Alginate Ototoxicity in the Mouse Model

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Abstract

Objective. To determine whether alginate exposure to the round window of the mouse causes any measurable ototoxicity.

Study Design. Prospective animal study.

Setting. Basic science laboratory affiliated with a tertiary care university medical center.

Subjects and Methods. After Institutional Animal Care and Use Committee approval, 5 adult mice were obtained and underwent bulbostomy and round window niche application of alginate. Auditory brainstem response (ABR) tests were completed at baseline prior to the procedure and also 5, 14, and 30 days postprocedure. Results were compared. At termination of procedure, the mice were sacrificed with harvest of the cochleae, which were viewed under histologic section.

Results. There were no significant increases in ABR thresholds in any of the test animals at all test periods after alginate exposure compared to baseline. There were also no observable behavioral changes after the procedure to indicate vestibular dysfunction. Cochlear sectioning revealed no evidence of histologic damage.

Conclusion. Exposure of alginate to the round window does not cause any obvious ototoxicity in the mouse model. Further clinical trials will be needed to elucidate the effect of alginate in the human middle ear.

Keywords
alginate, middle ear packing, resorbable material, ototoxicity

Methods

Experimental Design

After Institutional Animal Care and Use Committee (IACUC) approval by the University of Kansas Medical Center, animals (5 C57BL/6 mice) were obtained from Jackson Laboratories (Bar Harbor, Maine) between 8 and 10 weeks of age. Auditory brainstem response (ABR) tests were performed using Smart EP program (Intelligent Hearing Systems, Miami, Florida) on the mice to ensure hearing capability on bilateral ears, with a minimum of 128

The use of packing in the middle ear has been common practice in otologic surgery for many decades. A variety of different materials have been used. One of the most regularly used materials is absorbable gelatin sponge, Gelfoam (Pfizer, New York, New York). It has been applied as a middle ear packing in tympanoplasty and also as a seal for the oval window during stapedectomy. Previous formulations of commercially available absorbable gelatin sponge contained dilute formaldehyde, which in studies on cats was shown to cause fibrosis of the vestibule and destruction of the utricle and the saccule. In addition, retrospective series on stapedectomies have shown that closure with absorbable gelatin sponge resulted in a higher incidence of oval window fistula compared to vein grafts. Animal models have also shown the high incidence of fibrosis in the middle ear with absorbable gelatin sponge. Today, there is interest in finding potential alternatives. Alginate is a polysaccharide compound that has found numerous applications in biomedical science and engineering due to its favorable properties, including biocompatibility and ease of gelation. Alginate packing has been tested in the paranasal sinuses and shown to cause less mucosal edema and adhesions compared to conventional packing material. We aim to determine the potential ototoxicity of alginate on the mammalian inner ear by using the mouse model. We propose a commonly employed direct approach to the round window niche for application.

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sweeps. Stimulus tones were presented by using ear canal inserts in the mice while in an acoustic chamber. We obtained alginate sponge samples from Grace Medical (Memphis, Tennessee). The material was implanted in the test mice, after which the mice recovered for 5 to 7 days. ABR tests were then repeated at an interval of 5, 14, and 30 days after surgery. The mice were then sacrificed, and the cochleae were harvested. Cochleae were then fixed in paraffin and analyzed with hematoxylin and eosin (H&E) stain under light microscopy. Further details of the procedure can be seen as follows.

**Material Preparation**
Alginate sponge composed of both calcium and sodium alginate was obtained from Grace Medical, deposited in pre-made wells (Figure 1). One-eighth of each well of alginate was rehydrated in 0.5 cc of 0.9% sterile normal saline in an autoclaved 5-mL Eppendorf tube (Figure 2). Hydration of the alginate compound created a malleable, sponge-like consistency of material that was able to be surgically packed, similar to absorbable gelatin sponge.

**Hearing Testing (ABR)**
Animals were anesthetized with an intraperitoneally administered mixture of ketamine (100 mg/kg) and xylazine (5 mg/kg) and placed in a double-wall acoustic chamber. Testing was performed using the Smart EP program. Electrodes were placed on the vertex (+), behind the test ear (–), and behind the opposite ear (ground). Tone bursts were presented for ABRs at 4 to 32 kHz (pure-tone stimulation) prior to surgery (baseline) and at day 5, day 14, and day 30 postsurgery. The results over the study period were compared with statistical analysis in Microsoft Excel v16.0 (Microsoft Corporation, Redmond, Washington). An example of an ABR recording from mouse 1 can be seen in Figure 3.

**Surgical Procedure**
Animals were anesthetized with an intraperitoneally administered mixture of ketamine (100 mg/kg) and xylazine (5 mg/kg). The left postauricular skin was sterilized. With the aid of a microscope, a 1-cm postauricular incision was made behind the left ear using a scalpel. Blunt dissection was then used to tease through the subcutaneous tissue, exposing the cleidomastoidus muscle. The muscle was then retracted caudally, exposing the facial nerve, the posterior semicircular canal, and the bulla. An opening was made in the bulla with an insulin syringe. The stapedial artery and round window were identified after uncapping the bulla. The alginate sponge was then packed into the middle ear space until no more material could be inserted. This amount was approximately one-quarter of a rehydrated sample. The opening was then closed with surrounding subcutaneous tissue. The skin was reapproximated. The animals were allowed to recover for 5 days prior to audiometric testing. They were given ketoprofen on postoperative days 1 and 2 during the recovery period.

**Cochlear Histology**
After the last ABR tests were performed, mice were anesthetized with Beuthanasia-D (10 mg/kg; Schering-Plough Animal Health, Madison, New Jersey) and perfused with 4% paraformaldehyde in 0.1M phosphate-buffered saline (PBS) via cardiac perfusion. Temporal bones were extracted from each mouse and the tympanic bulla was exposed. The oval and round windows were opened to allow perfusion of fixative. Specimens were kept in fixative overnight at 4°C. After fixation, mouse temporal bones were decalciﬁed in RDO Gold de-calciﬁer (Apex Engineering Products Corporation, Aurora, Illinois) for 60
minutes. Tissues were processed in a paraffin processor and sectioned to an 8-micron thickness. Tissues were prepared with H&E stain to demonstrate morphology. Slides were then viewed under light microscopy.

**Results**

All 5 mice recovered uneventfully, without any observed behavioral changes to indicate potential gross vestibular dysfunction. On microscopic examination of the middle ear at 30 days, no remnant packing material was visible. ABR results in all 5 mice showed normal hearing thresholds at 5, 14, and 30 days after surgery. These data can be seen in Table 1. The ABR trends averaged for all 5 mice across the test period can be seen depicted in Figure 4, with error bars for 1 standard deviation included. No statistically significant increase was noted in ABR threshold at 30 days compared to baseline ABR. These results indicate that there was no significant drop in hearing after exposure to alginate, suggesting that there were no ototoxic effects on the auditory component of the inner ear from the compound.

After sacrificing the animals, the fixed cochleae for each of the test mice were sectioned and observed under light microscopy with H&E stain. Figure 5 shows representative sections from one of the animals. Figure 5A shows a mid-modiolar section through the cochlea with normal architecture noted of the cochlear turns. Figure 5B,C shows medium- and high-powered images, showing the scala media (Figure 5B) and the scala tympani with round window membrane (Figure 5C). There is no structural damage or inflammatory infiltrate present. In Figure 5B, there is some artifact involving the tectorial membrane and organ of Corti; however, this is within the expected range after paraffin processing. Typical histologic changes of the cochlea after exposure to ototoxic medications include cellular infiltrate and cystic separation of the stria vascularis,\(^7,^8\) which is not seen in our sections. These findings were consistent in all 5 test mice.

**Discussion**

Middle ear packing is frequently used in otologic surgery. The perceived benefits of packing include the ability to
provide structural support to prostheses or grafts, to maintain aeration, and to assist in hemostasis. However, conventional packing materials are not without their potential to cause harm. As stated, despite the widespread use of materials such as current commercially available absorbable gelatin sponge, there exist concerns regarding its role in potential adhesions and fibrosis in revision middle ear surgery. The material has been shown to produce fibrosis as well as vascular neogenesis in the rat middle ear mucosa. Liening et al conducted a study in which they compared the use of absorbable gelatin sponge, absorbable gelatin sheet, or absorbable collagen sheet prospectively in the rat middle ear. They found that gelatin sponge caused a significantly higher rate of fibrosis in the rat middle ear mucosa compared to the other 2 materials. Certainly, there is room for improvement and for the development of other potential agents.

The ideal agent would be one that is biologically inert so that it induces a minimal host reaction while still being capable of providing structural support. The material should also be resorbable so that no packing is left in the middle ear, which could cause sclerosis or dampening of sound wave transmission. Alginate is a natural occurring anionic polysaccharide polymer. It has been extensively investigated as a potential biomedical agent due to its biocompatibility and low toxicity, as well as ease of synthesis. The polymer can be combined with cations such as Ca$^{2+}$ or Na$^+$ to form a gel compound that can be used in wound healing or drug delivery. Numerous studies have investigated the use of alginate-based dressings in the treatment of chronic wounds such as diabetic foot ulcers. In addition to wound healing, investigators have also evaluated alginate’s effect on fibrosis. Back et al showed that alginate-impregnated dressing caused significantly less postoperative adhesion and fibrosis after experimental laparotomy in the rabbit model. Alginate-coated silicone sheeting has been used to deliver dexamethasone to the middle ear mucosa of mice.
guinea pigs, which showed favorable decreases in fibrosis.\textsuperscript{16} The material has also been structurally linked to other active biologic agents such as antibiotics.\textsuperscript{17} Thus, not only could alginate-based packing decrease fibrosis, but it could also potentially deliver sustained antibiotics to the middle ear during the postsurgical healing phase.

In human studies, alginate-based packing material has been studied in endoscopic sinus surgery. In a randomized study of 27 patients, Park et al\textsuperscript{5} showed that alginate packing caused less mucosal edema and adhesion on postoperative endoscopy compared to carboxymethyl cellulose packing. Other studies have also shown alginate-based packing to be well tolerated in the nasal cavities with less patient discomfort.\textsuperscript{18,19} This reduced edema and adhesion would be useful in middle ear surgery as well.

Prior to testing alginate in the human middle ear, a safety profile has to be established. Our study shows that in the mouse model, there is no obvious ototoxicity after exposure of the inner ear to this biologically inert polymer. ABR thresholds remained unchanged throughout the study duration, indicating that there was no significant hearing loss in any of the test mice. In addition, cochlear histology revealed no evidence of infiltrate or damage. Again, this suggests that there is no toxic damage on the inner ear from the alginate.

There are limitations to this study. Naturally, the results of an animal model may not translate to human subjects. In addition, healthy middle ear mucosa in mice may not accurately reflect the diseased middle ear mucosa of human patients undergoing chronic ear surgery. As this experiment addressed initial toxicity only, we did not study the supportive capability of the alginate sponge (eg, for grafts or prostheses). This could certainly be an avenue to explore in the future.

**Conclusion**

There was no evidence of any hearing loss either in the acute or late period after alginate placement. No evidence of balance problems was seen on behavioral evaluation. Evaluation of animals after termination of the experiment demonstrated that all the material had resorbed. Alginate does not appear to cause ototoxicity to the mouse ear when exposed to the round window membrane. Further clinical trials are required to elucidate the safety of alginate in the human middle ear.

**Author Contributions**

Sameer A. Alvi, analysis of data, manuscript drafting, final approval of manuscript, agrees to be accountable for all aspects of work; Jennifer Nelson-Brantley, acquisition and analysis of data, manuscript revision, final approval of manuscript, agrees to be accountable for all aspects of work; Hinrich Staecker, study design and analysis of data, manuscript revision, final approval of manuscript, agrees to be accountable for all aspects of work.

**Disclosures**

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**References**


