Original Paper

Audiology & Neuro-Otology

Audiol Neurootol 2005;10:53–63
DOI: 10.1159/000082575

Received: April 9, 2003
Accepted after revision: June 7, 2004
Published online: December 7, 2004

Novel Slow- and Fast-Type Drug Release Round-Window Microimplants for Local Drug Application to the Cochlea: An Experimental Study in Guinea Pigs

Wolfgang Arnold[a] Pascal Senn[c] Michael Hennig[b] Christiane Michaelis[a]
Kerstin Deingruber[a] Renate Scheler[a] Hans-Joachim Steinhoff[a]
Frits Riphagen[d] Kerstin Lamm[a]

[a]Clinic for Otorhinolaryngology, Head and Neck Surgery and [b]Institute for Medical Statistics and Epidemiology, Klinikum rechts der Isar, Technical University of Munich, Munich, Germany; [c]Clinic for Otorhinolaryngology, Head and Neck Surgery, Inselspital, University of Berne, Berne, Switzerland; [d]Schering OY, Turku, Finland

Key Words
Glucocorticoids · Corticosteroids · Evoked potentials, auditory, brain stem · Cytocochleogram · Round-window microimplant · Guinea pig cochlea

Abstract
Novel drug release microimplants (0.8 × 1.14 mm; custom-made by Leiras, now Schering OY, Finland) of slow- and fast-release types containing either 0.9 mg beclomethasone or no drug at all were placed unilaterally onto the round-window membrane (RWM) of 45 guinea pigs for a maximum duration of 28 days. The following parameters were tested on days 1, 14 and 28 after implantation: threshold levels of beclomethasone in the perilymph of the scala tympani, auditory brain stem responses (ABR thresholds and ABR threshold shifts), RWM morphology and hair cell loss (cytocochleograms). None of the animals in the non-implanted control group (n = 5) or placebo implant group (n = 15), but all animals in the slow-release-type implant group (n = 15) and fast-release-type implant group (n = 15) revealed the presence of beclomethasone on day 1 (34.9 and 64.3 pg/μl, respectively), day 14 (43.8 and 46.9 pg/μl, respectively) and day 28 after implantation (4.7 and 60.5 pg/μl, respectively). Histology of the RWMs appeared normal, and cytocochleograms revealed no inner hair cell loss and outer hair cell loss within normal ranges (from 0.5 ± 0.4 to 0.8 ± 0.2% per cochlea) in both ears in all experimental groups at any time during examination (days 1, 14 and 28). Initial values of ABR thresholds at 3, 6, 9 and 12 kHz did not differ significantly in any of the experimental groups. In non-implanted controls, no significant differences of ABR thresholds were observed in all frequencies tested in either ear on days 1, 14 and 28 compared to initial values, and ABR threshold shifts ranged from –3 ± 5 dB (min.) to +5 ± 7 dB (max.). On day 28 after implantation, there were no significant differences of ABR threshold shifts between this and the implant groups, except for 6 kHz of the slow-release device. Therefore, the placebo implants, the slow-release and the fast-release beclomethasone implants appear suitable for further experiments.

KARGER
Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1420–3030/05/0101–0053$22.00/0

Accessible online at:
www.karger.com/aud

Prof. Wolfgang Arnold, MD, PhD
Director of the Clinic for Otolaryngology, Head and Neck Surgery
Klinikum rechts der Isar, Technical University of Munich
Ismaninger Strasse 22, DE–81675 München (Germany)
Tel. +49 89 4140 2370, Fax +49 89 4140 4853, E-Mail w.arnold@lrz.tum.de

Introduction

There has recently been increasing interest in local drug delivery systems to the inner ear to achieve efficient therapeutic drug concentrations in cochlear tissues and to minimize or even obviate systemic side-effects. Of the various methods used, such as transtympanic injections or placement of a small drug-absorbing MicroWick [Silverstein, 1999] through a ventilation tube, drug-soaked gelfoam pledgets [Husmann et al., 1998] or fibrin-based sustained-release vehicles [Hoffer et al., 1997, 1999, 2001; Balough et al., 1998] placed onto the round-window membrane (RWM), osmotic pumps [Brown et al., 1993; Prieskorn and Miller, 2000] or bone-anchored totally implantable drug delivery systems [Lehner et al., 1997; Praetorius et al., 2001] connected with a microcatheter placed in the RW niche allow stable continuous or on-demand delivery of drugs penetrating the RWM [for a recent review, see Jackson and Silverstein, 2002]. In humans, however, transtympanic endoscopy, i.e. the use of a microendoscope [Plontke et al., 2002] and, if necessary, surgical removal of tissue overlying the RWM, is mandatory to achieve drug penetration through the RWM. According to studies in temporal bones [Nomura et al., 1983; Nomura, 1984; Proctor et al., 1986; Alzamil and Linthicum, 2000] and direct observation in vivo [Silverstein et al., 1997], the RW niche is completely covered by an extraneous (false) RWM (known as the RW niche membrane) in 13–21% and partially covered by a perforated RW niche membrane in 57% of all patients.

In the present study, novel sustained-release vehicles consisting of two types of siloxane-based polymers which differ in the daily release rate of the active ingredient (custom-made by Leiras, now Schering OY, Finland) were placed unilaterally onto the RWM of 45 guinea pigs. The slow- and fast-type drug release microimplants and the microimplants containing no drug at all (placebo implants) remained in vivo for a maximum of 28 days. The size of the microimplants (0.8 × 1.14 mm) was chosen according to quantitative anatomical data of the RW niche and membrane in guinea pigs [Fernandez, 1952; Nomura et al., 1983; Nomura, 1984; Ghiz et al., 2001]. The microimplants were filled with the beclomethasone glucocorticoid for the following reasons:

In vitro permeation tests performed by Leiras (now Schering) had previously shown that the release of beclomethasone from cylindrical implants of 5 mm length and 1.2–1.5 mm diameter was 15–20 μg per day for the low-dose or slow-release-type device and approximately 50 μg per day for the high-dose or fast-release-type implants.

Compared to the endogenous cortisol, the relative anti-inflammatory potency of dexamethasone or beclomethasone and prednisolone glucocorticoids is 25 and 4, respectively [Schimmer and Parker, 1996]. The duration of action (i.e. the biological half-life) of cortisol is 8–12 h, whereas dexamethasone, beclomethasone and prednisolone have long-term effects (36–72 h biological half-life) [Schimmer and Parker, 1996]. Since prednisolone is not stable for more than 48 h at 37 °C, it is not suitable for use in implanted long-term drug delivery systems [personal information, Merck KgaA, Germany].

After only a single application to the RWM, glucocorticoids were found to penetrate into the perilymph and endolymph in guinea pigs [Parnes et al., 1999; Chandrasekhar et al., 2000; Chandrasekhar, 2001; Bachmann et al., 2001].

Experimental and clinical studies on the therapeutic effect of systemic or intratympanic application of glucocorticoids in various inner ear diseases and tinnitus are promising [Lamm and Arnold, 1998, 1999a, b; Lamm et al., 1998].

The following parameters were tested in both ears on days 1, 14 and 28 after unilateral implantation: beclomethasone threshold levels in the perilymph of the scala tympani, auditory brain stem responses (ABR thresholds and ABR threshold shifts), RWM morphology and hair cell loss (cytocochleograms).

Material and Methods

Subjects

A total of 50 pigmented male guinea pigs were used (Charles River WIGA GmbH, Sulzfeld, Germany) that weighed between 270 and 370 g and exhibited normal Preyer's reflexes. The study was performed in accordance with the PHS Policy on Human Care and Use of Laboratory Animals, the NIH Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act (NIH Publication No. 86-23, revised 1985). The protocol permitting the use of animals was approved by the Government of Oberbayern (Munich) in accordance with the Animal Welfare Act (BGBI I, revised 5-25-1998) on March 21, 2002 (protocol No. 209.1/211-2531-16/02).

Anaesthesia and Analgesia

The animals were anaesthetized according to Erhardt et al. [2002] by intramuscular injection of a mixture containing 0.025 mg/kg fentanyl (Fentanyl® 0.5 mg, Curamed Pharma), 1.0 mg/kg midazolam (Midazolam-Ratiopharm® 15 mg/3 ml, Ratiopharm) and 0.2 mg/kg medetomidine (Domitor®, Pfizer). The anaesthesia was antagonized according to Erhardt et al. [2002] by a subcutaneous injection of a mixture containing 0.03 mg/kg naloxone (Naloselect® 0.4 mg/ml, Pharmaselect), 0.1 mg/kg flumazenile (Anexate® 0.5, Roche) and 1 mg/kg atipamezole (Antisedan®, Pfizer). The animals were analgized 4 days postoperatively by peroral administration of 0.2 ml

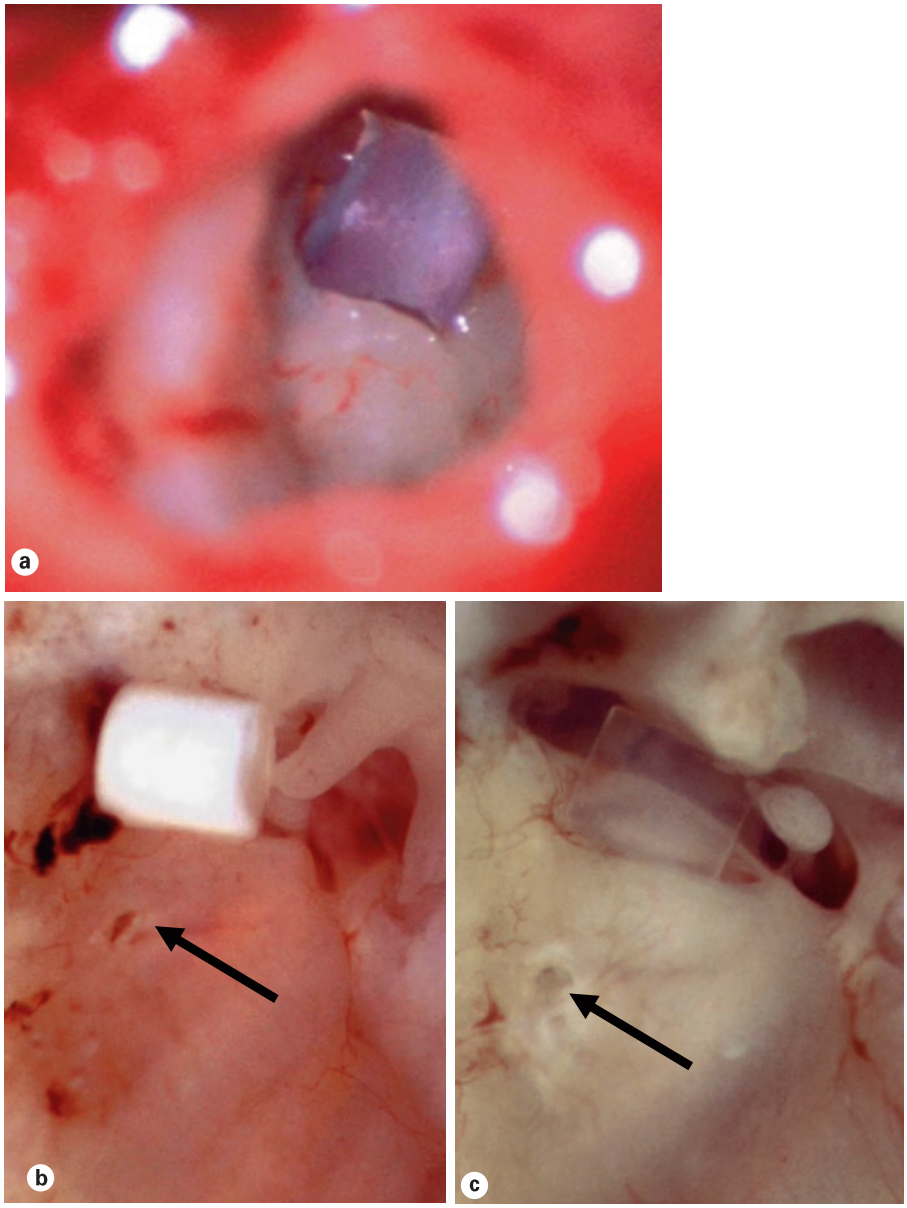

Fig. 1. a Microimplant (0.8 × 1.14 mm, custom-made by Leiras) containing no drug (placebo implant) placed onto the RW in vivo. **b** A slow-release beclomethasone implant. **c** A placebo implant after removal of the temporal bone on day 28 after implantation. Arrows indicate the burr hole in the lateral wall of the scala tympani made in order to sample perilymph before decapitation.

metamizol sodium (Novaminsulfon-Ratiopharm® 500 mg/1 ml, Ratiopharm). Prophylaxis against infection was given intraoperatively and on the 1st postoperative day by administering enrofloxacin (7 mg/kg, Baytril® 2.5%, Bayer) as a broad-spectrum antibiotic.

Surgery and Preparation

Core temperature was maintained at 37.5 °C using a heated blanket and infrared lamp. It was monitored using a rectal probe linked to a thermal sensor and universal monitor (Vicom SMU 612, Hellige). The electrocardiogram (ECG) was recorded continuously on the universal monitor (Vicom SMU 612, Hellige).

After local anaesthesia using 0.25 ml mepivacaine (Mecain® 1%, Curasan), the right bulla was exposed and opened via a retro-auricular approach under an operation microscope (Universal S2, Zeiss). The bony limb above the RW niche was carefully removed in order to gain free access to the RWM. Once the microimplant (see the following section) had been placed carefully onto the RW (fig. 1a–c), the bulla was surgically closed.

Slow- and Fast-Type Drug Release Microimplants

The slow- and fast-type drug release microimplants 0.8 mm in length and 1.14 mm in diameter (custom-made by Leiras, now Schering) consist of two types of siloxane-based polymers which differ in their daily release rate of active ingredient. The microimplants contained either 0.9 mg beclomethasone or no drug at all (fig. 1a–c). In vitro permeation tests performed by Leiras had previously shown that the release of beclomethasone from cylindrical implants of 5 mm length and 1.2–1.5 mm diameter was 15–20 µg/day for the slow-

release device and approximately 50 µg/day for the fast-release device.

Evaluation of Auditory Function

Acoustic stimulation and measurements of ABRs were performed using an electrophysiological recording system (ZLE-Systemtechnik) in an electrically shielded, sound-proof chamber (Industrial Acoustics Company) according to the method proposed by Steinhoff et al. [1995]. The stimuli (1.2-ms tone bursts with a rise and fall time of 0.2 ms each) that exist as a list in the memory of the 2-channel stimulator (UST/040, ZLE-Systemtechnik) were emitted via a 14-bit DAC at a rate of 85 kHz. Two ER-2 microphones (Etymotic Research) connected to a silicone tube 1 mm in diameter and 26 cm in length were used as the electroacoustic transducer. The distal ends of the tube were positioned in the entrance to the auditory canals on both sides. The bilateral deviation was derived using needle electrodes placed subcutaneously over the vertex (+) and mastoid process (–). The reference electrode was attached to the right rear extremity. The electrodes were connected to the measuring instrument via an electrode connector box featuring integrated electrode impedance measurement (EB 100-4, ZLE-Systemtechnik). The dual-channel data acquisition system (DAS 100/16, ZLE-Systemtechnik) regulated the stimulus response amplification controlled by the measuring programme, the signal filter and analog-digital conversion. Whereas supraliminal potentials were recorded in 10-dB steps, near-threshold potentials were recorded in 5-dB steps. The stimulus response thresholds were detected for the following frequencies: 3, 6, 9 and 12 kHz.

Qualitative and Quantitative Assays of Beclomethasone in the Perilymph

At the conclusion of the experiments, a small hole was burred in all animals 2–3 mm below the RWM on each side in order to sample 10 µl of perilymph using a reference pipette (Eppendorf, Germany) connected to a microloader of 0.5–20 µl volume (Eppendorf). Samples were centrifuged at 13000 *g* for 2 min (5415 C, Eppendorf), filled in PCR tubes (FT PP 0.2 ml, Treff Lab, Switzerland) and stored at –80°C.

The qualitative (present or not) and quantitative assays of beclomethasone in the perilymph samples were performed by Aster Cephac (Saint-Benoît, France) using liquid chromatography-mass spectrometry. The high-performance liquid chromatography (HPLC) system consisted of a 200 series autosampler (Perkin-Elmer, Foster City, Calif., USA) and a Zorbax Eclipse XDB C18, 3.5-µm column (Agilent technologie, Massy Palaiseau, France). The mobile phase was a mixture of methanol/water that was supplied at a flow rate of 1 ml/min at room temperature. Mass spectrometry determination was carried out using an API-365 triple-quadruple instrument (Sciex, Concord, Canada), equipped with a heated nebulisator source. Nitrogen was employed as auxiliary and nebuliser gas. The system was monitored by a personal computer running on a analyst 1.1 software. The chemicals used (beclomethasone and internal standard dexamethasone) were supplied by Sigma (Isle d'Abeau, France). Methanol and HPLC-grade water were purchased from Merck (Nogent-sur-Marne, France) and BDH (Chelles, France), respectively. Standard solutions were prepared by diluting beclomethasone and dexamethasone in methanol. Since it was difficult to obtain a blank matrix of perilymph samples, human cerebrospinal fluid was chosen to prepare the blank, negative samples and positive samples. The limit of detection was set at 2.25 pg/µl. Two calibration curves from 2.25 to 900 pg/µl were added before and after the study samples to estimate the beclomethasone concentration in the event of positive identification.

Since the implants were not exactly of the same size and therefore would not contain exactly the same quantities of beclomethasone, the assay had not been validated, and only a threshold level was defined. Conclusions on the exact concentration levels can therefore not be drawn.

Histology of the RWM and Cytocochleograms

After perilymph sampling, the anaesthetized animals were decapitated and the temporal bones removed. For histological examinations of the RWM, the cochleae were fixed in 4% formaldehyde with 0.1% glutaraldehyde, decalcified with 10% EDTA, dehydrated (Hypercenter XP, Shandon), embedded in paraffin wax (Tissue-Tek II, Vogel), sliced into a series of sections 5 µm thick using a microtome (HM 355, Microm), stained with haemalum-eosin and examined under a light microscope (Axiovert 135 TV, Zeiss).

For cytocochleograms, the organs of Corti were prepared according to the method proposed by Canlon and Fransson [1995] and then microsected into 8 regions. The specimens were rinsed with 0.01 *M* PBS, permeabilized with 0.3% Triton X-100 for 10 min, rinsed again with PBS and then incubated with rhodamine-phalloidin (TRITC 1:50, Molecular Probes) for 50 min. Inner and outer hair cell loss was calculated in each specimen using a fluorescence microscope (Axiovert 135 TV, Zeiss) equipped with a CCD camera (AT 200, Photometrics Ltd., USA) connected to image-processing software (3.0, PMIS™, USA).

Experimental Groups and Time Schedule for Data Collection

The animals were divided into four experimental groups: (1) the non-implanted controls (group 1, n = 5); (2) the placebo implant group (group 2, n = 15) received microimplants containing no drug; (3) the slow-release beclomethasone implant group (group 3, n = 15) received the slower-release microimplant containing 0.9 mg beclomethasone; (4) the fast-release beclomethasone implant group (group 4, n = 15) received the faster-release microimplant containing 0.9 mg beclomethasone.

The implant groups (groups 2–4) were subjected to ABR measurement 1 day pre-operatively (to establish initial values) and post-operatively on days 1, 14 and 28. The same procedure was used for the non-implanted control group (group 1) as well. Perilymph samples were taken postoperatively from 5 animals each in the implant groups (groups 2–4) on days 1, 14 and 28. Histological examination of the RWM was performed postoperatively on days 1, 14 and 28 in 3 animals each and cytocochleograms in 2 animals each of the implant groups (groups 2–4). In the case of the non-implanted control group (group 1), perilymph was sampled on day 28, and 3 animals were prepared for histological examination of the RWM, while 2 animals were prepared for cytocochleograms.

Statistical Analysis

In a first step, descriptive statistics – means, standard deviations (SDs), median, range – were calculated for all parameters of interest and all factors involved. Secondly, inductive statistics were performed separately for the levels of beclomethasone and ABRs. The following non-parametric analyses were performed for the levels of beclomethasone: (1) exact Kruskal-Wallis test to compare the four groups (controls, placebo, slow, fast) for each time point (day 1, 14, 28). In case of significant differences ($p < 0.05$ – no alpha correction was performed, as different animals were considered for each time

point) we continued by (2) pairwise comparisons using the exact Wilcoxon test for unpaired samples. Here a Bonferroni correction was used to adjust for the multiple test situation.

The significance of the differences in ABR thresholds of the non-implanted control group was tested as follows: values for each frequency on days 1, 14 and 28 were compared to initial values using the paired Student's t test. Initial values of mean ABR thresholds for each frequency and both ears of the four groups were compared using one-way analysis of variance (ANOVA). For ABR threshold shifts, we performed a multifactorial ANOVA, using the factors group (4 levels, groups 1–4), side (2 levels: right/left), frequency (4 levels: 3, 6, 9, 12 kHz) and time (3 levels: day 1, day 14, day 28). In addition, the groups were compared for each of the remaining 24 factor combinations starting with an overall exact Kruskal-Wallis test. In case of significant differences ($p < 0.05$) pairwise comparisons were performed using the exact Wilcoxon test.

The means and SDs were calculated for inner and outer hair cell loss in each experimental group for each of the 8 cochlear regions at the times indicated in the preceding section. Groups were compared using one-way ANOVA.

The histological findings of the RWM in each experimental group at the examination times were described qualitatively.

All calculations were performed using the software package SPSS®, version 11.0.

Results

Qualitative and Quantitative Assays of Beclomethasone in the Perilymph

No beclomethasone was detected in the perilymph samples at any time during sample collection in any cochleae in the non-implanted control group (group 1) or placebo implant group (group 2).

Beclomethasone was detected qualitatively in the perilymph samples drawn from the implanted (right) ear on days 1, 14 and 28 in all but 1 animal in the slow-release beclomethasone implant group (group 3) and in all animals in the fast-release beclomethasone implant group (group 4). No beclomethasone was detected on day 28 in 1 of the 5 animals provided with the slow-release device.

Individual perilymphatic beclomethasone levels ranged on day 1 after implantation from 16.6 pg/µl (min.) to 262 pg/µl (max.) in group 3, and from 43 pg/µl (min.) to 193 pg/µl (max.) in group 4. Similarly, on day 14 after implantation, data ranged from 2.9 pg/µl (min.) to 101 pg/µl (max.) in group 3, and from 7.6 pg/µl (min.) to 121 pg/µl (max.) in group 4. The largest variation was obtained on day 28 after implantation, when data ranged from 0.0 pg/µl (min.) to 172 pg/µl (max.) in group 3, and from 10.3 pg/µl (min.) to 731 pg/µl (max.) in group 4.

Means, SDs and medians are listed in table 1.

Comparing the concentration of beclomethasone within the four groups for each time point there were significant differences on all days (day 1: $p = 0.001$, day 14: $p = 0.001$, day 28: $p < 0.0001$). According to the pairwise comparisons on days 1 and 14 there were significant differences between groups 4 and 2 and between groups 3 and 2 (border = 0.05/3; $p = 0.008$). On day 28 there were significant differences between groups 4 and 2 (border = 0.05/6; $p = 0.002$) and between groups 4 and 1 (border = 0.05/6; $p = 0.008$).

No beclomethasone was detected in the perilymph of the contralateral non-implanted (left) ear except for 1 animal in group 3 on day 28. The value in this case amounted to 2.9 pg/µl; however, this was only slightly above the detection limit (2.25 pg/µl).

Table 1. Beclomethasone threshold levels (pg/µl)

	Day 1	Day 14	Day 28
Slow-release implant			
n	5	5	5
Mean	83.8	43.0	40.4
SD	101.9	40.7	74.0
SE	45.6	18.2	33.1
Median	34.9	43.8	4.7
Minimum	16.6	2.9	0.0
Maximum	262.0	101.0	172.0
Fast-release implant			
n	5	5	5
Mean	90.5	57.8	286.1
SD	60.6	45.5	350.7
SE	27.1	20.3	156.8
Median	64.3	46.9	60.5
Minimum	43.0	7.6	10.3
Maximum	193.0	121.0	731.0

Mean values, SD, standard errors (SE), median values and minimum and maximum values of beclomethasone threshold levels in the perilymph of the scala tympani on day 1, 14 and 28 after implantation of the slow-release beclomethasone implant (group 3) and of the fast-release beclomethasone implant (group 4). n = Number of samples.

Histology of the RWM and Cytocochleograms

The RWM of all implanted animals appeared histologically normal on days 1, 14 and 28 after implantation, as was seen in the non-implanted control group on day 28 (fig. 2a–d). There were no signs of any defects, scars or inflammatory tissue changes with subsequent membrane thickening due to the implantation. However, all microimplants were encapsulated by a thin mucous layer (fig. 2c, d).

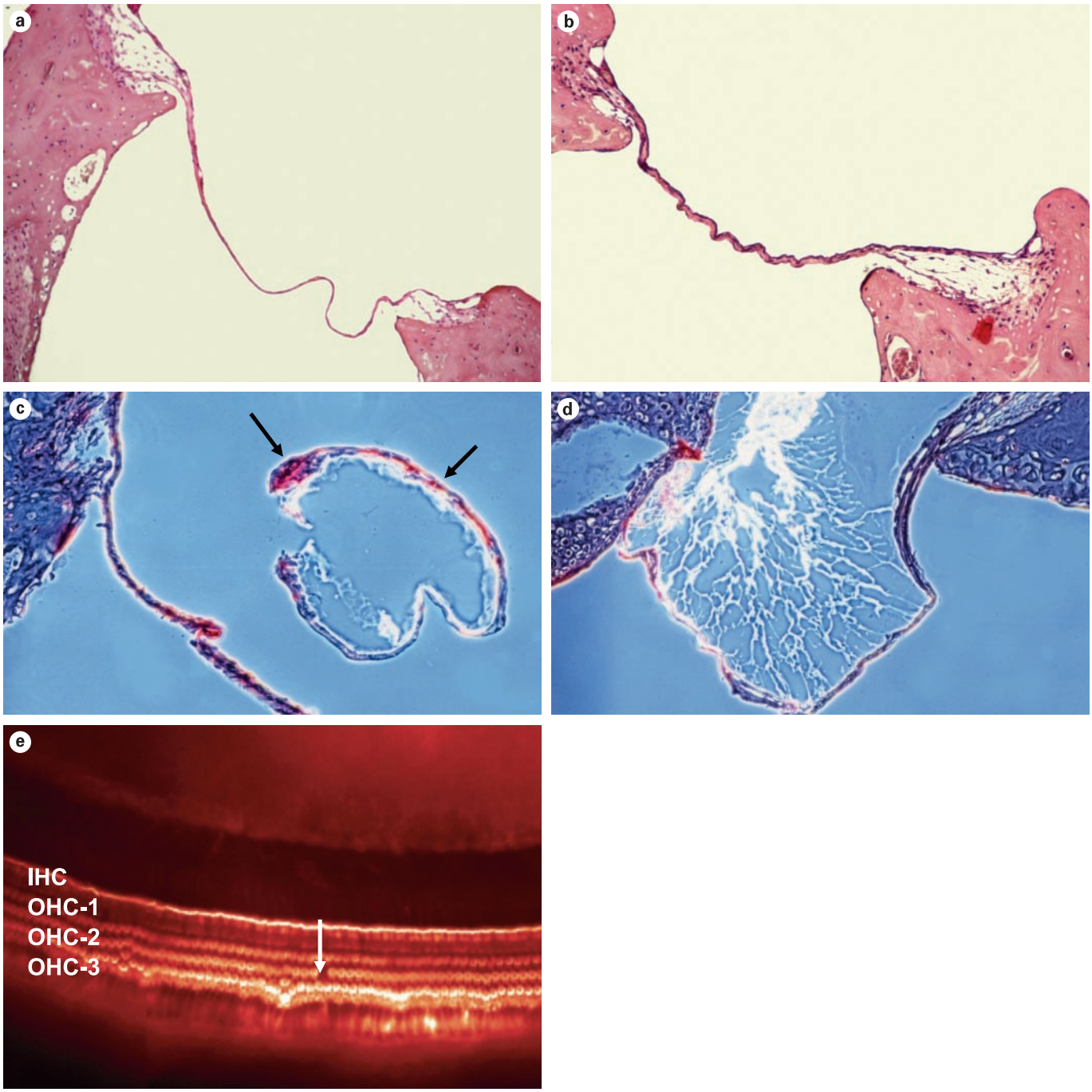

Fig. 2. Histological findings of the RWM in non-implanted controls (**a**), 28 days after implantation of the microimplants containing no drug (**b**), 28 days after implantation of the slow-release beclomethasone implant (**c**) and 28 days after implantation of the fast-release beclomethasone implant (**d**). There were no signs of any defects, scars or inflammatory tissue changes with subsequent membrane thickening. All microimplants, however, were encapsulated by a thin mucous layer (arrows). **e** Normal cytocochleogram of 1 out of 8 cochlea specimens corresponding to the 12- to 5-kHz region obtained from a non-implanted control animal on day 28. The arrow indicates 1 lost outer hair cell in the second row (OHC-2), while no inner hair cells (IHC) were missing. Similar results were obtained in both ears in all experimental groups at any time during examination (days 1, 14 and 28).

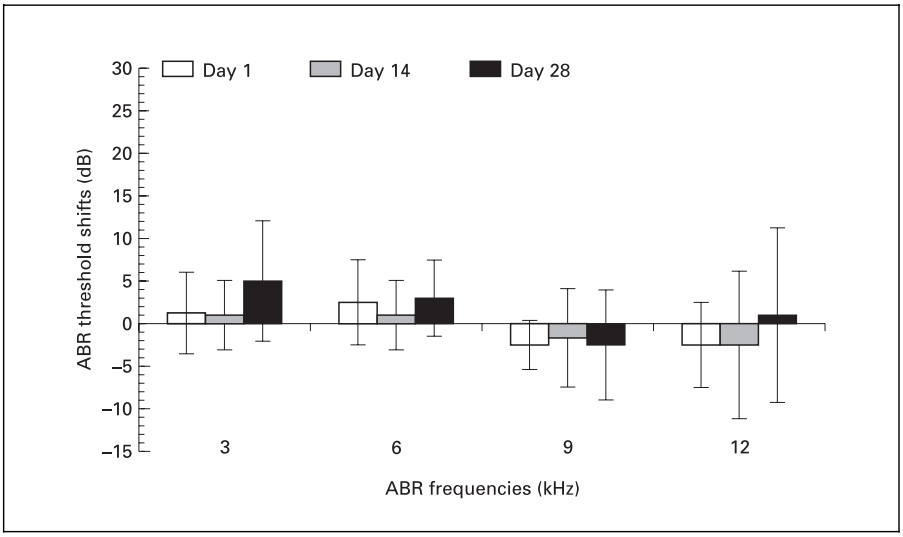

Fig. 3. Mean (±SD) frequency-specific (3, 6, 9 and 12 kHz) ABR threshold shifts (dB) in non-implanted controls (group 1, n = 5) on days 1, 14 and 28 compared to initial values. Compared to the slow-release beclomethasone implant (group 3) there were significant differences of ABR threshold shifts for 9 kHz on day 1 ($p = 0.006$) and for 6 kHz on day 28 ($p = 0.024$) (fig. 5). Compared to the fast-release beclomethasone implant (group 4) there were significant differences of ABR threshold shifts for 6 kHz on day 28 ($p = 0.044$) (fig. 6). For absolute initial values of ABR thresholds, see the Results.

The cytocochleograms obtained from the right cochlea revealed no inner hair cell loss and a mean outer hair cell loss of 0.8 ± 0.2% in the non-implanted control group (group 1), of 0.7 ± 0.8% in the placebo implant group (group 2), of 0.5 ± 0.4% in the slow-release beclomethasone implant group (group 3) and of 0.5 ± 0.3% in the fast-release beclomethasone implant group (group 4) in all of the 8 cochlear specimens corresponding to the regions of >26 kHz, 26–12 kHz, 12–5 kHz, 5–3 kHz, 3–1.5 kHz, 1.5–0.8 kHz, 0.8–0.4 kHz and <0.4 kHz on day 28 (fig. 2e). There was no statistically significant difference between the right and left cochleae in any of the experimental groups at each time of collection. Similarly, there were no statistically significant differences between data obtained from group 1 on day 28 compared to that obtained from group 2, group 3 and group 4 on day 1, 14 and 28 after implantation.

ABR Thresholds and ABR Threshold Shifts

Initial Values of ABR Thresholds. Initial values of mean ABR thresholds obtained from the right ear in the non-implanted control group (group 1) were 26 ± 4 dB SPL at 3 kHz, 26 ± 7 dB SPL at 6 kHz, 20 ± 5 dB SPL at 9 kHz and 26 ± 7 dB SPL at 12 kHz. There was no significant difference compared to the values obtained on days 1, 14 and 28 in this group. In the placebo implant group (group 2), initial values of mean ABR thresholds obtained from the right (implanted) ear were 27 ± 10 dB SPL at 3 kHz, 24 ± 7 dB SPL at 6 kHz, 21 ± 9 dB SPL at 9 kHz and 27 ± 8 dB SPL at 12 kHz. In the slow-release beclomethasone implant group (group 3), ABR thresholds were 24 ± 5 dB SPL at 3 kHz, 23 ± 6 dB SPL at 6 kHz, 21 ± 6 dB SPL at 9 kHz and 24 ± 4 dB SPL at 12 kHz, and in the fast-release beclomethasone implant group (group 4) data averaged 29 ± 4 dB SPL at 3 kHz, 27 ± 7 dB SPL at 6 kHz, 19 ± 6 dB SPL at 9 kHz and 24 ± 7 dB SPL at 12 kHz.

There was no significant difference of initial ABR thresholds between all four groups and between the right and left ears for all frequencies tested.

ABR Threshold Shifts. In the non-implanted control group (group 1) mean ABR threshold shifts (difference between initial values to those obtained on days 1, 14 and 28) varied in the right ear in all frequencies tested from –2.5 ± 5 dB SPL (min.) to +5.0 ± 7 dB (max.) (fig. 3), in the placebo implant group (group 2) from –3.3 ± 3 dB (min.) to +10.6 ± 14 dB (max.) (fig. 4), in the slow-release beclomethasone implant group (group 3) from +7.2 ± 11 dB (min.) to +17.5 ± 9 dB (max.) (fig. 5) and in the fast-release beclomethasone implant group (group 4) from +1.4 ± 11 dB (min.) to +9.1 ± 14 dB (max.) (fig. 6).

Only 17 (out of 52) animals with a complete set of observations could be included in the multifactorial ANOVA. Here the side (right vs. left) turned out to be a significant ($p = 0.029$) factor. In addition, there was a significant ($p < 0.0001$) difference between the four groups. Comparing the groups with an exact Kruskal-Wallis test, the following factor combinations resulted in a significant difference between the four groups: 6 kHz, right side, day 28 ($p = 0.009$, exact)/9 kHz, right side, day 1 ($p = 0.033$ – asymptotic only due to memory limitations of the PC). In the pairwise comparisons, significant differences could be

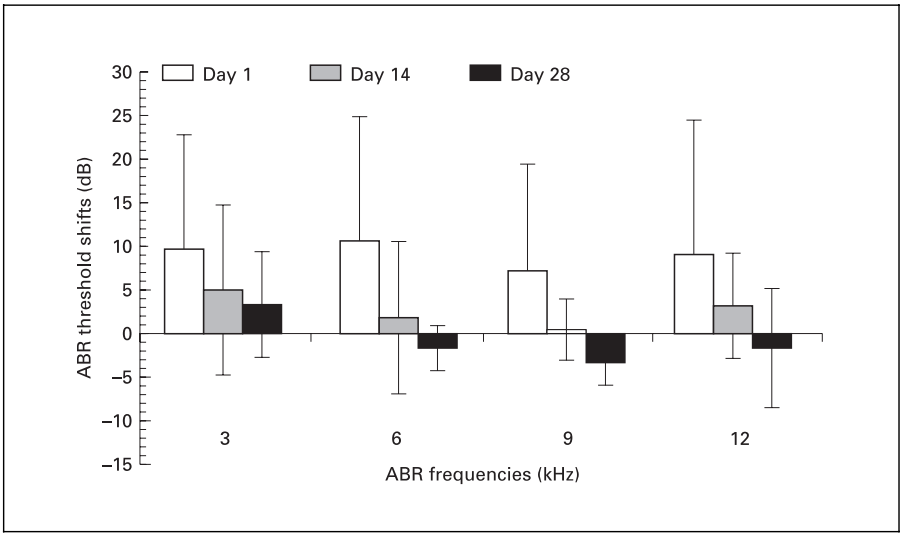

Fig. 4. Mean (±SD) frequency-specific (3, 6, 9 and 12 kHz) ABR threshold shifts (dB) on days 1 (n = 15), 14 (n = 10) and 28 (n = 5) after implantation of the microimplants containing no drug at all (placebo implants, group 2). There was no significant difference of ABR threshold shifts between this and the other groups, except for 6 kHz on day 28 after implantation of the slow-release device (fig. 5). For absolute initial values of ABR thresholds, see the Results.

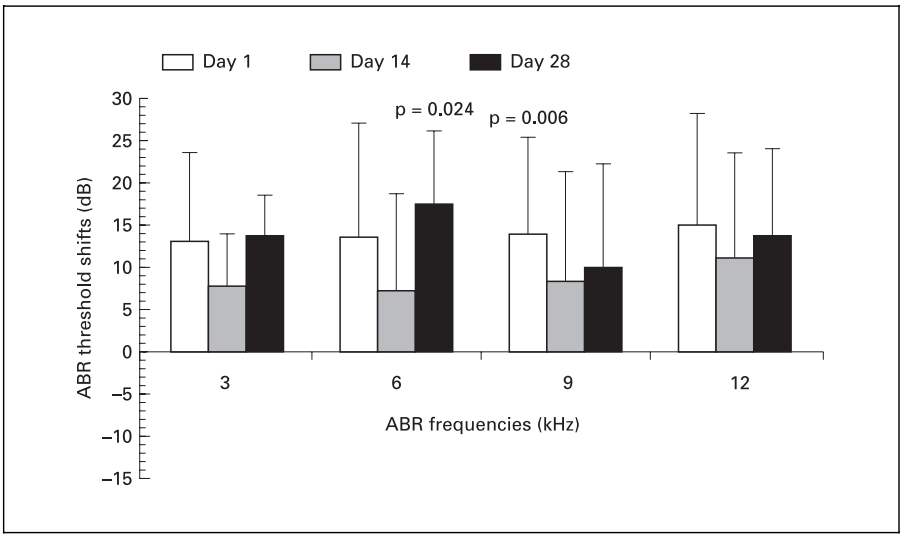

Fig. 5. Mean (±SD) frequency-specific (3, 6, 9 and 12 kHz) ABR threshold shifts (dB) on days 1 (n = 15), 14 (n = 10) and 28 (n = 5) after implantation of the slow-release beclomethasone implant (group 3). Compared to the non-implanted controls (group 1) there were significant differences of ABR threshold shifts for 9 kHz on day 1 (p = 0.006) and for 6 kHz on day 28 (p = 0.024). Compared to the placebo implants (group 2) there were significant differences of ABR threshold shifts for 6 kHz on day 28 (p = 0.005). For absolute initial values of ABR thresholds, see the Results.

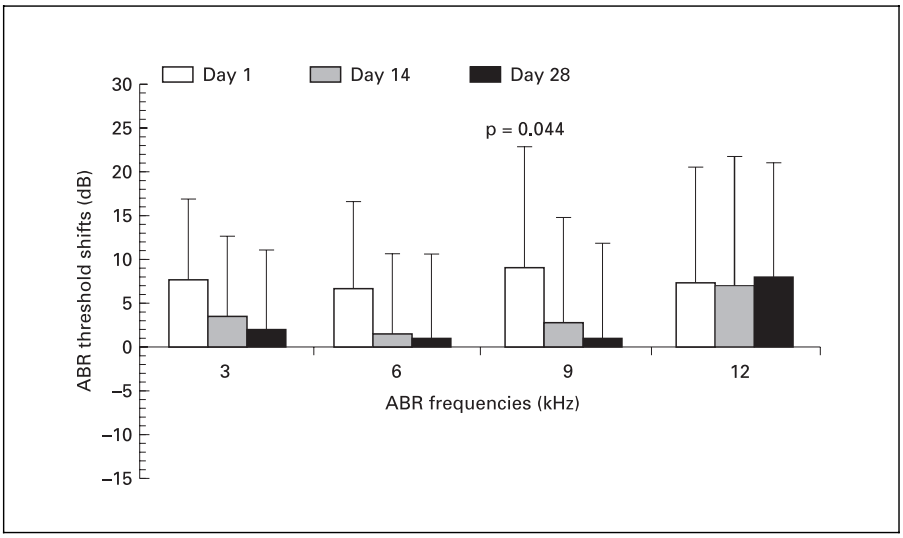

Fig. 6. Mean (±SD) frequency-specific (3, 6, 9 and 12 kHz) ABR threshold shifts (dB) on days 1 (n = 15), 14 (n = 10) and 28 (n = 5) after implantation of the fast-release beclomethasone implant (group 4). Compared to the non-implanted controls (group 1) there were significant differences of ABR threshold shifts for 9 kHz on day 1 (p = 0.044). For absolute initial values of ABR thresholds, see the Results.

seen at 6 kHz on day 28 on the right side between the non-implanted control group and the slow-release beclomethasone implant group ($p = 0.024$) and between the placebo implant group and the slow-release beclomethasone implant group ($p = 0.005$). In addition, significant differences could be seen at 9 kHz on day 1 on the right side between the non-implanted control group and the fast-release beclomethasone implant group ($p = 0.044$) and between the non-implanted control group and the slow-release beclomethasone implant group ($p = 0.006$).

Discussion

Compared to previous methods used to apply drugs to the RWM as described in the Introduction, the novel drug release RW microimplants presented here have major advantages both for short-term and for prolonged treatment regimens. Surgical intervention (tympanotomy) is minor, placing the microimplant into the RW niche is easy, the tympanic membrane is not perforated, and technical problems, such as change of position or obliteration of the microcatheter tip or dysfunction of micropumps, are avoided. Furthermore, the drug level which ought to be achieved in the inner ear within a given time, i.e. the daily release rate, may be varied by using different types of siloxane-based polymers. In vitro permeation tests performed by Leiras had previously shown that the release of beclomethasone from cylindrical implants of 5 mm length and 1.2–1.5 mm diameter was 15–20 μg/day for the low-dose or slow-release device and approximately 50 μg/day for the high-dose or fast-release implants.

The purpose of the present study was to confirm the release of beclomethasone into the perilymph of the scala tympani from both the slow-release and the fast-release microimplants adapted to the dimensions of the RW niche in guinea pigs (0.8×1.14 mm) in a yes-or-no fashion. This limitation was due to the fact that because of cutting imprecision, implants were not exactly of the same size and therefore would not contain exactly the same quantities of the drug. For this reason, the beclomethasone assay had not been validated, and only a threshold level was defined. The limit of detection was set at 2.25 pg/μl. Conclusions on the concentration levels can therefore not been drawn. This may explain the variability of individual perilymphatic beclomethasone threshold levels. However, on day 1 after implantation of the slow-release and the fast-release microimplant the median averaged 34.9 and 64.3 pg/μl, respectively, on day 14 after implantation 43.8 and 46.9 pg/μl, and at the end of the experiments on day 28 after implantation 4.7 and 60.5 pg/μl, respectively. A comparison of our data with endolymphatic and perilymphatic glucocorticoid concentrations or glucocorticoid tissue levels in the cochlea measured previously [Parnes et al., 1999; Chandrasekhar et al., 2000; Chandrasekhar, 2001; Bachmann et al., 2001; Tobita et al., 2002] is not possible, since these data were obtained after a single intratympanic injection.

However, whether or not the glucocorticoid levels achieved in these previous studies after a single application or the levels obtained in the present study using continuous sustained-release vehicles are efficient to treat various inner ear disorders must remain the subject of future experimental examination and clinical testing.

A toxic effect due to continuous long-term local administration of beclomethasone was excluded in the present study. The RWM appeared normal without any signs of defects, scars or inflammatory tissue alterations with subsequent membrane thickening. Similarly, cytocochleograms revealed no inner hair cell loss and an outer hair cell loss within normal ranges (from 0.5 ± 0.4 to $0.8 \pm 0.2\%$ per cochlea) in all experimental groups (non-implanted controls, placebo implant group, slow-release and fast-release beclomethasone implant groups) in both ears at any time of examination. Furthermore, on day 28 after implantation a significant difference of ABR threshold shifts remained only at 6 kHz in the slow-release type. In previous investigations, no changes of compound action potential thresholds, amplitudes and latencies were registered in chinchillas after intratympanic injection of triamcinolone [Ikeda and Morizono, 1991]. Similarly, transtympanal injection of dexamethasone caused no histological or ABR threshold changes in guinea pigs [Shirwany et al., 1998] or rats [Takeuchi and Anniko, 2000]. In addition, ultra-high doses (100 mg/kg) of intravenously administered prednisolone did not induce any changes in cochlear blood flow, cochlear microphonics, compound action potentials and ABRs in guinea pigs [Lamm and Arnold, 1998].

Furthermore, current experimental studies have shown significant beneficial prophylactic and therapeutic effects of glucocorticoids in immune-mediated progressive sensorineural hearing loss, viral and bacterial labyrinthitis, development of cholesteatoma, cochlear nerve injury, salicylate- and kanamycin-induced hearing loss, photochemically induced cochlear ischaemia, transient asphyxia and noise-induced cochlear damage and hearing loss [for reviews, see Lamm and Arnold, 1998, 1999b]. In clinical trials, positive experience with systemic or intratympanic glucocorticoid treatment was reported in im-

mune-mediated cochleovestibular diseases, Ménière's disease, sudden sensorineural hearing loss and tinnitus [for reviews, see Lamm et al., 1998; Lamm and Arnold, 1999b; Michel et al., 2000; Alexiou et al., 2001].

In summary, experimental and clinical studies have shown promising prophylactic and therapeutic effects of systemic and local application of glucocorticoids to the RWM in many cochlear disorders and potentially in tinnitus due to various underlying inner ear diseases. However, administration of a minimum of 250 mg glucocorticoids is required to achieve detectable drug levels in the perilymph of humans [Niedermeyer et al., 2003]. This can only be accomplished by intravenous infusion, not by oral administration. In contrast, local delivery methods require significantly less glucocorticoid doses and result in a significantly elevated inner ear drug level [Parnes et al., 1999; Chandrasekhar et al., 2000; Chandrasekhar, 2001; Bachmann et al., 2001; Tobita et al., 2002]. Therefore, introduction into clinical practice of the novel drug release RW microimplants presented here may be reasonable since they have certain advantages over previous methods and devices for short- and long-term treatment regimens.

Acknowledgements

F.R. (Leiras, now Schering OY, Finland) has provided the RW microimplants and further support. We would like to pay our special thanks to Arnaud Salvador and Cyriaque J. Sagan, PhD (Aster Cephac, Saint-Benoît, France), for conducting the beclomethasone assays in the perilymph samples, and to Bryant McEwen (Munich) for translation of the manuscript.

References

Alexiou C, Arnold W, Fauser C, Schratzenstaller B, Gloddek B, Fuhrmann S, Lamm K: Sudden sensorineural hearing loss: Does application of glucocorticoids make sense? Arch Otolaryngol Head Neck Surg 2001;127:253–258.

Alzamil KS, Linthicum FH Jr: Extraneous round window membranes and plugs: Possible effect on intratympanic therapy. Ann Otol Rhinol Laryngol 2000;109:30–32.

Bachmann G, Su J, Zumegen C, Wittekindt C, Michel O: Permeabilität der runden Fenstermembran für Prednisolon-21-Hydrogensuccinat: Prednisolongehalt der Perilymphe nach lokaler Applikation vs systemischer Injektion. HNO 2001;49:538–542.

Balough BJ, Hoffer ME, Wester D, O'Leary MJ, Brooker CR, Goto M: Kinetics of gentamicin uptake in the inner ear of *Chinchilla laniger* after middle-ear administration in a sustained-release vehicle. Otolaryngol Head Neck Surg 1998;119:427–431.

Brown JN, Miller JM, Altschuler RA, Nuttall AL: Osmotic pump implant for chronic infusion of drugs into the inner ear. Hear Res 1993;70: 167–172.

Canlon B, Fransson A: Morphologcial and functional preservation of the outer hair cells from noise trauma by sound conditioning. Hear Res 1995;84:112–124.

Chandrasekhar SS: Intratympanic dexamethasone for sudden sensorineural hearing loss: Clinical and laboratory evaluation. Otol Neurootol 2001;22:18–23.

Chandrasekhar SS, Rubinstein RY, Kwartler JA, Gatz M, Connelly PE, Huang E, Baredes S: Dexamethasone pharmacokinetics in the inner ear: Comparison of route of administration and use of facilitating agents. Otolaryngol Head Neck Surg 2000;122:521–528.

Erhardt W, Henke J, Lendl C: Narkose-Notfälle. Stuttgart, Enke, 2002, p 212.

Fernandez C: Dimensions of the cochlea (guinea pig). JASA 1952;24:519–523.

Ghiz AF, Salt AN, DeMott JE, Henson MM, Henson OW Jr, Gewalt SL: Quantitative anatomy of the round window and cochlear aqueduct in guinea pigs. Hear Res 2001;162:105–112.

Hoffer ME, Allen K, Kopke RD, Weisskopf P, Gottshall K, Wester D: Transtympanic versus sustained-release administration of gentamicin: Kinetics, morphology, and function. Laryngoscope 2001;111:1343–1357.

Hoffer ME, Balough B, Henderson J, DeCicco M, Wester D, O'Leary MJ, Kopke R: Use of sustained release vehicles in the treatment of Ménière's disease. Otolaryngol Clin North Am 1997;30:1159–1166.

Hoffer ME, Balough BJ, Kopke RD, Henderson J, DeCicco M, Wester DC, O'Leary MJ, Balaban C: Morphologic changes in the inner ear of *Chinchilla laniger* after middle ear administration of gentamicin in a sustained-release vehicle. Otolaryngol Head Neck Surg 1999;120: 643–648.

Husmann KR, Morgan AS, Girod DA, Durham D: Round window administration of gentamicin: A new method for the study of ototoxicity of cochlear hair cells. Hear Res 1998;125:109–119.

Ikeda K, Morizono T: Effect of ototopic application of a corticosteroid preparation on cochlear function. Am J Otolaryngol 1991;12:150–153.

Jackson LE, Silverstein H: Chemical perfusion of the inner ear. Otolaryngol Clin North Am 2002;35:639–653.

Lamm K, Arnold W: The effect of prednisolone and non-steroidal anti-inflammatory agents on the normal and noise-damaged guinea pig inner ear. Hear Res 1998;115:149–161.

Lamm K, Arnold W: Successful treatment of noise-induced cochlear ischemia, hypoxia and hearing loss. Ann NY Acad Sci 1999a;884:233–249.

Lamm K, Arnold W: How useful is corticosteroid treatment in cochlear disorders? Otorhinolaryngol Nova 1999b;9:203–216.

Lamm K, Lamm H, Arnold W: Effect of hyperbaric oxygen therapy in comparison to conventional or placebo therapy or no treatment in idiopathic sudden hearing loss, acoustic trauma, noise-induced hearing loss and tinnitus: A literature survey. Adv Otorhinolaryngol 1998;54:86–99.

Lehner R, Brugger H, Maassen MM, Zenner HP: A totally implantable drug delivery system for local therapy of the middle and inner ear. Ear Nose Throat J 1997;76:567–570.

Michel O, Jahns T, Joost-Enneking M, Neugebauer P, Streppel M, Stennert E: Das antiphlogistisch-rheologische Infusionsschema nach Stennert in der Behandlung von kochleovestibulären Störungen. HNO 2000;48:182–188.

Niedermeyer HP, Zahneisen G, Luppa P, Busch R, Arnold W: Cortisol levels in the human perilymph after intravenous administration of prednisolone. Audiol Neurootol 2003;8:316–321.

Nomura Y: Otological significance of the round window. Adv Otorhinolaryngol 1984;33:1–162.

Nomura Y, Okuno T, Kawabata I: The round window membrane. Adv Otorhinolaryngol 1983; 31:50–58.

Parnes LS, Sun AH, Freeman DJ: Corticosteroid pharmacokinetics in the inner ear fluids: An animal study followed by clinical application. Laryngoscope 1999;109(suppl 91):1–17.

Plontke SK, Plinkert PK, Plinkert B, Koitschev A, Zenner H-P, Löwenheim H: Transtympanic endoscopy for drug delivery to the inner ear using a new microendoscope. Adv Otorhinolaryngol 2002;59:149–155.

Praetorius M, Limberger A, Müller M, Lehner R, Schick B, Zenner HP, Plinkert P, Knipper M: A novel microperfusion system for the long-term local supply of drugs into the inner ear: Implantation and function in the rat model. Audiol Neurootol 2001;6:250–258.

Prieskorn DM, Miller JM: Technical report: Chronic and acute intracochlear infusion in rodents. Hear Res 2000;140:212–215.

Proctor B, Bollobas B, Niparko JK: Anatomy of the round window niche. Ann Otol Rhinol Laryngol 1986;95:444–446.

Schimmer BP, Parker KL: Adrenocorticotrophic hormone; adrenocortical steroids and their synthetic analogs; inhibitors of the synthesis and actions of adrenocortical hormones; in Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Goodman Gilman A (eds): Goodman and Gilman's Pharmacological Basis of Therapeutics, ed 9. New York, McGraw-Hill, 1996, pp 1459–1485.

Shirwany NA, Seidman MD, Tang W: Effect of transtympanic injection of steroids on cochlear blood flow, auditory sensitivity, and histology in the guinea pig. Am J Otol 1998;19:230–235.

Silverstein H: Use of a new device, the MicroWick, to deliver medication to the inner ear. Ear Nose Throat J 1999;78:595–598, 600.

Silverstein H, Rowan PT, Olds MJ, Rosenberg SI: Inner ear perfusion and the role of round window patency. Am J Otol 1997;18:586–589.

Steinhoff HJ, Janssen T, Arnold W: Frequenzspezifische Auslösung von Hirnstammpotentialen. Otorhinolaryngol Nova 1995;5:307–314.

Takeuchi N, Anniko M: Dexamethasone modifies the effect of *Pseudomonas aeruginosa* exotoxin A on hearing. Acta Otolaryngol (Stockh) 2000; 120:363–368.

Tobita T, Senarita M, Hara A, Kusakari J: Determination of prednisolone in the cochlear tissue. Hear Res 2002;165:30–34.