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Permeation Enhancers for Intratympanically-Applied Drugs studied using Fluorescent Dexamethasone as a Marker

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1. Introduction

Measurements of perilymph concentration achieved after intratympanic (IT) drug applications typically find the perilymph concentration to be substantially lower than the applied concentration. Following IT steroid applications in guinea pigs, perilymph levels were found to be less than 1.5 % of the applied concentration in a number of studies (1–3) with measurements often highly variable between individual animals. Amounts less than 0.3% of the applied concentration with high variability were also seen for steroid measurements from human ears (4,5). For gentamicin, perilymph levels were measured to be 1.4%, 1.7% and 2.5% following 1, 2 and 4 hr applications respectively (6). Lower perilymph concentrations, averaging 0.02% of the applied concentration were found when gentamicin was applied in humans (7).

Manipulations of the round window (RW) membrane have been shown to increase the entry of IT applied drugs. Exposure to dry air, solutions of high osmolarity, and to 1% benzyl alcohol increased the rate of marker entry from the RW niche into perilymph (8).

In the present study, we have used fluorescent dexamethasone (F-dex) as a marker agent to assess entry into perilymph. Fluorescent dexamethasone binds to glucocorticoid receptors but is a larger molecule with a formula weight of 835.9 compared to 392.5 for dexamethasone. It was selected because it enters perilymph very slowly from the middle ear and would therefore likely be influenced by permeation enhancement. Perilymph was collected with a sequential sampling technique, taking samples from the lateral semi-circular canal (LSCC) which allowed drug entry at both the round window and stapes to be quantified. We screened a variety of agents that have been used to improve drug penetration in other physiologic systems for their capacity to enhance entry into perilymph. The agents included:

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1. Benzyl Alcohol (BA). Shown to accelerate passive drug permeation across the plasma membrane (9) and to increase entry through the RW membrane (8).
2. Saponin is typically extracted from plants and has detergent-like properties. Saponin has been reported to remove membrane cholesterol leaving defects in the membrane. (10,11)
3. N-methyl-2-pyrrolidone (NMP) is a dipolar aprotic solvent used in the formulation of drugs for oral and transdermal delivery. It has been shown to increase the permeability of drugs through the skin (12,13)
4. Dimethylsulfoxide (DMSO) is a polar aprotic solvent often used to solubilize compounds with low water solubility. It also permeabilizes the skin (13).
5. Caprate. Also known as decanoic acid, caprate is a fatty acid present in the milk of mammals. Caprate has been shown to permeabilize the blood brain barrier (14) and to enhance permeation of macromolecules across tricellular tight junctions of intestinal cells (15).
6. Poloxamer 407 (P407) is a hydrophilic non-ionic surfactant comprised of repeating hydrophobic polypropylene glycol blocks flanked by hydrophilic polyethylene glycol blocks. Poloxamers, in solution or as micelles, have been reported to enhance drug transport across cellular barriers (16).

There are numerous other substances that have been used as permeation enhancers in other systems, such as to improve drug passage through the skin (17, 18). The hydrophobic terpene limonene and surfactant sodium dodecyl sulfate have been used to permeabilize the tympanic membrane to improve drug penetration from the ear canal to the middle ear (19). The agents tested here therefore do not include all substances with potential value to increase permeability. It is notable that many permeation enhancers are detergents or solvents that act by disrupting lipids in the membranes of cell layers forming physiologic boundaries in the body.

2. Materials and Methods

Animals

The study utilized 49 healthy, pigmented, NIH-strain guinea pigs of both sexes weighing 400 – 600 g (about 5 – 10 weeks old). 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 20130069 and 20160053 approved by the Institutional Animal Care and Use Committee of Washington University.

Animals were initially anesthetized with 100 mg/kg sodium thiobutabarbital (Inactin, Sigma, St Louis, MO) and then maintained on 0.8 to 1.2 % isoflurane in oxygen using a ventilator coupled to a tracheal cannula. The tidal volume of the ventilator was adjusted to maintain a 5 % end-tidal CO₂ level, monitored with a CapnoTrue AMP (Bluepoint Medical, The Netherlands), Heart rate and oxygen saturation were monitored with a pulse-oximeter

(Surgivet, Waukesha, WI). Body temperature was maintained at 38 °C with a thermistor-controlled heating blanket.

An incision was made behind the ear and the auditory bulla was exposed by a lateral exposure, allowing both the lateral semi-circular canal (LSCC) and the round window niche to be visualized. The LSCC was prepared for fluid sampling by thinning the bone over it with a dental burr. The bone was allowed to dry and a thin layer of cyanoacrylate glue (Permabond 101; Permabond, Pottstown, PA) was applied, followed by layers of two-part silicone adhesive (Kwik-Cast, World Precision Instruments, Sarasota, FL), built up at the edges to form a hydrophobic cup.

After the ear was prepared for fluid sampling a 20 μ L bolus of drug solution was applied to the round window niche with a hand-held micropipetter. The animals head was oriented to maximize the amount of fluid retained in the round window niche. The applied volume filled the niche and stapes area of the guinea pig but may have allowed some solution to reach the bulla near the apical region of the cochlea as it was propelled towards the Eustachian tube by the ciliated epithelium. Entry through the thin bone at the apex of the guinea pig has previously been demonstrated (20) and was therefore included in our analysis of data from this study. In all experiments, the solution contained 1 mM fluorescent dexamethasone (D1388 Invitrogen; ThermoFisher; <https://www.thermofisher.com/>) in phosphate-buffered saline. Control experiments (n=8) contained no additional component. In other experiments the applied solution also contained 10% dimethylsulfoxide (DMSO, Sigma, St Louis; n=3), 10% N-methylpyrrolidone (NMP, Sigma, St. Louis; n=3), 130 μ g/ml saponin (#558255, Calbiochem/Millipore Sigma; n=3), 1.94 mg/ml sodium caprate (Sigma, St. Louis; n=4), 1% benzyl alcohol (BA) (Sigma, St. Louis; n=3), 2% BA (n=4), 4% BA (n=6), or 17% poloxamer 407 (P407, Spectrum Chemicals, New Brunswick, NJ; n=5). In additional experiments, F-dex in P407 gel was given in combination with saponin (n=3) or BA (1% n=2; 2% n=3; 4% n=3) to determine whether the volume stabilization provided by P407 would enhance the permeabilization.

Sequential Sampling from the Lateral Semi-Circular Canal

Sampling perilymph from the LSCC allowed drug entry at the stapes and RW to be independently quantified. Sixty minutes after the drug solution had been applied, perilymph was collected from the LSCC as a series of 20 individual 1 μ L samples collected over a 20–40 min period in a technique we have previously described as “sequential sampling” (6,21,22). Sampling started by making a 30–40 μ m fenestration with a 30° House stapes pick (N1705 80, Bausch and Lomb Inc.) in the previously-thinned bony wall of the LSCC. Clear, uncontaminated fluid flowed from the fenestration, accumulating on the hydrophobic surface of the silicone. Fluid was collected with hand-held, blunt tipped capillary tubes (VWR 53432-706) marked for a nominal volume of 1 μ L and each taking 1–2 min to collect. The length of each sample in its capillary tube was measured with a calibrated dissecting microscope to establish the exact sample volume. Twenty individual samples were collected in this manner and pooled in pairs for analysis, resulting in 10 measurements each using a sample volume of ~2 μ L.

Samples collected in this manner allow drug concentrations in the perilymph from different regions of the inner ear to be quantified as illustrated in Figure 1A. In this study, we were specifically interested in the perilymph concentrations in scala tympani, indicating entry through the RW membrane, and in the vestibule, indicating entry at or near the stapes. When the LSCC is perforated, the escaping perilymph is driven by CSF entering the basal end of scala tympani through the cochlear aqueduct as indicated in the figure. This allows perilymph originating in spatially different regions along the perilymphatic spaces to be collected as different samples. In reality, the samples are not as cleanly differentiated as suggested by the figure, but interact with adjacent tissues and fluid spaces as they pass along the scalae. While this has minimal influence on perilymph from the SCC and vestibule it has greater influence on later samples. Perilymph originating near the base of scala tympani passes through the entire inner ear before it is collected. Computer simulations of the fluid movements during the sampling procedure permit such interactions to be taken into account in the interpretation of measured concentrations. The rationale for sampling at 60 mins after drug application was to take perilymph while middle ear drug concentration was still relatively high and to allow interpretation of how much drug entered at the RW and stapes before it was distorted by local intermixture between scala tympani and scala vestibuli.

After perilymph had been sampled, a single sample of the fluid remaining in the RW niche was collected. The average time for collecting this sample was 100.4 min (SD 18.6, n=29) after the solution had been applied. In addition, multiple samples of the original solution that had been applied to the RW niche were also taken.

Sample handling and analysis

Each of the samples collected was expelled from its collection capillary into 150 μ L of a diluent consisting of phosphate-buffered saline (PBS) with sodium azide preservative (Santa Cruz Biotechnology, Inc; <http://www.scbt.com/>). Injection solution was also used to make a dilution series, by adding 300 μ L of solution to 2.7 ml PBS diluent (a 10x dilution), repeated 8 times. Samples were loaded into a disposable 96 well plate and read in a SpectraMax i3 plate reader with SoftMax Pro 6.4 software with parameters optimized for FITC-dex (excitation 495–500 nm, 15 nM bandwidth; emission 535–540 nm, 25 nm bandwidth). A sigmoid curve (Hill function) was fitted to the dilution series measurements using the Solver function of Microsoft Excel and was used to convert fluorescence brightness to concentration.

Sample concentrations are shown graphically on logarithmic axes and averages calculated with the logarithms of F-dex concentrations.

Sequential sample interpretation through simulations of the experiments

Interpretation of sequential sampling measurements for some experiments was provided by our established simulation program, available for download at <http://oto.wustl.edu/saltlab>. Calculated sample concentrations from the simulator were fitted to the measured data by adjusting entry at the stapes to match samples 2 and 3 (originating from the vestibule), apical bone entry to match samples 4, 5 and 6 (originating from apical cochlear regions) and entry at the RW to match samples 7, 8 and 9 (originating from ST). Increasing entry at each site

increased the concentration in the corresponding samples. Entry was adjusted to minimize the differences between measured and calculated samples, allowing amounts entering at the 3 sites to be compared. Entry was dominated by the RW and stapes with only a minor amount entering at the apex. The simulator calculates solute distribution based only on established physical processes, such as diffusion, volume flow, elimination and exchange with adjacent compartments. Simulations take into account the dimensions of all the fluid and tissue spaces of the cochlea and vestibular system of the guinea pig. Simulation of the sequential sampling procedure takes into account the specific volumes and collection times for each sample, calculating the corresponding fluid flows through the perilymphatic spaces. Calculated data are shown as open symbols in the figures.

Statistical significance of measurements was assessed using Sigmaplot v13 software (Systat: systatsoftware.com).

3. Results

Sequential sampling of perilymph from the LSCC allows the entry from the middle ear into perilymph at different sites, primarily at the round window membrane and the stapes, to be measured and compared. Perilymph concentration measurements are presented as μM , with the applied solution containing 1000 μM F-dex. In control experiments, the application of 1000 μM F-dex to the RW niche for 1 hour resulted in low measured F-dex levels in perilymph, as shown in Figure 1B. In the average curve, highest concentrations were observed in samples 6 and 7 originating from scala tympani (0.10 to 0.14 μM ; 0.011 to 0.021 % of the applied concentration). As these samples had passed through regions previously only exposed to lower concentrations, indicated by the lower concentrations of preceding samples, their measured concentrations under-state the actual concentrations in scala tympani prior to sampling. Simulations taking the interactions during sampling into account for different entry conditions are shown in Figure 1C. Due to the higher losses from scala tympani perilymph due to interactions with CSF and adjacent tissue compartments (6,22) an equal rate of F-dex entry at the stapes and round window would be expected to generate higher concentrations in the vestibule (brown curve). In order to obtain samples comparable to those measured it was necessary to use proportionately greater entry at the RW membrane, with 89 % entry at the RW membrane and 8% entry at the stapes providing the best representation of the measured curve, with the balance, 3%, entering through the thin bone at the cochlear apex (20).

The sample curves measured when other compounds were included in the solution applied to the RW niche are shown in Figure 2. Substantially higher perilymph levels were measured compared to the control curves (mean control shown in blue) when saponin, benzyl alcohol or NMP were included in the media. In the case of benzyl alcohol, the increase occurred to a similar degree in samples 7 to 9 originating from ST (indicating entry at the RW membrane) and in samples 2 to 3 originating from the vestibule (indicating entry at or near the stapes). The relative rates of entry derived from simulation of the mean curve with benzyl alcohol were estimated to be 94% through the RW membrane and 3% through the stapes. In contrast, with NMP in the medium, samples 2 and 3, originating from the vestibule were higher than all later samples, indicating a proportionately greater entry at the stapes with

NMP treatment. From simulations, 75 % entry at the RW and 21% entry at the stapes accounted for the mean measured curve with NMP. Saponin was intermediate between these two cases, with 79% entry at the RW membrane and 17% entry at the stapes best representing the mean curve. NMP and saponin therefore appear to have a greater influence on entry at the stapes than on entry through the RW membrane. DMSO and caprate had minimal influence on F-dex concentrations, with results from many experiments close to the control condition. Poloxamer 407 gel also had no appreciable influence on F-dex entry, with measured perilymph levels closely overlying the control condition.

The overall entry of F-dex into each ear was determined by summing the total amount of F-dex collected in all samples (in pMoles) as a single metric representing the total amount of F-dex recovered. Figure 3 summarizes the amount of F-dex recovered in each experiment for the 7 application conditions shown in Figures 1 and 2. One-way ANOVA testing of all groups (Bonferroni t-tests, $n=31$) showed that NMP, saponin and 4% benzyl alcohol all significantly increased the amount of F-dex in perilymph by factors of 7.7x for NMP, 14.7x for saponin and 31.9x for benzyl alcohol respectively.

The dependence of F-dex entry on the concentration of benzyl alcohol in the medium was evaluated and is summarized in Figure 4. With 1% BA in the medium entry was increased a little (2.9 x control) but the increase was not statistically significant (ANOVA, Bonferroni t-test, $p=0.57$). Entry was increased significantly with 2% BA (10.2x control) and with 4% BA (31.9x control; as shown in Figure 3), indicating a dose dependence of the influence of BA. The inclusion of poloxamer gel in the applied solution dramatically reduced the influence of BA on F-dex entry, with only the highest concentration (4%) causing significant increase in F-dex entry compared to controls. BA also had a major influence on the gelling properties of 17% P407. At the 4% BA concentration, the P407 gel solidified in the cold and liquefied when warm which is the opposite of its normal properties.

The presence of P407 had no influence on the enhanced entry of F-dex caused by saponin, as shown in Figure 5. Saponin significantly increased perilymph F-dex both with P407 gel (ANOVA, Bonferroni t-test, $p=0.012$) and without the gel (ANOVA, Bonferroni t-test, $p<0.001$).

Drug solutions applied intratympanically typically decline in concentration rapidly with time. Figure 6A shows an experiment in which small fluid samples were taken from the RW niche at 20 min intervals for analysis, showing a progressive decline of concentration as a function of time after application. Simulation of the experiment with elimination from the middle ear set to a half time of 110 min best approximated the measured time course, as shown by the dotted line. In each perilymph sampling experiment, fluid from the RW niche was sampled at the end of the experiment. This was technically more difficult to do with Poloxamer gel in the solution, so only the liquid solution applications are presented here. Analysis of these samples with ANOVA (1-way, Bonferroni t-tests) showed that only one of the experimental treatments had an influence on the F-dex concentration of fluid in the middle ear, which for caprate were significantly higher ($p=0.017$). The final RW niche content of all experiments with liquid solutions that were unaffected by the treatment (i.e. excluding caprate) are summarized in Figure 6B. Here, middle ear concentration at the end

of the experiment is plotted against the total F-dex recovered from perilymph in each experiment. At the time of sampling from the RW niche, at a time which averaged 100.4 min after application, the RW niche F-dex content had fallen to 22.8% of the applied concentration (SD 13.0, n=29), as indicated by the red line on the figure. However, there was no positive correlation (coefficient 0.05) between the final middle ear F-dex level and perilymph levels achieved, suggesting that the varying rates of F-dex loss from the middle ear was not a major factor governing the perilymph levels achieved. This is probably because F-dex entering perilymph depends on the middle ear concentration over the entire initial 60 min period after application, when the middle ear concentration was substantially higher than at the post-sampling time point when the samples from the niche shown here were taken.

4. Discussion

In this study, we tested the influence of a variety of substances that are used as permeation enhancers in other systems to determine whether they would increase the entry of a relatively impermeable drug from the middle ear into perilymph. The application protocol was intended to screen for substances that would increase drug entry when given as a single application, combined with the intended drug to be delivered. This would make the permeation-enhancing substance more suitable for use in an intratympanic drug formulation, rather than requiring the agent to be given as a separate procedure prior to drug delivery. As a result, the protocol was strongly weighted towards those substances causing rapid permeability changes. Indeed, as the middle ear drug concentration was shown here to decline quite rapidly with time, any induced permeability change would need to occur rapidly, while the middle ear concentration was high, in order to be optimally effective.

We found BA to be the most effective permeabilizing agent when included at 4% concentration in the medium. BA has already been used intratympanically in humans. It is present at concentrations of up to 10 mg/ml (1%) in a number of steroid formulations that have been used clinically by intratympanic injection (8). It is used as a preservative in these formulations. There are, however, a number of problems associated with the use of BA to enhance drug entry into the inner ear. When solution containing just 1% BA is injected intratympanically it causes a burning or stinging sensation. The higher concentrations (2%, 4%) used in this study are unlikely to be well accepted and would probably need to be given under sedation or anesthesia in either humans or experimental animals. Other problems result if BA is combined in a formulation with P407. P407 works well to increase the residence time of the drug formulation in the middle ear. It forms a gel at body temperature so the injected solution cannot pass down the Eustachian tube and remains in the middle ear longer. BA completely changed the gelling properties of 17% P407 solutions so they did not gel at body temperature but did gel when cold. Furthermore, BA was far less effective when formulated in combination with P407 with a significant effect only at the 4% level (Figure 4).

Saponins are amphipathic glycosides (i.e. containing hydrophilic and lipophilic regions) classified as having non-ionic detergent properties. They are derived from specific species of plants, in this instance extracted from Quillaja bark. Saponin is used to permeabilize cell

membranes and can cause lysis of erythrocytes. Saponin has been reported to act by binding membrane cholesterol (11,23) inducing pits or defects of nanometer magnitude in the extracellular surface of the membrane (24), thereby compromising the barrier formed by the lipid bilayer. Unlike BA, saponin was compatible when 15% P407 was included in the medium. A lower concentration of P407 was used in our experiment as it was found to set adequately at body temperature. Although the saponin used in this study was the most effective agent when used in combination with P407, a form of saponin with higher purity would be required for any drug formulation.

The solvents DMSO and NMP were also effective at increasing entry into perilymph, but not to the same degree as BA and saponin. It was notable that both P407 and caprate were completely without effect on F-dex entry in this study.

As mentioned above, only those agents causing rapid permeability increase are likely to influence entry under the protocol used here. For example, it has been shown that the influence of caprate is progressive with time, having maximal effect on transepithelial resistance after 30–60 min (15). In our experiment, such a slow change in permeability would have little influence on perilymph F-dex concentration if the middle ear drug concentration had already fallen to a low level before the permeability increased. It also has to be considered that if the permeabilizing treatment also acted on the middle ear mucosa, allowing drug levels to fall faster with time than in the normal, untreated situation, perilymph levels would not increase to the degree expected. There was no evidence from the middle ear concentration measurements made at the end of each experiment that treatments influenced the elimination from the middle ear, except for caprate. Caprate was found to increase the retention of F-dex in the middle ear. The absence of influence of P407 on entry in the present study could also be related to the short application time used, although in other systems with longer observation times P407 has not been reported to influence permeability (25,26). The enhanced delivery across the blood-brain barrier with poloxamer micelles (16) appear to be largely explained by improved bioavailability, i.e. improved release characteristics of the drug, rather than an influence on permeability. We conclude from our study that only substances having a rapid influence on permeability (specifically solvents and detergents) would enhance drug entry into the inner ear from a single intratympanic application.

While the current study has evaluated the effectiveness of a number of agents, further studies are required to determine whether any of these substances would be suitable for inclusion in formulations for intratympanic therapy. Knowledge of the time course of permeability changes, specifically how fast permeability increase occurs and how quickly permeability recovers to normal, would help optimize their use. Longer-term experiments are also required to verify whether the agent is toxic to the inner ear or to structures of the middle ear, which are likely exposed to far higher concentrations than are tissues of the inner ear. Measures of high-frequency sensitivity and morphologic studies of the basal turn and tissues of the middle ear in the days to weeks following therapy are necessary. An absence of pathology of the cranial nerves in proximity or innervating the middle ear muscles, including the facial and glossopharyngeal nerves and, in humans, the chorda tympani, would also need to be demonstrated. Although small, nonpolar substances such as benzyl alcohol can pass

the membranous boundaries and will therefore enter perilymph, the perilymph concentration is expected to remain low as such substances also readily pass through the blood-labyrinth barrier and are quickly eliminated. Nevertheless, measurement of perilymph levels of the permeabilizing agent may be necessary if pathologic changes are associated with its use.

It is notable that the molecules found to be most effective in this study, benzyl alcohol, saponin and NMP are solvents and detergents that influence lipid membranes. This supports the view of Goycoolea (27) that entry permeability at the round window is limited by the epithelium at the middle ear side of the membrane, which is characterized by tight junctions between cells. It is unlikely that these agents would alter diffusion in other components of the round window. The stapes is similarly bounded on the middle ear side by an epithelial layer with tight junctions, which is likely to be the barrier influenced by our manipulations. Other components of the stapes, such as the annular ligament, has intercellular spaces and likely has a lesser influence on drug passage. If entry at the RW and stapes is primarily limited by the middle ear epithelia then many of the anatomic differences between animals and humans may play a lesser role in governing drug entry between the middle ear and perilymph.

In addition to chemical manipulations of permeability, mechanical methods to enhance drug entry at the RW membrane have been evaluated. One technique was reported in which microperforations were made mechanically in the RW membrane with microneedles (28). In an *in vitro* RW preparation, they found an increased entry across the microperforated membrane by a factor of 35x. This is comparable to the permeability increase caused by the highest concentration of BA (4%) used here.

The decline of F-dex concentration in the middle ear is comparable to declines of other substances previously reported. The substantial decline of F-dex, falling to 23 % of the applied concentration in 100 min, shows that drug levels cannot be assumed to remain stable in the middle ear when they are applied as a solution with no timed-release or suspended drug present. With real-time measurements, TMPA marker was found to decline to approximately 50% of the applied concentration within 30 min of application (20). Gentamicin was found to decline to 46% of the applied concentration at 1 hour after application. The concentration decline does not appear to result from fluid volume increase in the middle ear, causing dilution of the drug. Rather it appears to occur by loss of drug from the applied volume. Middle ear kinetics, influencing the concentration and time drug remains in the middle ear is a major factor influencing perilymph levels achieved and needs to be studied in more detail.

In summary, we have screened a number of agents at high concentrations to determine whether any would be suitable to increase drug entry into the inner ear. We have shown that drug entry can be substantially enhanced with the use of chemical permeation enhancers in the applied drug solution. When applied as a formulation for a single injection, BA, saponin, NMP and DMSO appear to be the most promising candidates for more detailed study.

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Figure 1.

A: Schematic of the origins of perilymph samples collected from the lateral semi-circular canal. Perilymph is pushed out by CSF entering at the base of scala tympani so the samples represent, in sequence, fluid from the LSCC, the vestibule, scala vestibuli, scala tympani and then CSF. **B:** Group mean curve with SD error bars summarizing the F-dex concentration of 10 sequentially-collected 2 μ L samples from 8 experiments. **C:** Sample curves calculated by simulation of F-dex entry and distribution followed by perilymph sampling for different entry conditions. The brown curve was calculated using similar amounts of entry at the round window and stapes. Higher concentrations are produced in vestibular perilymph (samples 1–3) compared to those from scala tympani (samples 6–9) because F-dex is lost more rapidly from the base of scala tympani due to interactions with CSF. To account for the concentrations measured in scala tympani (blue curve), F-dex entry through the round window membrane must be set to be much higher than that through the stapes (green curve), with ~89% of the F-dex entering at the round window best representing the measured curve.

Figure 2.

Red curves show the mean and SD of measured perilymph F-dex concentrations for 10 sequentially-collected samples in experiments where 1000 μM F-dex combined with the agent indicated was applied to the round window niche. Blue curves show the group mean for the control group, as shown in Figure 1B. Perilymph concentrations of F-dex were substantially increased for saponin, benzyl alcohol and NMP. Poloxamer gel (17%) had no influence on F-dex entry. The number of experiments with each agent were: saponin 3; benzyl alcohol 5; NMP 3; DMSO 3; Caprate 3; Poloxamer gel 5.

Figure 3.

Symbols: Total amount of F-Dex (in pMoles) summed across all 10 samples collected in each experiment. Black lines show the logarithmically-calculated group mean. Significance was assessed by one way ANOVA (Bonferroni t-tests, $p < 0.05$). NMP, saponin and benzyl alcohol all caused significantly higher amounts of F-dex in perilymph than the control application group.

Figure 4.

Upper panel: Total amount of F-Dex (in Pmoles) summed across all 10 samples collected in each experiment, shown for different amounts of benzyl alcohol in the medium. **Lower panel:** Total F-dex summed across all 10 samples as benzyl alcohol was varied in the presence of 17% poloxamer 407 gel. Benzyl alcohol had substantially lower influence on F-dex entry when combined with poloxamer gel. The gray line is the curve taken from the upper panel for comparison.

Figure 5.

Total amount of F-Dex (in Pmoles) summed across all 10 samples for each experiment shown for saponin with and without 15% poloxamer gel in the medium. F-dex entry remained significantly greater for saponin than controls, both with and without the poloxamer gel present.

Figure 6.

A: Change in middle ear concentration of F-dex with time after applying a 20 μL volume to the round window niche, measured by sampling the middle ear contents over time. Concentration declined with a half time of approximately 110 mins, as derived by simulation of the experiment, shown by the dotted line. **B:** F-dex concentration of fluid in the round window niche taken after perilymph sampling for all experiments not using poloxamer gel or caprate (see text) compared with the total perilymph content of F-dex in the samples. The concentration in the round window niche had fallen to an average of 22.8 % of the applied concentration. Animals with the highest amount of F-dex retained in the middle ear were not those with highest perilymph levels. The correlation coefficient of a fitted logarithmic function (not shown) was 0.05, suggesting that variations of middle ear kinetics did not have a major influence on perilymph levels achieved.