.

DISCUSSION

We have used fresh, cadaveric human temporal bone specimens to evaluate the distribution of a fluorescently labeled bisphosphonate administered through the oval window. The high affinity of 6-FAM-ZOL for bone allows us to readily identify the cumulative extent of diffusion. We found that 6-FAM-ZOL labeled the lateral cochlear wall and the modiolus of both the scala vestibuli and the scala tympani to the apex when administered to the oval window, with a steep baso-apical gradient in both scalae. The autofluorescent background with our confocal approach was extremely low. While the number of fresh cadaveric samples in this study is small ($n=3$ each for treated and untreated), our results are statistically significant.

Our identification of a baso-apical gradient in both scalae upon administration to the oval window and scala vestibuli suggests that drug diffused “radially”, in which inter-scalar communication allows for direct diffusion from the higher concentration within the scala

vestibuli into the scala tympani. Such a model would predict the observed baso-apical gradient in both the scala vestibuli and the scala tympani. In fact, this observed baso-apical gradient in both the scala vestibuli and the scala tympani following delivery into the scala vestibuli alone is consistent with work previously demonstrating radial diffusion between the scalae (19–21).

6-FAM-ZOL readily labeled both the lateral cochlear wall and the osseous spiral lamina of the cochlea. In our present study, the levels of fluorescence seen in the lateral cochlear wall and the osseous spiral lamina following 6-FAM-ZOL administration appear to be similar, although our previous guinea pig experiments showed that the modiolus appeared to be more avid for 6-FAM-ZOL when 6-FAM-ZOL was delivered directly into the scala tympani (10). Together, the data suggest that bisphosphonates could be used to facilitate drug delivery throughout the cochlea, including the spiral ganglion neurons within the modiolus, through routes between perilymph spaces and the osseous spiral lamina (22,23).

Although fresh cadaveric human temporal bones have clear advantages relative to animal models with respect to study of diffusion through native human inner ear anatomy, dynamics within the sealed living human cochlea are likely to be complex and may confound efforts at description in a cadaveric system. In the guinea pig, cerebrospinal fluid (CSF) entry into the scala tympani through the cochlear aqueduct has been described to have a diluting effect on substances delivered through the round window. This dilution has been theorized to derive both from entry of CSF into the scala tympani and from loss of drug into the CSF space (24), with CSF entering at a very slow rate in a sealed, normal cochlea (24,25). This likely contributes to the slow, apically-directed flow within the normal guinea pig cochlea, measured to be approximately 1.6 nL/min (25). It is not known how the less patent cochlear aqueduct in the human might influence these dynamics, nor is it known what the rate of intracochlear perilymph flow is in the human cochlea. Regardless, any apically-directed flow of perilymph would likely increase drug levels in the apex when delivered via either the round or oval window, relative to what would be expected for simple diffusion. Notably, an ongoing human clinical trial for gene delivery to the cochlea involves infusion of up to 90 μ L through the oval window (26), although the non-human primate data underlying the safety of introducing this amount remains unpublished. Future experiments in our system may therefore employ higher infused amounts and correspondingly higher levels of drug delivery.

CONCLUSION

A fluorescently labeled bisphosphonate derivative diffuses up to the apical turn of fresh cadaveric human cochlea when delivered through the oval window. Inter-scalar communication between the perilymphatic scalae of the cochlea likely plays an important role in inner ear drug delivery. These findings hold particular relevance for future strategies of drug delivery to the human inner ear.

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Figure 1. Regions selected for measurement of zoledrenate-associated fluorescence in the lateral cochlear wall and the modiolus

Depicted is a schematic of the human cochlea taken at a mid-modiolar section. Areas with reticular lines (lateral cochlear wall contacting the scala vestibuli), black solid areas (lateral cochlear wall contacting the scala tympani), dotted areas (modiolus contacting the scala vestibuli), and vertical lines (modiolus contacting the scala tympani) at each half-turn were measured for fluorescence signals using the ImageJ program.

Figure 2. Human temporal bones following administration of fluorescently labeled zoledronate (6-FAM-ZOL)

Confocal images taken at mid-modiolar cochlear sections are shown for the vestibule of a cochlea treated with artificial perilymph alone (A), the vestibule of a cochlea treated with 6-FAM-ZOL (B), a cochlea treated with artificial perilymph alone (C), and a cochlea treated with 6-FAM-ZOL (D). The images are representative of three independent experiments. The asterisk indicates the stapes footplate, the arrow the lateral wall of the vestibule, and the arrowhead the lateral wall of the basal cochlear turn.

Figure 3. Quantification of fluorescently labeled zoledronate (6-FAM-ZOL) following intracochlear administration

Using ImageJ, measurements were taken at each of five cochlear half-turns from base to apex in the human temporal bone specimens, along both the lateral cochlear wall (A and B) and the modiolus (C and D). The graphs show the average values generated from three independent experiments and standard error bars are shown. The effect of 6-FAM-ZOL administration is statistically significant along both the lateral cochlear wall and the modiolus contacting the SV and ST. For the lateral cochlear wall, the *p* values for the effect of 6-FAM-ZOL are 0.04 in the SV and 0.03 in the ST, whereas for the modiolus, the *p*

values for the effect of 6-FAM-ZOL are 0.001 in the SV and 0.002 in the ST. SV and ST indicate scala vestibuli and scala tympani, respectively.

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