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A Polymer-Based Extended Release System for Stable, Long-term Intracochlear Drug Delivery

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Objective: Investigate a new polymer-based drug coating suitability for safe intracochlear delivery and ability to maintain long-term physiologically active levels of the corticosteroid fluticasone propionate.
Study Design: In vitro dissolution study to evaluate release profiles of polymer-coated drug particles and in vivo studies using a guinea pig model to measure perilymph drug concentrations at specific time points after implantation with polymer-coated drug particles and evaluate their effect on hearing function.
Methods: Polymer-coated fluticasone propionate (FP) particles were surgically implanted in guinea pigs through the round window membrane into the cochlear scala tympani. In the pilot study, pre- and post-op hearing thresholds were conducted on days 7, 14, and 42. In a second study, post-op hearing thresholds were conducted on days 90, 120, and 180. Perilymph drug concentrations were measured on the same time points.
Results: In 15 of 16 animals from day 7 through day 90, drug levels were within the targeted range, with no initial burst release detected. Drug was present in all animals on day 90 and was detected in some animals at 120 and 180 days. Hearing was tested and compared with non-implanted ears. Very good hearing preservation was observed in ears implanted with intracochlear particles when compared with contralateral ears.
Conclusions: The polymer-based extended release system is effective in providing long-term, stable drug delivery for at least 90 days with good hearing outcomes. The results of this study support the potential for achieving long-term drug delivery with a single intracochlear administration. **Key Words:** Corticosteroids—Drug delivery—Guinea pig—Inner ear disorders—Polymer.

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Sensorineural hearing loss (SNHL) is a major medical problem with over 36 million affected Americans. Although the understanding of the molecular mechanisms responsible for various forms of hearing loss is in continuous progress, the pathophysiology of many common clinical conditions is still not completely understood. Consequently, there are currently no US Food and Drug Administration-approved pharmacological agents for the treatment of SNHL.

Corticosteroids, particularly glucocorticoids (GC), are commonly used in the clinic for the management of several inner ear disorders including sudden hearing loss, cochlear hydrops, Menière's disease, autoimmune inner ear disease, and certain vestibular disorders. GC receptors (GC-R) are abundant in the cochlea and the vestibular system of rodents and humans (1,2). It has been suggested that the oto-protective effects of GC could be associated, at least in part, with the activation of GC-R in the cochlea (2).

GC have multiple mechanisms of action in the inner ear, including immune suppression, anti-inflammatory action, membrane stabilization, and sodium transport regulation. GC are known to stimulate cochlear supporting cells to release molecular mediators that boost the resolution phase of inflammatory responses (3,4). They also act as vasodilators within the cochlea, increasing cochlear blood flow (5). Additionally, GCs may have a

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direct anti-oxidant effect on the cochlear tissue (6). All of these effects may contribute to the preservation of hearing and reverse hearing loss that is caused by diseases.

Pharmacokinetics studies using a computer simulation suggested that the GC prednisolone had a clearance half-life of 130 minutes in the cochlea, with almost all drug cleared by 24 hours (7). A more recent study estimated that the GC dexamethasone has a half-life in the cochlea of approximately 22.5 minutes (8). Therefore, sustained release formulations are necessary to extend the therapeutic dosage for longer durations and local administration is desired to increase drug exposure to the ear and decrease systemic side effects.

Various methods have been proposed to locally deliver different drugs to the inner ear. While intratympanic steroid injections facilitate absorption into the cochlea, much of the drug is lost through the Eustachian tube. This short period of absorption generates a large basal to apical drug gradient within the cochlea as well as large spikes in levels followed by rapid clearance of the drug from the perilymph. To overcome these issues, passive drug delivery systems, including gel-foams and hydrogels, have been used to concentrate and prolong the exposure to the round window membrane (RWM) (9,10). Active intracochlear drug delivery systems have also been developed to allow for better dosing control, scheduling delivery of multiple drugs, and producing higher drug concentrations and reduced base-to-apex concentration gradients (1,5,11,12). These intracochlear delivery devices include micropumps, microcatheters, and reciprocating fluid systems. While these systems have the ability to provide prolonged, stable drug dosing, they also carry the disadvantage of requiring surgical implantation and continued functioning of the hardware.

This study investigates a new polymer-based, extended release intracochlear drug delivery system's ability to maintain physiologically active levels of drug in the cochlea for long periods, in contrast to traditional forms of steroid administration to the ear (i.e., intravenous or intratympanic injection) which induce large spikes in levels followed by rapid clearance of the drug from the perilymph. The fabrication of the delivery system is highly tunable and can be tailored to develop release profiles of virtually any drug from the order of minutes to the order of years, depending on the desired kinetics. The versatility of the polymers used and potential of therapeutics packaged presents a unique opportunity for the treatment of myriad diseases of the ear from a single platform technology. The current study, using the GC fluticasone propionate (FP), was broken into three sections: 1) development of extended release FP particles in vitro, 2) a pilot pharmacokinetic animal study over 42 days, and 3) a long-term pharmacokinetic animal study over 180 days. We achieved prolonged dissolution rates in vitro and monitored drug release in the perilymph over a 6-month period while evaluating hearing in a guinea pig model.

MATERIALS AND METHODS

Polymer-Coated Steroid particles

Fluticasone propionate (FP) crystals were grown and coated with polyvinyl alcohol (PVA) polymer to produce extended release FP particles. The rate of drug release from the FP particles was modified by altering the thickness of the PVA coating and/or via thermal crosslinking of the PVA.

In vitro Dissolution Studies of Coated Particles

Coated FP particles (3 mg) or FP powder (3 mg), were stirred in 200 mL of 70% methanol:water (n = 3). At indicated time points drug levels were measured via absorbance at 239 nm. Ultraviolet-visible absorption spectra was recorded using a Model 8452A (Hewlett Packard, Palo Alto, CA) diode array spectrophotometer, 1 cm optical path. The final implant particles were selected from the batch of particles with the longest release.

Animal Studies

Thirty-five young albino guinea pigs (*Cavias porcellus*; either sex, 200–300 g) were used following procedures approved by House Research Institute's IACUC. Animal studies were divided into two parts: a Pilot Study lasting 42 days and a Long Term Study lasting from 90 to 180 days.

Hearing Assessments

Baseline hearing tests, consisting of standard auditory brain stem responses (ABR) were administered to all animals before surgery and immediately before euthanasia. Briefly, ABRs were measured under computer control in response to tone pips (5.6–45.2 kHz; 5-ms duration; 0.5-ms rise/fall; cos2 shaping; 30/s) with level adjusted in 5 dB steps over the range required to capture threshold (to 80 dB SPL max). Responses were detected with subcutaneous needle electrodes placed at the vertex and ventrolateral to the ipsilateral ear, with the ground electrode near the tail. Response was amplified (10,000 times), filtered (0.1–3 kHz bandpass) and averaged (across 512 sweeps at each frequency-level combination). All the electrical responses were saved and analyzed offline following termination of the experiments. The stored waveforms were visually inspected and auditory thresholds, defined as the lowest intensity to yield a reproducible deflection in the evoked response trace, were estimated for each ear, and threshold shifts were evaluated by comparison of the means calculated for the experimental groups with respect to the control by using analysis of variance techniques.

Surgery

At least 4 days after hearing assessments animals underwent surgery, receiving a FP particle in the right ear and using the left ear as internal control. Under general anesthesia (IM injection of ketamine 60 mg/kg and xylazine 5 mg/kg), a post-auricular incision was made over the bulla in the experimental ear. A small hole was drilled through the bulla using a posterior approach. A single implant particle was selected for desired 90+ day in vivo drug release duration based on size, picked-up with a 32-gauge covered needle covered with 1% sodium hyaluronate (HA) (10 mg/ml) and put in contact with the perilymph in the scala tympani by puncturing the RWM. The HA from the needle remained on the RWM, minimizing perilymph leakage. A suture was used to close the wound.

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Sampling of Perilymph

After euthanasia, the temporal bones corresponding to the implanted cochleae were removed, the bulla opened and placed in a microscope stage. The sampling instrument was prepared from a 10-μl 1701 LT Hamilton syringe (Sigma–Aldrich, St. Louis MO), using a 2 cm-long MicroFil nonmetallic needle (model MF34G, 0.16 mm OD, 0.1 mm ID; WPI, Sarasota, FL). The syringe was mounted on a PatchMan electronic micromanipulator (Eppendorf, Germany), and the needle was directly advanced through the RWM to approximately 1.0-mm into the perilymphatic cistern, and then withdrawn after collecting ~3 μl.

Fluticasone Propionate Levels in Perilymph Samples

Perilymph samples obtained at sacrifice were analyzed by liquid chromatography–mass spectrometry (LC-MS) using an API 3000 (AB-Sciex, Framingham, MA) working in Multiple Reaction mode. Results were compared with standardized FP concentrations to calculate FP concentrations in the perilymph.

LC Conditions

Agilent System: 1314 quaternary pump, 1367A autosampler, 1100 variable wavelength detector, 1200 solvent degasser (Agilent Technologies, Santa Clara, CA); analytical column: Kinetex C8 2.1 × 100 mm 2.6 μm (Phenomenex, Torrance, CA); guard column: Kinetex C8 2.1 × 10 mm 2.6 μm (Phenomenex, Torrance, CA); mobile phases: A: 0.1% acetic acid, B: 0.1% acetic acid in methanol; flow rate: 0.3 ml/min; injection volume 10 μl; LC Gradient Program: 3 minutes isocratic run, 30:70 A:B; Internal standard: rifampicin.

MS Conditions

Instrument: API 3000 (AB-Sciex, Framingham, MA); Mode: Multiple reaction monitoring; Extracted ion mode, *m/z*: 501 → 293.3 (FP), 822.3 → 98.4 (rifampicin); retention times: 2.3 minutes (FP), 1.2 minutes (rifampicin); ionization source: TIS+ (4500 V); Gas 1: 10 at 90 psig; Gas 2: 500 °C at 8 L/min and 90 psig.

RESULTS

In vitro Dissolution Studies

Sustained release fluticasone propionate (FP) particles were developed and used in these studies (Fig. 1). Different formulations of the coated FP particles were developed by altering polyvinyl alcohol polymer thickness and crosslinking. These were compared with uncoated FP crystals using an in vitro dissolution system to assess the controlled release functionality of the polymer coatings. The dissolution study was performed in 70% methanol, allowing for comparison of dissolution from the different coated particles over a period of a few hours as opposed to months (Fig. 2).

In vivo Pharmacokinetics

Guinea pigs were used for in vivo studies to assess the pharmacokinetics of drug release in the cochlea. According to the literature, FP has an effective dose of ~10 pg/mL (EC50 of ~7–30 pg/mL (13)) in cell culture. Based on this, we targeted doses three orders of magnitude above this, at least 10 ng/mL, designed to last for at least 90 days (Fig. 3).

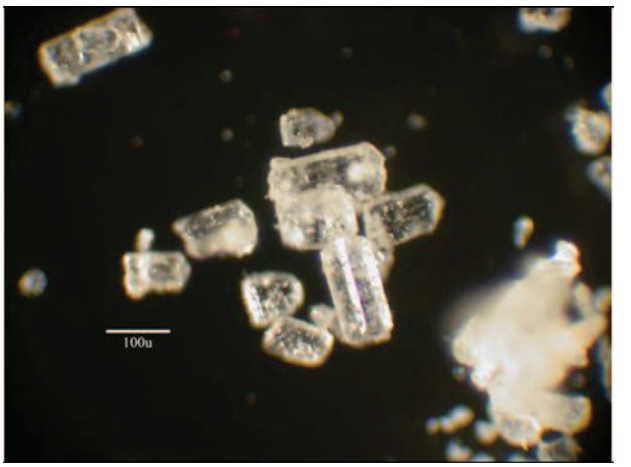

FIG. 1. Crystallization of fluticasone propionate. Fluticasone propionate was crystalized in methanol before coating with the sustained release polymer. Clear crystals were obtained, dried at 70 °C for 24 hours, and filtered through mesh sieves. Scale bar is 100 μm.

Two studies were undertaken to assess the performance of the FP particles: a Pilot Study aimed at monitoring drug release over the first 42 days following implantation to determine if FP could be delivered to the cochlear for up to 42 days, and a Long Term Study to monitor drug release at 90, 120, and 180 days after implantation for the Pilot Study, perilymph samples were collected at 1, 7, 14, and 42 days after implantation (Fig. 4). The results demonstrate reproducible drug release into the cochlea over a 42-day period with no burst release observed. There was some variability of drug levels observed in the cochlea in this Pilot Study, with detected levels ranging from 1 to 270 ng/ml.

Following the Pilot Study, a greater emphasis was made to normalize the size and shape of the coated FP particles. These carefully selected particles were used in the Long Term Study. This second study monitored drug release at 90, 120, and 180 days after implantation (Fig. 4). All animals at 90 days (n = 5) had quantifiable levels of FP. A more consistent level of drug was detected at day 90 in the long-term study as compared with the pilot study (Fig. 4), possibly due to the more robust selection criteria used to choose particles. Based on our delivery design, we expected a significant decrease in drug release after 90 days. Consistent with our expectations, we found that only two of the four animals at 120 days and one of the four animals at 180 days had quantifiable levels of FP. The perilymph of contralateral ears was tested for drug presence and none was detected in any subject (data not shown).

In vivo Hearing

In the Pilot Study, auditory function was assessed via ABRs in animals before drug implantation and at 1, 7, 14, and 42 days after implantation. All animals had normal hearing pre-implantation. At 1 day (n = 5), middle ear

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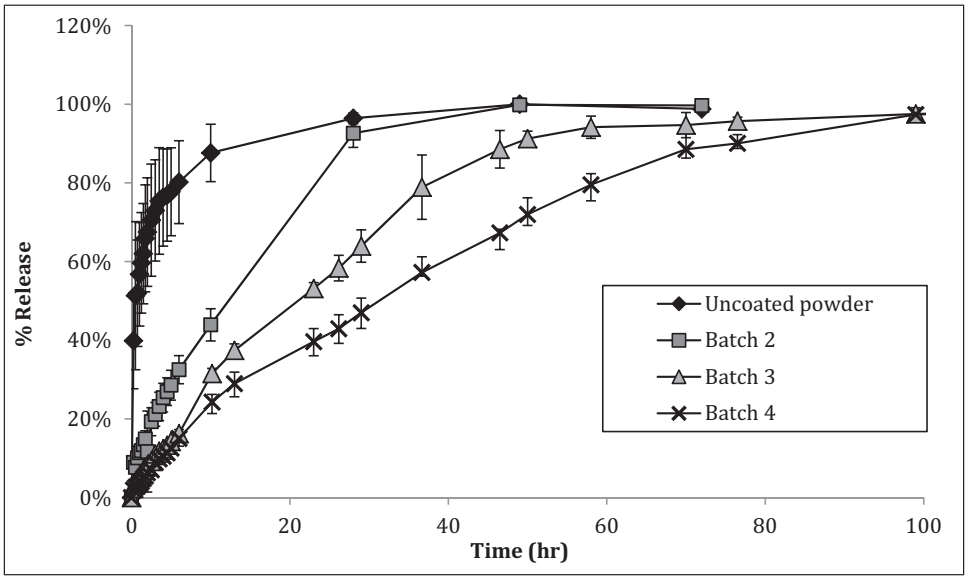

FIG. 2. In vitro dissolution of coated versus uncoated fluticasone propionate particles. Polyvinyl alcohol coated fluticasone propionate particles (3 mg) or fluticasone propionate powder (3 mg) were stirred in 200 ml of 70% methanol:water at room temperature (25 °C) at 30 RPM (n = 3). At the time points indicated samples were measured for drug levels at absorbance 239 nm. Batches 2, 3, and 4 refer to different polymer coating/crosslinking combinations.

effusion was found in all animals due to surgery. ABR measurements demonstrated a 15 to 30 dB change in threshold, possibly due to the effusion. At 7 days (n = 5), ABR measurements were within 10 dB of the pre-implantation threshold. A separate group of animals were tested at 14 days. One animal lost hearing after implantation and the rest were similar to the 7-day-old animals. At 42 days, one animal died during follow-up due to a surgical infection and was not able to be tested. The remaining four animals had ABR thresholds with an average of 10 dB difference from threshold (Fig. 5). At all time-points any difference in hearing was not considered statistically significant. Overall the hearing preservation rate was considered very good.

In the Long Term Study auditory function was assessed via ABRs in animals before drug implantation and at 90, 120, and 180 days after implantation. At 90, 120, and 180 days all animals demonstrated progressive hearing loss in both ears (implanted and control). The cause of this loss in hearing is not known but it may be consistent with progressive hearing loss previously observed in albino guinea pigs as they age (14). At 90 days (n = 5), 120 (n = 5), and 180 (n = 5) days post implantation, ABR measurements showed hearing was equivalent or better in ears implanted with extended release FP particles as compared with contralateral, control ears. At all time-points any difference in hearing was not considered statistically significant. This suggests good hearing preservation and tolerance of the FP particles. There were no adverse events with any of the study animals. The results of this second study show that a safe method for intracochlear drug administration can be developed. We think that with a surgical insertion tool, our technique for implantation could be standardized to allow consistent hearing preservation.

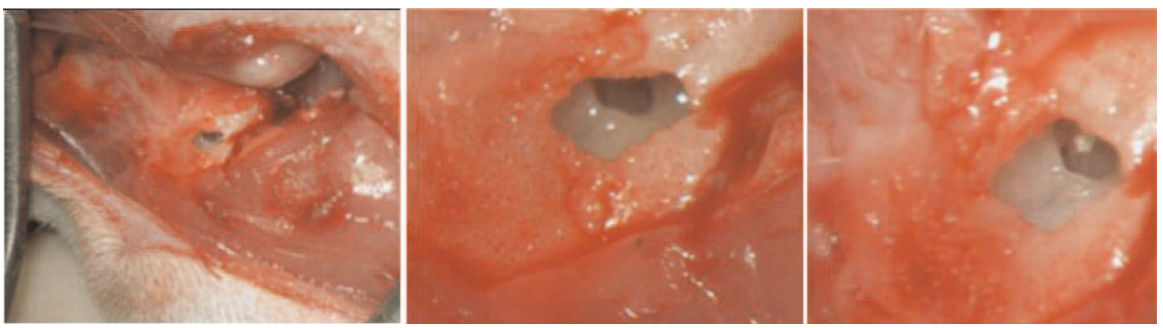

FIG. 3. Implantation of fluticasone propionate particles. Albino guinea pigs were implanted with a single intracochlear dose of coated fluticasone propionate particles in their right ear. The contralateral ear was used as internal control.

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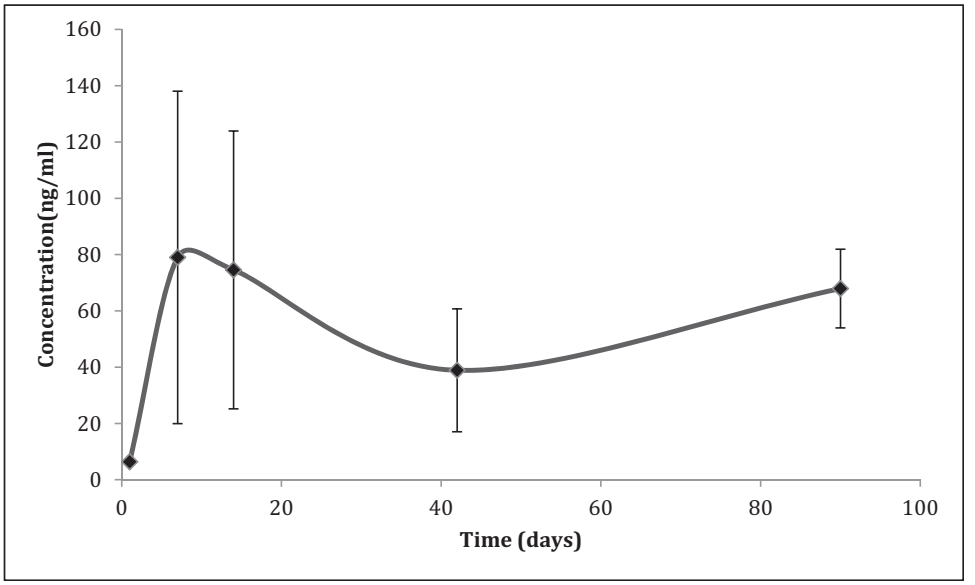

FIG. 4. Fluticasone propionate levels in the perilymph. Perilymph samples were obtained and analyzed as described in the Methods. Samples were obtained at sacrifice. Pilot Study: n = 1 (Day 1), n = 4 (Day 14, 42), n = 5 (Day 7); Long Term Study: n = 5 (Day 90), average drug levels at specific time-points are presented. Error bars represent standard error. Additionally, two of four animals at 120 days and one of four animals at 180 had detectable drug. These data are not included on the graph.

Safety

We had concerns about the potential local and systemic side effects from long-term steroid exposure. The hearing results in this study suggest that the FP–polymer combination is well tolerated in animals following long-term implantation. No behavioral changes, alterations in appetite, or weight loss were observed in any of the test animals, demonstrating that the steroid levels and surgery were well tolerated. This suggests that the polymer used in the implant particle and the levels of FP present in the cochlea following release from the particle appear to be non-toxic. This lays the foundation for further studies to corroborate the safety of the materials and implantation surgery in an animal model.

DISCUSSION

We have implanted a polymer-based drug delivery system into guinea pig cochleae, measured drug levels in the perilymph to assess pharmacokinetics, and evaluated the impact of this implant system on auditory function. All measured drug doses were above the targeted therapeutic range of 10 ng/ml as of day 7 with the exception of one animal at 42 days. There was no observed ''burst'' release of steroid and drug was detected in the cochlea of one animal for at least 180 days, far surpassing previous reports for other inner ear extended release delivery systems.

In the Pilot Study we found very good hearing preservation, with an average ABR threshold difference of 10 dB at 42 days respect to pre-implantation values. In the Long Term Study, ears containing the FP particle exhibited no hearing impairment when compared with contralateral ears at 90, 120, or 180 days post implantation. At no time point in either study were differences in hearing between control and implanted ears considered statistically significant (Fig. 5). We conclude that the intracochlear polymer-based extended release system described may achieve long-term steroid release in a manner that can safely preserve hearing without the reliance on multiple dosing, implanted, or external hardware.

Drug Delivery Systems

Previous studies investigating locally delivered GC for the treatment of inner ear disease have used both passive and active drug delivery systems. Pumps and wicks have disadvantages associated with their use, including difficulty reproducibly producing the desired release rate and effective concentration of the drug. This is largely due to a dependence upon the precise placement of the device, and/or a variability of drug release from the devices, in combination with the poorly predictable rate of diffusion of the drug into the inner ear through the RWM. The drug delivery particles described here bypass these issues through direct intracochlear implantation, eliminating the variable diffusion through the round window membrane and the need for implanted hardware. Unlike micropumps or reciprocating drug release systems, there is no fluid movement or fluid exchange between the cochlea and extracochlear space, further minimizing the risk of trauma. By maintaining a closed system within the cochlea after implantation, risk of infection and trauma to the inner ear is likely also reduced.

To avoid the invasive surgical procedures required for pumps, wicks, etc., improvements on passive

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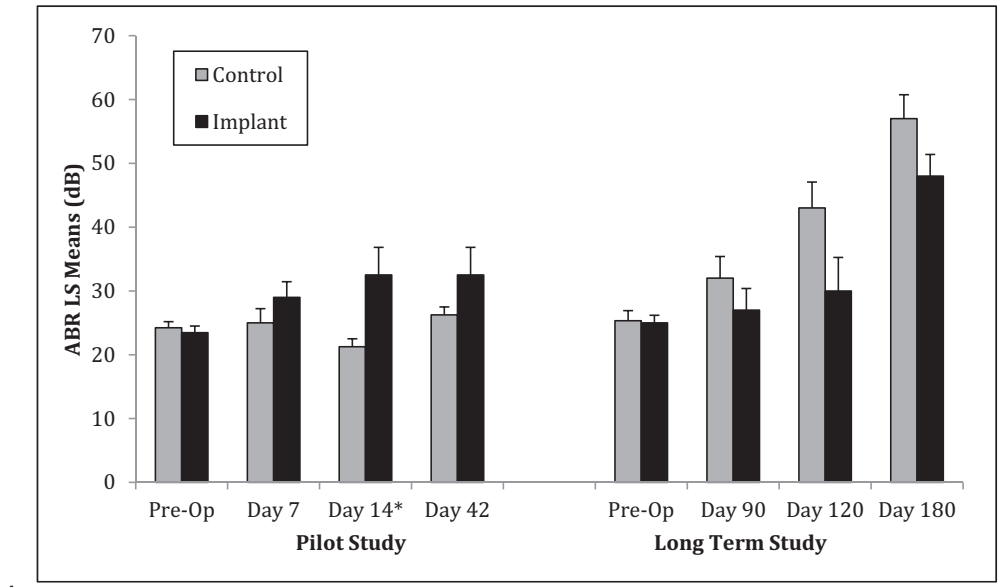

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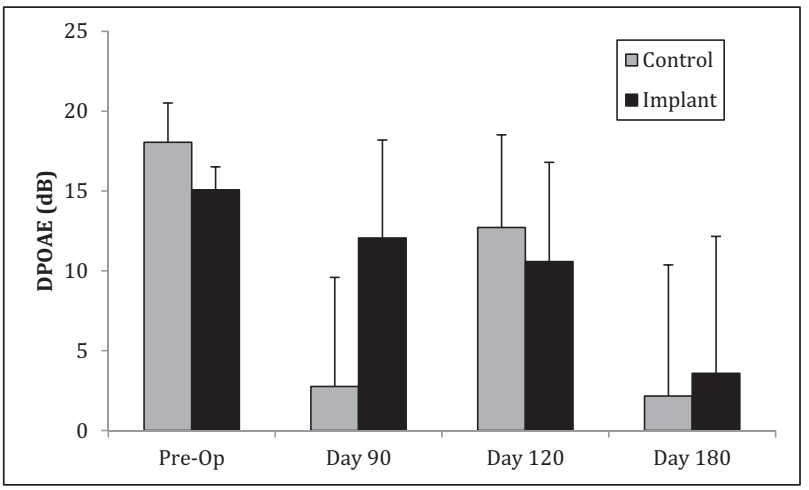

B

FIG. 5. *A*, Post-implantation hearing assessment—ABR. Hearing was monitored as described in the Methods before sacrifice. n = 15 (Pre-Op), n = 4 (Day 14, 42), n = 5 (Day 7, 90, 120, 180). Error bars represent the standard error. Control (C) and Implanted (I) ABR values ± standard error: Pre-Op (Pilot Study): 24.2 ± 1.0 (C), 23.5 ± 1.0 (I); 7 day: 25.0 ± 2.2 (C), 29.0 ± 2.4 (I); 14 day: 21.3 ± 1.3 (C), 32.5 ± 4.3 (I); 42 day: 26.3 ± 1.3 (C), 32.5 ± 4.3 (I). Pre-Op (Long Term): 25.3 ± 1.6 (C), 25.0 ± 1.2 (I); 90 day: 32.0 ± 3.4 (C), 27.0 ± 3.4 (I); 120 day: 43.0 ± 4.1 (C), 30.0 ± 5.2 (I); 180 day: 57.0 ± 3.7 (C), 48.0 ± 3.4 (I). * Day 14 data—one animal lost hearing and this was eliminated from the calculations. At no time point were differences in hearing between control and implanted ears considered statistically significant: Pre-Op (Pilot Study): $p = 0.5912$; 7 day: $p = 0.2623$; 14 day: $p = 0.0907$; 42 day: $p = 0.2148$. Pre-Op (Long Term): $p = 0.8275$; 90 day: $p = 0.3276$; 120 day: $p = 0.0857$; 180 day: $p = 0.1126$. *B*, Post-implantation hearing assessment, DPOAE—Long term study. Hearing was monitored as described in the Methods. Hearing was tested before sacrifice. n = 15 (Pre-Op), n = 5 (Day 90, 120, 180). Error bars represent the standard error. Control (C) and Implanted (I) DPOAE values ± Standard Error: Pre-Op: 18.1 ± 4.3 (C), 15.1 ± 2.5 (I); 90 day: 2.7 ± 5.8 (C), 12.1 ± 6.1 (I); 120 day: 12.7 ± 5.8 (C), 10.6 ± 6.2 (I); 180 day: 2.2 ± 8.2 (C), 3.6 ± 8.6 (I). At no time point were differences in hearing between control and implanted ears considered statistically significant. Pre-Op: $p = 0.3052$; 90 day: $p = 0.3409$; 120 day: $p = 0.8076$; 180 day: $p = 0.9078$. DPOAE indicates distortion product otoacoustic emissions.

intratympanic drug delivery have been made by developing technologies such as depots, hydrogels, and nanoparticles (15–18). Extended release hydrogel formulations containing dexamethasone have been developed for use in the ear. Wang et al. (16) showed that the drug concentration and duration of drug delivery can be altered by varying the concentrations of a poloxamer hydrogel and drug with significant levels of dexamethasone detected for at least 10 days after treatment. Piu et al. (17) continued work with this hydrogel and were able to achieve sustained levels of dexamethasone for 3 months in guinea pigs or 1 month in sheep. Despite the promising nature of these hydrogel formulations, their potential uses are somewhat restricted due

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to the large bolus release of drug followed by the orders of magnitude drop off and gradual decline of released drugs. Nanoparticles (NPs) continue to be explored for their potential in drug delivery to the inner ear. Du et al. (18) used supramagnetic poly(lactic-co-glycolic) acid-magnetite dexamethasone-acetate NP delivery under an external magnetic field to significantly increase round window membrane (RWM) permeation. In this study, the RWM became loaded with nanocomposites leading to continued drug release into the cochlea for weeks as PGLA degraded and drug continued to passively diffuse. These formulations also significantly decrease bolus levels of dexamethasone in the perilymph as compared with intratympanic injections of dexamethasone.

Although clinical trials have proven the efficacy of GC in the treatment of multiple inner ear diseases, the actual biochemical function of steroids within the inner ear has not been completely elucidated yet (19–25). GC pharmacokinetics in the cochlea varies greatly depending on the method of delivery. Research has shown that there is an early basal-apical concentration gradient when drug is required to diffuse across the RWM and there are substantial drug gradients across the scalae in the basal turn (12). However, sustained release systems of GCs have been shown to produce a more consistent distribution of drug throughout the inner ear (26). Sustained release allows for drug to continuously move from high to low concentration, thus diminishing gradients in the cochlea.

Safety/Toxicity

Further studies are needed to evaluate any histologic evidence of trauma or ototoxicity within the inner ear due to the implantation of FP particles. However, preliminary hearing results in this study suggest that the delivery system is safe as it did not produce any hearing impairment following long-term implantation when compared with contralateral ears. Progressive hearing loss was observed in both ears (implanted and non-operated) between pre-op measurements through 180 days. This may be consistent with progressive hearing loss due to age observed in a study by Nozawa et al. (14). A 15 to 17 dB shift in ABR thresholds was seen between animals 2 to 4 months and 13 to 15 months of age. Though consistent with the observations by Nozawa, the loss in hearing in this study was more significant and could be due, in part, to the surgical or pharmacological intervention. Additional work is needed to completely exclude this possibility.

There is a long history of placing implants into the middle and inner ear including stapes prostheses and CIs, but this is the first study to evaluate the implantation of a drug particle into the inner ear in a single procedure designed to provide long-term drug release. While the data indicate that the FP particles can release drug for up to 180 days, continued development is required to produce particles with longer release durations. Refinement of particle selection and modifications of the polymer coating(s) are ongoing. Additional studies out to 180 days demonstrated no additional adverse effects beyond the aforementioned progressive hearing loss in both ears (i.e., no infections, weight loss, or behavioral changes). Considering that our delivery system is far less invasive than many other implantable devices, the likelihood of it causing an increased risk of infection is felt to be low.

The polymer coating being studied has previously been used for intraocular drug delivery with successful drug release for periods of 2.5 to 3 years without evidence of ocular toxicity (27,28). While the formulation used in this study was designed to last for at least 90 days, it can be altered to produce shorter or longer durations of drug release. The surgical procedure required for implantation of the particles into the cochlea brings the added risk of infection and trauma, but with standardization of the implantation technique, it is felt that both infection and trauma can be minimized.

CONCLUSION

Both in vitro and in vivo studies have found that the polymer-based extended release drug delivery system is effective in providing long-term, stable drug delivery with good hearing outcomes. Even longer duration of intracochlear drug delivery than those reported in the present work could be achieved by altering the formulation of the polymer while still maintaining a single drug administration without reliance on implanted hardware.

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