Intratympanic Injection Drug Delivery System for Extended Steroid Delivery to the Inner Ear

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ABSTRACT SUMMARY
There are no FDA-approved drugs for the treatment of inner ear diseases that afflict millions of Americans every year. In their place, physicians often prescribe drugs off-label that – whether delivered orally or through local injection to the ear – lack safety data and show widely variable clinical responses.1,2 Here, we describe Orbis Biosciences’ innovative inner ear drug delivery platform – a system comprised of drug-loaded microspheres suspended in a Fast Film-forming Agent (FFA) that localizes microspheres to the Round Window Membrane (RWM) in the middle ear. This system could enable cost-effective, local delivery and extended-release of new and existing drugs, thereby providing physicians and patients new safe and effective treatments for debilitating diseases of the inner ear.

INTRODUCTION

New medicines are needed to better treat the millions of Americans affected by debilitating diseases of the inner ear, including sudden sensorineural hearing loss, autoimmune inner ear disease, tinnitus, and Meniere’s disease. Current approaches to local delivery of therapeutics to the inner ear offer either high control of dose levels or low administration costs - but not both. This significantly limits their commercial viability and the potential to improve patients’ lives.

We developed a novel microsphere-based intratympanic drug delivery system that uses a FFA to inject and subsequently secure drug-loaded microspheres to the RWM of mice. We demonstrate this system localizes microspheres to the RMW for greater than thirty-five (35) days with no evidence of inflammatory response, suggesting that this system is well-tolerated.

EXPERIMENTAL METHODS

Uniform betamethasone-loaded biodegradable microspheres were prepared using Orbis’ Precision Particle Fabrication technology. Briefly, betamethasone was dissolved in dichloromethane (DCM), to which a 50:50 poly (D,L-lactic-co-glycolic acid) (PLGA) was added such that the betamethasone comprised 1.0% w/w of the total solids content. The suspension was loaded into a syringe pump and used to produce microspheres using Orbis’ Precision Particle Fabrication (PPF) nozzle. The microspheres were collected in a solution of poly (vinyl alcohol) in deionized (DI) water. Following a 3-hour solvent evaporation step, the particles were filtered and lyophilized for 48 hours (Fig. 1).

In vitro betamethasone release from the PLGA microspheres was measured by depositing drug-loaded microspheres suspended in a proprietary FFA on a cellulose acetate membrane affixed to a Franz Cell. Franz cells were filled with receptor solution and sampled daily for eight (8) days.

Betamethasone-loaded microspheres suspended in the FFA were delivered to mice RWM as follows. C57/BL6 mice were anesthetized with a Ketamine/Xylazine cocktail, laid on their side, and immobilized. The skin and soft tissue was retracted, and an access hole to the tympanic cavity was created with a 28 GA needle. ~2.0 μL injections of 50 mg/mL fluorescent dye-loaded microspheres were then delivered directly above the RWM with a 10 μL Hamilton syringe. The mice were kept in this position for 5 minutes before being sutured and imaged on an IVIS in vivo Imaging System (Perkin-Elmer, Waltham MA) to confirm localization of the formulation to the inner ear space, and

Figure 1. Betamethasone-loaded PLGA microspheres.

Figure 2. Microsphere release kinetics versus size.
injected with fluorescent microspheres without the FFA component (saline vehicle). At 21 and 35 days timepoints, mice were sacrificed and necropsy was performed to evaluate microsphere localization. To measure potential inflammatory response, mice were euthanized at 28 days, and the inner ear anatomy was isolated, removed, decalcified, and paraffin-embedded. Samples were sectioned and immunohistochemically stained for two major inflammatory markers, interleukin (IL)-6 and tumor necrosis factor (TNF)-α, in addition to hematoxylin and eosin (H&E).

RESULTS AND DISCUSSION

In vitro dissolution testing of betamethasone-loaded microspheres demonstrated that microsphere size plays a role in controlling the release of drug from the microspheres.

At 21 days, mice treat with microspheres suspended in the FFA had microspheres localized directly on the RWM with a thin film as intended (Fig. 3A). There appeared to be only a slight loss of sphericity, indicating that some degradation of the particles occurred, but the overall integrity of the delivery system was maintained. Negative control mice displayed no visible microspheres, indicating that the particles had migrated away from the surgical site due to lack of an FFA component (Fig. 3B). Similarly, at 35 days, an analogous set of mice were euthanized and dissected. Once again, we were able to see a deposition of microspheres in the space directly adjacent to the RWM in mice treated with microspheres suspended in the FFA (Fig. 3C), and an absence of microspheres in negative control mice (Fig. 3D).

Staining indicated that the microspheres and FFA caused no significant inflammatory response. The intensity of TNF-α and IL-6 development was similar between the groups (data not shown), and H&E revealed no discernible changes in hair cell anatomy or apparent tissue reaction (Fig. 4A, B).

CONCLUSION

Using Precision Particle Fabrication, an injectable, extended release intratympanic delivery system was developed that can localize drug-loaded microspheres to the RWM of mice for greater than 35-days with minimal inflammatory response.

REFERENCES


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Figure 3. Microspheres visible upon necroscopy at (A) 21 and (C) 35 days. Omission of an FFA component will not adhere microspheres to the RWM at (B) 21 or (D) 35 days. Scalebar = 400 μm (A,B) and 300 μm (C,D).

Figure 4. Pathology evaluation following dosing with microspheres suspended in an FFA. Mice treated with this formulation (A) had no significant TNF-α presence compared to negative controls (B).