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Direct Drug Applications to Inner Ear Perilymph

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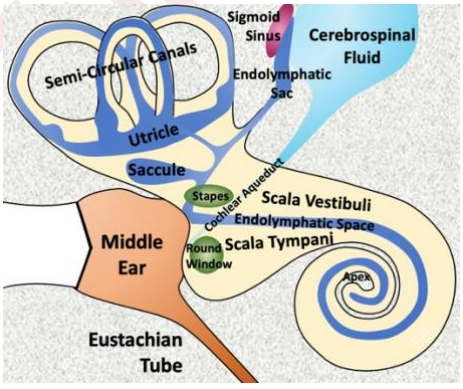
Injecting drug solutions directly into the perilymphatic space has the potential to greatly increase the reliability of dosing compared to less-invasive methods, such as intratympanic application. When performed correctly, drug injected directly can be distributed throughout the ear in a reliable, quantitative manner. However, there are some serious pitfalls that have been demonstrated over the years that can result in totally ineffective drug applications. The pitfalls are often not obvious without careful measurements. The applied drug can be rapidly washed away both during and immediately following the application, as can occur when drug is injected through the round window membrane of rodents (Plontke et al., 2016). There are a number of publications where negative results with a treatment have likely resulted from a technically ineffective delivery method (e.g. Salt & Konishi, 1982; Sellick et al., 2007). Some technical problems occur when delivering drugs to rodents that are not encountered in humans and other primates, due to the large, open cochlear aqueduct in rodents.

FluidSim simulations are included for some examples to show predicted drug time courses and distribution characteristics for different injection rates and volumes. In most cases, they represent the situation for a large molecule that is not easily lost from perilymph (no elimination from ST or SV), delivered at a concentration of 100 (arbitrary units). The plots are given as a general guide and FluidSim can readily be configured to simulate the specific situation for any specific molecule with its elimination characteristics.

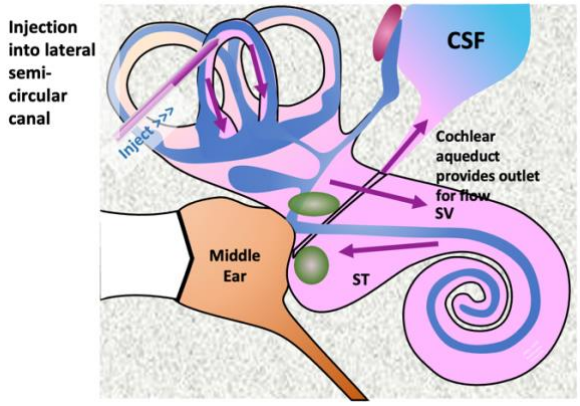
The figure below shows a diagram of the inner ear, labelled for reference, which will be used as the base for figures throughout this summary. For clarity, most of the labels are omitted in subsequent uses.



Schematic of the inner ear fluid compartments. Perilymph is show in pale yellow and endolymph is shown in dark blue. CSF is shown pale blue.

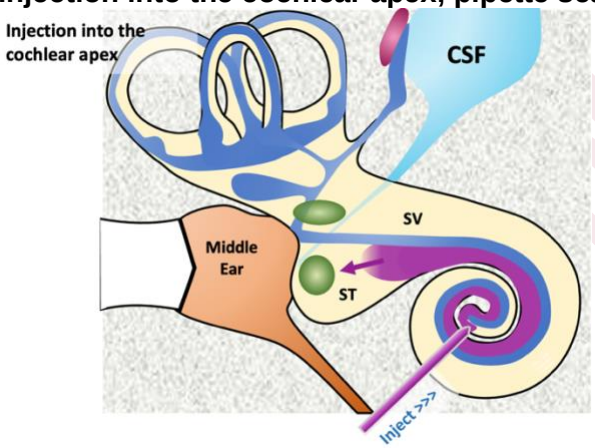
Brief Summary of the 3 Commonly-Used Injection Methods
(details of each are given further below)

1) Semi-circular canal injection, pipette sealed, no outlet



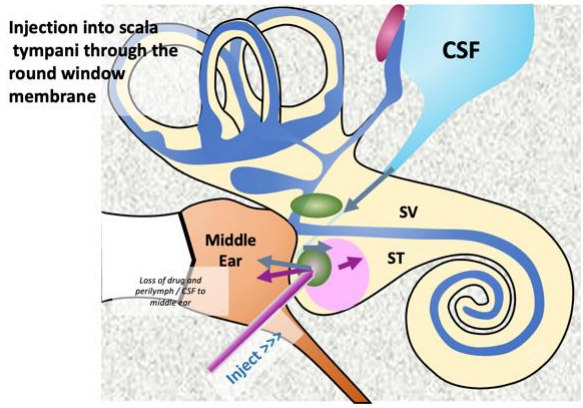
This is the most reliable way to load the entire perilymphatic space with drug. A limitation is that injecting enough drug to treat the basal regions of ST results in significant drug loss into the CSF.

2) Injection into the cochlear apex, pipette sealed, no outlet



This is the method of choice to deliver drug specifically to the hair cells and supporting cells of the organ of Corti and to the neural elements of the spiral ganglion.

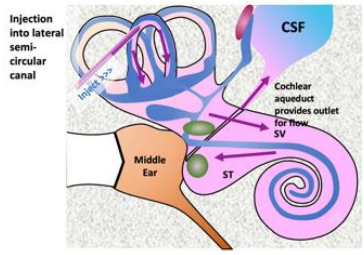
3) Injection into the basal turn of scala tympani with no outlet



Injection through the round window membrane is appealing based on its technical simplicity, but is fraught with complexity in the outcome due to fluid leakage at the injection site. It is generally used with large molecules or gene therapy, which can be effective with small injection volumes.

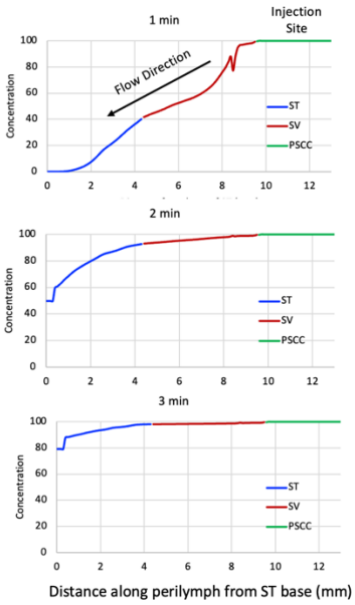
Details of Each Injection Method

1) Semi-circular canal injection, pipette sealed, no outlet



In rodents (mice and guinea pigs), this is an efficient method to deliver drug to the entire ear. The volume and rate injected depends on the species used, the drug's kinetic properties and where in the ear is to be treated. Unfortunately, the basal turn of ST is the most difficult region to treat, as shown by the following simulations.

In mice and rats, injection into the posterior semi-circular canal (PSCC) is used as it is superficial on the skull providing minimally-invasive access. Details of the method as used in mice is given in Ohlemiller at al., 2022.

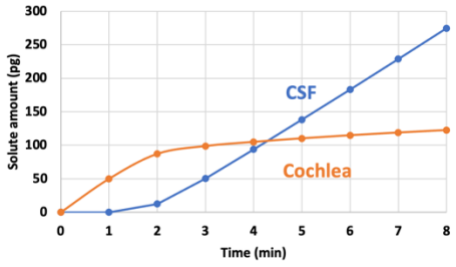


These plots **for mice** show the perilymph space "unrolled", from the base of ST to the apex (blue), from the apex down SV (red) and along the semi-circular canal (green). It shows the result of an injection at 0.5 uL/min with drug that is not rapidly eliminated and is delivered at an arbitrary concentration of 100.

After 1 minute (top panel) the SCC and basal part of SV is filled but little has reached ST. After 2 min (middle panel), ST concentration is rising but has only reached 50% of the applied concentration at the base.

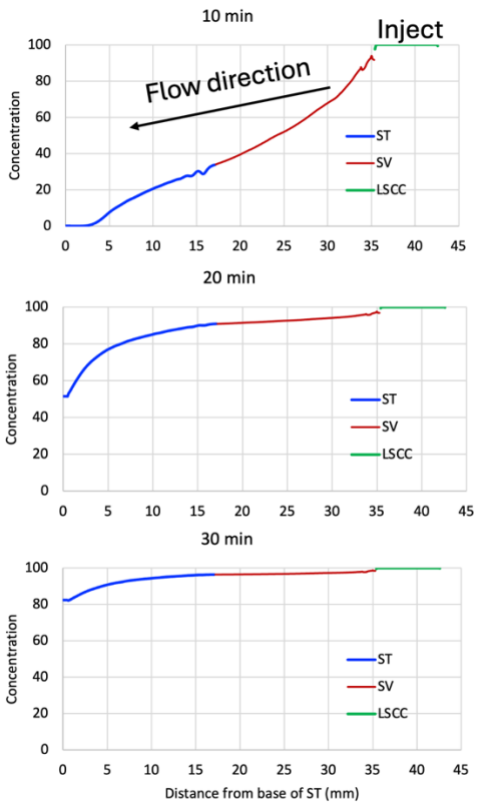
At 3 min (lower panel) the base of ST has reached 78% of the applied concentration.

At 4 min (the period typically used in our protocols, not shown), the base of ST reaches ~82% of the applied concentration



However, it is also sometimes necessary to consider how much drug is being driven into CSF by the injection. The plot (left) shows the total solute amount in the cochlea (all tissue and fluid spaces) and in CSF as a function of the injection duration.
A 4 min injection at 0.5 uL/min provides a near complete filling of the perilymph space with drug while minimizing the amount driven into CSF.

For guinea pigs, the situation is similar but perilymph volumes are larger in the guinea pig so the injected volume has to be larger. However, due to mechanical constraints, the injection rate cannot be increased too much, so the injection duration has to be longer. In our “standard” protocol we use a 30 min injection at 1 uL/min. As the lateral SCC is superficial and is easily exposed surgically in the guinea pig, this procedure can be performed reliably as a recovery procedure.



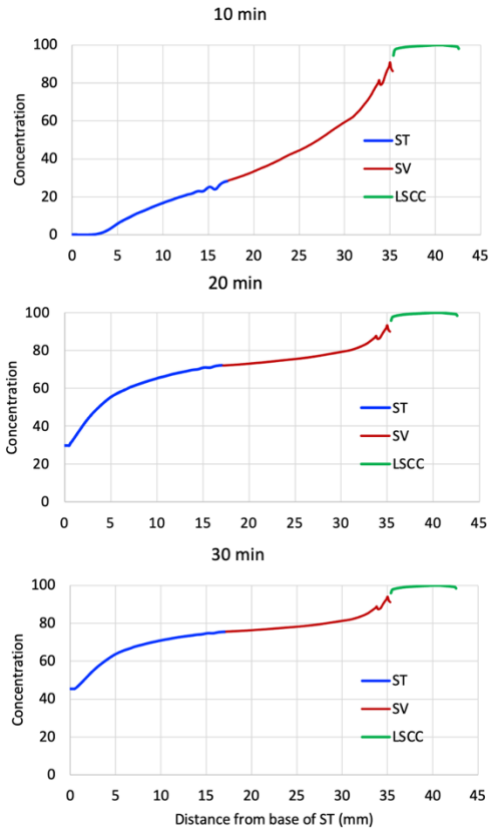
These figures show the perilymph spaces “unrolled” for the guinea pig during an injection at 1 uL/min.

It takes 10 min of injection for concentration to start rising in ST.

At 20 mins, the base of ST reaches 51% of the injected concentration. At this time just 9% of the injected drug has been driven into CSF.

At 30 min, the base of ST reaches 82% of the injected concentration with 29% of the injected drug being driven into CSF.

It is apparent that the entire perilymph space is reasonably well loaded by this protocol. However, this is the “best-case scenario” and assumes that none of the injected substance is eliminated (lost) to the vasculature.



In contrast, this simulation shows the “worst-case” scenario for a drug (triamcinolone-acetonide) that is quite rapidly eliminated from perilymph (SV half time 34 min, ST half time12 min).

As the drug solution moves along the fluid spaces the regions furthest from the injection site achieve lower concentrations.

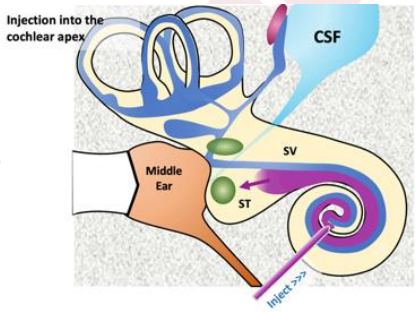
At 20 min the base of ST reaches 30% of the applied concentration vs. 51% with no elimination (above).

At 30 min, the base of ST reaches 45% of the applied concentration, vs. 82% with no elimination.

Thus, the ability to load the perilymphatic spaces with drug depends to some extent on the drug’s properties.

For Humans, it will never be possible to load perilymph with drug by this method. In humans the cochlear aqueduct is smaller and less patent, so the injection rates would need to be lower to avoid pressurizing the ear during injection. In addition, perilymph volumes in the human are much larger. These two factors make it impossible to replace perilymph in the human by injection from a pipette sealed into the ear.

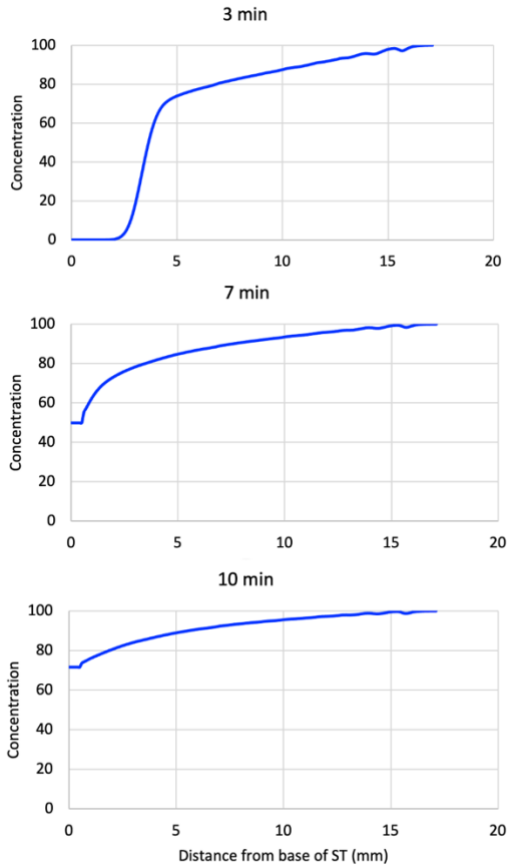
2) Injection into the cochlear apex, pipette sealed, no outlet



This method can be used in animals in which the apex can be readily accessed (rats, guinea pigs, mini-pigs, etc). It is more difficult to perform in mice and **cannot be performed in the human**, where the cochlear apex is embedded in thick bone.

It is performed as an acute, **non-recovery procedure**. Exposure of the cochlear apex is a major surgical procedure, so this cannot be performed as a recovery procedure.

It progressively fills ST with drug solution from apex to base, with the cochlear aqueduct providing the outlet for fluid flow.



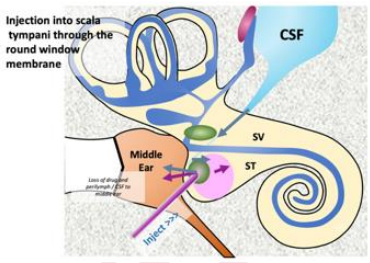
These plots show the progression of drug down ST with an injection rate of 1 uL/min.

Even at 10 mins, the basal perilymph concentration only reaches 71% of the applied concentration. This is primarily due to exchange with adjacent tissue compartments, including the spiral ligament, organ of Corti and spiral ganglion.

By changing pump speed as a function of time to correct for scala area changes, it is possible to force the leading edge of the drug solution to travel down ST at a fixed rate.

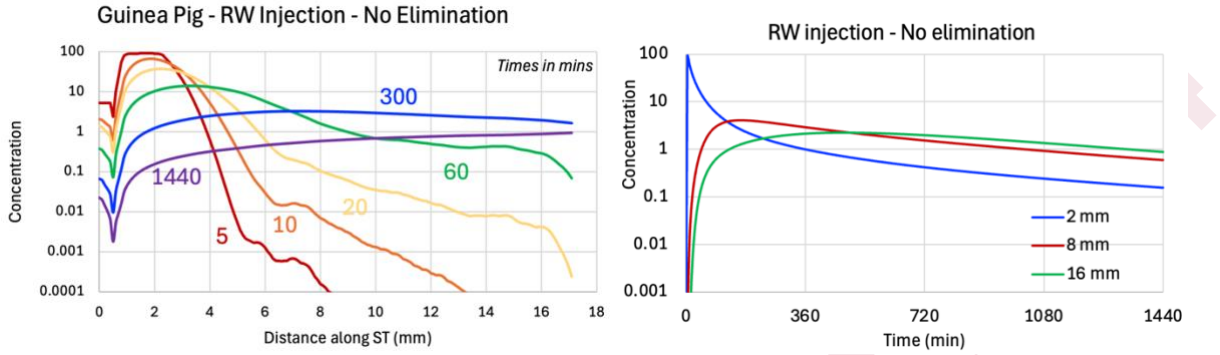
This has become the basis for experiments to study the spatial origins of the cochlear potentials. Inhibitors such as kainite progressively knock out neural function from the apex to the base, allowing the spatial origins of neural potential (CAP and ABR) to be identified as detailed in Lichtenhan et al, 2016.

3) Injection into the basal turn of scala tympani with no outlet



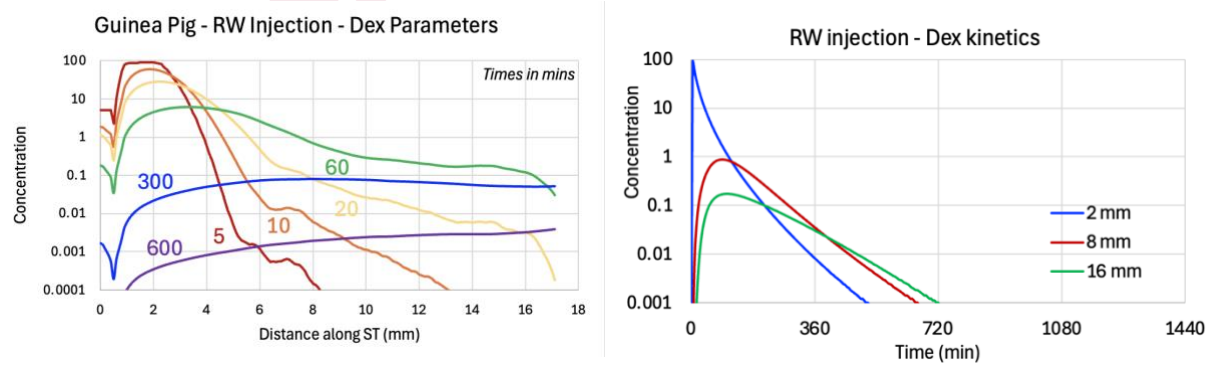
In this configuration the injection pipette is not sealed into the round window and fluid leaks around it before, during and after the injection procedure.
It is most effective when the injection pipette is inserted a short distance into ST, so the drug solution is injected as far (apically) as possible from the cochlear aqueduct and the resulting washout from flow between the aqueduct and the leaking injection site.
This procedure is typically only performed in species with larger ears, such as guinea pigs, mini-pigs and humans. It is not performed in mice due to the limited insertion distance of the injection pipette.

FluidSim set up to inject from a pipette inserted 1 mm form an insertion point of 0.8 mm from the base of ST (as indicated by the black lines). Note that drug rapidly spreads apical to the injection site by diffusion.



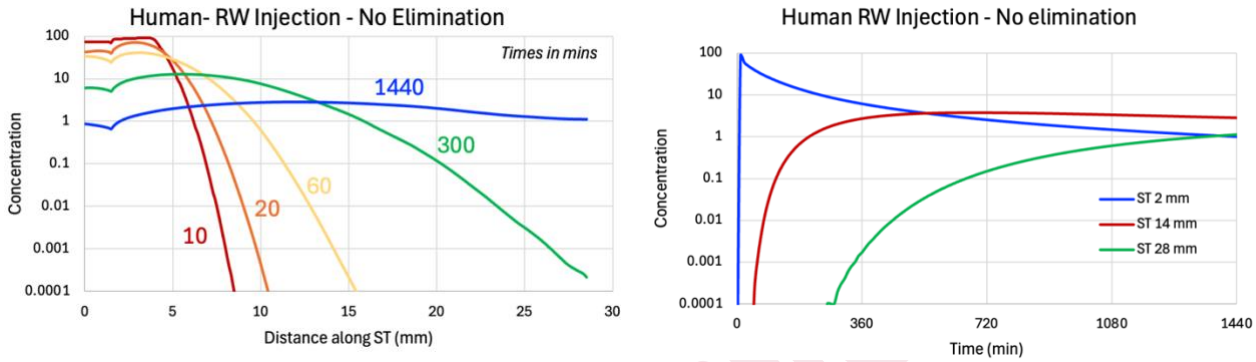
The figure (left) shows the distribution of drug along ST following RW injection of 5 uL solution (1 uL/min, 5 min), with an insertion depth of 1 mm from the insertion point of 0.8 mm.
The basal segment of ST is loaded which spreads apically by diffusion and is simultaneously washed out by CSF entering at the aqueduct and leaking from the injection site.
By 300 min, the drug is widely distributed along ST, albeit at approximately 30x lower concentration than that injected.
The time courses for 3 locations are shown at the right. The timing of C_{max} varies with cochlear location.
This calculation shows that RW injection provides a good delivery system for large, impermeable molecules, antibodies, adenoviruses, etc provided the agent is therapeutic at the diluted concentration reaching apical regions.

However, the method is less suitable for small molecules that are eliminated from perilymph more rapidly. The graphs below show comparable calculations for a molecule with properties similar to dexamethasone (SV elimination 87 min, ST elimination 40 min).

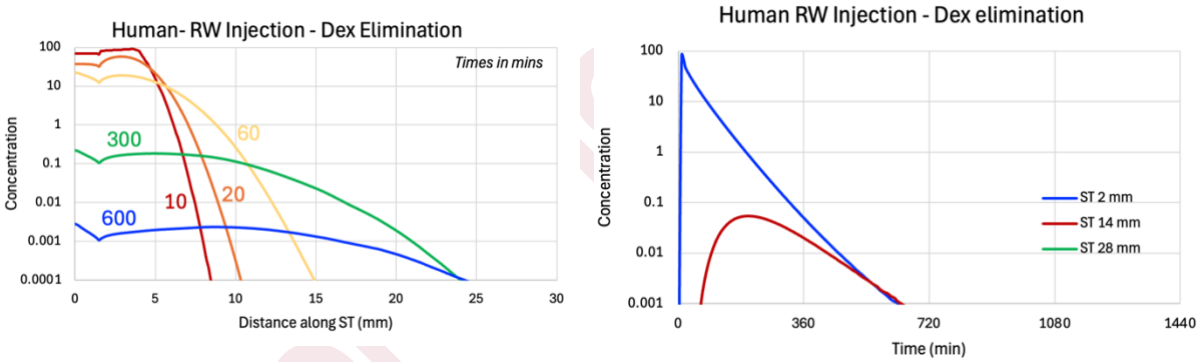


Distribution to apical regions is extremely limited (1000x below that injected), as is the period of drug exposure (just a few hours)

In Humans, the relationship is similar. In this case the simulation is set for the injection pipette inserted 2 mm from the RW injection site at 1.5 mm. A 10 minute, 2 uL/min injection (20 uL total) is used, which is sufficient to load the basal region close to the applied concentration (100 arbitrary units). At 24 hours the drug has diffused throughout the cochlea, albeit at substantially lower concentration (about 50 - 100 x lower) than that injected.



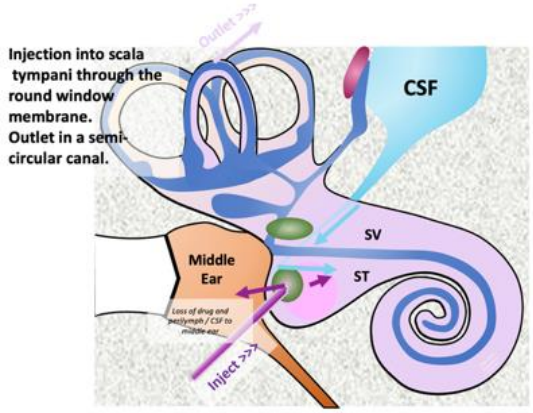
The method works less-well for smaller molecules that are eliminated from perilymph.



The above plots show the calculated distribution (left) and time course (right) for RW injection of a molecule with elimination characteristics similar to dexamethasone. The amount of drug reaching apical regions is expected to be extremely low with an exposure duration of just a few hours.

Additional (lesser used) methods

4) **Injection into the basal turn of scala tympani with an outlet in a semi-circular canal.**



This method is appealing to distribute drug throughout the entire ear. Specifically in humans and other primates, where the cochlear aqueduct does not provide an outlet for flow of the volume necessary to fill the entire ear.
It has also been used in mice as a method to increase drug distribution throughout the ear but is only semi-quantitative.

Drug concentration in the perilymph spaces depends on both the injection rate (easily defined) and the rate of CSF flowing through the cochlea due to the perforation of the semi-circular canal (at an unknown rate – between 0 and 1 uL/min).

As the CSF entry rate may change as a result of pressure changes associated with drug injection, it is not simple to predict the drug concentrations and exposure.

Similar methodology has been considered for use in humans (to deal with the larger perilymph volume of the human cochlea) but fenestration of a semi-circular canal is generally considered as excessively invasive with concerns about how well the defect heals without resulting in a canal dehisence.

5) **Intracochlear drug delivery devices**

There is strong interest in the development of devices to deliver drugs to the ear more effectively using custom devices. This includes drug eluting cochlear implants, drug eluting implantable polymers and a variety of cannulas. Drug levels and distribution from such devices can be calculated using the FluidSim program, incorporating the specific properties of the device.

References

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