Treatment of Chronic Suppurative Otitis Media With Topical Tobramycin and Dexamethasone

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Objectives: To investigate the safety and efficacy of a topical combination of tobramycin and dexamethasone in a primate model of chronic suppurative otitis media (CSOM) and to explore the contribution of the added topical steroid for the treatment of CSOM.

Design: Blinded, randomized, placebo-controlled trial.

Subjects: Sixty juvenile cynomolgus monkeys randomized into the following 6 treatment groups of 10 monkeys each: 0.3% tobramycin (group 1), combined 0.3% tobramycin–0.1% dexamethasone (group 2), combined 1.0% tobramycin–0.33% dexamethasone (group 3), 0.1% dexamethasone (group 4), vehicle (group 5), and phosphate-buffered saline solution (group 6).

Interventions: Chronic suppurative otitis media was established by inoculating the right ear with Pseudomonas aeruginosa. After 4 weeks of drainage, animals were treated according to the group assignment with 3 drops twice daily for 7 weeks. Hearing thresholds were monitored with repeated auditory brainstem response testing (ABR), and clinical response was monitored with repeated otoscopic examinations and cultures throughout the study. Cytocochleograms were evaluated for quantification of outer hair cell loss.

Results: Rapid resolution of otorrhea and eradication of P aeruginosa occurred in all groups receiving tobramycin. The inclusion of dexamethasone accelerated the resolution of otorrhea and negative yields of cultures compared with tobramycin alone. Otorrhea and positive culture findings persisted in the groups not treated with topical antibiotic. Results of ABRs at 4 and 8 weeks and cytocochleograms for outer hair cell loss were not affected by drug administration. Perilymph samples collected at the end of the study showed no detectable tobramycin.

Conclusions: Combined tobramycin-dexamethasone ear drops were safe and effective in the monkey CSOM model. Dexamethasone enhanced the efficacy of tobramycin.


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MATERIALS AND METHODS

Sixty juvenile cynomolgus monkeys of both sexes were included in the study. The animals were quarantined for 4 weeks before the experiment. All animals were examined using an operating microscope and tympanometry to confirm the health of the TM and ME. The animals were assigned randomly (10 animals per group) to 1 of the following 6 treatment groups: group 1, 0.3% tobramycin; group 2, 0.3% tobramycin-0.1% dexamethasone; group 3, high-dose 1.0% tobramycin-0.33% dexamethasone; group 4, 0.1% dexamethasone; group 5, vehicle containing benzalkonium chloride; and group 6, phosphate-buffered saline (PBS) solution. The animals were examined otomicroscopically twice a week, a culture was obtained, and the external ear canals were suctioned. A fourth ABR was performed on all right ears after the fourth week of treatment. The treatment was discontinued after 7 weeks, with continued otoscopic and culture evaluations until the animals were killed. A fifth ABR was performed 1 week after discontinuation of the treatment. All the animals were killed approximately 1 week after the last ABR. On the day the animals were killed, a drug penetration study was performed on selected animals to determine the amount of tobramycin in the perilymph. The animals were killed under heavy anesthesia using vital perfusion with a fixative. The temporal bones were dissected, gross macroscopic findings on the TM and ME were recorded, and biopsy specimens were obtained. The cochleae were perfused with fixative in situ through the round and oval windows and then dissected from the rest of the temporal bone and prepared for cytocochleogram.

SPECIFIC METHODS

Ketamine hydrochloride (10 mg/kg) was used for the anesthesia of the animals for brief procedures such as the otoscopic examinations with culturing and ear drop application. For the ABR, the animals were anesthetized with a mixture of ketamine hydrochloride (13.3 mg/kg), xylazine hydrochloride (2.7 mg/kg), and acepromazine maleate (0.4 mg/kg). The supplement was given as needed using half the initial dose.

Otomicroscopic examinations were performed with the operating microscope (M703F; Storz Instrument Co, St Louis, Mo) at 16× magnification. Sterile neonatal speculi (Storz Instrument Co) and pediatric disposable speculi (Kleen-spec; Welch Allyn, Skaneateles Falls, NY) were used during otomicroscopy. The otoscopic findings were recorded for presence of perforation (perforated, not perforated, or could not evaluate), presence of drainage (yes or no), quantity of drainage (0 indicates no drainage; 1, moist; 2, filling the ME; 3, filling the external auditory canal; and 4, draining out from the external auditory canal), and quality of drainage (serous, mucoid, mucopurulent, or purulent).

A culture was obtained during the otoscopic examination using a calcium alginate fiber–tipped aluminum applicator swab (Fisherbrand Sterile Swabs; Curtin Mathes son Scientific, Houston, Tex). The swab was streaked immediately on chocolate agar plates (for isolation of any bacteria present) and Pseudomonas isolation agar (Difco Laboratories, Detroit, Mich) plates (for P aeruginosa). The plates were placed in an incubator at 37°C and read at 24, 48, and 72 hours.

After 4 weeks without treatment, the ears were suctioned (sterile 5F or 3F cannulas; Storz Instrument Co) after delivering a uniform, color-coded dropper, blinding the study personnel and examiners to group assignment. The pH of the ear drops was 7.5, 5.5, 5.6, 6.6, 5.67, and 6.02 for the groups 1, 2, 3, 4, 5, and 6, respectively. Twice daily for 7 weeks, 3 drops were placed into the right ears. The animals were examined otomicroscopically twice a week, a culture was obtained, and the external ear canals were suctioned. A fourth ABR was performed on all right ears after the fourth week of treatment. The treatment was discontinued after 7 weeks, with continued otoscopic and culture evaluations until the animals were killed. A fifth ABR was performed 1 week after discontinuation of the treatment. All the animals were killed approximately 1 week after the last ABR. On the day the animals were killed, a drug penetration study was performed on selected animals to determine the amount of tobramycin in the perilymph. The animals were killed under heavy anesthesia using vital perfusion with a fixative. The temporal bones were dissected, gross macroscopic findings on the TM and ME were recorded, and biopsy specimens were obtained. The cochleae were perfused with fixative in situ through the round and oval windows and then dissected from the rest of the temporal bone and prepared for cytocochleogram.

STUDY DESIGN

This study enabled investigation of the efficacy and safety of tobramycin and dexamethasone at 2 different concentrations, as well as the components 0.3% tobramycin, 0.1% dexamethasone, and vehicle as separate treatments for the assessment of effects of each component on the outcome. A sixth group included as a negative control group was treated with PBS drops. Noninfected, uninfected, and untreated left ears were collected and are presented as separate control data for comparison of normative data. Investigators and study personnel were blinded to the treatment group assignment. The procedures on animals were approved by the Animal Research and Care Committee at the Children’s Hospital of Pittsburgh, Pittsburgh, Pa, and in accordance with the US Public Health Service Policy on Humane Care and Use of Laboratory Animals, the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Act.

Ototoxic effects in humans in a systematic way. The small number of studies that relate topical treatment to hearing loss in humans probably results from the difficulty in differentiating the effects of coexisting chronic or recurrent infection from that of topical aminoglycosides, since both potentially affect hearing.

Until recently, no ototopical antimicrobial preparation had been approved by the US Food and Drug Administration (FDA) for treatment of otorrhea in the face of a nonintact TM. Clinicians managing chronic and recurrent otorrhea have used such preparations without an FDA-approved otic indication, after warning the pa-
samples were obtained for cultures. The presence of the perforation was checked, and if the TM had healed, it was reperforated. If the size of the perforation was smaller than 30% of the TM area, it was enlarged. Reperforation of the TM was documented.

The ABRs were performed in a soundproof booth using a compact auditory electrodagnostic system (Nicolet Instrument Corp, Madison, Wis) for stimulus generation and potential recordings. Two types of stimuli were presented: clicks and tone bursts of 2000, 4000, and 8000 Hz. For each stimulus, 2000 sweeps were recorded, analyzed, and stored. Stimulus intensity was decreased from 100 dB, in steps of 20 dB down to 40 dB, in steps of 10 dB down to 20 dB, and in steps of 5 dB down to the level of hearing threshold. When ABR potentials disappeared, the ABR was repeated at 5-dB increments until the potential disappeared. The level of hearing was then confirmed by obtaining the potential again at the 5-dB lower threshold.

After the sedation with ketamine hydrochloride (10 mg/kg), animals were killed using intraperitoneal injection of pentobarbital sodium (35 mg/kg), followed by vital perfusion an hour later. Intracardiac perfusion with PBS for 3 minutes was followed by perfusion with Karnovsky fixation solution for 10 minutes. The temporal bones were dissected out within half an hour. The bony external ear canal was removed, and the TM thickness was graded (scale, 0-4+) using an operating microscope. Following removal of TM and malleus, the thickness of ME mucosa (scale, 0-4+) was assessed. Thicknesses were graded by the same investigator (C.M.A.), to have a subjective assessment of the differences between specimens. A punch biopsy specimen, including bone periosteum and mucosa, was obtained from the hypotympanum. The incus and stapes were removed and saved for histological examination. A separate bone and mucoperiosteal punch biopsy specimen was obtained from the mastoid, just peripheral to the antrum. The tissues were decalcified with 3% formic acid, embedded in paraffin, sectioned, and stained with hematoxylin and eosin using standard techniques. The thickness of the TM and the mucosa of ME and mastoid were measured as an indicator of the degree of inflammation, using an image analysis package.

Quantitative histological analysis was performed using a commercially available software (Metamorph Imaging System, Version 2; Universal Imaging Corporation, West Chester, Pa). Mucosal thickness was measured on the sections stained with hematoxylin and eosin. For each location, 3 measurements were randomly obtained, and their average was used in the analysis.

Immediately after the luxation of the stapes, the perilymph was aspirated through the oval window with a microliter pipette (Pipetman; Rainin Instrument Co, Woburn, Mass) and saved. The round window was punctured, and the labyrinth was perfused with fixative. Following trimming to remove the bone around the labyrinth, the cochlea were placed in the fixative in a refrigerator at 4°C overnight. The cochleae were placed into cacoodylate buffer and reperfused through the round window twice weekly until shipment to the Karolinska Institute, Stockholm, Sweden. There, the specimens were dehydrated and embedded in agar. They were processed in a routine manner for light microscopy and dissected for cytocochleogram.

On the day each animal was killed, the treatment code was broken and a perilymph penetration study was performed. The groups that had received drops with tobramycin during the treatment period were used for the study. The left ears of a few animals were included as controls. According to the protocol, the treatment had been discontinued at least 1 week before the animals were killed. To assess the penetration of tobramycin through the round window membrane, 3 drops of 0.3% tobramycin were applied in vivo to the external ear canals, similar to the method used throughout the experiment. To detect any potential time dependence, the animals were divided into 2 groups, and perilymph samples were collected approximately 1 or 3 hours after the dosing. After the animals were killed and the temporal bone was dissected, the ME was irrigated thoroughly to wash out any remaining tobramycin. The ME was dried, and the stapes was luxated. A micropipette with a pipet tip was used to aspirate the perilymph. The sample volume was measured, and it was stored at 80°C until the transfer and the analysis for the assay. To control for the effect of perilymph contamination from the ME during sampling, a group of left ears was dissected as described, and 3 drops of tobramycin were applied. The ME was irrigated and dried as in the experimental group just before obtaining the perilymph sample.

The perilymph samples were transferred to TexMS Analytical Services, Houston, Tex, for quantitative analysis of tobramycin. An internal standard was prepared with bekanamycin in perilymph fluid. Aliquots were analyzed by means of high-pressure liquid chromatography and mass spectrometry with electrospray ionization and selected ion monitoring. The protonated molecular ions for tobramycin (mass to charge ratio, 490) and the internal standard bekamycin (mass to charge ratio, 506) were monitored, and the resulting intensity ratio was used for quantitation. A 5-point calibration curve was then generated using control perilymph matrix spiked with tobramycin at 40, 60, 80, 100, and 120 pg/µL. Duplicate calibration standards were used at the high and low ends of the curve. Single standards were used for the 3 intermediate levels. Separately prepared perilymph samples spiked at 60 and 100 pg/µL were used as quality control samples and analyzed in duplicate.

A block-surface technique method described by Spoendlin and Brum13 was used for the histological evaluation of ototoxic effects. Surface preparations were examined under light microscopy for the quantification of hair cell loss. The total number of outer hair cells (OHCs) and of damaged OHCs were counted in 4 parts (each coil in 2 parts). Hair cells were counted separately for each position (first, second, and third OHC rows) in each part of each coil. The results are reported as percentage of the damaged hair cells for each part of the cochlea.

A combination of tobramycin and dexamethasone is currently marketed as an eye drop; however, it has been prescribed without an FDA-approved otic indication by otolaryngologists for the treatment of otorhea as well. We therefore conducted a study to investigate the efficacy and safety of topical tobramycin-dexamethasone in patients or parents of the potential ototoxic effects. Because this preference is substantiated by these controversial reports, there is a greater need today to avoid the potentially ototoxic, untested, and unapproved ear drops and to provide clinicians with the better, nontoxic, effective alternatives for the topical treatment of CSOM.
Pretreatment, interval, and end stages of testing for groups 1 through 6. Groups are described in the legend to Figure 1.

Figure 2. Percentage of ears with cultures yielding Pseudomonas aeruginosa for groups 1 through 6. Vertical line at week 0 depicts the initiation of treatment. Two of 6 available ears cultured beyond the eighth week yielded P aeruginosa in group 1. Groups are described in the legend to Figure 1.

Figure 3. Average hearing thresholds with auditory brainstem response testing at entry (baseline), after wide perforation of the tympanic membrane (perforation), just before the initiation of the treatment (pretreatment), after 4 weeks of treatment (interval), and at the eighth week (end) for the groups 1 through 6. Groups are described in the legend to Figure 1.

Figure 1. Average drainage scores for the groups treated with 0.3% tobramycin (group 1), combination of 0.3% tobramycin and 0.1% dexamethasone (group 2), high-dose combination of 1.0% tobramycin and 0.33% dexamethasone (group 3), 0.1% dexamethasone (group 4), vehicle containing benzalkonium chloride (group 5), and phosphate-buffered saline (group 6). Vertical line at week 0 depicts the initiation of treatment. The amount of otorrhea is scored as 4 for draining out of the external canal, 3 for filling the external canal, 2 for filling the middle ear, and 1 for minimal amount in the middle ear.

degree of drainage throughout the study, including the pretreatment and posttreatment periods, is illustrated in Figure 1. The drainage was present in more than 95% of the observations across all groups, before initiation of treatment. All study groups had an average of grade 3 corresponding to drainage at least filling the external ear canal. The amount of drainage remained constant for groups 4, 5, and 6 throughout the treatment period. However, there was a gradual decrease in drainage for the groups treated with drops that included tobramycin (groups 1, 2, and 3). In groups 2 and 3, the average score for drainage decreased in just 2 weeks to 2, corresponding to filling only the ME, and in 4 weeks to 1, corresponding to moisture in the ME. The rate of decrease in the amount of drainage was faster for groups 2 and 3 when compared with group 1. The average score for otorrhea in group 2 decreased to less than 1 by the fifth week of treatment and remained stable at that level for the remainder of the study. On the other hand, group 1 had a lower rate of decrease in the amount of drainage. Furthermore, the drainage increased in group 1 when the ear drops were discontinued at the end of the study.

CULTURE FOR P AERUGINOSA

Pseudomonas aeruginosa was isolated from ear cultures in 98% of the observations before initiation of the treatment. There were no differences in culture positivity for P aeruginosa between groups during the pretreatment period. The cultures continued to yield positive results throughout the treatment period for P aeruginosa for groups 4, 5, and 6 (Figure 2). On the other hand, the groups that received drops containing tobramycin, ie, groups 1, 2, and 3, had a rapid decrease in the percentage of ears with cultures yielding P aeruginosa. All of the ears in group 2 yielded negative culture findings after 3 weeks of treatment, and these remained negative for the rest of the study period. In group 3, the ears with culture-positive findings decreased rapidly to 20% in 1.5 weeks; however, complete eradication did not occur until another 4 weeks of treatment had passed. Group 1 had a gradual decrease across 6 weeks to reach 0% P aeruginosa in cultures. The

a primate model of CSOM due to P aeruginosa infection. A second objective was to determine whether the addition of a topical steroid provided additional benefit to ultimate treatment outcome.

RESULTS

One of the animals was killed during the study, owing to an illness unrelated to the study protocol. When the code was broken at the end of the study, it was found to belong to group 6.

OTOMICROSCOPY

All of the animals had normal TMs and MEs at entry. After the inoculation with P aeruginosa, the right ears were confirmed to be draining during biweekly otoscopic evaluations. The differences between the study groups in the

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cultures continued to yield negative findings during the last week of the study, when ear drops were not administered. However, a relapse for *P. aeruginosa* during the ninth week was observed in 2 of 6 ears in the interval between the end of treatment and killing.

**ABR TESTS**

The hearing thresholds of the right ears underwent ABR testing 5 times during the study. At entry, the right ears had average (± SD) hearing thresholds of 21.4 ± 7.4, 27.6 ± 7.6, 30.8 ± 10.8, and 34.5 ± 12.5 dB for click and 2000-, 4000-, and 8000-Hz stimuli, respectively. Perforating the TMs resulted in 29.0-, 31.8-, 25.9-, and 24.3-dB increases in the thresholds for click and 2000-, 4000-, and 8000-Hz stimuli, respectively. The average hearing thresholds for each treatment group at baseline, postperforation, pretreatment, treatment, and posttreatment ABRs are shown in Table 1. Over 4 weeks of treatment determined by otomicroscopy are presented in Figure 3.

<table>
<thead>
<tr>
<th>ABR No. (Stage)</th>
<th>Treatment Group†</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hearing thresholds (dB)</td>
<td>†Group 1 was treated with 0.3% tobramycin; group 2, combined 0.3% tobramycin–0.1% dexamethasone; group 3, combined 1.0% tobramycin–0.33% dexamethasone; group 4, 0.1% dexamethasone; group 5, vehicle containing benzalkonium chloride; and group 6, phosphate-buffered saline solution.</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (baseline)</td>
<td>35.50 ± 12.77</td>
<td>27.88 ± 6.50</td>
<td>27.62 ± 5.77</td>
<td>27.25 ± 5.39</td>
<td>25.25 ± 3.66</td>
<td>27.88 ± 8.06</td>
<td></td>
</tr>
<tr>
<td>2 (post perforation)</td>
<td>60.88 ± 5.89</td>
<td>56.38 ± 9.44</td>
<td>55.25 ± 6.42</td>
<td>56.75 ± 5.51</td>
<td>56.75 ± 6.40</td>
<td>51.88 ± 10.51</td>
<td></td>
</tr>
<tr>
<td>3 (pretreatment)</td>
<td>56.00 ± 9.37</td>
<td>54.00 ± 6.04</td>
<td>60.13 ± 12.28</td>
<td>48.25 ± 8.75</td>
<td>55.25 ± 15.17</td>
<td>51.38 ± 14.85</td>
<td></td>
</tr>
<tr>
<td>4 (interval-treatment)</td>
<td>46.50 ± 8.38</td>
<td>48.50 ± 6.56</td>
<td>56.00 ± 10.49</td>
<td>53.25 ± 6.74</td>
<td>55.75 ± 17.56</td>
<td>49.86 ± 12.39</td>
<td></td>
</tr>
<tr>
<td>5 (posttreatment)</td>
<td>46.75 ± 7.21</td>
<td>55.63 ± 6.55</td>
<td>53.75 ± 7.87</td>
<td>55.00 ± 9.49</td>
<td>53.88 ± 12.90</td>
<td>59.58 ± 15.05</td>
<td></td>
</tr>
</tbody>
</table>

*Unless otherwise indicated, data are given as average ± SD decibels. ABR indicates auditory brainstem response.

**PERILYMPH FINDINGS**

The correlation coefficient for the standard curve was 0.99. The accuracy for the individual quality control samples ranged from 81% to 105%, with an overall accuracy of 94%. Two of the samples were consumed during the development of the testing conditions that would yield maximum electrospray ionization response for tobramycin. Four samples were inadvertently damaged during the shipment. One analytical run was sufficient to assay all of the remaining 33 perilymph fluid samples. All of the samples of perilymph assayed below the limit of quantitation (both lower quadrants, <40 pg/µL) for tobramycin.

**ME FINDINGS**

Average scores for the degree of TM and ME inflammation determined by otomicroscopy are presented in Table 2. The uninfected, untreated left ears served as controls, with an average score of 0 for TM and ME. Groups 3, 4, and 6 had higher scores for inflammation. Group 1 had the lowest score among the infected study groups.

Histological assessment of the TM, ME, and mastoid mucosal thickness is summarized in Table 2. The thickness of the TM could not be assessed in 5 of 12 control group specimens because of technical difficulties related to the very thin TMs. Among the infected ears, the thickness of TM was the highest in group 5 and the lowest in group 2. Mucosal thickness of ME was the highest in group 5 and the lowest in group 2. Mucosal thickness of ME was the highest in group 6, followed by groups 4 and 5, and lowest in group 2, followed by groups 3 and 1. Mastoid mucosal thickness in group 2 was the lowest, followed by group 3.

**COCHLEAR HISTOPATHOLOGICAL FINDINGS**

The light microscopic evaluation demonstrated purulent material within 2 cochleae, 1 in groups 5 and 6 each. In those specimens, the inner ear structures were grossly

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Table 1. Average Hearing Thresholds in 6 Treatment Groups With 2000, 4000, and 8000 Hz, and Click Stimuli on 5 ABR Tests Throughout the Study*

<table>
<thead>
<tr>
<th>ABR No. (Stage)</th>
<th>Treatment Group†</th>
</tr>
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<tbody>
<tr>
<td>Hearing thresholds (dB)</td>
<td>†Group 1 was treated with 0.3% tobramycin; group 2, combined 0.3% tobramycin–0.1% dexamethasone; group 3, combined 1.0% tobramycin–0.33% dexamethasone; group 4, 0.1% dexamethasone; group 5, vehicle containing benzalkonium chloride; and group 6, phosphate-buffered saline solution.</td>
</tr>
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<td>1 (baseline)</td>
<td>35.50 ± 12.77</td>
</tr>
<tr>
<td>2 (post perforation)</td>
<td>60.88 ± 5.89</td>
</tr>
<tr>
<td>3 (pretreatment)</td>
<td>56.00 ± 9.37</td>
</tr>
<tr>
<td>4 (interval-treatment)</td>
<td>46.50 ± 8.38</td>
</tr>
<tr>
<td>5 (posttreatment)</td>
<td>46.75 ± 7.21</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Change with treatment</th>
<th>No. of ears with change</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABR 5 – ABR 3 (dB)</td>
<td>–9.25 ± 6.74</td>
</tr>
<tr>
<td>≥10 dB</td>
<td>0</td>
</tr>
<tr>
<td>≥20 dB</td>
<td>0</td>
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identified; however, damage to the organ of Corti and significant loss of hair cells prohibited any count from being performed. The data of the 2 cochleae with pus are not included in the calculation of the average OHCL loss (OHCL) of these treatment groups. The cytocochleogram revealed partial damage, probably as a result of processing, in 2 other cochlea. The OHCs in the first coil of a cochlea in group 6 and all the OHCs except the first part of the first coil in another cochlea in group 5 could not be read. Only the data on undamaged sections of these 2 cochleae are included in the calculation of the averages of the treatment groups.

Results of light microscopy demonstrated normal basilar and Reissner membranes, stria vascularis, and organ of Corti (Figure 4, A). There was no damage to the inner hair cells (Figure 4, B and C). The OHCL did not show considerable differences between the various parts of cochlea (Table 3). The OHCL in the first coil was slightly higher than in the second coil, a finding consistent throughout the groups, including the control group. The average OHCL was 0.90%, 0.86%, 1.11%, 1.09%, 0.90%, 0.82%, and 0.65% of the total number of hair cells for groups 1, 2, 3, 4, 5, and 6 and the control group, respectively. None of the cochleae in any treatment group, except the 2 with pus, had more than 3% OHCL. Although OHCL below the levels of 5% is considered insignificant, the distribution of the number of ears that had at least 1% and 2% OHCL in each treatment group are presented in Table 3. These results suggest no significant OHCL during the topical treatment of CSOM with tobramycin or dexamethasone.

### Table 2. Tympanic Membrane, Middle Ear, and Mastoid Findings by Otoscopy at Time of Dissection of Temporal Bone After Killing and With Histologic Examination

<table>
<thead>
<tr>
<th>Inflammation score</th>
<th>Treatment Groups</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness, mm</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>TM</td>
<td>0.90 ± 0.70</td>
<td>1.40 ± 0.66</td>
</tr>
<tr>
<td>ME</td>
<td>0.70 ± 0.46</td>
<td>1.00 ± 0.45</td>
</tr>
</tbody>
</table>

*Groups are described in the second footnote to Table 1. TM indicates tympanic membrane; ME, middle ear. Data are given as average ± SD. Inflammation score is explained in the “Specific Methods” subsection of the “Materials and Methods” section.

### COMMENT

The ethical and methodological limitations of obtaining reliable information on ototoxic effects in humans leads to research in animals. Most of the studies on ototoxic effects have been performed in chinchilla or guinea pig models. Several studies in various animals have demonstrated hearing loss or cochlear damage due to ear drops. Various antibiotics, antifungals, and solvents penetrated the perilymph and produced hair cell loss in animals. When the same ear drops were placed into the ME of higher species, such as baboons, a more limited sensory cell loss was seen. On the other hand, topical dosing of ciprofloxacin hydrochloride to chinchillas demonstrated no significant effect on ABRs or the cytocochleogram. The outcome differences in these studies may result from active ingredients in the drops, duration of the treatment, degree of inflammation, and differences in size, thickness, location, and permeability of the round window membrane between species. Applicability of these safety results to humans, therefore, have been questioned.

The differences in human ME architecture has implications not only for safety but also for treatment efficacy. Studies on ototoxicity are rarely conducted in an infectious model. This results in part from the lack of a good infectious model in rodents. Significant anatomic interspecies differences in the external ear canal, eustachian tube, mastoid pneumatization, and access to mastoid from ME have limited the use of animals for efficacy studies. Mastoid involvement in human CSOM has been claimed to be a major factor in the chronicity. The restricted access between the ME and mastoid air cells was thought to limit the efficacy of ear drops. These concerns, besides the concerns about applicability of rodent safety results, support basic studies in primates. Therefore, a monkey animal model was developed by Dohar et al to study efficacy and safety of ear drops in CSOM.

The primary reason for preferring a noninfectious model for evaluating safety is to test the ototoxicity of an ear drop when the ear is in its most vulnerable state. Although ear drops are typically prescribed to treat drainage, drops may be used after the resolution of drainage. The persistence of inflammation in the ME may continue to limit the penetration of drops into the perilymph. The undetectable levels of tobramycin in the perilymph in our study may reflect restricted permeability due to residual ME inflammation, despite presumed cure seen on otoscopy and culture findings. In our study, the degree of inflammation as assessed by mucosal thickness in the groups with persistent infection was up to 8 times that of the control side for the TM, 5 times that for the ME mucosa, and 3.5 times that for the mastoid mucosa. The degree of ME inflammation in our study appeared to be mainly due to infection. However, although the otorrhea had resolved before the end of the study in the groups treated with tobramycin-containing drops, inflammation (mucosal thickness) persisted in TM, ME, and mastoid mucosal specimens. Even in the group with the least degree of inflammation, group 2, TM thickness was 4 times and ME mucosa thickness was 1.5 times the control side. The thick-
ness of the mastoid mucosa in group 2 was the same as that of the control group. Group 1, however, had relatively higher average thickness at the time the animals were killed. The only group with recurrent *P aeruginosa* infection (group 1) had a similar amount of mastoid inflammation as the groups with persistent drainage. This is reflected in the thicker mastoid mucosa. On the other hand, some investigators linked the biofilm state of the bacteria with the persistence or recurrence in CSOM and other chronic infectious diseases. These bacteria are distinct from their planktonic forms, very resistant to antibiotics and host defense mechanisms, and difficult to isolate using routine culture techniques. However, it is unclear, given this explanation, why only a few ears in group 1 had recurrence in our study.

In a study on susceptibility patterns of aural *Pseudomonas* isolates, Dohar et al reported that tobramycin had significantly better in vitro activity (94%) compared with gentamicin (79%). Piperacillin sodium was the only intravenous agent with better (96%) in vitro activity. Our study clearly demonstrates the efficacy of tobramycin in the resolution of otorrhea and elimination of *P aeruginosa*. All 3 groups treated with tobramycin (groups 1, 2, and 3) had gradual resolution in the amount of otorrhea over several weeks, whereas there were no differences in the amount of otorrhea in the other 3 groups (groups 4, 5, and 6). Both groups receiving tobramycin-dexamethasone (groups 2 and 3) experienced more rapid resolution of otorrhea than did the group receiving tobramycin alone (group 1). These results may in part be consistent with the study by Fradis et al that compared the efficacy of topical tobramycin and ciprofloxacin for the treatment of CSOM in humans. In their study, tobramycin (without dexamethasone) was found to be equally effective (66.7%) in bacteriologic response but worse in clinical response (72.2% vs 78.9%) when compared with ciprofloxacin ear drops. In another study, otopical ciprofloxacin was found to be effective in nearly 70% of patients with otorrhea associated with *P aeruginosa*, previously unresponsive to other antimicrobials.

In a study of children with acute purulent otorrhea, topical ofloxacin demonstrated an 84.4% cure in subjects evaluated clinically, and it eradicated 96.3% of all baseline pathogens in subjects evaluated microbiologically.

Our study clearly demonstrates an added benefit of dexamethasone when combined with tobramycin for resolving otorrhea and eradicating *P aeruginosa*. The duration of treatment necessary to achieve complete eradication of *P aeruginosa* from the ears was 3, 5.5, and 6 weeks for groups 2, 3, and 1, respectively. Although not quite different than the 66.7% bacteriologic response reported with 3 weeks of topical treatment of human CSOM with ciprofloxacin or tobramycin in the study by Fradis et al, prolonged need for treatment in the monkey model may result from less frequent (twice a day) administration of the drops, limited access of the dropped medications into the ME because of the narrow ear canals, and anticipated natural nonhygienic habits of monkeys that may lead to constant or recurrent contamination of their perforated ears.

Differences in the responses for culture and clinical outcome measures were observed in a previous study using this model. In that study, a rapid eradication of *P aeruginosa* with topical ciprofloxacin, its vehicle, or Cortisporin (a combination product of hydrocortisone, neomycin sulfate, and polymyxin B sulfate) was not followed by a clinical response as assessed by otoscopy. Although the treatment period in that study was only 4 weeks, no decrease in the amount of otorrhea was observed. However, in our study, a response was apparent even in the first 4 weeks of the 7-week treatment period. In fact, for groups 2 and 3, the drainage score was halved in the first 2 weeks.

Monitoring of hearing loss is conducted when medications with potential ototoxicity are used in clinical practice or in animal studies. Hearing loss was monitored with ABR at 5 different stages throughout our study, to better identify, besides the histological features, the contribution of each of the potential factors, ie, TM perforation.
otitis media with effusion.28 When effective medications are used, this complication of CSOM is rarely seen.

Table 3. Inner Ear Findings in 6 Treatment Groups and Controls*  

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer hair cell loss, %†</td>
<td>1.08 ± 0.86</td>
<td>0.80 ± 0.48</td>
<td>1.16 ± 0.43</td>
<td>1.23 ± 0.27</td>
<td>0.96 ± 0.29</td>
<td>0.71 ± 0.26</td>
<td>0.91 ± 0.42</td>
</tr>
<tr>
<td>First coil, first part</td>
<td>1.31 ± 0.76</td>
<td>1.17 ± 0.56</td>
<td>1.46 ± 0.98</td>
<td>1.78 ± 1.47</td>
<td>1.29 ± 0.69</td>
<td>1.00 ± 0.48</td>
<td>0.93 ± 0.48</td>
</tr>
<tr>
<td>First coil, second part</td>
<td>0.73 ± 0.48</td>
<td>0.94 ± 0.87</td>
<td>1.06 ± 1.00</td>
<td>0.98 ± 0.63</td>
<td>0.64 ± 0.31</td>
<td>0.85 ± 0.57</td>
<td>0.27 ± 0.22</td>
</tr>
<tr>
<td>Second coil, first part</td>
<td>0.47 ± 0.20</td>
<td>0.50 ± 0.32</td>
<td>0.76 ± 0.43</td>
<td>0.48 ± 0.38</td>
<td>0.72 ± 0.68</td>
<td>0.77 ± 0.63</td>
<td>0.50 ± 0.36</td>
</tr>
<tr>
<td>Second coil, second part</td>
<td>0.90 ± 0.44</td>
<td>0.86 ± 0.43</td>
<td>1.11 ± 0.55</td>
<td>1.09 ± 0.57</td>
<td>0.90 ± 0.30</td>
<td>0.82 ± 0.44</td>
<td>0.65 ± 0.20</td>
</tr>
<tr>
<td>Entire cochlea</td>
<td>2.57 ± 1.06</td>
<td>2.86 ± 1.23</td>
<td>3.23 ± 1.50</td>
<td>3.46 ± 1.78</td>
<td>3.23 ± 1.50</td>
<td>3.09 ± 1.47</td>
<td>3.09 ± 1.47</td>
</tr>
<tr>
<td>No. of cochleae with loss</td>
<td>2</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>≥1%</td>
<td>2</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>≥2%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>≥3%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pus</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Partially damaged</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

*Groups are described in the second footnote to Table 1. Controls consisted of intact left ears.
†Data are given as average ± SD.

The histological evaluation of the cochleae demonstrated purulent labyrinthitis in 2 of the ears. Neither of these ears were in the groups that were treated with drops including tobramycin or dexamethasone. These data suggest that hearing loss or OHCL may be due to the infection. The degree of SNHL in humans has been found to correlate with the duration of chronic otitis media.27 Presence of SNHL has also been found to be related etiologically to otitis media with effusion.28 When effective medications are used, this complication of CSOM is rarely seen. This significant complication in our study reminds us of all the potential complications of CSOM, some of which are life threatening, and the importance of developing a safe and effective treatment for this condition.

The data on the OHCL did not include the 2 ears with labyrinthitis. If included, a 100% OHCL for those 3 ears would increase the average and the SD of the OHCL for those groups. These extreme values, although appropriate for the purpose of the analysis of the study, would obscure the homogeneously low OHCL that was comparable to that of the other groups. Moreover, the OHCL in these 2 ears did not result from the ototoxicity of the ear drops, but, ironically, because their drops lacked any antibiotic or anti-inflammatory agent.

The OHCL results in our study were well below the accepted limits for ototoxic effects. Average OHCL up to 5% is considered normal.22 Our study revealed an average OHCL of less than 1.11% in all treatment groups. None of the sections in any treatment group exceeded 1.78% OHCL in average. A comparison was made between hearing and the histological data in the ears that exceeded thresholds in hearing tests or OHCL, to assess whether there were individual correlations between these factors. In group 4, the ear that had 20-dB worsening had 2.36% OHCL, whereas the ear with 22.5-dB worsening had only 0.75% OHCL. In all of the other ears in group 4 that had at least a 10-dB hearing loss, the OHCL was less than 2%. One of the 2 ears in group 5 that had a 15-dB hearing loss was found to have 0.35% OHCL. The second ear with a 12.5-dB deterioration, however, was the ear that had damage in certain sections of the cochlea. However, the OHCL in the preserved sections was only 0.83%. The cochlea with pus in the vehicle group had significant hearing loss after the inoculation of the animal with P aeruginosa, but before the initiation of the treatment. The analysis of hearing loss and histological results for each individual animal with relatively outlying data did not demonstrate an ototoxic effect of tobramycin or dexamethasone.

A Pearson correlation analysis between OHCL and change in hearing with treatment in all of the animals did not demonstrate a statistically significant (r = −0.12; P = .39) relationship. It should be remembered that the data for cochlea with pus or with damaged sections were excluded from the initial analysis. To assess the effect of this on the
correlation, 100% of OHCL was entered to the sections of missing data because of pus or damage. This brought the correlation coefficient to 0.33 at \( P = .01 \). It seems that the persistence of otorrhea and the chronicity of the suppurative otitis media may increase the OHCL. The SNHL in some animals seems to be due to the infection itself. At least in the 2 cochleae with pus, this relationship is clear. The correlation between hearing and histological findings was analyzed separately only for the 3 groups that were given tobramycin. This correlation between hearing and OHCL for the groups was significant (\( P = .03 \)) for groups 1, 2, and 3. However, the correlation for these groups was negative (\( r = -0.40 \)), suggesting that for groups that received tobramycin, more pronounced hearing loss was associated with less OHCL. This negative correlation may also mean that with the resolution of otorrhea, there was an improvement in hearing, but that this may be increasing the risk for OHCL. Although the degree of OHCL in this study was well below the thresholds for ototoxic effects, this slight trend is consistent with other studies that have demonstrated the effect of inflammation in reducing the risk for ototoxic effects.

Our study monitored otoxicity, similar to other animal studies, by assessing the hearing loss and OHCL. This approach ignores potential vestibulotoxicity. No apparent signs of vestibulotoxic effects developed in any of the animals during the study period; however, a systematic and objective method of assessment was not part of the study protocol. Since many topical agents, such as aminoglycosides, may have prominent toxic effects on the vestibule, some while sparing the cochlea, standard test batteries should be developed to monitor vestibulotoxicity and included in the animal models for ototoxic effects.

Our study suggests that tobramycin with dexamethasone is a very effective treatment for otorrhea and eradication of \( P \) a e r u g i n o s a in a monkey CSOM model. When comparing the treatment groups for hearing threshold changes and OHCL, our study suggests that neither tobramycin nor dexamethasone is ototoxic, individually or in a combined formulation. Dexamethasone clearly contributes to the efficacy of the combination ear drop, leading to more rapid resolution of the CSOM and eliminating the risk of recurrence after the discontinuation of drops. The tobramycin-dexamethasone ear drop presents a promising choice for the treatment of otorrhea due to \( P \) a e r u g i n o s a infection. Future studies should address potential vestibulotoxicity of aminoglycosides as well as assess the safety and efficacy of this ear drop in humans.

Accepted for publication September 21, 1999.

This study was supported in part by Alcon Laboratories Inc, Fort Worth, Tex.


We thank Marie-Louise Spangenberg, Julie Banks, and Jean Betsch for their technical support and outstanding effort in completing this project.

REFERENCES