Intra-cochlear trafficking of aminoglycosides

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Cochlear sensory hair cells are pharmacologically sensitive to aminoglycoside antibiotics that are used for treating life-threatening bacterial sepsis. Cochlear tissues are compartmentalized behind an impermeable paracellular barrier called the blood-labyrinth barrier (BLB). Most macromolecules cannot cross the blood-labyrinth barrier; however, aminoglycosides can cross this barrier into the cochlear fluids and enter hair cells, inducing hair cell death and consequent permanent hearing loss or deafness. The trafficking routes and cellular mechanisms required for aminoglycoside trafficking across the blood-labyrinth barrier remain unknown.

Aminoglycosides enter cochlear hair cells across their apical membranes that are bathed in endolymph, a hitherto unexpected trafficking route. The stria vascularis, a component of the blood-labyrinth barrier, preferentially loads with aminoglycosides. Our recent work demonstrates that the stria vascularis exhibits high expression of the cation-selective ion channel TRPV4, and that this channel is permeable to aminoglycosides. However, aminoglycosides must employ more than one cellular mechanism to cross the blood-labyrinth barrier into endolymph against the electrical gradient.

Aminoglycoside antibiotics are clinically essential for treating life-threatening bacterial infections (like meningitis) in neonatal and post-natal patients. It has long been known that aminoglycosides kill sensory hair cells in the inner ear, causing deafness and vestibular disorders. Yet how aminoglycosides are trafficked across the cell-based blood-labyrinth barrier (similar to the blood-brain barrier, see Fig. 1A) to these sensory hair cells is unknown. Early pharmacokinetic studies determined that perilymph concentrations of aminoglycosides more closely mimic serum concentrations than the endolymphatic lumen of the scala media, circumscibing the endolymphatic fluid space.

Aminoglycosides have long been known to block the mechanosensitive transduction channels of sensory hair cells in the inner ear. More recently, it was demonstrated that aminoglycosides permeate the mechanosensitive transduction channels located in the apical hair bundle of cochlear hair cells. Although these experiments were performed in vitro, these findings implicate that, in vivo, aminoglycosides cross the blood-labyrinth barrier into endolymph prior to entering hair cells. This is a radical notion, yet one that fits the data from a variety of published studies.

It is important to identify aminoglycoside trafficking mechanisms across the blood-labyrinth barrier into the inner ear fluids and block these trafficking routes. With this blocking strategy, aminoglycoside therapy will be used more efficaciously without fear of permanent hearing loss and deafness. We use fluorescent conjugates of one clinically relevant aminoglycoside—gentamicin—because of its continued use in the clinical setting, to identify these trafficking mechanisms.

We typically conjugate the fluorophore Texas Red to gentamicin and determine its cellular distribution microscopically using both in vivo and in vitro preparations. We found that gentamicin-Texas Red (GTTR) can rapidly enter cells through non-endocytotic mechanisms, including the permeation through non-selective cation channels. More importantly, we also determined that GTTR preferentially loads the stria vascularis compared to the lateral wall, and that GTTR entry into the marginal cells of the stria vascularis could be competitively inhibited. This preferential strial loading with GTTR suggests that aminoglycosides traffic from the strial capillaries to marginal cells.

Although the cationic aminoglycosides permeate non-selective cation channels with a pore diameter sufficiently large to permit drug permeation (approx. 1 nm), information about which cation channels are aminoglycoside-permissive remains limited. The transient receptor potential (TRP) vanilloid receptor 1 (V1) channel, and the mechanosensitive inner ear transduction channels, are candidates. We wanted to determine which aminoglycoside-permissive channels are present in the stria vascularis. Recent studies show that another TRP channel, the mechanosensitive TRPV4, is present in the stria vascularis. Given this background, we set out to determine if TRPV4 is aminoglycoside-permissive, and its cellular distribution in the stria vascularis.

Our data showed that GTTR can penetrate TRPV4, as determined by loss of TRPV4-mediated uptake in cells that express
a TRPV4 mutant. In wild-type mice, TRPV4 was extensively localized in the intermediate cells of the stria vascularis, and near the lumenal membrane of marginal cells that is bathed in endolymph. This distribution of immunocytochemical expression of TRPV4 initially suggested that TRPV4 could play a role in the efflux of aminoglycosides from intermediate cells into the intrastrial space, and also in aminoglycoside efflux from the marginal cell into endolymph, following the electrical and concentration gradients. This transport route is similar to the route of potassium cycling into endolymph.

However, our in vitro data did not support this hypothesis. TRPV4 expression reduced the cellular efflux of GTTR into endolymph-like media. TRPV4-null mice did not display cochlear GTTR distribution patterns in vivo that were different to those in wild-type mice (unpublished data). On the other hand, TRPV4 could cause aminoglycoside uptake and retention in cochlear hair cells and strial marginal cells that are exposed to endolymph at the apical membrane. Importantly, GTTR permeation through TRPV4 channels is complicated by the heterometric nature of TRP channels in vivo. For example, a recent study has shown that TRPV4 and TRPP2 form a channel complex in the kidney. This may add another layer of possible permutations for cation channel permeability to aminoglycosides, further enhancing the potential for redundancy in the variety of aminoglycoside-permissive cation channels expressed in the cochlea.

Although some aspects of aminoglycoside uptake and clearance by the cell may be explained by the drug permeation through channels composed of TRPV4 and/or its related cation channels, this mechanism alone is probably insufficient to explain aminoglycoside trafficking mechanisms across the multi-cellular layered, blood-labyrinth barrier in the cochlea. Transport of the cationic aminoglycosides from strial endothelial and intermediate cells into the intra-strial space and marginal cells is against the electrical gradient, and may require an active mechanism (Fig. 1B). Transport from marginal cells into endolymph could be passive through aminoglycoside-permissive cation channels down the electrical and concentration gradients (Fig. 1B).

Future studies will be directed toward identifying the aminoglycoside trafficking mechanisms across the blood-labyrinth barrier in the cochlea, which will facilitate the development of new strategies to block cochlear uptake of aminoglycosides and reduce the prevalence of aminoglycoside-induced hearing loss.

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