

Physiologic Effects of Microneedle-Mediated Intracochlear Dexamethasone Injection in the Guinea Pig

Sharon J. Feng, BA, BS ; Stephen Leong, MD ; Aykut Aksit, PhD; Daniella Hébert, BE, PhD; Elizabeth S. Olson, PhD; Jeffrey W. Kysar, PhD; Anil K. Lalwani, MD

Objectives: Oral or intratympanic corticosteroids are commonly used to treat sudden sensorineural hearing loss (SSHL), tinnitus, and Meniere disease. Direct intracochlear delivery has been proposed to overcome the variability in bioavailability and efficacy of systemic or middle ear delivery. In this study, we aim to characterize the physiologic consequences of microneedle-mediated direct intracochlear injection of dexamethasone through the round window membrane (RWM).

Methods: In Hartley guinea pigs ($n = 5$), a post-auricular incision followed by bullostomy was made to access the round window membrane. Using 100 μm diameter hollow microneedles, 1.0 μl of 10 mg/ml dexamethasone was injected through the RWM over 1 min. Compound action potential (CAP) and distortion product otoacoustic action emissions (DPOAE) were measured before perforation, at 1 h, and at 5 h following injection. CAP hearing thresholds were measured from 0.5 to 40 kHz, and DPOAE f2 frequencies ranged from 1.0 and 32 kHz. Repeated measures ANOVA followed by pairwise t -tests were used for statistical analysis.

Results: ANOVA identified significant CAP threshold shifts at four frequencies (4, 16, 36, and 40 kHz) and differences in DPOAE at 1 frequency (6 kHz). Paired t -tests revealed differences between the pre-perforation and 1 h time point. By 5 h post injection, both CAP hearing thresholds and DPOAE recover and are not significantly different from baseline thresholds.

Conclusion: Direct intracochlear delivery of dexamethasone via microneedles results in temporary shifts in hearing thresholds that resolve by 5 hours, thus supporting microneedle technology for the treatment of inner ear disorders.

Key Words: dexamethasone, inner ear drug delivery, intracochlear drug delivery, microneedle, round window membrane, steroids.

Level of Evidence: N/a

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INTRODUCTION

Sudden sensorineural hearing loss (SSNHL) is characterized by an acute onset of hearing loss, typically within

a 72 h time period.¹ In the United States, the incidence of SSNHL ranges from 11 to 77 cases per 100,000, with the majority of cases occurring in patients older than 65 years.² The etiology of SSNHL is varied, but most cases are considered idiopathic, with an underlying viral or vascular event producing inner ear inflammation and subsequent hearing threshold elevation.^{1,3} The standard treatment for SSNHL is a 10-day course of oral glucocorticoid therapy⁴; an alternative to systemic therapy is intratympanic injection of glucocorticoids over the span of 3 weeks.⁵ Therapeutic response to glucocorticoids, which is achieved in 60–70% of patients, is thought to occur due to the anti-inflammatory effects of the medication.³ The utility of glucocorticoids in autoimmune inner ear disease, Meniere disease, and other cochleovestibular disorders is also based on this anti-inflammatory mechanism.

Although intratympanic injection has been shown to have a greater impact on gene expression than systemic therapy,⁶ the technique is limited by the fact that agents must remain in the middle ear space for a period of time before sufficient diffusion across the round window membrane (RWM) occurs. Patients also are required to remain still and in supine positioning for approximately 30 min after injection to assist diffusion into the inner ear.⁷ Nonetheless, a significant portion is subject to clearance by the Eustachian tube, which can limit the therapeutic effect.⁸ Intratympanic glucocorticoids are also potentially toxic to middle ear structures such as the ossicles,

From the Vagelos College of Physicians and Surgeons (S.J.F., S.L., A.K.L.), Columbia University Irving Medical Center, New York, New York, U.S.A.; Department of Otolaryngology-Head and Neck Surgery (S.J.F., S.L., E.S.O., A.K.L.), NewYork-Presbyterian/Columbia University Irving Medical Center, New York, New York, U.S.A.; Department of Mechanical Engineering (A.A., D.H., J.W.K.), Columbia University, New York, New York, U.S.A.; and the Department of Biomedical Engineering (E.S.O.), Columbia University, New York, New York, U.S.A.

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Sharon J. Feng and Stephen Leong contributed equally to this work.

Anil K. Lalwani, MD, Jeffrey W. Kysar, PhD and Aykut Aksit, PhD are the co-founders of Haystack Medical, Inc., that will commercialize microneedle and associated technology to facilitate medical access to the middle and inner ear. A conflicts of interest committee has reviewed and approved all documents.

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Send correspondence to Anil K. Lalwani, MD, Division of Otolaryngology and Skull Base Surgery, Columbia Cochlear Implant Program, Department of Otolaryngology—Head & Neck Surgery, NewYork-Presbyterian/Columbia University Irving Medical Center; Columbia University Vagelos College of Physicians & Surgeons, 180 Fort Washington Avenue, HP8/New York, NY 10032.

Email: akl2144@cumc.columbia.edu

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associated muscles, and nerve branches.⁸ Last, the polar nature of commonly used compounds like dexamethasone sodium phosphate may limit passive diffusion across the RWM; nonpolar compounds are not routinely used because they do not readily solubilize and thus lack therapeutic effect. A 2016 clinical trial investigating the efficacy of an intratympanic sustained-release dexamethasone injection (OTO-104) demonstrated no significant benefit over baseline, which may be attributed to limited diffusion of the compound through the RWM.⁹

Recently, advances in intracochlear delivery have demonstrated efficacy in bypassing the issues seen in intratympanic injection. Earlier attempts at intracochlear access involved cochleostomy and injection or implantation of agents into the inner ear space; however, cochleostomy risks inner ear damage and potential hearing loss.^{10,11} More recent attempts at intracochlear delivery have focused on minimally invasive access through the oval or round window, which allows for the precise delivery of therapeutics without the potential risks of cochleostomy. Techniques that have been developed thus far include drug-eluting cochlear implants,^{12,13} silicone-based implants connecting the middle and inner ear spaces,¹⁴ and direct injection through the RWM.^{15,16} Less invasive methods include RWM perforations to increase diffusion across the RWM into the inner ear.^{17,18} However, each of these techniques involves significant, often irreversible, trauma to the RWM.

To circumvent the current issues apparent in intracochlear administration, our laboratory has developed microneedles that allow for perforation of the RWM with minimal trauma and full reconstitution of the RWM structure within 72 h.^{19–24} Several of these studies used an *in vivo* guinea pig model to assess the physiological consequences of perforation, aspiration, and injection through the RWM with follow-up hearing studies between 0 and 2 h, 48 h, 72 h, and 1 week post-perforation. Between 0 and 2 h post-perforation, our laboratory has found threshold shifts of 5–10 dB in the 22–40 kHz frequency range at 0–2 h post-perforation and resolution of these findings within 1 week. At 48 h post-perforation,²¹ we did not find threshold changes following microneedle-mediated injection of 1.0 μ l of artificial perilymph, equivalent in volume to about 20% of the scala tympani volume.¹⁹ Similarly, at 72 h post-perforation, we found no hearing loss following microneedle-mediated aspiration.²⁵ Additionally, we have established that the RWM fully heals within 48 to 72 h following perforation, demonstrating that no residual anatomic or functional changes persist as a consequence of microneedle-mediated perforation.^{19,21,25}

We have successfully aspirated perilymph fluid—again in a guinea pig model—using a hollow, lumenized microneedle, and we have used this technique to characterize the perilymph proteome following glucocorticoid treatment.^{8,25} Specifically, we showed that systemic and intratympanic glucocorticoid treatment induced significant changes in the inner ear proteome.²⁵ Interestingly, the proteomic changes were more pronounced with systemic glucocorticoids than with intratympanic glucocorticoids, which may indicate a greater degree of Eustachian tube clearance for intratympanic glucocorticoids than previously believed.

Recently, we have demonstrated the safety and efficacy of microneedle-mediated direct intracochlear injection.¹⁹ Using an artificial perilymph injectate, we found that injection of up to 1.0 μ l of fluid (which is about 20% of the volume of the scala tympani) into the guinea pig inner ear did not produce hearing loss. In addition, injection of 1.0 μ l of the fluorescent compound FM 1–43 FX led to substantial fluorescence in the basal and middle turns of the cochlea.¹⁹ In total, these results support the use of microneedles for diagnostic aspiration and therapeutic delivery. In this study, we use microneedles to directly inject dexamethasone sodium phosphate into the guinea pig inner ear *in vivo*, thus demonstrating, for the first time, that our microneedles may be used to safely deliver therapeutics into the inner ear. We aim to elucidate the minimum amount of time necessary for hearing to recover after intracochlear injection of dexamethasone.

MATERIALS AND METHODS

Microneedles

Microneedles were designed in SolidWorks (Dassault Systems SolidWorks Corporation, Concord, NH), and stereolithography files were fabricated using Describe software (Nanoscribe GmbH, Karlsruhe, Germany) with a 1 μ m slicing distance and synthesized with Photonic Professional GT 2PP system using photoresist IP-S (Nanoscribe GmbH). The result is a 100 μ m outer diameter, 35 μ m inner diameter, single bevel microneedle (Fig. 1). These microneedles have previously been shown to perforate the round window membrane without causing changes in hearing. Perforations are lens-shaped, generated by separation rather than scission of membrane fibers, and completely heal within 72 h. Details of microneedle design, synthesis, and properties have been previously reported.^{19,21–23,25,26} These microneedles are mounted on a 30-gauge stainless steel Hamilton syringe (Hamilton Company, Reno, NV), which are then loaded onto a 10 μ l Gastight Hamilton syringe (Model 1701 RN, Hamilton Company, Reno, NV). This syringe is placed on an UMP3 UltraMicroPump (World Precision Instruments, Sarasota, FL), mounted to a micropositioner (Model 1350M, World Precision Instruments). Altogether, the microneedle apparatus allows for precise perforation of the RWM with the exact injection of a specified volume and rate of dexamethasone sodium phosphate solution.

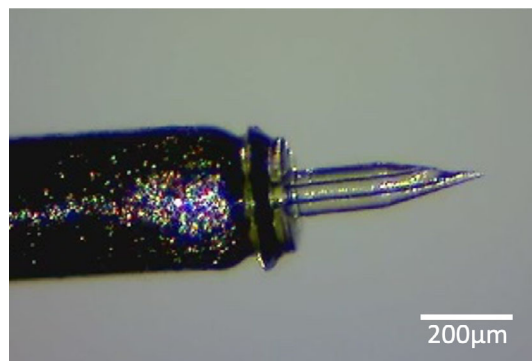


Fig. 1. Light microscope image of a single-lumen, hollow microneedle mounted to the tip of a 30-gauge blunt Hamilton needle. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

Surgery and Microneedle Injection

All procedures in this study were reviewed and approved by the Columbia University Institutional Animal Care and Use Committee (IACUC).

Hartley guinea pigs ($n = 5$) were procured from Charles River Laboratories (Wilmington, MA). Animals weighed between 225 and 300 g at the time of surgery. Animals were first anesthetized with 3.0% isoflurane via an induction chamber and subsequently maintained on 1.5–3.5% isoflurane delivered via a nosecone. For analgesia, animals received Buprenorphine SR (0.1 mg/kg) and Meloxicam (0.5 mg/kg) delivered subcutaneously. Lidocaine was also injected at the post-auricular incision site and at the two sites where the head-holder attaches to the head.²⁶

A 5–8 mm incision was made post-auricularly above the right bulla, allowing for exposure of the bulla using blunt dissection. The facial nerve was identified, and a 2–3 mm bullostomy was performed with fine forceps just posterior to the styloidmastoid foramen. Once the round window membrane was exposed, the microneedle apparatus was introduced. The microneedle is mounted to a 30-gauge Hamilton needle (Hamilton Company, Reno, NV), which is attached to a 10 μ l Hamilton syringe (Model 1701 RN, Hamilton Company, Reno, NV). The Hamilton syringe is placed on a UMP3 UltraMicroPump (World Precision Instruments, Sarasota, FL), which is mounted to a micropositioner (Model 1350 M, World Precision Instruments). The microneedle was advanced using the micropositioner to create a perforation. 1.0 μ l of a 10 mg/ml solution of pharmaceutical grade dexamethasone sodium phosphate solution (West-Ward, Eatontown, NJ) was injected through the RWM of the cochlea using the UMP pump at a rate of 1.0 μ l/min. The microneedle apparatus was removed, and the animal maintained on isoflurane for 5 h after perforation. Hearing tests were conducted using CAP and DPOAE at 1 h post-perforation and at 5 h post-perforation. After the final hearing test, animals were euthanized with phenytoin/pentobarbital.

Hearing Tests

We used compound action potential (CAP) and distortion product otoacoustic action emissions (DPOAE) to evaluate hearing in the anesthetized guinea pig. Baseline hearing tests were conducted after surgical opening of the bulla, at 1 h post-injection, and at 5 h post-injection.

CAP detects the minimum hearing threshold via action potentials measured at the cochlear base and is a measure of cochlear nerve function. We tested 18 frequencies ranging from 0.5 to 40 kHz (Fig. 2). Tone intensities were increased 5 dB SPL at a time and the minimum amplitude to generate the characteristic response curve was recorded as the hearing threshold.

DPOAE measures the outer hair cell response, and thus function, by playing two simultaneous pure tone frequencies and measuring the response. We inserted a hollow ear-tube, fitted with a speaker and Sokolich ultrasonic probe microphone, just above the right external auditory canal of an anesthetized guinea pig. Sound stimuli is played at 70 and 80 dB SPL with a fixed frequency ratio of $f_2/f_1 = 1.2$, wherein f_1 increases from 1.0 to 32 kHz in 1.0 kHz intervals (Fig. 3). Measurements at $2f_1 - f_2$ were considered positive responses if ≥ 3 dB SPL above the noise level.

Statistical Tests

Repeated measures ANOVA was conducted at each frequency for all CAP and DPOAE hearing tests. Significance was set as $p < 0.05$. For frequencies with significant changes in

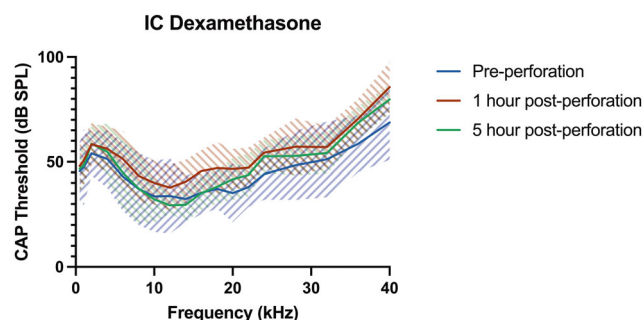


Fig. 2. Mean CAP thresholds (blue line), 1 h after (red line), and 5 h after (green line) microneedle-mediated injection of 1.0 μ l dexamethasone sodium phosphate through the round window membrane. Shaded areas represent the 95% confidence interval. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

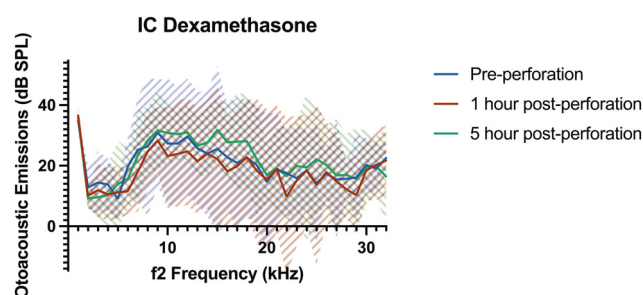


Fig. 3. Mean DPOAE results before (blue line), 1 h after (red line), and 5 h after (green line) microneedle-mediated injection of 1.0 μ l dexamethasone sodium phosphate through the round window membrane. Primary frequencies are 80 dB SPL. Shaded areas represent the 95% confidence interval. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

hearing, pairwise t-tests were used to further evaluate differences between each of the three timepoints: pre-perforation versus 1 h post-perforation, pre-perforation versus 5 h post-perforation, and 1 h post-perforation versus 5 h post-perforation. Statistical tests were performed in RStudio (Posit, Boston, MA) and Excel (Microsoft, Redmond, WA).

RESULTS

Repeated measures ANOVA identified four frequencies with significant changes in CAP thresholds: 4 kHz ($p = 0.0457$), 16 kHz ($p = 0.0245$), 36 kHz ($p = 0.0485$), and 40 kHz ($p = 0.0348$). Pairwise t-tests revealed significance at pre-perforation versus 1 h post-perforation for all four frequencies: 4 kHz (mean shift 4.8 dB, $p = 0.0261$), 16 kHz (mean shift 10.2 dB, $p = 0.0015$), 36 kHz (mean shift 12.0 dB, $p = 0.0214$), and 40 kHz (mean shift 16.8 dB, $p = 0.0293$). No significance was found when comparing pre-perforation versus 5 h post-perforation.

For DPOAE, repeated measures ANOVA found one frequency with significant hearing change: 6 kHz ($p = 0.0119$). Similarly, pairwise t-tests demonstrate this significance comes from the pre-perforation versus 1 h post-perforation timepoint: 6 kHz (mean shift 8.1 dB,

$p = 0.0018$). No significance was found when comparing pre-perforation versus 5 h post-perforation.

DISCUSSION

Our laboratory has previously demonstrated microneedle-mediated injection of artificial perilymph of 1.0 μl , which is about 20% of the volume of the scala tympani, is safe for intracochlear delivery in a guinea pig model. In this study, we demonstrate this technology can be safely used to deliver 1.0 μl of conventional inner ear therapeutics, namely dexamethasone sodium phosphate, directly into the cochlea of a guinea pig model. There is a slight threshold shift in some frequencies at 1 h post-injection that resolves by 5 h post injection, indicating that the minor hearing loss associated with microneedle-mediated injection is likely transient. Safe and effective injection of glucocorticoids sets the groundwork for injection of other inner ear therapeutics, namely gene therapies, which have previously been limited by the inaccessibility of the bony labyrinth. Our microneedle technology is enabling in the field of cochlear drug delivery because it allows us to bypass these anatomic barriers in a minimally traumatic fashion.

At the 1 h timepoint, we observed 5–17 dB threshold shifts at 4 frequencies between 0.5 and 40 kHz, which mostly recovers by the 5 h timepoint. At 36 and 40 kHz, there remained a mean shift of 10–11 dB after 5 h, although these shifts were no longer significant on statistical analysis. This study was designed to detect a significant difference at $\alpha = 0.05$ with 90% power and was underpowered to detect threshold shifts with low magnitude or high variance, which remains a limitation of this study. A potential cause of these shifts may be pressure changes following the injection and subsequent perilymph reflux. A similar phenomenon is observed in humans after stapedectomy, which necessarily creates a perilymph leak that generally closes via an endolymphatic seal but may form a perilymphatic fistula.²⁷ A previous retrospective study found slight threshold shifts at 2 and 4 kHz following stapedectomy which resolved within a few days.²⁸ Perforations created by our microneedle are likely smaller than those created by stapedectomy, which may result in a shorter course of perilymph leak that resolves after several hours, not several days, as observed after stapedectomy. Another potential cause of temporary threshold shifts may be serous labyrinthitis, which is noninfectious inflammation of the inner ear that can occur following stapedectomy, but whose course is on the order of days and not hours.²⁷ This is unlikely to be a major factor contributing to changes in hearing thresholds following microneedle injection, as the hearing loss we observe resolves after several hours. Finally, hearing loss could also be due to the preparation of the dexamethasone sodium phosphate solution causing irritation.²⁹

In addition to testing hearing at 1 and 5 h post-injection, our laboratory has conducted hearing tests immediately after perforation and up to 1 week post-perforation.^{19–22} In total, these studies, in combination with the data presented here, show temporary shifts in hearing thresholds at 0–2 h post-perforation that are no longer present at 5 h, 48 h, 72 h, and 1 week post-

perforation. These results suggest that the shifts in hearing thresholds seen with microneedle-mediated dexamethasone injection are at least partially attributable to the perforation of the RWM itself, which is transient in nature.

Our study has several limitations. In previous studies, we evaluated hearing following microneedle perforation over longer timeframes, ranging from 1 h post perforation to 1 week post perforation. In this study, we evaluated hearing at 1 and 5 h post dexamethasone injection, which offers a unique, but limited perspective on hearing changes following therapeutic injection, as we do not expect RWM to heal until around 48 to 72 h post injection. The purpose of this study was to evaluate the amount of time necessary for hearing to recover after dexamethasone injection, despite a persistent perforation in the RWM. Future studies may attempt to characterize hearing over a longer timeframe to ensure the long-term safety of our technique. An additional limitation is the single concentration and volume of dexamethasone used for injection. For this study, we choose 1.0 μl as the injection volume, as we have previously demonstrated that 1.0 μl injections are safe and not associated with hearing changes at 48 h following injection.¹⁹ In the same study, we demonstrated that larger injection volumes (2.5 and 5.0 μl) were associated with changes in hearing at 48 h.¹⁹ In this study, temporary hearing loss and possible toxicity may have been related to the formulation of dexamethasone itself and may be exacerbated in a dose-dependent fashion, although the maximum concentration of dexamethasone therapeutically available (10 mg/ml) was used. To further elucidate this potential toxicity, future studies may utilize a range of dexamethasone concentrations for injection and characterize the effects on hearing and hair cell function. Quantification of the amount of dexamethasone reaching the basal, middle, and apical turns of the cochlea may also help to elucidate its effects on hearing. Though our findings are based on a single concentration of dexamethasone over the course of 5 h, this study adds to the growing body of evidence that microneedles are safe and effective for therapeutic intracochlear injection and may be used for the delivery of advanced inner ear therapies, such as gene therapy.

CONCLUSION

In this study, we demonstrate that microneedle-mediated injection of 1.0 μl , which is about 20% of the volume of the scala tympani, of dexamethasone sodium phosphate is a safe and feasible technique for inner ear drug delivery in a guinea pig model. One hour after dexamethasone injection, we measure a mild hearing loss at four frequencies between 0.5 and 40 kHz that resolves within 5 h. We suggest that self-limited perilymph reflux following microneedle-mediated injection is the cause of this transient hearing loss; a less likely explanation is serous labyrinthitis. Thus, we demonstrate that microneedle technology is safe for direct intracochlear injection of therapeutics. We anticipate that this technology will allow for minimally traumatic delivery of advanced cochlear therapies, such as gene therapy, first in guinea pig models and eventually in human subjects.

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