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Research Paper

Perilymph pharmacokinetics of locally-applied gentamicin in the guinea pig

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ABSTRACT

Intratympanic gentamicin therapy is widely used clinically to suppress the vestibular symptoms of Meniere's disease. Dosing in humans was empirically established and we still know remarkably little about where gentamicin enters the inner ear, where it reaches in the inner ear and what time course it follows after local applications. In this study, gentamicin was applied to the round window niche as a 20 μL bolus of 40 mg/ml solution. Ten 2 μL samples of perilymph were collected sequentially from the lateral semi-circular canal (LSCC) at times from 1 to 4 h after application. Gentamicin concentration was typically highest in samples originating from the vestibule and was lower in samples originating from scala tympani. To interpret these results, perilymph elimination kinetics for gentamicin was quantified by loading the entire perilymph space by injection at the LSCC with a 500 μg/ml gentamicin solution followed by sequential perilymph sampling from the LSCC after different delay times. This allowed concentration decline in perilymph to be followed with time. Gentamicin was retained well in scala vestibuli and the vestibule but declined rapidly at the base of scala tympani, dominated by interactions of perilymph with CSF, as reported for other substances. Quantitative analysis, taking into account perilymph kinetics for gentamicin, showed that more gentamicin entered at the round window membrane (57%) than at the stapes (35%) but the lower concentrations found in scala tympani were due to greater losses there. The gentamicin levels found in perilymph of the vestibule, which are higher than would be expected from round window entry alone, undoubtedly contribute to the vestibulotoxic effects of the drug. Furthermore, calculations of gentamicin distribution following targeted applications to the RW or stapes are more consistent with cochleotoxicity depending on the gentamicin concentration in scala vestibuli rather than that in scala tympani.

1. Introduction

For years, it has been widely assumed that intratympanically applied drugs such as gentamicin primarily entered perilymph of the inner ear through the round window (RW) membrane (Smith and Myers, 1979; Okuno and Nomura, 1984; Lundman et al., 1987; Goycoolea et al., 1988). Some histological studies had shown that substances such as tetracycline or horseradish peroxidase were found in tissues of the vestibule, suggesting they may have entered through the stapes footplate (Maass and Stupp, 1974; Tanaka and Motomura, 1981; Saijo and Kimura, 1984). However, the demonstration that small molecules could readily exchange between perilymph of scala tympani (ST) and scala vestibuli (SV) by local, "radial" fluid pathways (Salt et al., 1991) meant that it was difficult to establish the route by which substances reached the vestibule.

Pharmacokinetic studies of locally-applied gentamicin in chinchillas (Hoffer et al., 1997, 1999; Balough et al., 1998) were interpreted quantitatively through a computer model by Plontke et al. (2002). In that analysis, entry at the RW membrane was assumed and the passage of drug from ST to SV and the vestibule within the cochlea was able to account for the measured concentration time course of samples taken from the vestibule. A major finding of this analysis was that large basal-apical concentration gradients along

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ST were predicted to exist. The subsequent development of the sequential sampling technique for perilymph (in which multiple perilymph samples are collected and analyzed individually) allowed the gradients along ST to be confirmed experimentally (Plontke et al., 2007). The existence of gradients was supported by morphological studies showing greater hair cell losses at the base of ST after local applications (Wagner et al., 2005) and by gradients in gentamicin measured by immunochemical methods that were described as "striking" (Imamura and Adams, 2003). The existence of basal-apical gradients along the cochlea has high clinical relevance as it explains how vestibular function can be ablated in humans while preserving the function of speech frequency regions of the cochlea. In contrast to local RWM delivery, systemic application of gentamicin resulted in highest perilymph levels in the apex of the cochlea with decreasing concentrations towards the basal regions of ST (Hahn et al., 2013).

In a recent study, gentamicin application was targeted to the RW or stapes followed by functional and histological assessment after 7 days (King et al., 2013). These studies found that vestibular hair cell loss, cochlear hair cell loss and hearing loss (measured by ABR) were all substantially higher when gentamicin was delivered to the stapes rather than to the RW. This result was interpreted as suggesting that gentamicin entered the inner ear more readily through the oval window and stapes than through the RW. Previous studies have demonstrated that other substances, such as gadolinium, enter the inner ear more readily at the stapes than at the round window. In the case of gadolinium applied to guinea pigs, it was estimated that 90% of the total entry occurred at the stapes (King et al., 2011). A similar, prominent entry into the vestibule was demonstrated in humans (Zou et al., 2005, 2010). These combined studies raise questions about where gentamicin enters the inner ear and how it distributes within the inner ear following intratympanic applications. Even though the drug is widely used to treat humans, this has never been assessed before. In the present study, we have directly measured gentamicin entry into perilymph of the vestibule and into ST following local applications to the RW niche.

Interpretation of measured perilymph concentrations in terms of the relative entry at the RW and stapes is complicated by the existence of homeostatic processes for perilymph which vary with cochlear location. A recent study using fluorescent dextran as a marker showed that marker levels declined in perilymph at the base of ST far faster than in perilymph of the vestibule (Salt et al., 2015). The decline was thought to result partly from a slow (~30 nl/min) influx of CSF through the aqueduct, diluting ST perilymph contents. It was also shown that dextran or fluorescein decline at the base of ST occurred more slowly when cyanoacrylate glue was applied to the RW membrane (Salt et al., 2015, Plontke et al., 2016). As there was no indication that dextran leaks from the normal RW membrane, this result was thought to result from a mechanical stiffening of the normally compliant RW membrane by the cyanoacrylate. This would reduce fluid oscillations across the cochlear aqueduct driven by respiratory CSF pressure changes. Such an exchange would cause marker loss from ST perilymph in the normal, RW compliant state. A comparable oscillatory fluid exchange across a cannula sealed into ST is used in a device to deliver drugs to perilymph (Fiering et al., 2009). Both of these interactions between perilymph and CSF in the basal turn of ST are incorporated into the computer model of the inner ear fluids that was used to interpret the results of gentamicin applications.

2. Materials and methods

2.1. Animals

The study utilized 26 pigmented, NIH-strain guinea pigs weighing 400–600 g. Experiments were conducted in accordance with policies of the United States Department of Agriculture, the National Institutes of Health guidelines for the handling and use of laboratory animals, and under protocols 20101035 and 20130069 approved by the Animal Care Committee of Washington University.

Animals were anesthetized with 100 mg/kg sodium thiobutabarbital (Inactin, Sigma, St Louis, MO) and maintained on 0.8–1.2% isofluorane in oxygen using a mechanical ventilator combined with a tracheal cannula. A 5% end-tidal CO_2 level was maintained, monitored with a CapnoTrue AMP (Bluepoint Medical, The Netherlands), through adjustment of the ventilator's tidal volume. Heart rate and oxygen saturation were monitored with a (Surgivet. Waukesha, WI) pulse-oximeter. Body temperature was maintained at 38 °C with a thermistor-controlled heating blanket.

2.2. Experimental conditions and surgical approach

Gentamicin was applied to the ear either by an injection into the RW niche (n = 12), or by direct injection into perilymph at the lateral semi circular canal (LSCC)(n = 14). Under both protocols, perilymph was subsequently sampled from the LSCC for analysis. Access to the inner ear was gained by an incision behind the pinna and exposure of the lateral portion of the bulla. The LSCC for was prepared for injection and/or fluid sampling by thinning the bone over the canal with a dental burr. A branch of the facial nerve was removed in those animals where it ran parallel to the LSCC. When the canal lumen was visible through the thinned bone, the bone was dried and a layer of thin cyanoacrylate glue (Permabond 101; Permabond, Pottstown, PA) was applied followed by layers of two-part silicone adhesive (Kwik-Cast, World Precision Instruments, Sarasota, FL). The silicone was applied thinly over the canal wall but multiple layers were built up at the edges to form a hydrophobic cup. A 30–40 μm fenestration into the bony canal wall was made through the adhesives and canal wall using a 30° House stapes pick (N1705 80, Bausch and Lomb Inc.). The conical shape of the pick prevented substantial entry of the tip into the canal, resulting in minimal damage to the membranous boundaries of endolymph. In mice, the fluid collected in this manner has been shown to be perilymph (Hirose et al., 2014).

For drug injections into the LSCC, an injection pipette was inserted into the fenestration in the canal wall and a tissue wick used to remove the fluid bolus accumulating on the hydrophobic surface. A droplet of cyanoacrylate glue was applied to seal the pipette in place. Following this procedure there was no visible fluid leakage at the injection site. "Invisible" fluid leakage below the adhesives (i.e. between the adhesives and the bone) was prevented by this method as the cyanoacrylate was applied to a dry bone surface, to which it bonded extremely well.

2.3. Application of gentamicin solutions

Gentamicin was applied to the RW niche as a 20 μL volume of 40 mg/ml Refobacin (Merck, Darmstadt, Germany). When this volume of liquid was applied it immediately contacted both the RW membrane and the stapes footplate, and over time could spread to other areas of the bulla. The ear was orientated so that most fluid remained in the niche in an attempt to minimize the amount reaching the bulla. In experiments where perilymph was sampled at 2 or 4 h after application, drug solution was reapplied every hour after wicking away the previously-applied solution, avoiding any contact with the RW membrane or stapes. This was performed as we knew middle ear gentamicin was declining quite rapidly and we needed to maintain perilymph levels in a measurable range. Drug solution remained in the RW niche during perilymph sampling from the LSCC, kept clear of the sampling site by silicone adhesive

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