

Quantitative interpretation of corticosteroid pharmacokinetics in inner fluids using computer simulations

Stefan K.R. Plontke^a, Alec N. Salt^{b,*}

^a *Tübingen Hearing Research Center and Department of Otorhinolaryngology, Head and Neck Surgery, University of Tübingen, D-72076 Tübingen, Germany*

^b *Department of Otolaryngology, Box 8115, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, MO 63110, USA*

Received 30 October 2002; accepted 23 March 2003

Abstract

The delivery of drugs to the inner ear by applying them directly onto the round window membrane is a promising way to treat human inner ear disorders. To further develop this strategy, and to design controlled clinical trials, additional preclinical studies are necessary. It is especially important to derive the time course and total dose for the various target regions within the inner ear. Since direct pharmacokinetic measurements in the human cochlea are not possible, simulations provide a valuable tool for the interpretation and planning of animal studies, for evaluating changes of application protocols and drug delivery systems, and for extrapolating the results from animal studies to the human. The present study has analyzed two previously published data sets in which concentration time courses of corticosteroids in the cochlear fluids were reported. Drug movements were simulated with a finite element computer model of the inner ear fluids. The time course of corticosteroid pharmacokinetics could be approximated for each study by consideration of the specific experimental paradigm. Although the experimental studies reported considerably different drug levels in the fluid samples taken from the cochlea, these differences were largely explained by considering the experimental design of the respective studies. After correction for experimental differences, the calculated perilymph levels of drug were within a factor of two of each other. The simulations demonstrated that an important factor controlling the drug level achieved is the time the drug solution remains in the middle ear. It can be concluded that small differences in delivery protocols may cause large variations in the drug levels achieved in the inner ear fluids.

© 2003 Elsevier B.V. All rights reserved.

Key words: Cochlea; Corticosteroid; Perilymph; Round window; Pharmacokinetics; Inner ear drug delivery

1. Introduction

There is an increasing interest in the treatment of inner ear disorders by topical application of drugs to the inner ear. Using different local delivery strategies, drugs can be applied that cannot be given systemically

due to unfavorable risk–benefit ratios or systemic side effects.

Apart from new investigational drugs showing promising results for treatment of inner ear diseases or for otoprotection (Li et al., 2001; Korver et al., 2002; Lalwani et al., 2002), there is a strong interest in topically administered corticosteroids which have been used both in animal research (Bachmann et al., 2001; Chandrasekhar et al., 2000; Parnes et al., 1999; Shirwany et al., 1998; Spandow et al., 1988) and clinically in humans (Arriaga and Goldman, 1998; Blakley, 1997; Chandrasekhar, 2001; Gianoli and Li, 2001; Kopke et al., 2001; Parnes et al., 1999; Sakata et al., 1996; Shea and Ge, 1996; Shea, 1999; Silverstein et al., 1996, 1998). It is important to note, however, that at present no drug including corticosteroids is yet approved for local ap-

* Corresponding author. Tel.: +1 (314) 362-7560; Fax: +1 (314) 362-7522.

E-mail address: salta@msnotes.wustl.edu (A.N. Salt).

Abbreviations: AUC, area under the curve; C_{est} , calculated concentration; C_{meas} , measured concentration; C_{max} , maximum or peak concentration; CSF, cerebrospinal fluid; RWM, round window membrane; ST, scala tympani

plication to the round window membrane (RWM) with the indication of treatment of inner ear diseases or otoprotection.

For further development and transfer of this promising therapeutic strategy into controlled clinical trials it is necessary to undergo preclinical studies including pharmacokinetic and toxicity studies to establish dose–effect relationships for both therapeutic and toxic effects (Lamm and Arnold, 1999; Shirwany et al., 1998; Spandow et al., 1988). It is especially important to derive the time course and total dose at the various target regions within the inner ear. Direct studies of inner ear pharmacokinetic profiles after intratympanic application in humans (i.e. phase I clinical trials) are not possible, because human inner ear fluids cannot be safely sampled without damage to the ear. The alternative is to predict drug levels from results obtained in animal experiments. To make quantitative predictions a methodology must be established that is applicable to the unique situation of the inner ear fluid spaces. The purpose of the present study is to demonstrate one approach to how cochlear fluid kinetics can be analyzed. From experimental studies (Hoffer et al., 2001) and mathematical simulations based on general principles of drug dispersal in inner ear fluids (Salt and Ma, 2001; Plontke et al., 2002) it is known that the pharmacokinetic profile of a drug in the inner ear depends highly on the specific application protocol, on the cochlear dimensions, on the delivery strategy, and on the physico-chemical factors affecting agent diffusion. In addition, the measured drug levels in the inner ear are influenced by the method used to sample the cochlear fluids (Hara et al., 1989; Salt et al., 2003). It has already been documented that fluid sampling methods are critical to obtain valid results although quantification of sample contamination due to sampling is difficult (Scheibe et al., 1984; Hara et al., 1989; Salt and Thalmann, 1988).

A number of studies have documented the pharmacokinetic profiles for various corticosteroids after topical application to the RWM (Bachmann et al., 2001; Chandrasekhar et al., 2000; Parnes et al., 1999). For one of the papers (Chandrasekhar et al., 2000) the detail of description of the experimental design and presentation of the results did not allow any simulation of the experiment. Data from the other two studies (Parnes et al., 1999; Bachmann et al., 2001, hereafter abbreviated as Parnes' and Bachmann's data respectively) use conventional sampling techniques and lend themselves to quantitative analysis. Although similar drugs were delivered in the same animal species at similar concentrations in the two studies, the measured peak concentrations of steroid in perilymph differed by a factor of approximately 20 between the two studies. This difference causes uncertainty in drawing any

conclusion with regard to drug levels in the human. Therefore, these studies have been analyzed in detail using computer simulation techniques.

2. Methods

Corticosteroid pharmacokinetics in the cochlear fluids were simulated with a finite element computer model, the Washington University Cochlear Fluids Simulator, version 1.6, which is available in the public domain at <http://oto.wustl.edu/cochlea/>. The simulator considers the geometric dimensions of the inner ear, specifically the cross-sectional area of each scala as a function of distance (Thorne et al., 1999), the area of the RWM and its geometric relationship to scala tympani (ST) (Ghiz et al., 2001), the size of the vestibule and of the helicotrema. It incorporates passive physical processes of drug dispersal in fluids (predominantly diffusion) as well as inner ear-specific pharmacokinetic parameters, including the permeability of the RWM, the rate of clearance from the inner ear fluids to external compartments (such as to blood), and the rate of local exchange between the cochlear scalae (on a segmental basis as seen in a radial cochlear section) (Salt, 2002; Plontke et al., 2002). In addition, solute loss from the middle ear to the cochlear fluids, or to the middle ear mucosa and Eustachian tube, was incorporated.

In the model, each scala is represented in 0.1 mm segments, each with an associated area and volume. Diffusion between 0.1 mm scala segments and volume flow effects are calculated for 0.1 s time periods, while other processes (clearances, drug movements across the RWM) were calculated for 1 s time increments. The diffusion coefficients used were estimated on the basis of the formula weight of the drug used. The quantitative accuracy of the model has been validated by comparisons with direct solute measurements from the cochlea made with ion-selective electrodes (Ohyama et al., 1988; Salt et al., 1991, 2003; Salt and Ma, 2001). A more detailed description of the operation of the model is given elsewhere (Salt, 2002).

An important factor in experimental studies of inner ear fluid pharmacokinetics is the sampling method used. It has been shown experimentally that the act of aspirating a perilymph sample can markedly influence the concentration of drug in the inner ear fluids, so that the sample concentration may not always be a good indicator of the drug level at the site prior to sampling (Scheibe et al., 1984; Hara et al., 1989; Salt and Thalmann, 1988; Salt et al., 2003). Calculations for samples taken from intact cochleae (as in Parnes' study) therefore incorporated a detailed simulation of the sampling procedures, in which volume movements and fluid flows associated with sample aspiration were calculated. The

simulated sample represented the summed perilymph components drawn into the pipette. The sample concentration therefore depended on the sample size, the sampling rate and the location of the sampling pipette. Important in this regard is the location of sampling relative to the location of the cochlear aqueduct, through which cerebrospinal fluid (CSF) replaces the perilymph that is drawn from ST in the sampling process.

Simulations of corticosteroid time course data reported by Parnes and Bachmann replicated the drug delivery procedures and protocols used in their experiments, as summarized in Table 1. A best fit of the calculated time courses to the experimental data was obtained by determining sums of squared differences between the measured concentrations (C_{meas}) and those estimated by the simulation (C_{est}) and systematically changing parameters until the sum of squared differences $[\sum(C_{\text{est}} - C_{\text{meas}})^2]$ was minimized (Plontke et al., 2002). The duration of the simulations of Bachmann's data was set equal to their sampling time points (15, 20, 80, 180, 330, or 960 min). This interval also determined how long the drug was left in the middle ear before washout. Since Bachmann obtained 5–10 μl samples of perilymph by taking fluid from the apex when the basal turns of ST and scala vestibuli were opened, their data were compared with the total perilymph concentration calculated by summing all cochlear perilymph segments, for a combined total volume of 8.6 μl .

Simulation of Parnes' data specifically considered the time the solution remained in the middle ear, the sampling time points (at 60, 120, 240, and 360 min after

drug application), the sample location, volume and rate of aspiration (1.5 $\mu\text{l}/\text{min}$ at 60 and 120 min and 1 $\mu\text{l}/\text{min}$ at 240 and 360 min) as shown in Table 1. Since samples in Parnes' study were taken from the base of ST, with negligible contribution from apical locations or perilymph in scala vestibuli, the data were fit by systematically changing RWM permeability using parameters for clearance and inter-scala communication derived from the simulation of Bachmann's data.

Drug levels were also calculated for cochlear dimensions corresponding to those of the human cochlea, using pharmacokinetic parameters derived from the guinea pig. Scala length, volume and cross-sectional area as a function of distance for the human were based on those documented by Thorne et al. (1999). The area of the human RW was taken as 2.22 mm^2 which is an average of two published values (Okuno and Sando, 1988; Su et al., 1982). The vestibule was approximated as a volume of 30 μl over a length of 6.5 mm (Igarashi et al., 1986; Buckingham and Valvassori, 2001). Using parameters derived from simulation of Parnes' data, concentration time courses for four drug application paradigms were then calculated. The first protocol was a brief intratympanic injection of 0.5 ml methylprednisolone at a concentration of 40 mg/ml for 10 min representing a single application at an outpatient setting with the drug likely to remain in the middle ear for only a short time. The second protocol represented an intratympanic injection where patient movement was controlled to the extent that the applied drug stayed in contact with the RWM for 30 min. The third paradigm mimicked an intratympanic injection with volume sta-

Table 1

Comparison of the experimental protocol and the characteristics of the measured pharmacokinetic profiles as published by Parnes et al. (1999) and Bachmann et al. (2001)

	Parnes et al., 1999	Bachmann et al., 2001	Component changed in Fig. 5
Animal	Guinea pig	Guinea Pig	
Application method	Injection of solution into the middle ear	Injection of solution into the middle ear	
Concentration in middle ear	40 mg/ml	50 mg/ml	Fig. 5E
Volume in middle ear	110 μl	100 μl	Fig. 5F
Applied drug	Methylprednisolone succinate	Prednisolone-21-hydrogen succinate	Fig. 5G
Drug formula weight	374.48 g/mol	360.45 g/mol	
Drug diffusion coefficient	$0.784 \times 10^{-5} \text{ cm}^2/\text{s}$	$0.795 \times 10^{-5} \text{ cm}^2/\text{s}$	
Duration of application	Washout from middle ear after 30 min	Washout from middle ear before sampling	Fig. 5D
Samples per animal	Multiple, 10 μl each	Single, 5–10 μl	Fig. 5B
Sample location	Scala tympani, approx. 1 mm from base	Apex, stapes and round window opened, CSF pressure released	Fig. 5C
Average measured peak concentration (C_{max})	50.37 $\mu\text{g}/\text{ml}$	952.3 $\mu\text{g}/\text{ml}$	Fig. 5A/H
Time at peak concentration (T_{max})	60 min	180 min	Fig. 5A/H
RWM permeability*	$2.0 \times 10^{-11} \text{ m/s}$	$3.5 \times 10^{-11} \text{ m/s}$	Fig. 5A/H
Clearance half time*	130 min	130 min	Fig. 5A/H
Inter-scala communication half-time*	45 min	45 min	Fig. 5A/H

Included are also the parameters calculated in this study (*) that provided a best fit to the published data.

bilization such as described by Hoffer et al. (1997) and Shea (1999). In these studies, the drugs were dissolved in fibrin glue or hyaluronic acid respectively, to prevent loss of drug through the Eustachian tube. Drug levels for the above paradigms were calculated for a total time of 960 min (16 h). A fourth protocol simulated the continuous delivery of drug to the middle ear, as with a pump, for 10080 min (7 days) with a further 3 days with the pump off, for for a total time of 14400 min (10 days). This extracochlear application to the RWM should not be confused with the widespread use of intracochlear drug application with pumps, such as that described by Brown et al. (1993). The method simulated in this protocol applies a constant concentration to the exterior surface of the RWM. For each of the protocols, drug levels at different locations in ST were compared using the peak concentration reached during the entire delivery period (C_{\max}) and using the total dose, determined as the area under the curve (AUC), which is the summed drug concentration with time, integrated over the entire delivery and recovery periods.

3. Results

Calculated drug time course curves were compared quantitatively with Bachmann's data while parameters defining RWM permeability, radial inter-scala communication and clearance were systematically varied. A best fit was established with a RWM permeability of 3.5×10^{-11} m/s, an inter-scala communication half-time of 45 min and a clearance of 130 min, as shown in Fig. 1. Bachmann's data were reasonably approximated by this calculated curve, with the peak concentration

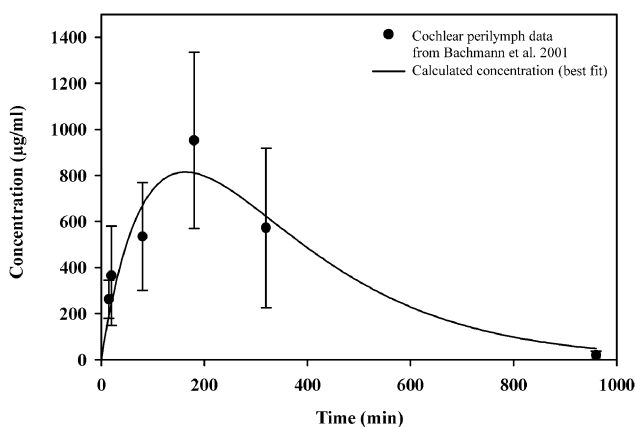


Fig. 1. Filled circles: Experimental measurement of prednisolone levels in perilymph samples taken from the apex and following round window application (data replotted from Bachmann et al., 2001). Solid line: Time course of total perilymph prednisolone concentration calculated by the finite element computer model that best fits the experimental data. Numerical parameters for the model are given in Table 1.

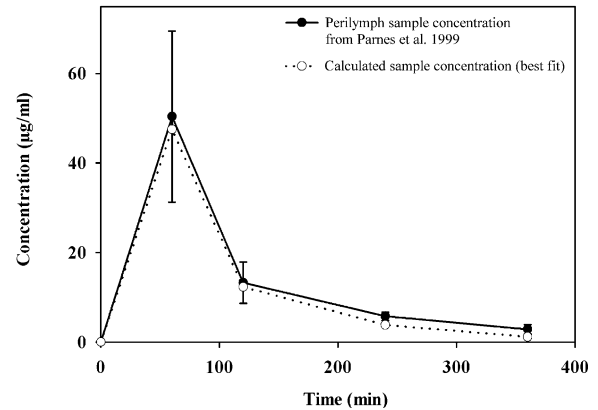


Fig. 2. Filled circles: Experimental measurement of methylprednisolone levels in repeated perilymph samples taken from ST following round window application (data replotted from Parnes et al., 1999). Open circles: Calculated concentration for samples derived from the model following the experimental protocol used by Parnes et al., 1999. RWM permeability was adjusted to best fit the calculated data to that obtained experimentally, as shown in Table 1.

of the simulation occurring at 165 min. It has to be noted that the experimental design of this study, in which ST perilymph and scala vestibuli perilymph were combined into one sample, was not ideal to quantify the radial communication between the scalae and calculated curves were relatively insensitive to small changes in the radial communication parameter.

A similar analysis of Parnes' data was performed. Using the application and sampling protocol shown in Table 1, RWM permeability (which primarily affects the amount of drug in perilymph, but not the curve shape), was adjusted until the calculated data best fit the experimental data, as shown in Fig. 2. The best fit occurred with a RWM permeability of 2.0×10^{-11} m/s. In this simulation, only discrete time points corresponding to Parnes' experiments were available, since each data point represents the calculated sample composition of a fluid sample aspirated at the indicated time. The close fit to Parnes' data is apparent, with adjustment of only the RWM permeability. The peak sample concentration with this protocol occurs with the sample at 60 min, which contrasts with the time courses shown in Fig. 1. The differences between the two studies can be best appreciated by considering Parnes' experimental protocol in detail, as shown in Figs. 3 and 4. The heavy line in Fig. 3 shows the calculated concentration of the methylprednisolone in ST at a location of 1 mm from the base of ST (i.e. at the approximate location of sampling). Thirty minutes after the application, when the solution is washed out from the middle ear, the drug level begins to decline. An even more striking effect on drug level is caused by the first sample aspiration after 60 min. The volume of fluid aspirated is replaced by CSF, drawn in through the cochlear aqueduct as the sample is aspirated. The cochlear aqueduct is patent

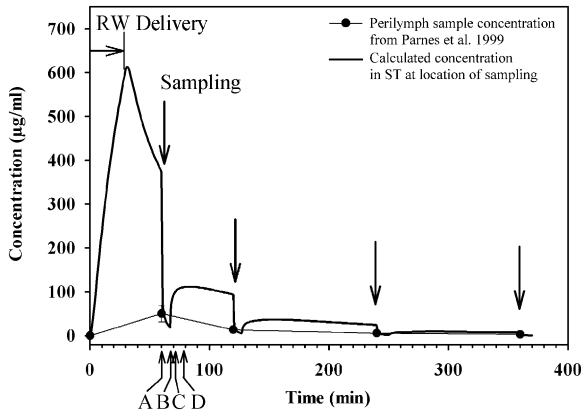


Fig. 3. Calculated time course of ST perilymph concentration at the location of sampling, 1 mm from the basal tip of the scala. Repeated 10 μ l fluid samples were taken which are presumed to contain substantial amounts of CSF, drawn into ST through the cochlear aqueduct, which enters close to the sampling location. The concentration profiles along ST at the time points A, B, C, and D are shown in Fig. 4.

in guinea pigs and opens into ST approximately 1.1 mm from the base (Ghiz et al., 2001). CSF entry causes a dilution of the drug level in ST perilymph as indicated by the drop in concentration of methylprednisolone in ST and also a dilution of the methylprednisolone levels in the sample, depending on the volume of CSF drawn into the pipette. When sample aspiration finishes, the perilymph concentration at the measurement location increases again due to drug diffusion from regions with a higher concentration, basal and apical to the sampling point. The calculated concentration profiles along the length of the cochlea at specific time points during sampling, indicated A–D, are illustrated in Fig. 4. During sample aspiration there is a dramatic decrease in drug concentration close to the site of aqueduct entry, which is partially replaced by diffusion from adjacent regions when sampling stops. The replacement of perilymph by CSF also explains how it is possible that after taking a 10 μ l sample, a volume that is larger than the total cochlear perilymph volume, methylprednisolone remains in ST as shown by the measured concentrations in subsequent samples (Figs. 2 and 3). Each subsequent sample only locally reduces the drug concentration leaving more distant regions of ST relatively undisturbed.

The influence of different elements in the experiment on the time course of drug in the perilymph is illustrated by progressively changing parameters from the protocol used in Parnes' study (Fig. 5A) to that used in Bachmann's study (Fig. 5H). The first parameter considered was the sample aspiration used in Parnes' study. By plotting the fluid concentration at the sampling site in the absence of sample aspiration (Fig. 5B) the time course of the curve still shows an early peak but the peak level is more than 10 times the concentration that

was measured and published. This clearly demonstrates that the sampling procedure itself adds an artificial clearance and exerts a substantial influence on the concentration time course. While Fig. 5B still plots the calculated concentration time course at the sampling location at 1 mm from the base of ST, the next panel (Fig. 5C) shows the calculated drug level for the entire cochlear perilymph, corresponding to the sample protocol used by Bachmann. Since apical cochlear regions, with lower concentration, are now included in the sample, the time course reaches a level of only three times the concentration in Fig. 5A, but the peak is still early. The next parameter changed concerns the time the drug is left in the middle ear. When the volume applied to the middle ear (110 μ l) is allowed to remain there, rather than being washed out after 30 min, the peak perilymph level becomes much greater and the peak occurs later (Fig. 5D). The washout after 30 min in Parnes' study causes a break in the time course and the early commencement of decrease in methylprednisolone levels in the perilymph. Taking into account the remaining parameters, including differences in applied concentration (Fig. 5E), the volume applied to the middle ear (Fig. 5F) and the difference in diffusion coefficient of prednisolone relative to methylprednisolone due to slightly different molecular size (Fig. 5G), made only minor changes of the absolute levels of the drug time course.

After all apparent differences in the experimental protocols of the two studies were taken into account, concentration time courses of the two studies could be

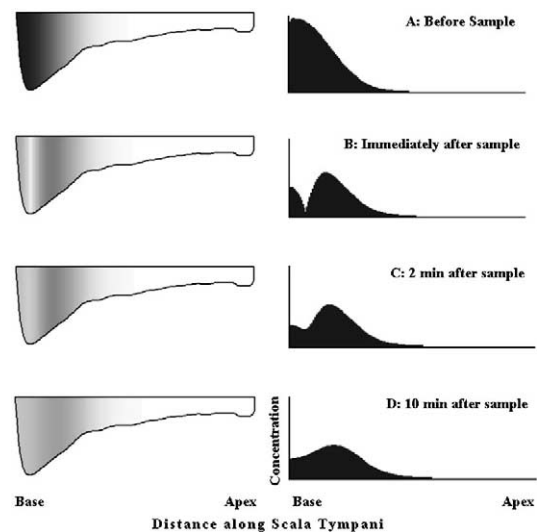


Fig. 4. Calculated concentration profiles of methylprednisolone along ST before, during and at different time points after the perilymph sampling procedure. Sample aspiration causes a decrease in ST perilymph concentration at the sampling site (B). When sample aspiration ceases, the drug diffuses back into the region of low concentration (C, D). Panels A–D correspond to the time points A–D in Fig. 3.

substantially reconciled, resulting in a difference in the peak level concentration of a factor of less than two (comparing Fig. 5G and H and accounted for in our analysis by differences in the RWM permeability values for the two studies). Contrary to the large difference in cochlear perilymph drug levels that would have been inferred from the respective publications, the concentration time course of the drugs in perilymph would have been similar, had the application protocol not differed substantially. Based on these data, the simulation program permits the drug time course to be approximated for a variety of application protocols.

Since in clinical practice intratympanically applied

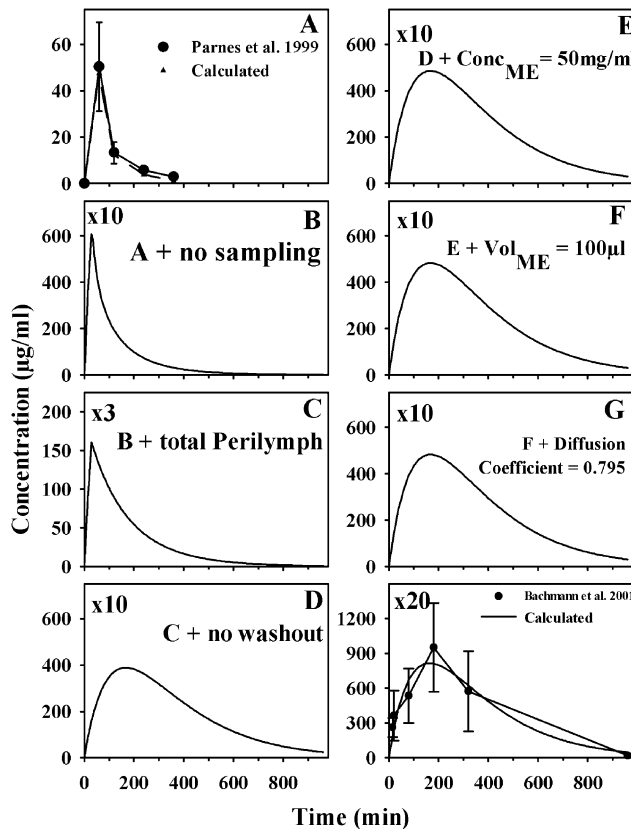


Fig. 5. Although similar experimental studies concluded substantially different drug levels in the cochlear perilymph, the differences could be almost totally explained by considering the specific experimental designs of the respective studies. Panel A and H show published concentration time courses for methylprednisolone and prednisolone after intratympanic application (replotted from Parnes et al., 1999 and Bachmann et al., 2001) and the best fit of the calculated concentration time course. Note the difference of an approximate factor 20 in peak concentration and the differences in time of peak concentration in the two studies (A,H). Panels B–G show the calculated concentration time courses while sequentially taking into account each of the differences in the experimental protocol between the two studies (as indicated in Table 1). Each panel shows the results of changing a single parameter, so that the influence of each can be appreciated. A more complete description of each condition is given in the text. The numbers in the upper left corners provide the scaling factor of the y-axes with respect to panel A.

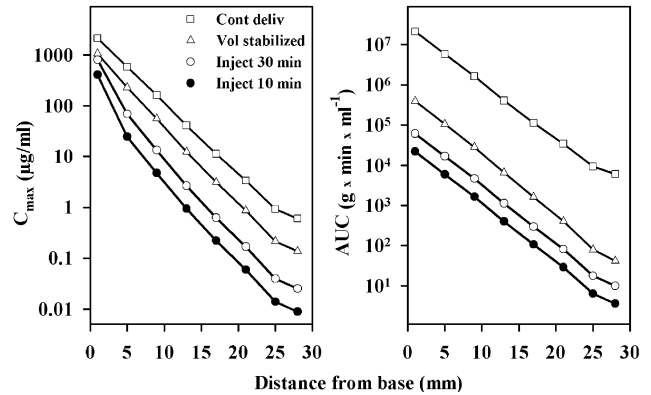


Fig. 6. Calculated drug distribution of methylprednisolone for cochlear dimensions representing the human, using parameters derived from the simulation of the guinea pig experiments by Parnes et al. (1999). Comparison of the drug distribution along ST for application by four delivery strategies. Left: C_{\max} . Right: AUC. Open squares: Continuous delivery of drug to the RWM for 7 days. Open triangles: Single drug application with volume in the middle ear stabilized (such as by fibrin glue). Circles: Single, brief application followed by a washout from the middle ear after 30 min (open circles) and 10 min (filled circles). For both C_{\max} and AUC, very large gradients along ST are expected to be present in the human although the slope of the curves depends on the actual inner ear clearance.

corticosteroids are already being used, the drug distributions in the inner ear for cochlear dimensions representing the human were also calculated using parameters derived from the above simulations of guinea pig experiments. Fig. 6 summarizes the peak methylprednisolone concentration (C_{\max} , left panel) and the AUC (right panel) as a function of distance along ST for the four application protocols described in Section 2, specifically a simulated continuous delivery for 7 days (open squares), a single RW application with volume in the middle ear stabilized to prevent volume loss through the Eustachian tube (open triangles), a single application without volume stabilization for 30 min (open circles) or a single brief application for 10 min (filled circles). It is predicted that for all application protocols the basal region of the cochlea will be exposed to significantly higher steroid levels than the apical regions of the cochlea. Continuous delivery results in substantially higher C_{\max} and AUC, while the brief one-shot application give the lowest absolute drug levels. The relative distribution of the steroid is almost the same for all application methods, indicating that for drugs with relatively fast perilymphatic clearance half-times the application method may have little influence on the relative distribution of drug along the cochlea.

4. Discussion

Drug delivery to the inner ear through the RWM is a

promising way for the treatment of inner ear disorders. Experimental animal studies investigating perilymph drug levels after topical application are important studies for the advancement of this therapeutic strategy, but they are technically difficult to perform. The animal experiments analyzed in this study incorporated an extensive amount of experimental work. Since animal studies are used to guide the clinician with regard to applications in humans (Chandrasekhar et al., 2000; Gianoli and Li, 2001; Kopke et al., 2001; Parnes et al., 1999), careful interpretation of experimental data is necessary. Using data from two different animal studies the time course of corticosteroid pharmacokinetics could be approximated for each study using an established finite element computer model. Although the experimental studies measured largely different drug levels in the cochlear perilymph these differences could be explained by considering the details of the experimental design. Our analysis demonstrates the high level of detail that is necessary for the interpretation of experimental studies. The present model provides a useful approach, based on physical principles, by which the relevance of different elements in an experimental study can be assessed.

One issue that needs to be considered is the semantics of the term ‘perilymph concentration’. Often, concentration measurements are represented as ‘cochlear perilymph concentration’ (Bachmann et al., 2001), ‘scala tympani/scala vestibuli concentration’ (Parnes et al., 1999) or ‘vestibular perilymph concentration’ (Hoffer et al., 1997). To be precise, these measurements more correctly describe the ‘sample concentration’ obtained from the location in question. It should be made clear that the contents of the sample pipette, into which fluid has been aspirated, may not be all perilymph. Especially when larger samples are taken, fluid in the cochlea is replaced by CSF, which dilutes the perilymph (assuming the drug is not present in CSF) and thus influences the sample concentration. Sampling of any form artificially contributes to inner ear clearance. The dilution of the sample by CSF may lead to an erroneous estimation of the cochlear perilymph level at the specific sampling location.

Since in Parnes’ study each of the four 10 μl samples aspirated was larger than total cochlear perilymph volume, which is approximately 8.6 μl for the guinea pig, and sampling occurred from a location near the entrance of the cochlear aqueduct, it is apparent that substantial sample contamination by CSF must have occurred. This is consistent with prior studies that have demonstrated that perilymph samples may be contaminated by CSF (Scheibe et al., 1984; Salt and Thalmann, 1988; Hara et al., 1989; Salt et al., 2003). However, the present study shows that by simulation of sampling procedures used in a specific experiment

with the finite element computer model, quantitative interpretation of such experimental results is still possible.

It is known that drugs topically applied to the RWM or delivered to the basal turn of ST are not equally distributed throughout the inner ear (Salt and Ma, 2001; Stover et al., 1999). The distribution along the length of the cochlea is dominated by the rate of diffusion of the drug (faster for small molecules and slower for large molecules) relative to the rate of clearance of the drug from the scala. If the drug is rapidly cleared then it will not spread as far along the scala. Our simulations of steroid time courses are consistent with a clearance half-time of approximately 130 min. With this rate of clearance it can be calculated that drug levels reaching the apex of the guinea pig cochlea are low. This situation is even more pronounced for longer cochlear scalae corresponding to the human (Fig. 6). For both C_{max} and AUC, very large and therefore clinically relevant gradients along ST are expected to be present in the human. Based on our simulations and on experimental findings (Salt and Ma, 2001), we believe it is not technically possible to achieve a uniform drug distribution along the perilymphatic spaces using present round window application methods.

As clearance from perilymph plays a major role in drug concentration gradients along ST, it is of importance to design experiments that permit perilymph clearance to be clearly differentiated from middle ear clearance. When fluid remains in contact with the round window, this allows substances to diffuse into or out of the perilymph according to the prevailing concentration gradient. If drugs are cleared more rapidly from the middle ear than from perilymph, loss of drug from perilymph to a fluid-filled middle ear can contribute to the apparent perilymph kinetics. The experimental design, especially the application protocol and the sampling procedure, determines which pharmacokinetic parameters can be derived with accuracy from the experiment. In both studies analyzed here it is not possible to clearly differentiate perilymph and middle ear clearances, so there is a level of uncertainty regarding the true perilymphatic clearance rate. In order to establish the perilymph clearance rate definitively, additional data are necessary in which a sustained drug level is maintained on the RWM so that perilymph kinetics are not influenced by middle ear clearance.

The analysis of different application protocols shows that the relative distribution of drugs in the ear is unlikely to be markedly affected by the application protocol. This is somewhat counter-intuitive as it would seem reasonable to assume that prolonged application would result in the drug becoming more evenly distributed in the ear. On the contrary, if clearance half-times are on the order of hours, then within a matter of hours a

steady state is established that will remain little changed by further application. In contrast, the analysis shows that the application protocol has a marked effect on the drug levels achieved in perilymph, with different delivery strategies resulting in very different amounts of drugs in the perilymph. A key factor controlling the drug level achieved is the time the drug remains in contact with the RWM. It therefore follows that in order to control perilymph drug levels more precisely, application methods must be developed that carefully control both the level and the duration of drug in the middle ear.

Acknowledgements

This work was supported by BgVV/ZEBET Grant WK1-1328-162 from the Federal Institute for Risk Assessment, Germany and AKF-UKT Grant 50-1-0 from the University of Tübingen (S.K.R.P.) and by Research Grant DC01368 from the National Institutes on Deafness and Other Communication Disorders (NIDCD), National Institutes of Health (A.N.S.).

References

- Arriaga, M.A., Goldman, S., 1998. Hearing results of intratympanic steroid treatment of endolymphatic hydrops. *Laryngoscope* 108, 1682–1685.
- Bachmann, G., Su, J., Zumegen, C., Wittekindt, C., Michel, O., 2001. Permeabilität der runden Fenstermembran für Prednisolon-21-Hydrogensuccinat. *HNO* 49, 538–542.
- Blakley, B.W., 1997. Clinical forum. A review of intratympanic therapy. *Am. J. Otol.* 18, 520–526.
- Brown, J.N., Miller, J.M., Altschuler, R.A., Nuttall, A.L., 1993. Osmotic pump implant for chronic infusion of drugs into the inner ear. *Hear. Res.* 70, 167–172.
- Buckingham, R.A., Valvassori, G.E., 2001. Inner ear fluid volumes and the resolving power of magnetic resonance imaging: can it differentiate endolymphatic structures? *Ann. Otol. Rhinol. Laryngol.* 110, 113–117.
- Chandrasekhar, S.S., 2001. Intratympanic dexamethasone for sudden sensorineural hearing loss: clinical and laboratory evaluation. *Otol. Neurotol.* 22, 18–23.
- Chandrasekhar, S.S., Rubinstein, R.Y., Kwartler, J.A., Gatz, M., Connelly, P.E., Huang, E., Baredes, S., 2000. Dexamethasone pharmacokinetics in the inner ear: comparison of route of administration and use of facilitating agents. *Otolaryngol. Head Neck Surg.* 122, 521–528.
- Ghiz, A.F., Salt, A.N., DeMott, J.E., Henson, M.M., Henson, O.W., Jr., Gewalt, S.L., 2001. Quantitative anatomy of the round window and cochlear aqueduct in guinea pigs. *Hear. Res.* 162, 105–112.
- Gianoli, G.J., Li, J.C., 2001. Transtympanic steroids for treatment of sudden hearing loss. *Otolaryngol. Head Neck Surg.* 125, 142–146.
- Hara, A., Salt, A.N., Thalmann, R., 1989. Perilymph composition in scala tympani of the cochlea: influence of cerebrospinal fluid. *Hear. Res.* 42, 265–271.
- Hoffer, M.E., Balough, B., Henderson, J., DeCicco, M., Wester, D., O'Leary, M.J., Kopke, R., 1997. Use of sustained release vehicles in the treatment of Meniere's disease. *Otolaryngol. Clin. North Am.* 30, 1159–1166.
- Hoffer, M.E., Allen, K., Kopke, R.D., Weisskopf, P., Gottshall, K., Wester, D., 2001. Transtympanic versus sustained-release administration of gentamicin: kinetics, morphology, and function. *Laryngoscope* 111, 1343–1357.
- Igarashi, M., Ohashi, K., Ishii, M., 1986. Morphometric comparison of endolymphatic and perilymphatic spaces in human temporal bones. *Acta Otolaryngol.* 101, 161–164.
- Kopke, R.D., Hoffer, M.E., Wester, D., O'Leary, M.J., Jackson, R.L., 2001. Targeted topical steroid therapy in sudden sensorineural hearing loss. *Otol. Neurotol.* 22, 475–479.
- Korver, K.D., Rybak, L.P., Whitworth, C., Campbell, K.M., 2002. Round window application of D-methionine provides complete cisplatin otoprotection. *Otolaryngol. Head Neck Surg.* 126, 683–689.
- Lalwani, A.K., Jero, J., Mhatre, A.N., 2002. Current issues in cochlear gene transfer. *Audiol. Neurootol.* 7, 146–151.
- Lamm, K., Arnold, W., 1999. How useful is corticosteroid treatment in cochlear disorders? *Otorhinolaryngol. Nova* 8, 203–216.
- Li, G., Frenz, D.A., Brahmblatt, S., Feghali, J.G., Ruben, R.J., Berggren, D., Arezzo, J., Van De Water, T.R., 2001. Round window membrane delivery of L-methionine provides protection from cisplatin ototoxicity without compromising chemotherapeutic efficacy. *Neurotoxicology* 22, 163–176.
- Ohyama, K., Salt, A.N., Thalmann, R., 1988. Volume flow rate of perilymph in the guinea-pig cochlea. *Hear. Res.* 35, 119–129.
- Okuno, H., Sando, I., 1988. Anatomy of the round window. A histopathological study with a graphic reconstruction method. *Acta Otolaryngol.* 106, 55–63.
- Parnes, L.S., Sun, A.H., Freeman, D.J., 1999. Corticosteroid pharmacokinetics in the inner ear fluids: An animal study followed by clinical application. *Laryngoscope* 109, 1–17.
- Plontke, S., Wood, A.W., Salt, A.N., 2002. Analysis of gentamicin kinetics in fluids of the inner ear with round window administration. *Otol. Neurotol.* 23, 967–974.
- Sakata, E., Itoh, A., Itoh, Y., 1996. Treatment of cochlear-tinnitus with dexamethasone infusion into the tympanic cavity. *Int. Tinnitus J.* 2, 129–135.
- Salt, A.N., 2002. Simulation of methods for drug delivery to the cochlear fluids. *Adv. Otorhinolaryngol.* 59, 140–148.
- Salt, A.N., Ma, Y., 2001. Quantification of solute entry into cochlear perilymph through the round window membrane. *Hear. Res.* 154, 88–97.
- Salt, A.N., Thalmann, R., 1988. Cochlear fluid dynamics. In: Jahn, A.F., Santos-Sacchi, J. (Eds.), *Physiology of the Ear*. Raven Press, New York, pp. 341–357.
- Salt, A.N., Ohyama, K., Thalmann, R., 1991. Radial communication between the perilymphatic scalae of the cochlea. II: Estimation by bolus injection of tracer into the sealed cochlea. *Hear. Res.* 56, 37–43.
- Salt, A.N., Kellner, C., Hale, S., 2003. Contamination of perilymph samples from the basal cochlear fluid with cerebrospinal fluid. *Hear. Res.* S0378-5955(03)00137-0.
- Scheibe, F., Haupt, H., Bergmann, K., 1984. On sources of error in the biochemical study of perilymph (guinea pig). *Arch. Otorhinolaryngol.* 240, 43–48.
- Shea, J.J., 1999. Dexamethasone perfusion of the inner ear through the round window membrane for Meniere's disease. In: Harris, J.P. (Ed.), *Meniere's Disease*. Kugler, The Hague, pp. 395–398.
- Shea, J.J., Ge, X., 1996. Dexamethasone perfusion of the labyrinth plus intravenous dexamethasone for Meniere's disease. *Otolaryngol. Clin. North Am.* 29, 353–358.

- Shirwany, N.A., Seidman, M.D., Tang, W., 1998. Effect of transtympanic injection of steroids on cochlear blood flow, auditory sensitivity, and histology in the guinea pig. *Am. J. Otol.* 19, 230–235.
- Silverstein, H., Choo, D., Rosenberg, S.I., Kuhn, J., Seidman, M., Stein, I., 1996. Intratympanic steroid treatment of inner ear disease and tinnitus (preliminary report). *Ear Nose Throat J.* 75, 468–471.
- Silverstein, H., Isaacson, J.E., Olds, M.J., Rowan, P.T., Rosenberg, S., 1998. Dexamethasone inner ear perfusion for the treatment of Meniere's disease: a prospective, randomized, double-blind, crossover trial. *Am. J. Otol.* 19, 196–201.
- Spandow, O., Hellstrom, S., Anniko, M., 1988. Impaired hearing following instillation of hydrocortison into the middle ear. Preliminary report from an animal study. *Acta Otolaryngol.* 455 (Suppl.), 90–93.
- Stover, T., Yagi, M., Raphael, Y., 1999. Cochlear gene transfer: round window versus cochleostomy inoculation. *Hear. Res.* 136, 124–130.
- Su, W.Y., Marion, M.S., Hinojosa, R., Matz, G.J., 1982. Anatomical measurements of the cochlear aqueduct, round window membrane, round window niche, and facial recess. *Laryngoscope* 92, 483–486.
- Thorne, M., Salt, A.N., DeMott, J.E., Henson, M.M., Henson, O.W., Jr., Gewalt, S.L., 1999. Cochlear fluid space dimensions for six species derived from reconstructions of three-dimensional magnetic resonance images. *Laryngoscope* 109, 1661–1668.