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Local drug delivery to the entire cochlea without breaching its boundaries

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SUMMARY

The mammalian cochlea is one of the least accessible organs for drug delivery. Systemic administration of many drugs is severely limited by the blood-labyrinth barrier. Local intratympanic administration into the middle ear would be a preferable option in this case, and the only option for many newly emerging classes of drugs, but it leads to the formation of drug concentration gradients along the extensive, narrow cochlea. The gradients are orders of magnitude and well outside the therapeutic windows. Here we present an efficient, quick and simple method of cochlear pumping, through large amplitude, low-frequency reciprocal oscillations of the stapes and round window that can consistently and uniformly deliver drugs along the entire length of the intact cochlea within minutes without disrupting cochlear boundaries. The method should facilitate novel ways of approaching the treatment of inner ear disorders since it overcomes the challenge of delivering therapeutics along the entire cochlear length.

KEYWORDS: cochlea, round window, drug delivery, intratympanic administration, cochlear pumping

INTRODUCTION

Reliable, efficient and uniform drug delivery to the cochlea remains an unsolved challenge and is a major barrier to the prevention or treatment of inner ear disorders. The mammalian
cochlea is one of the least accessible organs for drug delivery (Salt and Plontke, 2009; Rivera et al., 2012; El Kechai et al., 2015; Hao and Li, 2019). Systemic administration of many drugs, notably the most frequently used corticosteroids and aminoglycoside antibiotics, is severely limited by the blood-labyrinth barrier (Salt and Hirose, 2018). Direct injection into the cochlea is limited by the requirement for surgery for access and does not guarantee uniform drug delivery along the cochlea.

Several potential therapeutic compounds to treat inner ear disorders are under clinical investigation. This comprises old and newly emerging classes of drugs and therapies including corticosteroids, local anaesthetics, antioxidants, apoptosis inhibitors, neurotransmitters and their antagonists, monoclonal antibodies, growth factors, signalling pathway regulators and genetic material (see Devare et al., 2018; Hao and Li, 2019). A recent review identified 43 biotech companies currently pursuing experimental compounds for inner ear therapy (Schilder et al., 2019). All such efforts are, however, restricted by our inability to reliably deliver such compounds into the cochlea.

Intratympanic administration of drugs (Schuknecht, 1956) relies on their remaining in contact with the round window (RW) (a membranous opening in the bony wall of the cochlear into the middle ear) long enough to allow their diffusion into the perilymph of the scala tympani (ST). The ability of drugs to pass through the RW does not, however, guarantee their effective distribution along the cochlear spiral. Drug distribution in the ST is limited by the low flow rate of perilymph within the cochlea and by cochlear geometry. The longitudinal flow of perilymph in the cochlea has been shown to be relatively slow, if present at all (Ohyama et al., 1988), and drug distribution in the perilymph is dominated by passive diffusion. Passive diffusion along the ST is, however, constrained because the cochlea is a
relatively long and narrow tube with a cochlear cross-section that decreases gradually from
the RW at the base to the apex.

Direct measurements of the distribution of marker ions and contrasting agents (Saijo and
Kimura, 1984; Salt and Ma, 2001; Haghpanahi et al., 2013), corticosteroids (Hargunani et al.,
2006; Plontke et al., 2008; Grewal et al., 2013; Creber et al., 2018) and antibiotics (Imamura
and Adams, 2003; Mynatt et al., 2006; Plontke et al., 2007) or measurements of the
physiological effects of drugs (Chen et al., 2005; Borkholder et al., 2010) have demonstrated
that the concentration of substances applied to the RW is much higher in the cochlear base
than in the apex.

A large number of methods, including intracochlear administration, cochleostomy and
canalostomy, have been proposed for solving the problem of uniform drug distribution along
the cochlea but only two current strategies address this problem without breaching the
boundaries of the intact cochlea (e.g. see El Kechai et al., 2015). The first strategy relies on
retaining drugs in contact with the RW to allow drug diffusion into the cochlea apex. Notable
examples of devices designed for this purpose include Microwicks, osmotic pumps etc.

Hydrogel-based drug delivery systems also allow retention of therapeutics in the middle ear
in contact with the RW. The problem with this strategy is that retention of drugs at the RW
leads to their establishing steady-state concentration gradients along the cochlea which
depend on the relationship between diffusion and clearing (Salt and Ma, 2001; Sadreev et al.,
2019), but the base-to-apex gradients can still be very pronounced.

The second strategy, although relatively non-invasive to the cochlea, requires development of
more complex drug formulations. The technique employs drug loaded nanoparticles, which
could be used to take advantage of anatomical and cellular feature of the cochlea which
enable drug uptake through routes and pathways other than the ST route (Buckiová et al.,
2012; Glueckert et al., 2018). Magnetically driven, drug-loaded magnetic nanoparticle can also be actively distributed along the entire cochlea (Ramaswamy et al., 2017).

Here we demonstrate that cochlear pumping (CP), through pressure oscillations in the ear canal at frequencies low enough to avoid damage to the cochlear sensory apparatus, can consistently and uniformly deliver drugs along the entire length of the intact cochlea within minutes without disrupting cochlear boundaries.

**RESULTS**

**Cochlear pumping at low frequencies does not cause elevation of hearing thresholds**

When inaudible low-frequency air pressure oscillations are presented at the ear canal, they are transmitted to the stapes which causes back and forth cochlear fluid movements through the scala vestibuli (SV) and ST coupled via helicotrema (Graphical Abstract). The RW works as a pressure relief valve during these movements and moves in counter phase with the stapes because the cochlear bony wall and fluid are poorly compressible. The low-frequency pressure changes within the cochlea are, however, shunted by the helicotrema and do not cause excitation of the travelling wave and stimulation of the sensory epithelium. In fact, shunting of low-frequency perilymph flow, which occurs for example during middle ear muscle reflex, thereby preventing cochlear overstimulation, is the previse role of the helicotrema (von Békésy, 1960). The high-frequency slope of the helicotrema mechanical filter is not, however, infinitely steep (6 dB/octave in guinea pigs and 12 dB/octave in humans (Marquardt et al., 2007)) and the choice of CP frequency for drug delivery is critical to prevent cochlear damage. In our experiments, low-frequency air pressure oscillations applied to the ear canal at 4 Hz, which caused large amplitude (~ 80 µm peak-to-peak) stapes displacement (see Transparent Methods), did not elevate the threshold of the compound action potential (CAP) of the auditory nerve (Figure 1A).
Cochlear pumping promotes even distribution of drugs along the cochlear spiral

The ability to distribute drugs uniformly along the entire cochlea spiral using relatively large, low-frequency periodic displacement of fluid in the ST and SV (Graphical Abstract) was demonstrated in our experiments with application of salicylate to the RW. Salicylate readily passes through the RW (Borkholder et al., 2014; Sadreev et al., 2019). To monitor salicylate diffusion along an intact guinea pig cochlea in vivo, we utilized the suppressive effect of salicylate on cochlear amplification via block of the outer hair cell somatic motility (Russell and Schauz, 1995; Hallworth, 1997). We measured elevation of the CAP thresholds caused by salicylate at different frequencies, which, due to cochlear tonotopicity, corresponds to different distances from the RW (Greenwood, 1990).

Salicylate did not cause elevation of the CAP threshold responses for frequencies below 5 kHz, which corresponds to about 45% of the total cochlear length from the base, when it diffused through the cochlea passively (Sadreev et al., 2019). The calculated gradient of base-to-apex salicylate concentration was about 13 orders of magnitude. When, however, placement of salicylate solution on the RW was followed by CP, i.e. by 5-minute cycles of large-amplitude (80 µm peak-to-peak), low-frequency (4 Hz) stapes movements caused by pressure oscillations in the ear canal, the CAP threshold was elevated throughout the entire 1 kHz - 30 kHz frequency range tested (Figures 1B; 2; 3). This corresponds to about 75% of the total cochlear length from the base (Greenwood, 1990). Partial recovery of the CAP thresholds after washing out salicylate from the RW (Figure 1B) provided confirmation that the integrity of the sensory cells was preserved and the threshold elevation after joint salicylate application and CP was not caused by the low-frequency pressure oscillations (Figure 1A).
The technique had no observable influence, compared to passive diffusion, on responses to RW salicylate application for locations close to the RW at the base of the cochlea (Figure 2, 27 kHz). CP, however, led to more rapid threshold elevation for locations which were distal and apical to the RW, even if the maximal threshold elevations were similar for both experimental paradigms (Figure 2, 15 kHz). The threshold elevation saturated after 4-5 cycles of stimulation even for the low frequencies of the most apical locations (Figure 1B; 2, 1-3 kHz) where passive diffusion produced no effect. Smaller threshold elevations at low frequencies were due only to the reduced contribution of cochlear amplification to cochlear responses at these frequencies (Sadreev et al., 2019, Robles and Ruggero, 2001) and, in fact, reached almost maximal possible elevations for those frequencies (Figure 3).

**DISCUSSION**

A stapes displacement of 80 µm in our experiments corresponds to 1.6 mm linear displacement of the fluid in the apical parts of ST, because in guinea pigs the apical ST cross-sectional area is almost 20 times smaller (Thorne et al., 1999) than the stapes area (Sim et al., 2013) and both the cochlear bony wall and fluid are poorly compressible. Therefore, while salicylate effect in the most apical 25% of the cochlear length was not measured due to poor hearing sensitivity of guinea pigs below 1 kHz, most of the fluid in this region was replaced by fluid from more basal regions during a single cycle of CP. Hence, estimates of salicylate distribution derived for the basal regions are valid for the most apical 25% of the cochlea.

The cochlear bony wall and fluid are poorly compressible. The poor compressibility results in the fluid volume velocity along the SV and ST to be the same. Consequently, fluid linear displacement and velocity are much higher in the narrow apical parts of the scalae than at the base. Specifically, the apical ST cross-sectional area in guinea pigs is almost 20 times smaller than in the basal cochlear region (it is almost 6 times smaller in humans) (Thorne et al.,
This proportional increase in fluid displacement and velocity cannot alone produce effective drug mixing along the cochlea. It will, however, facilitate mechanisms discussed below which can enhance the distribution to the cochlear apex of a drug that originally diffuses through the RW and oval window into the cochlear base.

The larger fluid linear velocity at the apex is still not sufficient to cause turbulent mixing of drugs. Due to the small diameter of the cochlear scalae, the fluid flow along them is dominated by fluid viscosity (i.e. it occurs at low Reynolds numbers). Thus, the fluid flow is laminar even over uneven inner surface (Cervo et al., 2013) of the scalae and turbulent mixing will not contribute to uniform drug distribution. The boundary layer driven acoustic streaming (i.e. Rayleigh streaming) can potentially contribute to drug mixing in our experiments (Boluriaan and Morris, 2003; Squires and Quake, 2005). Its role, however, should not be significant because the boundary layer thickness in water/perilymph at 4 Hz is comparable with the scala tympani diameter. Nevertheless, the cochlear helical structure (Graphical Abstract) should lead to additional drug mixing due to the formation of Dean vortexes (e.g. Nivedita et al., 2017) and to chaotic mixing/advection, both transversal and longitudinal, observed for laminar fluid flows in helical pipes (Jones et al., 1989; Nguyen, 2011), which can be further facilitated by periodic changes of the flow direction (Ottino and Wiggins, 2004). Therefore, under specific condition of cochlear stimulation, these mechanisms may well be major factors contributing to the mixing and distribution of drugs along the ST and SV. None of the tentative mechanisms discussed above, except for acoustic streaming, causes net flow of perilymph along the scalae. We argue, however, that enhanced drug diffusion along the cochlea in our experiments could be observed without net forward motion of perilymph. Namely, we propose that the suggested mechanisms help to achieve more efficient mixing at the drug solution/pure perilymph borderline. Hence, this borderline moves much faster towards the apex than it is observed in experiments when only molecular
diffusion is involved which helps to achieve even drug distribution along the entire cochlear
within relatively short time.

The tentative physical principles which govern the uniform distribution of salicylate along the
cochlea are universal and should be valid for the distribution of an arbitrary substance,
including nanoparticles, in the human cochlea. Salicylate was used in these initial
experiments because of its well-documented physiological effect which allows estimation of
the drug distribution along the intact cochlea without sampling perilymph. It was also used
because it challenged the CP method. Salicylate is a difficult drug to distribute along the
cochlea because it is cleared rapidly from the ST (Sadreev et al., 2019). It is anticipated that
drugs, which are better retained in the ST, will be redistributed along the cochlea even more
quickly and efficiently (Salt and Ma, 2001; Sadreev et al., 2019).

Limitations of the Study

While the human cochlea has the same helical structure as the guinea pig cochlea, which
should promote drug mixing due to the mechanisms discussed above, there are differences in
the geometry of the cochlea which need be considered for application of the CP in human
patients. The human cochlea is longer and the apical decrease in the cross-section of scalae is
smaller than in guinea pigs (Thorne et al., 1999). This may result in a longer time required to
deliver drugs along the more extensive cochlea where increase in the liner fluid velocity at
the apical part is less pronounced. On the other hand, the higher cut-off frequency and steeper
low-frequency slope of the helicotrema’s filter in humans (Marquardt et al., 2007) would
permit the use of higher CP frequencies without damaging the cochlear sensory apparatus.
This, together, with the possibility of using larger stapes displacements, which are limited to
~ 80 µm in guinea pigs by the crista stapedius, could result in higher CP efficiency.
While prolonged, high-amplitude, low-frequency stimulation did not cause elevations of hearing thresholds (Figure 1A), this stimulation might potentially affect the vestibular system, especially the saccule. In fact, excitation of the saccule enables mice to detect low-frequency sounds which are outside the frequency range of their cochleae (Jones et al., 2010). This possibility, however, could not be assessed under the neurolept anaesthetic technique used in the study. Therefore, a thorough safety study of the CP method is needed for its translation to human patients.

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AUTHOR CONTRIBUTIONS

A.N.L., N.Z., Y.M.Y. and I.J.R. conceived and designed the study. A.N.L. performed the experiments. A.N.L. and I.I.S. analyzed experimental results. All authors contributed to analysis and discussion of the results. A.N.L. and I.J.R. wrote the manuscript with contribution from all authors.

DECLARATION OF INTERESTS

A.L., N.Z. and Y.Y. are inventors on a United Kingdom Patent Application No. 1908260.1 submitted by The University of Brighton that covers method and device for substance delivery to the inner ear. N.Z. is employed by the company Otophysica Ltd, Uckfield, UK. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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FIGURE CAPTIONS

FIGURE 1. Effect of the CP on the neural responses in the absence and presence of salicylate on the RW.

(A) A representative examples of CAP threshold curves in the presence of the CP alone. Pressure oscillations at 4 Hz that caused ~ 80 µm peak-to-peak stapes displacement were applied to the ear canal during 5 minutes before each non-zero time point plotted and the CAP thresholds were measure during the following 5-minute interval without CP. The legend indicates the cumulative pumping time for each curve. Solid black line indicates mean ± SD for all 7 curves, each representing a different cumulative pumping time.

(B) A representative examples of CAP threshold elevation when the same pumping protocol as in (A) was used after the application of 5 µl of 100 mM salicylate solution to the RW at time zero and recovery of the thresholds after washing out the salicylate. The frequency of the pure tone acoustic stimulation used for eliciting the CAP is indicated in the figure legend for each curve.

FIGURE 2. Comparison of the efficiency of the CP technique and passive diffusion in the distribution of salicylate along the cochlea.
Pooled data for experiments with CP (red symbols, 5 preparations) and passive diffusion (black symbols, 5 preparations (Sadreev et al., 2019). Pressure oscillations at 4 Hz were applied to the ear canal during 5 minutes before each red non-zero time point plotted to cause large-amplitude (~ 80 µm peak-to-peak) stapes movement and the CAP thresholds were measure during the following 5-minute interval without pressure oscillation. Frequency of acoustic stimulation is indicated within each panel. 5 µl of 100 mM salicylate solution was applied to the RW at time zero. Solid red lines indicate mean CP data and solid black lines indicate 5-point running averages for passive diffusion data. Grey lines near the horizontal axis indicate statistically significant (p < 0.05, unpaired t-test) differences between data for the CP and passive diffusion within consecutive 10-minute intervals. Some of the passive diffusion data have been presented before (Sadreev et al., 2019).

**FIGURE 3. Comparison of frequency dependence of the CP and passive diffusion effects.**

Frequency dependence of the CAP threshold elevation after 60 minutes of salicylate application during CP (35 minutes of the total pumping time) (mean±SD, n=5) and its comparison with the frequency dependence for passive diffusion (mean±SD, n=5). Open circles show maximal increase of the CAP thresholds after complete block of the cochlear amplifier (mean±SD, n=3) (Sadreev et al., 2019). Data for passive diffusion have been partially presented before (Sadreev et al., 2019).
Highlights

- Systemic delivery of drugs to the inner ear is limited by the blood-labyrinth barrier
- Middle ear administration results in pronounced drug gradients along the cochlea
- Cochlear pumping distributes drugs evenly along the entire cochlea within minutes