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The Early Kinetics of Gentamicin Uptake into the Inner Ear

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Abstract: Transtympanic gentamicin administration has become a popular modality in the treatment of Ménière's disease. This modality and other inner-ear medical therapy are gaining increased clinical and scientific attention. We previously described the kinetics and effects of gentamicin uptake into the inner ear after delivery of the medicine into the middle ear using a variety of different techniques and sustained-release modalities [1]. In our previous work, we reported an early peak perilymph concentration and the presence of intracellular gentamicin at the 4-hour time point. We also demonstrated the activation of inner-ear damage pathways at this early time point. In this report, we examine the kinetics of gentamicin at very early time points, 1 and 2 hours after administration. Healthy adult chinchillas underwent implantation of middle-ear sustained-release devices (one to each ear) containing gentamicin. The animals then were maintained in a neutral position and underwent perilymph gentamicin sampling at the two predetermined time points. This technique allowed us to assess accurately very early time point inner-ear gentamicin kinetics and to compare the activity. The samples then were run for concentration using mass spectrometry. The information gained from this study may increase our scientific understanding about the effects of gentamicin on the inner ear and may allow clinicians to treat patients more effectively for inner-ear disorders.

Key Words: fluorescent polar immunoassay; gentamicin; mass spectrometry; Ménière's disease; sustained-release

The use of intratympanic gentamicin therapy to treat Ménière's disease has become increasingly popular and accepted over the last decade. Despite the increasing use and visibility of this type of therapy for Ménière's disease, a number of significant issues remain to be addressed. There are still significant questions concerning the best dosing scheme, the best total dose, the best method of administration, the best end point of therapy, and the best techniques for measuring short- and long-term effects. It is likely that much of the confusion in this field is based on a misunderstanding of the kinetics and activity of transtympanic gentamicin. The transtympanic route is inherently inaccurate. The amount of medicine that leaks down the eustachian tube, escapes into the external auditory canal, or is sequestered in a portion of the middle ear where it is unavailable for uptake into the inner ear varies with each dose and with each patient. Even if the amount of medicine available for uptake into the inner ear could be controlled, the kinetics and activity of the medicine once it reaches the inner ear after a transtympanic administration are not well understood.

In an attempt to answer some of the questions surrounding transtympanic medical therapy, our laboratory has concentrated attention on the use of sustained-release devices to deliver medicine from the middle ear to the inner ear. The premise has been that these vehicles allow us to control the amount of medicine in contact with the round window. With the input of medicine controlled, we have been able to study both the amount

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of medicine that crosses the round window membrane and the effects of this medicine on the morphology and function of the inner ear. We have constructed a perilymph kinetics concentration curve for gentamicin after delivery to the inner ear, examining time points from 4 hours to 2 weeks after the onset of administration [1]. However, many investigators studying systemic administration of gentamicin have shown significant inner-ear tissue levels 1–3 hours after the onset of medicine administration [2, 3].

The goal of this study was to examine the perilymphatic kinetics of gentamicin after middle-ear administration in a sustained-release vehicle at these earlier time points. In this study, we specifically examine the 1- to 2-hour time points. In addition, in our previous work [1], the concentration of gentamicin has varied considerably among animals at each time point. It is possible that this variation reflects interanimal differences in gentamicin absorption into the inner ear. It is also possible that this variation is related to the fluorescent polar immunoassay (FPIA) technique that was used to measure the gentamicin concentrations. A second goal of this study was to validate the use of a new measurement technique (mass spectrometry) as a more accurate way of measuring gentamicin concentrations in the very small amount of perilymph that can be obtained from each of the study animals.

MATERIALS AND METHODS

A group of *Chinchilla laniger* were divided into two equal groups. One group was assigned to the 1-hour time point, and the other group was assigned to the 2-hour time point. Implantation of the microdose catheter was accomplished as follows: Each animal was sedated by injectable anesthetic (described in our earlier work [1]). The tissue around the right auditory meatus was injected with epinephrine (1:100,000) to control bleeding. An incision was made longitudinally along the tragus leading to the meatus. Once divided, the tissue plane orthogonal to the axis of the incision was dissected down to the underlying bone. This section of cleared bone, which is located inferior to the auditory meatus, serves as the shelf onto which the forthcoming catheter is glued. The concave bulla of the ear was removed using a sharp probe, with care taken not to touch the underlying ossicles. Once removed, the stapes and malleoincudal complex were disarticulated using a Rosen needle by pressing away from the oval window in line with the axis of stapedial travel. This method resulted in oval-window rupture in approximately 1 of 15 operations.

At this point, the round window was visible to the unaided eye, although the remainder of the procedure was performed under magnification through a binocular dissection scope. A hole slightly larger than the outer diameter of the catheter was made in the aforementioned bony shelf of the marginal bulla, and the catheter was inserted into the hole so that the tip of the catheter rested lightly on the margin of the round window. The catheter was temporarily affixed in place by applying VetBond cyanoacrylate cement to the interface between the catheter and the hole. Durelon bone cement was then added to the same area to reinforce the catheter. With the glue cured, an Eppendorf 20-200 μl pipet was dialed to 100 μl, and a gel-loading tip was applied. One hundred microliters of gentamicin sulfate (10 mg/ml) were drawn into the pipet tip and injected into one of the exposed lumens of the catheter. This volume of fluid was sufficient to fill the catheter and produce a droplet that was visible on the round window membrane. The clock was started when this injection was complete. To reduce evaporative loss, the ear was cursorily closed with strategically placed silk sutures.

At the 1-hour and 2-hour time points, respectively, these sutures were removed, and the round window was visualized. The ear was irrigated with 10 ml of normal saline and was suctioned. Latent moisture was dried using desiccated oxygen. Once all visible moisture was removed, a gel-loading pipet tip attached to an Eppendorf 0.5- to 2.5-μl pipet was plunged through the round window membrane to a depth of approximately 3 mm. Two microliters of perilymph then were withdrawn and placed in a 150-μl microcentrifuge tube with 98 μl of sterile diionized water. Animals were dispatched via intracardiac injection of 5 ml Euthasol (a pentobarbital-based euthanasia agent) according to procedures established by our laboratory animal care and use committee. The samples were analyzed using mass spectrometry (Pharmout, Inc., Sunnyvale, CA) and then were corrected for dilution to yield a concentration of gentamicin in micrograms per milliliter.

RESULTS

Six ears from three different animals were analyzed at the 1-hour time point, and seven ears from four different animals were analyzed at the 2-hour time point. The 1-hour time points ranged from 3,899 μg/ml to 4,502 μg/ml (mean, 4,068 μg/ml; range, 602 μg/ml; standard deviation, 205). The 2-hour time points ranged from 3,670 μg/ml to 4,592 μg/ml (mean, 4,153 μg/ml; range, 892 μg/ml; standard deviation, 304).

DISCUSSION

The first of this study's two goals was to begin to examine the very early kinetics of gentamicin absorption

into the inner ear after delivery in a sustained-release vehicle. In our previous work, we demonstrated the kinetics of gentamicin delivery to the inner ear for time points greater than 4 hours after administration. We found that there was a slightly higher concentration 4 hours after administration as compared to 8 hours after administration, with peak concentrations occurring 24 hours after administration of the medicine. This early 4-hour subpeak initially confused us. These pilot data begin to suggest that there is a much higher concentration of gentamicin at 1 and 2 hours than at 4 hours. This very early high peak concentration is in keeping with the findings of other investigators who showed relatively rapid gentamicin absorption and clearance from the perilymph [2–4]. It is possible that the act of implanting the sustained-release device into the round window membrane produces a significant ingress of gentamicin across the round window membrane, owing to the surgical manipulation. This may be especially true because the catheter is first implanted and then loaded with gentamicin until a "flash" of medicine is seen at the end of the device. There is no difference seen between the 1- and 2-hour time points, so it appears that this initial peak may have a slight shoulder. After the initial peak is cleared, the gentamicin concentration in the perilymph returns to a more steady-state level that (with the use of a sustained-release device delivering 1 μl/hr) takes 24 hours to achieve and lasts for at least several days. Certainly, because these are only pilot data resulting from work with a very few animals, we can only suggest this conclusion. More animals must be examined, and the same measurement technique has to be applied to all the animal time points.

A second goal of our study was to validate the use of a new measurement technique for measuring gentamicin concentrations in the perilymph of the chinchilla. Mass spectrometry appears to be an excellent and very reliable measurement technique. The mass spectrometry measurements yielded ranges that were less than one-fourth as large as the mean concentrations at both the 1- and 2-hour time points, whereas using FPIA, the range of values seen at the 4-hour time point was nearly twice the mean of 125 μg/ml. It is possible that there is an increase in physiological variability between the 2-hour and 4-hour time points, especially because a significant amount of gentamicin needs to be cleared during that 2-hour period. However, mass spectrometry appears to be at least as accurate as FPIA, if not more reliable, because the 1- and 2-hour standard deviation and range as a function of the mean are lower than are the same values for the 4-, 8-, and 24-hour time points using FPIA analysis. Of course, the best comparison is to divide each animal sample in half and send one set for mass spectrometry and one set for FPIA analysis. Plans are under way to do this in our laboratory.

CONCLUSION

This pilot study was designed to examine two questions. First, is the gentamicin concentration in the inner ear at the 1- and 2-hour time points significantly greater than at the 4-hour time point? Second, is mass spectrometry a reliable method for analyzing gentamicin concentration in the perilymph? The study has begun to provide answers to both questions. It appears that very early gentamicin concentrations are fairly high using a sustained-release device, perhaps as a function of the implantation technique, and it appears that mass spectrometry provides relatively tight values at these early time points. Of course, much more work must be done. In particular, time points before 1 hour and between 2 and 4 hours must be analyzed, and mass spectrometry must be compared directly to FPIA using the same set of samples. Nonetheless, this pilot work seems to justify pursuing answers to these questions.

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