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Dexamethasone concentration gradients along scala tympani after application to the round window membrane

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Abstract

Hypothesis—Local application of dexamethasone-21-dihydrogene-phosphate (Dex-P) to the round window membrane (RWM) of guinea pigs produces a substantial basal-apical concentration gradient in scala tympani (ST) perilymph.

Background—In recent years, intratympanically-applied glucocorticoids are increasingly being used for the treatment of inner ear disease. Although measurements of intracochlear concentrations after round window (RW) application exist, there is limited information on the distribution of these drugs in the inner ear fluids. It has been predicted from computer simulations that substantial concentration gradients will occur with lower concentrations expected in apical turns after RW application. Concentration gradients of other substances along the cochlea have recently been confirmed using a sequential apical sampling method to obtain perilymph.

Methods—Dex-P (10mg/ml) was administered to the RWM of guinea pigs (n=9) *in vivo* for 2 to 3 hours. Perilymph was then collected using a protocol in which ten samples, each of approximately 1μl, were taken sequentially from the cochlear apex into capillary tubes. Dex-P concentration of the samples was determined by HPLC. Interpretation of sample data using a finite element model allowed the longitudinal gradients of Dex-P in scala tympani to be quantified.

Results—The Dex-P content of the first sample in each experiment (dominated by perilymph from apical regions) was substantially lower than that of the third and fourth sample (dominated by basal turn perilymph). These findings qualitatively demonstrated the existence of a concentration gradient along scala tympani (ST). After detailed analysis of the measured sample concentrations using an established finite element computer model, the mean basal-apical concentration gradient was estimated to be $17 \cdot 10^3$. Both absolute concentrations of Dex-P in ST and the basal-apical gradients were found to vary substantially.

Conclusion—The existence of substantial basal-apical concentration gradients of Dex-P in ST perilymph was demonstrated experimentally. If the variability in peak concentration and gradient is also present under clinical conditions this may contribute to the heterogeneity of outcome that is observed after intratympanic application of glucocorticoids for various inner ear diseases.

Keywords

cochlea; drug delivery; dexamethasone; glucocorticoids; guinea pig; intratympanic; perilymph; pharmacokinetics; round window membrane; steroids

Introduction

Over the past decade there have been a rapidly growing number of clinical reports on local inner ear drug delivery for the treatment of inner ear diseases like idiopathic sudden sensorineural hearing loss (ISSHL), tinnitus, Menière's disease, autoimmune inner ear disease or to diminish cochlear implant insertion trauma. The rationale behind this is that despite the lower total amount of drug given, medications applied to the round window membrane (RWM)

can result in higher concentrations in the inner ear fluids than with systemic application. Several pharmacokinetic animal studies have confirmed this principle (1).

Strategies for local drug delivery to the inner ear include *intracochlear* (as in cochlear implantation) and *extracochlear (intratympanic)* application routes. For the latter, a variety of drug delivery systems have been described for use in humans and animals. Single or repeated intratympanic injections are performed with or without volume stabilization and with or without visualization of the round window membrane. In other cases, controlled drug release devices are used for continuous or discontinuous drug application via partly or fully implantable pump systems or biodegradable biopolymers (1;2).

A growing number of publications focus on the treatment of ISSHL with glucocorticosteroids. Either dexamethasone or methylprednisolone are applied as a first line or (more often) second line (i.e. salvage or rescue) therapy using various drug delivery protocols. The treatment results in these reports vary considerably and the evidence provided is modest since most of the clinical reports are case series (3). Few studies compared their findings with a control group (4–10) and only one randomized trial has so far been published (11).

Several factors can account for variations in outcome or even treatment failures. The first possibility is that the applied drug may not be effective. By definition, the etiology of ISSHL is not known and different pathophysiological pathways are likely to account for the same clinical presentation. Therefore, the substance chosen may not be the appropriate drug for all patients included in a study. A second possibility is that mucosal adhesions or false membranes in front of the human round window membrane may prevent the drug getting into contact with the RWM (12). The third possibility is that following application to the RWM, the drug might not be reaching the target, possibly caused by variations in RWM permeability, with insufficient drug doses entering the cochleae of some patients. Alternatively, it could be due to the existence of concentration gradients within the inner ear with different regions being exposed to significantly different drug levels. The existence of these concentration gradients had already been implicated in studies using computer simulations to interpret pharmacokinetic studies with gentamicin and glucocorticoid administration to the round window membrane (13–15). In the guinea pig, gradients have been demonstrated experimentally for an ionic marker and for gentamicin (16;17). Furthermore, previous pharmacokinetic studies in the cochlea that involved fluid sampling have faced the problem of significant sampling artifacts due to contamination of perilymph samples with CSF entering the base of scala tympani as soon as the cochlear bony wall is opened for fluid sampling (1). For the development of effective pharmacologically-based inner ear therapies however, it is important to apply a quantitative approach towards dose-effect relationships. With the development of the rather straightforward experimental method of sequential apical sampling it has become possible to quantify drug distribution throughout cochlear perilymph (16;18). The aim of the present study was to measure concentration gradients of dexamethasone-phosphate along the length of scala tympani perilymph of the guinea pig after short-term application to the round window membrane.

Methods

Animal preparation and procedures

The method of animal preparation and drug application is described in detail elsewhere (19). In brief, nine specific pathogen free guinea pigs (mean weight: 423g; 300–600g; Charles River, Kißlegg, Germany) were anesthetized by intraperitoneal injection of an initial dose of 8 mg/kg xylazine (Bayer, Leverkusen, Germany) plus 140 mg/kg ketamine hydrochloride (Pharmacia & Upjohn, Erlangen, Germany). Secretions were controlled by 0.3 mg/kg atropine sulphate (Braun, Melsungen, Germany). Anesthesia was maintained by repeated intramuscular

injections of 25–33% of the initial dose. Further preparation followed as described in Hahn et al. (2006) and in Figure 1. The animal experiments were approved by the animal studies committee of the University of Tübingen.

Dexamethasone-21-dihydrogen-phosphate disodium salt (Dex-P, Fortecortin® Inject, Merck, Darmstadt, Germany, 10 mg/ml) was applied continuously to the RWM for 2 to 3 hours. Fluid sampling was started approximately 10 min after the drug application stopped. The method of apical sampling and its modification for obtaining perilymph samples from different regions of scala tympani has previously been described (16–18), and is shown schematically in Figure 1. Ten approximately equal samples of 1 µl each were taken from the cochlear apex. In two of the experiments, the first two samples were 0.5 µl in volume, but 1 µl samples were collected in later experiments as the drug concentration in smaller samples was undetectable. The time required to take 1 µl samples averaged 50 s (SD: 14 s, n=90). Samples were diluted into 7 to 13 µl sterile filtered water and stored at –20°C together with four dilutions of the Dex-P solution.

Quantitative analysis of dexamethasone-21-dihydrogen phosphate and dexamethasone

Dex-P and Dex were quantitatively analyzed by a Sykam high performance liquid chromatography (HPLC) system equipped with Grom Saphir 110 C18 (5 µm, 10×4 mm) precolumn and a Grom-Sil 120 ODS-3 CP (5 µm, 250×4 mm) column (Grom Analytik, Rottenburg, Germany). For further HPLC-details see Hahn et al. (19). The peaks for Dex-P and Dex appeared at 7.0 min and 26.1 min, respectively. The limit of detection (LOD) for Dex-P was approximately 0.100 µg/ml (S/N = 1:5) in a 5 µl injection volume of diluted perilymph sample which corresponds to 0.5 ng of Dex-P. The limit of quantification (LOQ) was 0.195 µg/ml.

Interpretation of sequential sample data

Sample data were interpreted using a finite element model (<http://oto.wustl.edu/cochlea/>), modified to incorporate fluid movements, drug diffusion and volume accumulation corresponding to those during apical sampling (described in detail in: (13;16;17)). The sample concentrations were fitted by adjusting clearance (general clearance, including binding to lipids and proteins and drug conversion), RWM permeability, perilymph flow (before sampling) and accessibility to compartments parallel to ST, such as the spiral ligament and modiolus. With this analysis it was possible to derive the concentration profile along ST immediately prior to sampling, which best accounted for the sample values.

Results

Measured Dex-P concentrations in the 10 samples sequentially taken from the cochlear apex are shown in Figure 2. In one animal, all sample concentrations were below the detection limit so the experiment was excluded. The peak concentrations varied from <1 µg/ml to approximately 600 µg/ml (mean: 136 µg/ml, SD: ±185 µg/ml), corresponding to <0.01% to 6% (mean: 1.36%) of the applied concentration.

The higher concentrations in the 3rd and 4th samples (originating from the basal part of the cochlea) relative to the first sample (originating from the apical region) confirmed the existence of significant basal-apical concentration gradients along scala tympani. This is further demonstrated in Figure 3, where the measured sample concentrations of the first four samples from each experiment are plotted against their estimated region of origin in scala tympani.

The gradients in the sample concentrations, quantified by the ratio of the first and fourth sample in each experiment varied considerably (table 1). However, this calculation greatly underestimates the actual gradient along ST. Due to a CSF-dilution effect, the concentrations

near the base underestimate the basal concentration. In addition, the first sample overestimates the apical concentration because: i) The first sample represents the average concentration of the fluid within approximately the apical 10 mm of scala tympani (to account for the 1 μ l volume taken). ii) In five animals (marked with “§” in table 1) the concentration of Dex-P in the first sample was below the limit of detection. To calculate the concentration ratio in these experiments the LOD (1 μ g/ml) was used, although the actual concentration might have been much lower. For these reasons, we performed a more detailed analysis of the sample data using computer simulations of drug delivery and sampling (Fig. 4). With the parameters that provided the best fit, it was possible to calculate the drug concentration along scala tympani prior to sampling. The computer analysis increased the spatial resolution for drug distribution and showed that drug gradients necessary to generate the observed sample concentrations were substantially higher than suggested by the raw sample concentrations (Table 1).

Dex-P is a prodrug that is hydrolyzed to the active moiety (dexamethasone base, Dex). Since Dex concentrations were lower than those of Dex-P, quantitative analysis of Dex was only possible in a limited number of experiments. The measured concentrations for Dex were lower but the similarity of the sample concentration curves suggests that the distributions along ST were similar to that of Dex-P (Fig. 5).

Discussion

Absolute glucocorticoid concentrations

The peak concentration levels of Dex-P at samples 3 and 4 in our study (136 μ g/ml $\pm$ 185 μ g/ml) were similar to measurements in a recent microdialysis study (19) but showed much higher levels than previously reported (20;21). This is mainly due to significant sampling artifacts with sample dilution by CSF when perilymph is collected from the base of scala tympani, which was the method used in the prior studies. It has been shown that with that technique the actual perilymph concentration is underestimated (1;14;22). With the new and relatively straightforward sequential apical sampling method, effects of sampling artifacts were largely reduced, giving more accurate representations of intracochlear concentrations prior to sampling (16;18).

The high variability in intracochlear glucocorticoid concentration reached after RWM application is not a new observation but has been described previously in several studies (Dexamethasone: (19–21), (Methyl-)prednisolone: (21;23), Hydrocortisone: (21)). A high variability in RWM permeability is commonly assumed to be the underlying cause. Variability of intracochlear drug concentrations after local application is likely contributing to inconsistencies in therapeutic efficacy. Correlations of efficacy with pharmacokinetic measurements of intracochlear drug levels would be valuable to confirm this.

Basal-apical concentration gradients

An important finding confirmed experimentally in this study was the existence of substantial basal-apical glucocorticoid concentration gradients in ST perilymph, as previously described for other substances (16;17). A recent immunohistological study on the pharmacokinetics of Dex-P and Dex after RWM application in the mouse has qualitatively demonstrated a widespread distribution of the glucocorticoid throughout the cochlea, including the apical regions (24). It has to be noted that scalae lengths in the mouse inner ear are much shorter, with scala tympani less than 4.6 mm long (25). Basal-apical gradients are therefore expected to be much smaller in the mouse cochlea than in rodents with larger cochleae, such as the guinea pig. The opposite would be expected for human cochleae in which the scala tympani is about twice the length of that of the guinea pig. For the human the basal-apical gradients are expected to be even larger than those measured in our experiments (13;14). The existence of large

intracochlear concentration gradients and their variability appears to be less problematic for substances like glucocorticoids since they are known to have a wide therapeutic range. Therefore, it would more likely be possible to use high doses in therapeutic settings with minimal toxicity to the base of the cochlea. In those cochleae demonstrating very large gradients, however, the amount of drug reaching the cochlear apex after short application times would probably be too low to be effective. For drugs with a narrow therapeutic range, a high variability in the intracochlear concentrations and in concentration gradients may be problematic and better control of drug delivery becomes necessary.

Pharmacokinetic parameters

While the sample concentration data qualitatively demonstrate the existence of substantial gradients for Dex-P and Dex following local applications, these data can be interpreted qualitatively by simulations of the experiment. Simulations provided a good representation of the drug gradients along ST but with the present data there was a unique difficulty which confounded accurate parameter identification. This arises from the interaction between RWM permeability and drug clearance from ST, which in the simulations both similarly influenced the amount of drug in the samples. Prior studies with TMPA showed a rate of clearance that was rapid enough to affect the distribution of TMPA along the scala (16) while clearance of gentamicin was so low that it could be neglected (17). For Dex-P, clearance occurred at an intermediate rate so it was not possible to resolve clearance and RWM permeability independently. The mean RWM permeability values ($\bullet 10^{-8}$ m/s) were 1.69 with no clearance, or 1.89, 2.06, 2.56 and 3.87 respectively for clearance half times of 500, 250, 125 and 65 mins. Experiments with longer application times than those used here would necessary to characterize these two parameters independently. The other important parameter derived by the analysis was the rate of perilymph flow along ST prior to sampling. The average of 9 nl/min (Table 1) is less than that reported recently in similar apical sampling experiments using an ion marker (19 nl/min (16)) and gentamicin (21 nl/min (17)) but more than the rates reported in previous studies with fixed ion electrodes: 1.6 nl/min (26) and 4.4 nl/min (27). Volume flow of perilymph in the sealed cochlea would act as an additional transport mechanism, allowing drugs to spread towards the apex faster than by diffusion alone. This may merit further investigation as any mechanism influencing longitudinal drug distribution is of high significance for clinical applications.

It has to be emphasized that in this study concentration gradients were determined for application times of two to three hours. It is expected that with time the drug levels in the inner ear will “equilibrate”. However, since drug is constantly removed from the inner ear by clearance processes, drug levels as the system approaches equilibrium are far lower than those immediately following application (2;13–15). With our experimental and analytical approach, measurements of Dex-P and Dex in fluid samples at later time points after drug application are not possible since sample concentrations would be too low to be detectable.

Future studies are necessary directed towards i) long-term measurements of intracochlear concentrations after drug application, ii) increasing substance uptake through the round window membrane, iii) reducing the variability of absolute concentrations in the inner ear, iv) reducing the variability of longitudinal drug distribution (i.e. basal-apical concentration gradients), v) correlation of intracochlear concentration and therapeutic efficacy and vi) controlling substance distribution in the cochlea.

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Fig. 1.

Sequential apical sampling: **A:** Cartoon drawing of the view to the opened guinea pig otic bulla through a ventrolateral surgical approach showing the tympanic membrane (TM) and the cochlea (I, II, III: cochlear turns) and drug application pipette (AP) in the RW niche. After removal of the mucosa, cyanoacrylate glue (Aesculap, Tuttlingen, Germany) was applied to the apex and a silicone “cup” (two-part silicone elastomer sealant, KWIK-Cast, WPI, Sarasota, USA) was constructed (1). **B:** Cross section of the cochlear apex with the layer of cyanoacrylate (CA) and silicone over the bony surface covering the perilymphatic space (PL). **C:** At the end of the application (2), the pump was stopped and the RWM niche was carefully wicked dry avoiding any contact with the round window membrane (RWM). When the cochlear apex is perforated, perilymph is slowly expelled, driven by cerebrospinal fluid pressure. **D:** The expelled fluid was collected in calibrated glass capillaries (Blaubrand® intraMARK, BRAND GmbH, Wertheim, Germany) used as sampling pipettes (SP), (3).

Fig. 2.

Left: When the cochlear apex is perforated, cerebrospinal fluid (CSF) is slowly expelled through the cochlear aqueduct entering at the base of scala tympani (black arrow). Sequential samples taken from the apex contain perilymph from different regions (1–4) from scala tympani. During the drug application and the sampling process substance is also partitioned and repartitioned to and from other compartments like the modiolus, scala vestibule, endolymphatic space and lateral wall (dotted arrows). **Middle/Right:** Each curve shows the measured Dex-P concentrations in 10 samples taken sequentially from the cochlear apex. The first 4 samples (grey area) contain mainly perilymph from different regions of scala tympani (1–4 on left panel). The higher concentration of the 3rd and 4th samples (basal region) relative to the first sample (apical region) indicates a significant basal-apical concentration gradient along ST. The following samples (numbers 5 to 10) mainly contain cerebrospinal fluid which has replaced and diluted ST perilymph during the sampling process. The measured Dex-P levels in these later samples can be explained by repartitioning of the drug from other cochlear tissues back into scala tympani. A: absolute concentrations, B: normalized to concentration in the 4th (most basal) sample.

Fig. 3.

Concentrations of the first four samples taken in each experiment shown as absolute values (A), on a logarithmic scale (B), and normalized to the concentration in sample number 4 (C). The distance along scala tympani plotted is that of the mid-point of the estimated region of origin (half the sample volume apical and half the volume basal to the location), as indicated by the insert in panel A. Despite the limited spatial resolution based on the four samples, all graphs clearly demonstrate the existence of significant basal-apical concentration gradients.

Fig. 4.

Left: Interpretation of sample concentrations using a computer model: An example of a best fit between measured and simulated sample concentrations is shown. **Middle and right:** Dex-P gradients along scala tympani derived from simulations of each experiment: Concentration gradients are plotted on an absolute concentration scale (A) and a logarithmic scale (B).

Fig. 5.

At the time of sampling part of the applied prodrug (Dex-P) was converted into the active moiety (Dex). The sample concentration courses between the active moiety (Dex) and the applied prodrug (Dex) were compared in three experiments (A, B, C). Although concentrations for Dex were lower, the sample concentration course followed the one for Dex-P.