

Sustained-Release Delivery of Leupeptin in the Chinchilla: Hearing Results

Michael E. Hoffer,¹ J. Clay Finley,¹ Peter Killian,¹ Kim R. Gottshall,¹ Ben J. Balough,¹ Derin Wester,¹ and Abraham Shulman²

¹Department of Defense Spatial Orientation Center, Department of Otolaryngology, Naval Medical Center San Diego, and ²Martha Entenmann Tinnitus Center, Health Science Center, State University of New York, Brooklyn, NY

Abstract: Transtympanic medical therapy is becoming an increasingly popular modality for the treatment of “inner-ear disorders.” While investigators continue to examine the best dosing paradigms for gentamicin in the treatment of Ménière’s disease and for steroids in the treatment of hearing loss, they have also begun to focus on the use of other agents. In particular, transtympanic therapy has been advocated as a plausible route for the treatment of tinnitus. Transtympanic therapy for tinnitus is not new, and a number of groups have reported success in the past. Despite this success, a number of laboratories have been focusing on newer agents that might yield higher success rates in the treatment of tinnitus and other inner-ear disorders. Many of these agents could have systemic side effects when delivered in high enough doses; therefore, they are ideal candidates for transtympanic administration. The goal of this study is to begin to define the effects of one of these agents—leupeptin, a calpain antagonist—on the normal inner ear of an animal model. In this investigation, we demonstrate the effects of sustained-release delivery of leupeptin (2.5 µg/ml) on the hearing of chinchillas. The medicine produced no hearing loss at the early time points but did produce some hearing loss at later time points. We discuss these results and begin to outline the next steps in the investigation of this agent.

Key Words: inner ear; kinetics; neuroprotective agents; noise-induced hearing loss; toxic damage

The success of transtympanic therapy for the treatment of Ménière’s disease and hearing loss has encouraged investigators to begin to examine the use of more novel agents for the treatment of these and other “inner-ear” pathologies. Much of this work takes advantage of our increasing knowledge of toxically mediated cell-death pathways, such as apoptosis.

One of the primary mediators in these cell-death pathways is activation of calpains. Calpains are naturally occurring calcium-activated cysteine proteases. Calpains have been demonstrated to play an active role in a num-

ber of pathological neurodegenerative conditions [1]. Calpain activation may play a role in promoting neurological degeneration in such disorders as Alzheimer’s disease and multiple sclerosis [1]. In addition, calpain activation plays a role in neurological damage after anoxic or traumatic brain injury [2]. Calpain antagonists have been demonstrated to be neuroprotective and reduce damage that occurs after a variety of acquired traumatic or anoxic brain injuries [1,2]. In addition, a number of studies have demonstrated that calpain inhibitors may protect neurons of the inner ear from toxic damage.

Leupeptin is a well-studied calpain inhibitor. This drug appears to be an excellent candidate for treating toxic damage of the inner ear, because of its extreme solubility, availability, and extensive previous in vivo studies [1]. One of the important issues with the use of calpain inhibitors is their route of administration. Systemic administration of the medicine may produce un-

Reprint requests: CDR Michael E. Hoffer, Department of Defense, Spatial Orientation Center, Department of Otolaryngology, Naval Medical Center San Diego, San Diego, CA 92134-2200. Phone: 619-532-9604; Fax: 619-532-6088; E-mail: mehoffer@nmcscd.med.navy.mil

wanted side effects over the long term, so local administration of the medicine may offer the safest and most efficacious route to treat inner-ear damage. To date, very little work has been done in examining the effects of leupeptin on the inner ear.

Seidman's group demonstrated that guinea pigs tolerated 0.5 $\mu\text{g/ml}$ (up to 10 ml total) local administration of the medicine, evincing no changes in cochlear blood flow or hearing threshold over an extended period (8 weeks) [2]. This study examined the kinetics, electrophysiology, and morphology of local administration of the medicine at higher (and possibly more therapeutic) levels. In addition, no group has examined how the cells of the inner ear respond to leupeptin administration. These basic questions must be examined before the medicine can be applied in a therapeutic manner. We have published hearing results, kinetics curves, and inner-ear cellular response patterns seen with another medicine, gentamicin [3–5]. In this project, we have used the same basic laboratory setting to begin to examine these issues, using leupeptin as the test medicine.

MATERIALS AND METHODS

The basic methods used in this project have been detailed previously [3]. Briefly, 14 adult *Chinchilla laniger* were used in this phase of the project. They were divided into three groups: group A, 4-hour time point (four animals); group B, 24-hour time point (five animals); and group C, 7-day time point (five animals). Each animal underwent preoperative audiometry to ensure normal hearing before implantation of the sustained-release device. After this hearing test, the Silverstein Microwick (Micromedics, Inc, Eagan, MN) was implanted into the round-window niche through a transbullar approach. After implantation, the wick was saturated with 200 μl of leupeptin (2.5 $\mu\text{g/ml}$). At the preset time points, the animals underwent sampling of the perilymph in the untreated ear (as previously described) [3]. After this sampling, a labyrinthectomy was performed on the nontreated ear. At this point, a bone-conducted auditory brainstem response (ABR) test was performed on each animal to obtain its end-point hearing level. The wick was then removed, and both the perilymph of the treated ear and a sample of blood were obtained for analysis of leupeptin kinetics data. All the work in this experiment was performed in accordance with the regulations of our institution's Laboratory Animal Care and Use Committee (LACUC), which approved this protocol.

RESULTS

All 14 experimental animals had normal hearing at the beginning of the experiment and did well from the point

of wick insertion until their end-point hearing test and perilymph measurement. The four animals at the 4-hour time point and the five animals at the 24-hour time point demonstrated no change in hearing. However, two of the five animals that had the 1-week implantation demonstrated complete deafness in the treated ear, whereas the other three animals at this time point had normal hearing. Control animals who were implanted with a saline-impregnated wick did not demonstrate hearing loss at any of these time points.

DISCUSSION

Our results indicate that, in normal ears, leupeptin produces no damage at the very early (4-hour) and early (24-hour) time points but that two of the five animals that had the 1-week implantation demonstrated complete deafness in the treated ear, whereas the other three animals at this time point had normal hearing. Our findings agree, to some extent, with one previously published leupeptin study that demonstrated no detrimental effects of leupeptin on hearing functions 4 hours after direct injection into the scala tympani [6]. Seidman's group placed leupeptin into the middle ear via a different sustained-release device and found no hearing loss up to 8 weeks after administration [2].

A number of possibilities exist to explain the difference in results. First, we used a concentration of leupeptin higher than that used by Seidman's group (2.5 $\mu\text{g/ml}$ in our study versus 0.5 $\mu\text{g/ml}$ in Seidman's study), a different animal, and a different delivery method. Also to be considered is that our results are an aberration related to possible infection, trauma, and the like. However, despite our higher concentration and different delivery method, Seidman's group delivered more total leupeptin to the round window than we did in our study. The answers may lie in the kinetics and histology data, which will be available at a later date. It will be instructive to examine the level of leupeptin in the 7-day animals that demonstrated a complete hearing loss as compared to those that did not. This analysis, however, may not help to solve the dilemma, because all the animals might have no leupeptin (or low levels) remaining at 7 days, but the deafened animals may have had high levels at some intermediate point between 0 and 7 days, whereas the animals without a hearing loss may never have achieved high levels in their perilymph. Several sets of animals at intermediate time points are being examined to help to resolve this question.

One of our interesting findings was the "all-or-none" effect seen at the 7-day time point. The animals were either deaf or had completely normal hearing; no animal had intermediate effects (partial hearing loss). We cannot explain this effect but again look to the forth-

coming kinetics and morphological data to help to resolve this dilemma.

CONCLUSION

Inner-ear medical therapy has become a popular treatment modality for a variety of inner-ear disorders. To advance this therapy and treat such conditions as chronic tinnitus, new methods and new medicines must be developed. In this experiment, we demonstrated that leupeptin can be administered to the inner ear in a sustained-release device but that this administration may be associated with hearing loss in higher concentrations. These results highlight the incumbency of those of us who work in this field to perform careful basic science work before beginning work on our patient populations. Also important is for researchers to continue to examine new medicines and to describe new delivery techniques and devices to allow this important field to advance and thereby provide treatments for patients who are in need of this type of therapy.

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