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Research paper

Gentamicin administration on the stapes footplate causes greater hearing loss and vestibulotoxicity than round window administration in guinea pigs

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ABSTRACT

Clinically, gentamicin has been used extensively to treat the debilitating symptoms of Mèniére's disease and is well known for its vestibulotoxic properties. Until recently, it was widely accepted that the round window membrane (RWM) was the primary entry route into the inner ear following intratympanic drug administration. In the current study, gentamicin was delivered to either the RWM or the stapes footplate of guinea pigs (GPs) to assess the associated hearing loss and histopathology associated with each procedure. Vestibulotoxicity of the utricular macula, saccular macula, and crista ampullaris in the posterior semicircular canal were assessed quantitatively with density counts of hair cells, supporting cells, and stereocilia in histological sections. Cochleotoxicity was assessed quantitatively by changes in threshold of auditory brainstem responses (ABR), along with hair cell and spiral ganglion cell counts in the basal and second turns of the cochlea. Animals receiving gentamicin applied to the stapes footplate exhibited markedly higher levels of hearing loss between 8 and 32 kHz, a greater reduction of outer hair cells in the basal turn of the cochlea and fewer normal type I cells in the utricle in the vestibule than those receiving gentamicin on the RWM or saline controls. This suggests that gentamicin more readily enters the ear when applied to the stapes footplate compared with RWM application. These data provide a potential explanation for why gentamicin preferentially ablates vestibular function while preserving hearing following transtympanic administration in humans.

1. Introduction

Local drug delivery has distinct advantages for the treatment of inner ear disease, including targeted therapy and elimination of the risk of systemic drug-related side effects. This approach has a place in the clinical treatment of Mèniére's disease and sudden sensorineural hearing loss. It has been widely accepted that drugs delivered to the middle ear space during local delivery primarily enter the inner ear via permeation through the round window membrane (RWM) into scala tympani (ST), with subsequent diffusion into scala vestibuli (SV) and the vestibule through the interstitial spaces of the spiral ligament (Salt and Ma, 2001; Salt et al., 2003; Plontke et al., 2007) and into Rosenthal's canal via caniculi perforantes (Shepherd and Colreavy, 2004). Transosseous drug entry has also been reported to occur through the bone of the otic capsule in rodent models (Mikulec et al., 2009). Qualitative data from some investigators has suggested that substances could enter vestibular perilymph through the oval window (OW) (Tanaka and Motomura, 1981; Saijo and Kimura, 1984; Zou et al., 2005). We have previously reported results consistent with this. In a Magnetic Resonance Imaging (MRI) study (King et al., 2011), significantly higher Gadolinium (Gd) based contrast agent was observed in the vestibule and SV than expected from round window entry, following intratympanic delivery in the guinea pig model. The amount of Gd in SV and the vestibule could not be explained by entry through the RWM

List of abbreviations: ABR, auditory brainstem responses; Gd, gadolinium; GPs, guinea pigs; HCs, hair cells; IHCs, inner hair cells; MRI, Magnetic Resonance Imaging; OHCs, outer hair cells; OW, oval window (OW); RW, round window; RWM, round window membrane; ST, scala tympani; SV, scala vestibuli; SVJ, stapediovestibular joint; TMPA, trimethylphenylammonium

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alone. Rather, it was calculated that up to 90% of the Gd had entered the vestibule directly in the vicinity of the stapes footplate. It is evident in other MRI studies in rats and humans that Gd signal is higher in the vestibule than in ST. This is consistent with the interpretation that the Gd marker may have entered the inner ear via the annular ligament of the stapediovestibular joint (SVJ) (Zou et al., 2005, 2012).

Direct drug entry into the vestibule in the vicinity of the stapes footplate was subsequently confirmed using ionic marker trimethylphenylammonium (TMPA) following intracochlear injections or applications to the round window (RW) niche, with or without occlusion of the RW membrane or stapes area (Salt et al., 2012). In that study, perilymph TMPA concentrations were monitored either in real time with TMPA-selective microelectrodes sealed into ST and SV, or by the collection of sequential samples of perilymph from the lateral semi-circular canal. When the RWM was occluded, round window niche irrigation produced higher concentrations in SV compared to ST, confirming direct TMPA entry into the vestibule in the region of the stapes. Additionally, the TMPA levels of initial samples (originating from the vestibule) were markedly lower when the stapes area was occluded. This demonstrated that entry through the oval window greatly influences the level of drug in vestibular perilymph. As a result, the concentration of drugs in the vestibule may be considerably higher following intratympanic administration than previously recognized based on entry through the RWM alone.

Clinically, aminoglycoside antibiotic gentamicin may be administered intratympanically to relieve the debilitating symptoms of vertigo associated with Mèniére's disease (Lange, 1977; Silverstein et al., 1999). The symptoms of Mèniére's disease largely result from fluctuations of vestibular and auditory function associated with endolymphatic hydrops. In severe cases of Mèniére's disease, one approach has been to control vertigo attacks by suppressing vestibular function with gentamicin. Gentamicin is toxic to the cochlea and vestibular organs, affecting both hearing and balance (Govaerts et al., 1990). In the vestibular neuroepithelium, type I hair cells are known to be most susceptible to gentamicin as these cells more avidly take up or retain the drug in the early period after administration (Lopez et al., 1997; Lyford-Pike et al., 2007; Hirvonen et al., 2005; Tanyeri et al., 1995). Ototoxicity is an undesirable potential side effect of intratympanic gentamicin therapy, and the risk of hearing loss increases proportionately with gentamicin dosage (concentration, volume and administration time) (Plontke et al., 2002; Salt et al., 2008). Attempts to deliver gentamicin more specifically to the vestibule, by surgically occluding the RWM with connective tissue before intratympanic injection of a high drug concentration, did not markedly affect patient outcome; specifically a significant number of patients (27%) still experienced hearing loss (Quaranta et al., 1999).

In light of these considerations and the findings from our previous studies, it is of clinical interest to understand whether gentamicin enters the inner ear via the oval window. If so, then there is a possibility that with optimization of the delivery technique, it may be possible to target the vestibule directly with gentamicin thereby potentially minimizing the risk of ototoxicity during aminoglycoside therapy for Mèniére's disease. Here we explore functional and morphological effects of aminoglycoside toxicity upon the cochlea and vestibular systems when gentamicin is targeted to either the OW or to the RWM. Hearing thresholds and histological damage to cells in the cochlea and vestibule were compared after a highly concentrated gentamicin solution was delivered directly onto the RWM or OW. Saline control groups for each treatment group were used to control for the effects of the surgery.

2. Materials and methods

2.1. Animal preparation

The study was approved by the Royal Victorian Eye and Ear Hospital (Melbourne, Australia) Animal Ethics Committee (Ethics Approval 11/238AR). Twenty-two tri-color adult guinea pigs (Dunkin-Hartley strain) of either sex, weighing between 397 and 851 g, were used in the study and randomly assigned to a treatment group. The age of the animals in both gentamicin treatment groups were similar. The animals were anaesthetized with ketamine (Troy Laboratories Pty Ltd, Australia; 60 mg/kg) and xylazil-20 (Troy Laboratories Pty Ltd, Australia; 4 mg/kg) administered intramuscularly. Anesthesia was monitored during the experiment using pedal and ocular reflexes and supplemented as necessary with 67% of the initial dose. 0.5–1 ml of Lignocaine-20 (Troy Laboratories Pty Ltd, Australia) was administered subcutaneously to the surgical site prior to making the incision. Temgesic (0.05 mg/kg) and Endotril (Troy Laboratories Pty Ltd, Australia; 20 mg/kg) were administered sub-cutaneously following surgery for analgesia and infection control respectively.

2.2. Materials

Three microliters of a 337 mg/ml solution (1 mg total) of gentamicin sulfate (G3632-5g, Sigma Aldrich) in phosphate buffered saline was administered either onto the stapes footplate or onto the RWM. The solution was delivered with a 5 μL Hamilton syringe fitted with a with 32 gauge flat tip needle (SGE Analytical Science Pty Ltd, Australia) connected to a syringe driver (Micro4™ Microsyringe Pump, World Precision Instruments, USA). For the control cohorts, 3 μL of 0.9% normal saline (Promed, Thermofisher Scientific) was administered onto the stapes footplate or onto the RWM.

2.3. Surgical procedure

Using a dorsolateral posterior-auricular surgical approach, the bulla of one ear was opened using a 1.5 mm diameter cutting burr under the operating microscope to expose the round window and stapes footplate. The treatment solution was administered directly on to the footplate of the stapes (gentamicin $n = 5$, saline $n = 4$) or RWM (gentamicin $n = 4$, saline $n = 4$). Following delivery, the animal was left in position for 30 min to allow the drug to permeate the structure. Any visible excess fluid was wicked away with a paper tissue wick, the wound was sutured closed. At one week after treatment, animals were anesthetized for auditory brainstem response recording and then euthanized with an intraperitoneal injection of 0.5 mg/kg Lethabarb (Virbac Pty Ltd, Australia). The inner ears were removed for histological analysis.

2.4. Auditory brainstem response recordings

Acoustically evoked auditory brainstem responses (ABR) were measured prior to the first surgery and a final ABR was performed one week after treatment to measure auditory function in the treated ear. The change in ABR threshold was calculated as the difference between the two measurements. ABR recordings were made in a sound-proof Faraday cage whilst the contralateral ear was occluded with an ear mould compound (Otoform, Dreve Germany) to attenuate hearing. Computer generated acoustic stimuli (5 ms tone pips with 1 ms rise/fall times at frequencies 2, 8, 16, 24 and 32 kHz) were delivered free-field from a loudspeaker (Richard Allen DT-20, UK) placed 0.1 m from the ipsilateral pinna. Stimulus intensity was attenuated in 5 dB steps. Brainstem responses were