Hydrogen peroxide ototoxicity in unblocking ventilation tubes: A chinchilla pilot study

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OBJECTIVE: Some clinicians use hydrogen peroxide (H₂O₂) to clear the lumen of ventilation tubes that become blocked postoperatively. The ototoxicity associated with H₂O₂ has been controversial.

STUDY DESIGN: We designed an experiment to test if H₂O₂ damages the cochlear hair cells using a Chinchilla laniger animal model.

METHODS: Nine chinchillas (18 ears) were included in this study. Each animal was used as its own control. Following the insertion of ventilation tubes in both ears and baseline recordings of the auditory brain stem responses (ABR), we instilled 2 ml of 3 percent H₂O₂ into their right external auditory canals (experimental ears). H₂O₂ was left in the external auditory canal for a total of 5 minutes and then was drained. We instilled a normal saline control solution in their left ears (control ears) in a similar fashion. ABR recordings were performed 1 day after the last instillation of H₂O₂ and 5 days later.

RESULTS: There was no statistically significant difference in the ABR thresholds of the experimental and control ears.

CONCLUSION: H₂O₂ did not appear to cause ototoxicity in chinchilla ears with tympanostomy tubes exposed to H₂O₂ instillation using a standard clinical protocol. © 2007 American Academy of Otolaryngology–Head and Neck Surgery Foundation. All rights reserved.

Insertion of ventilation tubes is the most common pediatric surgery, with more than 1 million operations performed in the United States and Canada every year.¹ It is the treatment of choice for children with recurrent ear infections or serous otitis, as it enables aeration of the middle ear. This can reverse the hearing loss caused by fluid in the middle ear and reduces the likelihood of acute otitis media. However, the ventilation tube can occasionally become blocked postoperatively by a plug of mucinous or suppurative middle ear secretions or by a blood clot. Clinically, hydrogen peroxide (H₂O₂) is widely used to clear the lumen of a blocked ventilation tube by instillation into the external auditory canal, but the ototoxicity of this agent has not been clearly assessed in humans. Previous in vitro studies have shown that H₂O₂ may affect the morphology of cochlear outer and inner hair cells of the guinea pig.²,³ Furthermore, another study showed that H₂O₂ directly instilled through the wall of the scala tympani in live guinea pigs could have deleterious effects on cochlear function.⁴ More recently, it was determined that H₂O₂ directly injected into the middle ear of the fat sand rat could disrupt the function of the cochlear and vestibular systems.⁵ However, a retrospective study determined that H₂O₂ used to unplug tympanostomy tubes in humans would not result in an immediate change in bone conduction thresholds, and it was concluded that this agent could be safely used for this clinical procedure.⁶

The discrepancy between the clinical findings and the results obtained through experiments on live animals and on culture cells was discussed by Perez et al.⁵ It was postulated that this inconsistency could be explained by anatomical differences between the human ear and the animals used as experimental models. It was also suggested that the route of administration could play an important role.⁵ In the previous in vivo studies, H₂O₂ was directly injected inside the middle or inner ear, whereas clinically, it is usually instilled into the...
external auditory canal, left inside for a few minutes, and then drained. We assume that an animal study that follows a protocol more reflective of the clinical use of H$_2$O$_2$ can provide a better assessment of the safety of this agent for use in humans. Therefore, following a procedure that is similar to the one used in the clinical setting to clear ventilation tubes, we were able to determine whether the quantity of H$_2$O$_2$ that passes through the tympanostomy tube is sufficient to cause ototoxic damage to the cochlear hair cells and to affect the function of the peripheral auditory system.

**MATERIALS AND METHODS**

**Animal Model and Agent Tested**

The study was performed on nine female chinchillas (*Chinchilla laniger*) with a mean body weight of 454 ± 31 g. The chinchilla was chosen because it is a well-established animal model for hearing loss studies, and our laboratory has extensive experience with this animal. Moreover, the chinchilla possesses a wide tympanic membrane; a cochlea that is readily accessible for microsurgical procedures, and a middle ear that can be easily accessed through a large and very thin bulla. The sample size calculation took into account the previous results of Perez et al that showed a standard deviation of 18 dB in auditory brain stem response (ABR) recordings. The minimum detectable difference between the means was set to 30 dB, while type I and type II errors were set to 0.05 and 0.1, respectively.

Ventilation tubes were inserted in both ears of all the animals, and each animal was used as its own control. It was decided that one ear would receive the agent to be tested while we would instill the control solution in the other ear. After having anesthetized the chinchillas, we positioned their heads so that the right external auditory canals would face upward. Then we instilled 2 ml of 3 percent H$_2$O$_2$ into their right external auditory canals. We maintained their heads in this position for 5 minutes. Afterward, we positioned their heads so that their right external auditory canals would face downward. This position enabled us to drain their ear for 5 minutes. This procedure was repeated for 7 consecutive days at 24-hour intervals. The control group consisted of the left ears into which we instilled osmotic saline solution in the same way that we applied H$_2$O$_2$ in the tested ears.

While the ear that received the H$_2$O$_2$ was not randomized, the audiologist who recorded and analyzed the ABRs was blinded as to which product was instilled in the ears to avoid assessment bias.

All the animals were taken care of following the guidelines of the Canadian Council on Animal Care and the standards of the institutional animal care committee.

**Anesthetic Protocol**

Induction was achieved with intramuscular injection of atropine sulfate (0.04 mg/kg), xylazine hydrochloride (2.4 mg/kg), and ketamine hydrochloride (25 mg/kg). Maintenance of anesthesia is achieved with xylazine hydrochloride (0.81 mg/kg) and ketamine hydrochloride (5 mg/kg) at 60-minute intervals. Body temperature was maintained at 36°C.

**Surgical Procedure: Insertion of the Ventilation Tube**

The external auditory canal and the tympanic membrane were cleared of all debris. Under the operating microscope, an incision was performed in the anterior lower quadrant of the tympanic membrane. Afterward, we inserted a 1.14-mm-wide Reuter Bobbin tube. The following day, before the first instillation of H$_2$O$_2$, we confirmed that the tube was correctly placed by otoscopic evaluation.

**Assessment of Ototoxicity: Auditory Brain Stem Response**

To assess the functional state of the peripheral auditory system, we recorded the ABR at various stages in the protocol using a SmartEP device (Intelligent Hearing Systems, Miami, FL). The first ABR testing was performed after the insertion of the ventilation tubes and served as the baseline measurement. ABR recordings were then carried out 1 day after the last instillation of H$_2$O$_2$. The final recordings were performed 5 days later.

The acoustic stimuli were presented through ER3A insert earphones. They consisted of 8,000-, 16,000-, and 20,000-Hz tone bursts (5 msec rise/fall, Blackman envelope) presented at a rate of 39.30 bursts per second with alternating polarity through filtered high-frequency transducers. For each given frequency, the tone burst intensity started at 0 dB nHL, increasing in steps of 5 dB until threshold was reached. We defined the threshold as the lowest intensity for which a response could be recorded at least three times. Because each animal was used as its own control, the contralateral ear was masked using broadband noise while recording the ABR. The elicited responses were therefore representative of the function of the stimulated ear only.

The electrical activity was recorded using needle electrodes placed subdermally. The negative end was placed on the pinna of the ear for which ABR was recorded. The ground electrode was placed on the pinna of the contralateral ear, and the positive electrode was placed on the vertex. The response was amplified 100,000 times, band filtered in the range of 100-1500 Hz, and averaged 800 times.

**Statistical Analysis**

The Mann-Whitney U test was used to compare ABR thresholds between the baseline recordings and the measurements made after product instillation for both control and experimental groups separately. Using the same test, increases in ABR thresholds were compared between the control and tested ears. In both analyses, comparisons were done for each frequency separately, as well as for all frequen-
cies combined. A value of $P < 0.05$ was considered statistically significant. Confidence intervals were also calculated.

## RESULTS

### Saline (Control Group)

At baseline, all frequencies included, the ABR thresholds were between 0 and 60 dB (mean value, 22.4 ± 15.5 dB) for the control group. After saline instillation, the average threshold rose to 48.5 ± 23.7 dB ($P < 0.05$) 1 day post-treatment and remained at 46.9 ± 22.4 dB on the fifth day post-treatment. ABR thresholds with respect to frequency are illustrated in Table 1.

### Hydrogen Peroxide (Experimental Group)

At baseline, for all frequencies, the ABR thresholds were between 0 and 40 dB (mean value, 19.6 ± 11.2 dB) for the experimental group. After H$_2$O$_2$ instillation, the average threshold rose to 39.8 ± 19.4 dB ($P < 0.05$) 1 day post-treatment and remained at 41.5 ± 21.7 dB on the fifth day post-treatment. ABR thresholds with respect to frequency are illustrated in Table 1. The average threshold increases between the experimental and control groups were not found to be statistically significant ($P$ values at 1 and 5 days post-treatment, 0.35 and 0.65, respectively). Similarly, there was no statistically significant difference between the threshold increases of both groups when evaluating each frequency separately (Table 2).

## DISCUSSION

The ototoxicity of H$_2$O$_2$ was previously assessed in vivo and in vitro. Clerici et al$^3$ determined that H$_2$O$_2$ caused outer hair cell shortening and bleb formations in a concentration-dependent manner. They also showed that H$_2$O$_2$ could affect the transduction of acoustic stimuli at the level of the cochlear hair cells after the injection of this liquid directly into the cochlea of live guinea pigs.$^4$ Similarly, Perez et al$^5$ found that H$_2$O$_2$ could have deleterious effects on the cochlear function when it was directly injected into the middle ear through a polyethylene tube placed in the bone of the cortex. They reported an increase of greater than 50 dB in the ears treated with H$_2$O$_2$.

### Table 1

<table>
<thead>
<tr>
<th>Hydrogen peroxide</th>
<th>Day 1 (Baseline)</th>
<th>Threshold increase (95% CI)*</th>
<th>Day 5 (Post-treatment)</th>
<th>Threshold increase (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>All frequencies†</td>
<td>19.6</td>
<td>20.2 (13.3-27.1)</td>
<td>41.5</td>
<td>21.9 (14.5-29.2)</td>
</tr>
<tr>
<td>8 kHz</td>
<td>6.67</td>
<td>16.7 (5.30-28.0)</td>
<td>12.4</td>
<td>13.9 (2.72-25.1)</td>
</tr>
<tr>
<td>16 kHz</td>
<td>27.2</td>
<td>23.3 (11.0-35.6)</td>
<td>54.4</td>
<td>27.2 (13.8-40.7)</td>
</tr>
<tr>
<td>20 kHz</td>
<td>25.0</td>
<td>20.6 (3.54-37.6)</td>
<td>49.4</td>
<td>24.4 (7.42-41.5)</td>
</tr>
</tbody>
</table>

| Saline | All frequencies | 22.4 | 26.1 | 0.05 | 21.9 (14.5-29.2) |
| 8 kHz  | 6.11           | 27.8  | 23.9 | <0.05 | 23.9 (6.51-41.3) |
| 16 kHz | 32.2           | 58.9  | <0.05 | 57.8 | 25.6 (9.31-41.8) |
| 20 kHz | 28.9           | 52.8  | <0.05 | 52.8 | 23.9 (7.27-40.5) |

*CI, confidence interval.
†All frequencies refer to the average of the auditory brain stem response thresholds obtained at 8, 16, and 20 kHz.

### Table 2

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Day 1</th>
<th>Difference in threshold increase (95% CI)*</th>
<th>Day 5</th>
<th>Difference in threshold increase (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$O$_2$</td>
<td>20.2</td>
<td>26.1</td>
<td>0.35</td>
<td>21.9</td>
</tr>
<tr>
<td>Saline</td>
<td>16.7</td>
<td>27.8</td>
<td>0.44</td>
<td>13.9</td>
</tr>
<tr>
<td>8 kHz</td>
<td>23.3</td>
<td>26.7</td>
<td>0.76</td>
<td>27.2</td>
</tr>
<tr>
<td>20 kHz</td>
<td>20.6</td>
<td>23.9</td>
<td>0.69</td>
<td>24.4</td>
</tr>
</tbody>
</table>

*CI, confidence interval.
†All frequencies refer to the average of the auditory brain stem response threshold increase obtained at 8, 16, and 20 kHz.
However, none of these studies accurately reflect the clinical situation in which H$_2$O$_2$ remains only transiently in the middle ear in small quantities before it is drained out. Under the conditions described by Perez et al, H$_2$O$_2$ cannot drain from the middle ear. Furthermore, in the protocol of Perez et al, H$_2$O$_2$ was directly injected into the middle ear through a long polyethylene tube inserted opposite the round window, where it can easily penetrate into the inner ear. On the other hand, when H$_2$O$_2$ passes though a ventilation tube in small quantities, it must drip along the mucosa of the middle ear, where it can be absorbed before it reaches the oval and round windows.

Our results show a similar increase in the ABR thresholds for both groups during the week after the last instillation of the test and control solutions. The threshold increases were 26.1 dB and 24.4 dB for the control ears at 1 and 5 days post-treatment, respectively. The threshold increases were 20.2 dB and 21.9 dB for the tested ears at 1 and 5 days post-treatment, respectively. No significant difference was found between the threshold increases for the control and tested ears. The 95 percent confidence intervals for the hearing difference are −16.3 to 4.5 and −12.6 to 7.4 at 1 and 5 days post-treatment, respectively. Therefore, H$_2$O$_2$ affected the cochlear function in a way similar to that of the control solution. Furthermore, it has previously been found that early signs of ototoxicity can first be detected in responses to high stimulus frequencies (≥8 kHz). No statistically significant difference was found between the threshold increases of both groups when examining each frequency separately.

In the initial sample size calculation, the type II error was set to 10 percent and the sample size was calculated based on the previous study of Perez et al showing a standard deviation of 18 dB in ABR recordings of their experimental group. In the present study, the standard deviation varies from 15.8 to 20.5 dB when comparing threshold increases with all frequencies combined thus obtaining a type II error varying from 5 to 16 percent. The standard deviation varied from 18.1 to 22.5 dB when comparing ABR threshold increases while examining each frequency individually, thus obtaining a type II error between 10 and 23 percent.

Thus, following a protocol similar to what is done clinically, H$_2$O$_2$ entering the middle ear through a tympanostomy tube produces a similar rise in ABR thresholds as the control saline solution, and this increase is much less impressive than the 50-dB increase reported by Perez et al. Furthermore, the chinchilla animal model is a very sensitive model for toxicity, as the thickness of the round window is only 10 to 14 μm compared with 40 to 70 μm in humans, thereby allowing a much easier penetration of the toxic agents into the cochlea. Our findings correlate with those of Brennan et al., who determined that H$_2$O$_2$ was an effective treatment to clear the lumens of ventilation tubes in humans without impairing the peripheral hearing system. However, as pointed out by Perez et al, the effectiveness of H$_2$O$_2$ for unblocking ventilation tubes still appears to be controversial. On the one hand, both 3 percent H$_2$O$_2$ and 10 percent sodium bicarbonate were found to be effective ceruminolytic agents, and they were shown to effectively clear blocked tympanostomy tubes. On the other hand, in a more recent study, Westine et al determined that H$_2$O$_2$ was no more effective than water for clearing tubes blocked specifically by mucoid effusions. They explained that the discrepancies between their findings and the results obtained in previous studies may be explained by the nature of the plug. They also speculated that H$_2$O$_2$ may be more effective in dissolving blood-based clogs because of catalase enzymes found in blood that may help in the degradation reaction. Thus, 3 percent H$_2$O$_2$ was found to be effective at dissolving cerumen in a previous study and at clearing ventilation tubes in two other studies.

**CONCLUSION**

In this pilot study, H$_2$O$_2$ did not cause a significant threshold change in ABR recordings compared with a control saline solution when instilled in chinchilla ears with tympanostomy tubes following a standard clinical protocol. This is despite the fact that the chinchilla animal model is very sensitive in detecting ototoxic damage. A larger study with historical analysis of the cochlea is currently being performed.

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**REFERENCES**


