Development and Characterization of Chemical Cochleostomy in the Guinea Pig

Jennifer C. Alyono, MD1, C. Eduardo Corrales, MD2, Markus E. Huth, MD3, Nikolas H. Blevins, MD1, and Anthony J. Ricci, PhD4

Abstract

Objectives. Creation of an atraumatic, hearing-preservation cochleostomy is integral to the future of minimally invasive inner ear surgery. The goal of this study was to develop and characterize a novel chemical approach to cochleostomy.

Study Design. Prospective animal study.

Setting. Laboratory.

Methods. Experimental animal study in which phosphoric acid gel (PAG) was used to decalcify the otic capsule in 25 Hartley guinea pigs. Five animals in each of 5 surgical groups were studied: (1) mechanically opening the auditory bulla alone, (2) PAG thinning of the basal turn otic capsule, leaving endosteum covered by a layer of bone, (3) micro-pick manual cochleostomy, (4) PAG chemical cochleostomy, exposing the endosteum, and (5) combined PAG/micro-pick cochleostomy, with initial chemical thinning and subsequent manual removal of the last osseous layer. Preoperative and postoperative auditory brainstem responses and otoacoustic emissions were obtained at 2, 6, 10, and 16 kHz. Hematoxylin and eosin–stained paraffin sections were compared.

Results. Surgical and histologic findings confirmed that application of PAG provided reproducible local bone removal, and cochlear access was enabled. Statistically significant auditory threshold shifts were observed at 10 kHz (P = .048) and 16 kHz (P = .0013) following cochleostomy using PAG alone (group 4) and at 16 kHz using manual cochleostomy (group 3) (P = .028). No statistically significant, postoperative auditory threshold shifts were observed in the other groups, including PAG thinning with manual completion cochleostomy (group 5).

Conclusion. Hearing preservation cochleostomy can be performed in an animal model using a novel technique of thinning cochlear bone with PAG and manually completing cochleostomy.

Keywords

cochleostomy, hearing preservation, atraumatic cochleostomy

Introduction

Minimally traumatic, hearing preservation cochleostomy will be integral to the future of inner ear surgery. Currently, most cochleostomies are performed for the purposes of cochlear implantation in patients who have severe or profound sensorineural hearing loss; however, with the advent of electric acoustic stimulation devices, preservation of residual hearing has become increasingly important. Minimally traumatic cochleostomy is also important for furthering in vivo hearing research. The ability to access the inner ear without perturbation is critical to ensuring that studies of the cochlea are reliable and accurate.

Current cochleostomy techniques generally fall into 1 of 2 categories: mechanical with a drill, or thermal with a laser. We introduce the technique of chemical cochleostomy, which represents a novel approach using phosphoric acid gel (PAG). In a manner similar to decalcification protocols for histology, the acidic environment is thought to increase the solubility of the calcium and phosphate crystalline salts that are the building blocks of bone. We hypothesized that using an acid gel would allow for resorption of the otic capsule in a localized area. Potential advantages of this approach include avoiding the following: exposure of

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1Department of Otolaryngology–Head and Neck Surgery, Stanford University School of Medicine Stanford, California, USA
2Department of Otology and Laryngology, Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts, USA
3Department of Otorhinolaryngology, Head and Neck Surgery, Inselspital, University of Bern, Freiburgstrasse, Bern, Switzerland
4Department of Otolaryngology–Head and Neck Surgery and Department of Molecular and Cellular Physiology, Stanford University School of Medicine Stanford, California, USA

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Corresponding Author:
Anthony J. Ricci, PhD, Otolaryngology–Head & Neck Surgery, Stanford University School of Medicine, 801 Welch Road, Stanford, CA 94305, USA. Email: aricci@stanford.edu
the inner ear to mechanical injury from the drill, the introduction of free bone fragments within the scalae, the potential for uncontrolled fracture of the otic capsule, \(^1\) thermal injury generated by high-speed drills and lasers, \(^2\) noise-induced injury \(^3,^4\) photo-acoustic injury secondary to pressure waves generated by laser use, \(^2,^5\) and mechanical or thermal injury to vulnerable adjacent surrounding structures. \(^6,^7\) Application of chemical agents could also potentially be performed at locations and from angles not normally accessible using a large instrument like a drill.

**Methods**

**Animal Preparation**

All studies were performed in accordance with methods approved by the Stanford University Administrative Panel on Laboratory Animal Care. Twenty-five female Hartley guinea pigs were studied, with 5 animals in each of 5 groups: (1) mechanically opening the auditory bulla alone; (2) PAG thinning of the basal turn otic capsule, leaving endosteum covered by a layer of bone; (3) micro-pick manual cochleostomy; (4) PAG chemical cochleostomy, exposing the endosteum; and (5) combined PAG/micro-pick cochleostomy, with initial chemical thinning and subsequent manual removal of the last osseous layer.

Anesthesia was achieved using intraperitoneal injections of ketamine (40 mg/kg) and xylazine (5 mg/kg). While animals were anesthetized, temperature, heart rate, oxygen saturation, and breathing pattern were monitored (Oxypulse, Oxivet MD300m, Phoenix, Arizona). Following conclusion of procedures and audiometry, animals were euthanized under anesthesia by decapitation. Two animals from group 5 were sacrificed immediately following postoperative audiometry for purposes of histologic sectioning. The remaining 3 animals were studied longitudinally, with audiometric measurements performed on postoperative days 2 and 9 under anesthesia. The skin and soft tissue overlying the auditory bulla were closed using 2 layers of simple interrupted suture. No closure of the bony bulla or cochlea was performed. These animals were given carprofen (2.5 mg/kg) postoperatively for analgesia.

**Surgery**

Surgical sites were shaved and prepped with betadine. For local anesthesia, 1% lidocaine with 1:100,000 epinephrine was infiltrated subcutaneously. A 1.5-cm postauricular incision was made overlying the auditory bulla. The dorsolateral surface of the auditory bulla was opened by use of a pick and cupped forceps as far anteriorly as the tympanic membrane, as far posteriorly as the neck, and as far medially as the vestibular system (Figure 1). Anatomic landmarks were identified, including the incudostapedial joint, round window, and basal turn of the cochlea (Figure 1A).

All cochleostomies were performed along the basal turn of the cochlea, overlying the scala tympani. For those animals undergoing mechanical cochleostomy, this was achieved with a right-angle pick. Chemical cochleostomy was achieved by applying approximately 15 μL of 34% PAG (Dentsply, Philadelphia, Pennsylvania) to the basal turn of the cochlea for 3 separate 5-minute applications using a blunt 25-gauge needle (Figure 1B). After each application, PAG was removed by suction and rinsing with artificial perilymph (145 mmol/L NaCl, 2.7 mmol/L KCl, 2.0 mmol/LMgSO4, 1.2 mmol/L CaCl2, 5.0 mmol/L HEPES buffer, pH 7.4). Combined PAG/micro-pick cochleostomy, with initial chemical thinning and subsequent manual removal of the last osseous layer, was performed by applying PAG for 2 separate 5-minute applications and then using a right-angle pick to complete cochleostomy. Those animals that underwent PAG thinning of the basal turn otic capsule, leaving endosteum covered by a layer of bone, similarly had applications of PAG for 2 separate 5-minute periods.

**Audiometry**

Audiometric measurements were performed in a sound-proofed booth. Auditory evoked brainstem responses (ABRs) were obtained at 2, 6, 10, and 16 kHz using custom hardware and MATLAB software described previously. \(^8\) Briefly, needle electrodes were placed subcutaneously at the vertex and behind the ipsilateral ear for measurement and on a hind limb as a ground electrode. For those animals undergoing cochleostomies, the cochleostomy site was left...
open during audiometric measurements. The sound intensity level was raised in 10-dB steps from 10 to 80 dB SPL, and 500 responses at each sound level were recorded and averaged. The peak value of the ABR was measured, and the threshold at each frequency was calculated to be when this value was 5 standard deviations above the noise floor.

**Histologic Assessment**

After the temporal bones were isolated, cochleae were removed by opening the auditory bulla and removing the ossicles. Cochleae were then fixed in 4% paraformaldehyde solution for 4 to 8 hours. Decalcification was performed in 0.12 M EDTA in 0.1 M phosphate-buffered solution for 5 to 7 days until bones were soft on manual palpation. Cochleae were then embedded in paraffin, sectioned with a microscope, and stained with hematoxylin and eosin. Sections were assessed for the degree of bony resorption.

**Statistical Analysis**

Statistical analyses were performed with Excel (Microsoft, Seattle, Washington). Comparisons of averaged data were performed with 1-way analysis of variance and Student’s t tests. P values < .05 were considered statistically significant. Sample size calculations were based on an α of 0.05 and power value of 0.8.

**Results**

Power calculations revealed that with 5 animals in each cohort, the study was powered to detect a threshold shift of 14 dB. To detect a shift of 10 dB, 9 subjects would be required in each cohort.

For each of the 5 cochleae tested in group 4, PAG provided reproducible local bone removal, and cochlear access was enabled as seen on both microscopic observations and histopathologic assessment (Figure 2). Lack of bony covering could be confirmed both visually and via gentle palpation under microscopic observation.

Microscopic observation revealed that when PAG was used to thin the cochlear wall without breach of the endosteum, veins within the endosteum directly under the cochleostomy site remained patent. When PAG was used to breach the cochlear wall, endosteal vessels appeared coagulated. This was indicated by a change in color from red to brown in each of the 5 cochleae in group 4 (Supplemental Figure S1, available online only). In preliminary studies, vessels undergoing such a color change demonstrated diminished or absent bleeding when mechanically breached with a micro-pick. Conversely, vessels of those animals in group 5, which underwent completion micro-pick cochleostomy after thinning the cochlea with PAG, each demonstrated no color change and had expected bleeding from endosteal vessels when breached.

No statistically significant auditory threshold shifts were observed after opening of the auditory bulla alone (group 1) (Figure 3A) or after thinning of the basal turn of the otic capsule with PAG, leaving endosteum covered by a layer of bone (group 2) (Figure 3B).

Statistically significant auditory threshold shifts were observed at 16 kHz using manual cochleostomy (group 3) (P = .028) and at 10 (P = .048) and 16 kHz (P = .0013) following cochleostomy using PAG alone (group 4) (Figure 4). No statistically significant postoperative auditory threshold shifts were observed when cochleostomy was performed by first thinning the otic capsule with PAG (group 5) (Figure 5A). For the 3 animals in group 5 that were followed longitudinally for 9 days, no threshold shifts were observed at postoperative days 2 or 9 (Figure 5B).

**Discussion**

The ability to perform minimally traumatic cochleostomy will be integral to the future of inner ear surgery. Current approaches to the cochlea are largely mechanical, using a drill to penetrate the otic capsule or a micro-pick to open the round window. The inner ear can also be accessed thermally using a laser, as is common during stapes surgery. Numerous studies have examined methods to preserve hearing following cochleostomy and cochlear implantation. One meta-analysis found that better hearing preservation was associated with the use of postoperative systemic steroids, with the use of soft tissue rather than fibrin glue for cochleostomy seal, and with cochleostomy rather than round window approaches. Lasers of various wavelengths (eg, carbon dioxide, erbium:yttrium-aluminum-garnet, and potassium-titanyl-phosphate) and piezoelectric devices have been used as alternatives to diamond burs. In this study we describe a novel approach using PAG to perform chemical cochleostomy.

One concern of using PAG is its corrosive strength and thus potential for damaging nearby structures, vasculature,
or the cochlea itself. Although an English-language literature search did not reveal its prior use in otologic surgery, PAG is routinely used by dentists in the oral cavity. Developed in the 1950s, PAG is used to condition human enamel so that it can be bonded to resins in restorative and orthodontic dentistry.\textsuperscript{18-20} In the same way that dentists take care not to allow the acid to come into contact with mucosal surfaces,\textsuperscript{20} we were careful to allow the PAG contact with only the intended cochleostomy site. Following each application, we suctioned all visible gel and rinsed it away with

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**Figure 3.** Auditory evoked brainstem response (ABR) thresholds following bulla opening (A) and thinning of the cochlea with 2 separate 5-minute applications of phosphoric acid gel (B). No significant threshold shifts were observed.

**Figure 4.** Auditory evoked brainstem response (ABR) thresholds after chemical cochleostomy (A) and micro-pick manual cochleostomy (B). Significant threshold shifts (*) were observed at 10 (\(P = .048\)) and 16 kHz (\(P = .0013\)) following cochleostomy using phosphoric acid and at 16 kHz using manual cochleostomy (\(P = .028\)).

**Figure 5.** Auditory evoked brainstem response (ABR) thresholds following combined phosphoric acid gel/micro-pick cochleostomy, with initial chemical thinning and subsequent manual removal of the last osseous layer. No significant shifts were seen following cochleostomy (A) or on postoperative day 2 or 9 (B).
a buffered solution to ensure that it would not spread to unintended sites.

We were able to completely resorb the otic capsule over the basal turn of the guinea pig cochlea with phosphoric acid alone. However, this technique resulted in statistically significant threshold shifts at the higher frequencies, with only a trend toward so at lower frequencies. It is reasonable that the higher frequencies were more severely affected, as our cochleostomy site lay over the basal turn, and this area corresponds to the higher frequencies on a tonotopic map of the guinea pig cochlea. Steps intermediate to full chemical cochleostomy resulted in preserved hearing. Manually opening the bulla with or without thinning the otic capsule with PAG resulted in no statistically significant threshold shifts (Figure 3).

Fully manual micro-pick cochleostomy resulted in a statistically significant threshold shift at 16 kHz (Figure 4B), with great variability between ears. Some ears had preserved hearing following cochleostomy; however, others had maximal hearing loss (Supplemental Figure S2, available online only). It is possible to perform manual cochleostomy in the guinea pig without significant hearing loss. However, learning to do so reliably can be challenging and time-consuming due to the small size and delicate nature of the cochlea. Development of techniques that are both easy to learn and easy for multiple operators to reproduce would be useful. Accordingly, application of PAG is simple and straightforward, and stray gel can be easily suctioned or wiped off unwanted surfaces. Thinning the cochlear wall first allows for a more controlled manual cochleostomy that requires less force to perform than fully manual cochleostomy and thus is easier to complete.

This combined approach of using PAG to thin the cochlea, followed by manual completion micro-pick cochleostomy, proved to be a minimally traumatic, hearing preservation method (Figure 5A). Hearing preservation persisted up to 9 days following surgery (Figure 5B).

Damage to vascular structures has been hypothesized to be a contributor to hearing loss during cochlear implantation. In a guinea pig study, intrascalar blood has been shown to cause both transient and long-term hearing loss. In this study, because all cochleostomies were performed along the basal turn of the cochlea just proximal to the round window, endosteal veins overlying the scala tympani in this area were inevitably encountered. Endosteal vessels were consistently observed at our cochleostomy site, although their exact location and orientation were variable (Figure 1D and Supplemental Figure S1). In the case of fully chemical cochleostomy, we observed coagulation of these endosteal vessels. Whether this vascular effect or direct PAG entry into the scala tympani was a larger contributor to the observed high-frequency hearing loss was not determined.

User experience has been shown to significantly affect surgical results in numerous otolaryngologic procedures. Thus, when techniques are compared, the order in which surgeries are performed is relevant. One might hypothesize that those surgeries performed later in sequence might result in better hearing results simply due to the surgeon acquiring more experience. In this study, the surgeries performed for group 5 (combined PAG/micro-pick cochleostomy) were performed prior to 4 of the 5 fully manual surgeries performed in group 3. In other words, combined PAG/micro-pick cochleostomy had hearing results superior to those of fully manual cochleostomy despite being performed when the surgeon had less experience.

Because cochleostomies were left open during all postoperative measurements, it is possible that a third window effect increased measured thresholds. However, as the cochleostomy sizes were comparable, this pseudcondutive hearing loss would have affected each cohort similarly.

A limitation of this study is its sample size. The study was adequately powered to detect a threshold shift of 14 dB; however, to detect a shift of 10 dB, 9 subjects would be required in each cohort. Another limitation is the number of animals followed longitudinally. Only 3 animals in group 5 were followed for 9 days, as the other 2 animals in this group were sacrificed immediately after surgery for histologic study. Animals in the fully manual and fully PAG cochleostomy groups were not followed longitudinally. It is possible that the immediate threshold shifts seen were only temporary, as can be observed clinically following stapedotomy. In future studies, groups 3, 4, and 5 could be followed postoperatively for a longer period than in this pilot study, such as for up to 3 months.

Several hurdles would be expected in translating this technique for eventual use in humans. Modifications would be required, because the increase in time needed to resorb the thicker human otic capsule would render this approach impractical in an operating room setting. One solution might be to increase the strength of the PAG by compounding it with a stronger acid. Another tactic might be to start the cochleostomy using a drill, follow with PAG, and then finish manually with a micro-pick. To increase safety, a neutralizing agent or buffer could be prophylactically applied as a physical barrier surrounding the intended cochleostomy site to prevent spread to unintended sites. Refinements in technique, larger cohorts, and longer longitudinal study will be useful for further development of chemical cochleostomy.

Conclusion

We describe a novel technique using PAG to perform chemical cochleostomy in a guinea pig model. When PAG was used to thin the cochlear wall followed by manual completion cochleostomy, this technique consistently preserved normal hearing. Developing easy-to-learn, reproducible cochleostomy techniques is critical for hearing research and for future application in otologic surgery. These data represent proof of concept that alternative means of accessing the cochlea are possible. Future technologies need to be adapted to be both practical and efficient for the thicker human otic capsule.
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Jennifer C. Alyono, conception, study design, analysis, acquisition, interpretation, drafting, final approval; C. Eduardo Corrales, conception, study design, analysis, acquisition, interpretation, drafting, final approval; Markus E. Huth, conception, study design, analysis, acquisition, interpretation, drafting, final approval; Anthony J. Ricci, conception, study design, analysis, interpretation, drafting, final approval.

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Supplemental Material
Additional supporting information may be found at http://otojournal.org/supplemental.

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