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Measurement of ototoxicity following intracochlear bisphosphonate delivery

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Abstract

Hypothesis—Assessing the maximum safe dose for local bisphosphonate delivery to the cochlea enables efficient delivery without ototoxicity.

Background—Otosclerosis is a disease of abnormal bone metabolism affecting the otic capsule, which can cause conductive hearing loss. Larger otosclerotic lesions involving the cochlear endosteum and spiral ligament can result in sensorineural hearing loss. Bisphosphonates are used to treat patients with metabolic bone diseases, including otosclerosis. Local delivery is the most efficient way of delivery to the cochlea while avoiding systemic side effects. To attain intracochlear bisphosphonate delivery without ototoxicity, the maximum safe dose of bisphosphonates requires definition. In the present study, we tested increasing concentrations of zoledronate, a third-generation bisphosphonate in an intracochlear delivery system. We measured ototoxicity by monitoring distortion product otoacoustic emissions (DPOAE) and compound action potentials (CAP).

Methods—Artificial perilymph and increasing molar concentrations of zoledronate were administered to the cochlea in guinea pigs via a cochleostomy. Hearing was measured at multiple time points. A fluorescently labeled zoledronate derivative (6-FAM-ZOL) was co-administered as an internal control for drug delivery. Specimens embedded in the resin blocks were ground to a mid-modiolar section and fluorescent photomicrographs were taken.

Results—No significant shift in hearing was observed in animals treated either with artificial perilymph or with 4% of the human systemic zoledronate dose. However, CAP thresholds increased during infusion of 8% of the human systemic zoledronate dose, improved four hours later, and then increased again four weeks later. Using fluorescent photomicrography, intracochlear bisphosphonate delivery up to the apical cochlear turn was confirmed by visualizing 6-FAM-ZOL.

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Conclusions—These findings provide reference values for intracochlear bisphosphonate delivery in the treatment of cochlear otosclerosis and describe a useful method for tracking cochlear drug delivery.

Keywords

Bisphosphonates; Fluorescence imaging; Inner ear drug delivery; Otosclerosis; Ototoxicity

INTRODUCTION

Otosclerosis is a metabolic bone disorder involving inappropriate bony remodeling of the otic capsule. Clinically, otosclerosis typically presents as a conductive hearing loss due to a fixed stapes footplate. Stapedectomy can address the conductive hearing loss observed in otosclerosis. Advanced otosclerotic lesions can involve the cochlear endosteum and spiral ligament (1), which is thought to lead to the additional sensorineural hearing loss seen in cochlear otosclerosis. While estimates vary, the incidence of clinical otosclerosis is thought to be approximately 1% among Caucasians, with 10% of these patients presenting with a sensorineural hearing loss in addition to a conductive hearing loss (2). A significant number of patients with otosclerosis therefore present with relatively advanced lesions and could potentially benefit from treatment of the underlying disease process beyond addressing the conductive hearing loss alone.

Currently, third generation nitrogen-containing bisphosphonates such as zoledronate and risedronate are widely used in the clinic to treat patients with bone metabolic disorders such as osteoporosis, Paget's disease of bone, multiple myeloma, and bone metastasis. These potent nitrogen-containing bisphosphonates bind selectively to bone matrix and are believed to inhibit bone resorption by blocking farnesyl diphosphate synthase in the mevalonate pathway within osteoclasts (3). The systemic use of bisphosphonates has been associated with rare but potentially severe side effects including osteonecrosis of the jaw, atrial fibrillation, and atypical fractures (4). Moreover, bisphosphonates are contraindicated in pregnancy (5). We have reported on a small cohort of cochlear otosclerosis patients, in whom treatment with bisphosphonate halted the progression of sensorineural hearing loss (6). However, the off-label systemic use of bisphosphonates for cochlear otosclerosis remains limited due to the potential for side effects.

Local delivery of bisphosphonate could avoid potential systemic side effects while providing a high local concentration to the targeted organ. Using a fluorescently labeled zoledronate, 6-FAM-ZOL (7,8), we have previously compared the efficacy of bisphosphonate delivery to the cochlea in guinea pigs following systemic administration, local delivery across the round window membrane, and intracochlear delivery via a cochleostomy. Intracochlear delivery was the most efficient means of delivery to the inner ear; only 2% of the systemic dose was required to produce similar deposition of the labeled bisphosphonate in cochlear bone. We were able to achieve levels higher than possible with systemic delivery, and which did not cause ototoxicity as measured by stable hearing levels (9). In the present study, we assessed the maximum safe dose of zoledronate delivered to the cochlea in guinea pigs.

MATERIALS AND METHODS

1. Animals and administered drugs

Male albino guinea pigs (Hartley strain; Charles River Laboratories, Inc., Wilmington, MA) were used, each weighing approximately 350g. Pentobarbital (12.5 mg/kg intraperitoneally), fentanyl (0.1 mg/kg intramuscularly), and haloperidol (5 mg/kg intramuscularly) were given for anesthesia. Supplemental doses of 0.07 mg/kg fentanyl and 3 mg/kg haloperidol alternating every hour with 6.25 mg/kg pentobarbital were administered as needed. Fatal-Plus, a highly concentrated pentobarbital solution, was intraperitoneally injected for euthanizing animals. All animal experiments were approved by the Massachusetts Eye and Ear Infirmary Institutional Animal Care and Use Committee.

2. Molar concentrations of zoledronate solution mixed with 6-FAM-ZOL

To monitor the delivery of zoledronate into the cochlea, we added 6-FAM-ZOL, a fluorescently labeled derivative of zoledronate (7) to the infusion solution. 6-FAM-ZOL has been demonstrated to mimic the pharmacological properties of the parent drug, zoledronate (7). Our goal was to have the minimum concentration of 6-FAM-ZOL (177 μM) thought to be necessary to visualize the distribution of the infused solution (9). We previously demonstrated that 6-FAM-ZOL at 354 μM (0.02 $\times$ of the human systemic zoledronate dose) did not affect thresholds of distortion product otoacoustic emissions (DPOAE) and compound action potentials (CAP) (9). We tested two concentrations of the zoledronate + 6-FAM-ZOL solution equivalent to 0.04 and 0.08 times the human systemic dose of the drug (for convenience, we designated these mixtures as “0.04 $\times$ solution” and “0.08 $\times$ solution” respectively). The total concentration of zoledronate + 6-FAM-ZOL in these solutions was 710 μM and 1420 μM respectively; the molar ratios of zoledronate to 6-FAM-ZOL were 3 and 7 respectively. Our previous study showed that 1.25 μg of 6-FAM-ZOL could be infused over 40 minutes directly into the scala tympani via a cochleostomy in guinea pigs without affecting the DPOAE and CAP (9). In each animal treated either with 0.04 $\times$ or with 0.08 $\times$ solution, 0.625 μg of 6-FAM-ZOL was administered together with zoledronate over 40 minutes into the scala tympani as a fluorescent tracer for cochlear delivery. The content of artificial perilymph (AP) was 120 mM NaCl, 3.5 mM KCl, 1.5 mM CaCl_2 , 5.5 mM glucose, 20 mM HEPES. NaOH was added to adjust the pH to 7.5.

3. Zoledronate infusion into the scala tympani via a cochleostomy

A Harvard Apparatus PHD 2000 Infusion Syringe Pump was used to introduce the zoledronate / 6-FAM-ZOL mixture into the scala tympani of guinea pigs via a cochleostomy approximately 0.5 mm distal to the round window, made by a hand drill. The size of the cochleostomy is approximately 250 μm in diameter. A 500 μL glass syringe was connected via polyetherether ketone (PEEK; Upchurch Scientific) tubing to an 11 mm length of PTFE (teflon) tubing (201 μm od, 101 μm id). A small bleb was made 3 mm from the distal end of the tubing using methyltriacetoxysilane to prevent over-insertion.

We infused 1 μL over one minute five times, spaced nine minutes apart, as per our previous study (9). Study animals were treated with zoledronate solution mixed with 6-FAM-ZOL either at 0.04 $\times$ or at 0.08 $\times$ total. Control animals were treated with AP alone. Animals were

immediately sacrificed at the end of the acute ototoxicity experiments. For the chronic ototoxicity experiments, the infusion line was removed after completion of the infusion, a blood clot was used to seal the cochleostomy, the surgical site was closed, and the animals were allowed to recover for 4 weeks before re-analysis of hearing. Both the acute and the chronic ototoxicity experiments had three subgroups -- control animals treated with AP alone, animals treated with 0.04× solution, and animals treated with 0.08× solution.

4. Hearing measurement and analysis

DPOAE and CAP were measured as described in a previous paper (9). CAP was chosen over auditory brainstem response (ABR) testing because of its greater speed and better signal-to-noise ratio. In the acute ototoxicity experiments, DPOAE and CAP were measured multiple times over 4 hours following administration of solution, after which the animals were euthanized. In the chronic ototoxicity experiments, DPOAE and CAP were measured during the drug infusion, after which the animals were allowed to emerge from anesthesia. Hearing was measured again 4 weeks later and the animals were euthanized.

5. Specimen processing and confocal photomicrography

Temporal bones were harvested and fixed in an excess volume of neutral buffered formalin (10%, Fisher Scientific, Pittsburgh PA) at room temperature for 24 hours. The fixation continued in fresh neutral buffered formalin for another 24 hours. Specimens were dehydrated in ethanol, followed by xylene, after which they were infiltrated and embedded in methyl methacrylate (MMA, Acrylosin soft, Dorn and Hart, Villa Park, IL). Curing of the specimens was performed at room temperature in the dark to prevent photobleaching. The hardened block was ground to a mid-modiolar section of the cochlea.

Images (1024 by 1024; 8 bit) were obtained using a Leica TCS-SP2 confocal microscope over a depth of 500 µm to produce maximum fluorescence images with the section of the strongest signal selected in the middle. The same microscope settings were used in all experiments and all specimens from a single experiment were photographed at the same sitting.

RESULTS

Consistent with our previous experiments, the fluorescent signal was strongest within the scala tympani of the first basal turn, next to the cochleostomy and delivery site. Fluorescent signal was detected up to the apical turn both along the modiolus and on the scalar surface of the lateral cochlear wall in treated specimens (Fig. 1B, 1C, 1E, and 1F), while the autofluorescent signal of the control specimens remained weak (Fig. 1A and 1D). Fluorescent signal strength remained stable with increasing concentration of zoledronate in both acute and chronic experiments, indicating that the amount delivered to the cochlea did not vary significantly between experiments. Surgical middle ear inflammation had largely subsided by four weeks after the cochleostomy, although we found a small amount of granulation tissue and mucosal synechiae around the cochleostomy and the bullectomy site in a few animals. These findings did not correlate with any observed changes in CAP or DPOAE.

Both acutely and at four weeks, CAP remained within 10 dB of baseline in animals treated with AP or with 0.04× solution (Fig. 2D–E). In addition, DPOAE remained within 20 dB across all animals at all frequencies acutely and at four weeks (Fig. 2A–C). These results suggest that zoledronate is not ototoxic with intracochlear delivery up to 4% of the human systemic dose.

Treatment with 0.08× solution, however, resulted in acute CAP shifts between 12 and 16 kHz, in the 20–30 dB range (Fig. 2F). These frequencies corresponded to the approximate location of the tubing within the cochlea and, therefore, the highest delivered concentration of zoledronate. We first detected the CAP shift in acute experiments within 30 minutes after the start of drug delivery. The CAP shift partially resolved over four hours. However, when we evaluated chronic experimental animals at four weeks, the shift had recurred between 12–16 kHz (Fig. 2F). We re-plotted these results for several representative frequencies with CAP shift vs time, showing that the shift occurred rapidly after initial drug delivery and localized to the 12–16 kHz region corresponding to the highest levels of drug delivery (Fig. 3).

DISCUSSION

In this study, we sought to identify the maximum dose of zoledronate that can be locally delivered to the cochlea without incurring ototoxicity. We previously demonstrated that 2% of the systemic dose, delivered locally, could achieve cochlear zoledronate concentrations similar to that achieved with systemic administration. We found that delivery of 4% of the human systemic dose adjusted by weight is possible without affecting the hearing, as measured by CAP and DPOAE. Delivery of 8% of the human systemic dose resulted in an early shift in CAP, most notable at frequencies adjacent to the delivery catheter, which improved over the four-hour course of the acute experiments. However, when evaluated at four weeks in the chronic experiments, animals treated with 8% of the human systemic dose showed a recurrent CAP shift at frequencies corresponding to the highest levels of drug delivery. There was no shift in DPOAE attributable to zoledronate treatment.

Different species of laboratory animal are differentially sensitive to ototoxic drugs (10), with guinea pigs being one of the more sensitive rodent species (11,12). Guinea pigs have frequently been used to predict the ototoxicity of drugs in human administration. For example, the ototoxicity of cisplatin in guinea pigs has been reported to be similar to that in humans (13). The guinea pig is therefore a reasonable rodent model in which to assess ototoxicity.

The ototoxicity we observed was present in the CAP, generated in the auditory nerve, but not in the DPOAE, generated in the outer hair cells, suggesting that the ototoxicity at high (8%) zoledronate doses is generated in the inner hair cells or auditory nerve. It is striking that the initial shift in CAP improves over several hours but then returns at four weeks. The acute, reversible, effects of zoledronate may be associated with its ability to bind free calcium (Ca^{2+}) (14), which plays a critical role in neurotransmitter release at the hair cell synapse, as well as in other biological activities of the cochlea (15,16). Reducing free calcium levels in the cochlea may attenuate synaptic transmission and signaling between inner hair cells and

auditory neurons; outer hair cell function in this setting is unlikely to be affected. It is unclear whether the acute and chronic CAP shifts are mechanistically related. The acute effects reverse very quickly on cessation of drug delivery, while the chronic effects are not evident until the measurement of cochlear responses 4 weeks later. Both the acute and chronic effects occur at frequency ranges near the cannula outflow, where drug concentration is highest. Our zoledronate solutions included 6-FAM-ZOL as a monitor, which may slightly underestimate the ototoxicity of zoledronate because 6-FAM-ZOL appears to be slightly less active than native zoledronate (7,9).

Our experiments provide a window of safety within which zoledronate could be administered locally to the cochlea. Previous clinical trials in patients with multiple myeloma and bone metastases had to be modified from 8 mg to 4 mg zoledronate treatments because of renal toxicity (17). Our previous work found that the amount of zoledronate delivered to the cochlea with the human systemic dose (4 mg) adjusted for weight was roughly equivalent to 30% of the dose when delivered to the round window membrane, or 2% of the dose when infused into the cochlea via a cochleostomy (9). With respect to delivery in humans, one way in which bisphosphonate might be delivered directly to cochlear fluids in this manner is via a drug-eluting stapes prosthesis (9). Since our present experiments suggest that 4% of the human systemic dose is non-ototoxic when infused directly into the cochlea, local delivery methods may provide a way to increase local concentration within the cochlea while avoiding systemic toxicity. In addition, establishing parameters for non-ototoxic delivery of bisphosphonates is critical for the possible use of bisphosphonates themselves as otologic drug delivery vehicles (18).

We have previously characterized the use of 6-FAM-ZOL as a non-ototoxic marker for the extent of cochlear drug delivery. In the present study, we have added a small amount of 6-FAM-ZOL in each experiment as an easily visualized and measured internal control for the extent of cochlear drug exposure. This method could be used to standardize cochlear drug delivery levels across a series of experiments. Other current methods of measuring comparative levels of cochlear drug delivery include direct sampling from the apex (19,20), which has the advantage of directly sampling perilymph in real time. 6-FAM-ZOL provides another tool for assessing the cumulative amount of drug delivered to the various regions of the cochlea.

CONCLUSION

Four percent of the human systemic zoledronate dose can be delivered directly to the cochlea via a cochleostomy in guinea pigs without damaging hearing. Our findings provide reference parameters for intracochlear bisphosphonate delivery and describe a useful method for comparing various methods of cochlear drug delivery using a fluorescently labeled bisphosphonate compound.

Acknowledgments

Conflict of Interest: Dr C. E. McKenna is a founding member of BioVinc LLC, which is producing 6-FAM-ZOL for commercial use.

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Fig. 1. Fluorescent photomicrographs following direct intracochlear infusion of artificial perilymph, 0.04× solution, and 0.08× solution

Fluorescent photomicrographs taken at mid-modiolar sections of the cochlea are shown for cochleas treated with artificial perilymph (A and D), cochleas treated with 0.04× solution (B and E), and cochleas treated with 0.08× solution (C and F). The upper row shows fluorescent photomicrographs of guinea pigs sacrificed 4 hours after infusion, and the lower row those of guinea pigs sacrificed 4 weeks after infusion. 0.04× and 0.08× solution, respectively, indicate 4% and 8% of the human systemic zoledronate dose mixed with a small amount of 6-FAM-ZOL at corresponding molar concentrations.

Fig. 2. DPOAE and CAP measurements following cochlear infusion

The upper row indicates distortion product otoacoustic emissions (DPOAE) and the lower row compound action potentials (CAP). The chronic experiments are graphed along the curve labeled 4 weeks, while the other curves are from acute experiments. Averages from independent experiments are shown. 0.04× and 0.08× solution indicate 4% and 8% of human systemic zoledronate dose, respectively, mixed with a small amount of 6-FAM-ZOL at corresponding molar concentrations. No significant DPOAE shift was observed during the course of the experiments. The CAP shift in the 0.08× solution group occurred at 30 minutes between 8 kHz and 24 kHz, partially normalized at 260 minutes, and increased again four weeks later.

Fig. 3. Threshold shift of compound action potentials (CAP) following infusion of 0.08x solution at the frequencies of 24 (A), 16 (B), 12 (C), and 8 kHz (D)

The graphs present the average values generated from independent experiments of either acute or chronic experiments. The acute experiments were performed in one day, while the chronic experiment animals were kept alive for 4 weeks after surgery. The vertical and the horizontal standard error bars are shown for CAP threshold shift and the time point when the CAP was measured, respectively. The rectangular grey-shaded areas indicate the time period over which drug was infused. CAP shift in 0.08x solution peaked between 12 and 16 kHz.

0.08× solution indicates 8% of the human systemic zoledronate dose mixed with a small amount of 6-FAM-ZOL at corresponding molar concentrations.

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