Ototoxicity of Topical Azithromycin Solutions in the Guinea Pig

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Objective: To investigate possible ototoxic effects of topical azithromycin (AZ) in the guinea pig.

Design: A prospective, controlled animal study.

Setting: The University of Texas Southwestern Medical Center at Dallas.

Participants: Twenty-three pigmented guinea pigs were given single, unilateral middle ear applications of a solution containing 3% (n=3), 2% (n=5), 1% (n=5), or 0.5% (n=5) AZ or saline (n=5). The contralateral ear served as the untreated control.

Main Outcome Measures: The animals were observed for behavioral changes for 2 weeks and then humanely killed. The ears were processed for anatomical evaluation. Morphologic changes were analyzed by quantitation of middle ear changes and cochlear inner and outer hair cell loss. Statistical analysis was performed to examine effects by dose.

Results: Analysis revealed extensive middle and inner ear changes associated with all formulations of AZ. Moderate correlation was found between the extent of middle ear changes and AZ concentration ($r^2=0.59$), whereas a strong correlation was seen between inner ear damage and AZ concentration ($r^2=0.94$). Both inner and outer hair cells were affected, with inner hair cell damage consistently greater than outer hair cell damage.

Conclusions: The results of this study demonstrate that ototopical AZ can cause middle ear changes and significant hair cell loss in the guinea pig. This finding, together with previous clinical reports, indicates that topical AZ should be used with caution in the clinical setting.


Acute otitis media is one of the most common otologic disorders requiring outpatient antibiotic therapy. Infections in acute otitis media have been associated with the formation of bacterial biofilms, which make such infections difficult to treat. Research continues into the development of new antibiotic formulations for otologic use that can effectively treat ear infections and stem the rise in bacterial resistance. There is long-standing interest in the development of formulations that would be simpler to administer and would limit systemic exposure to antibiotics.

Long-term, systemic administration of macrolide antibiotics has been an effective alternative to the more commonly used antibiotics in the treatment of otitis media, with few ototoxic effects in otherwise healthy patients. Macrolide antibiotic treatment is also effective against mycoplasma, mycobacteria, and other non-bacterial agents. Macrolide antibiotics can penetrate deeply into tissues and have been shown to interfere with quorum sensing of bacterial biofilms similar to those found in some types of otitis media. Azithromycin (AZ) has been shown to decrease Haemophilus influenzae biofilms in vitro. Therefore, ototopical formulations of AZ may potentially be used to treat external otitis or otitis media.

To our knowledge, the safety of ototopical formulations of AZ has not been systematically studied. Because AZ has been shown to be capable of rapid penetration into tissues, ototopical administration could increase inner ear concentration relative to what could be achieved by systemic dosing, thereby increasing the potential for ototoxicity. The focus of the present study was to determine whether a topical application of a therapeutic dose of AZ is likely to be safe to middle and inner ear tissues.

METHODS

At The University of Texas Southwestern Medical Center at Dallas (UT Southwestern), 23 pigmented male guinea pigs, each weighing 330 to 540 g, were given different concentrations of AZ in a proprietary solution ototopically to determine the solution’s effect on the middle and inner ear. To reduce variability in drug penetration, all animals were hydrated with 1 mL of saline, subcu-
The anatomy of the middle ear and organ of Corti were further processed by rinsing in phosphate-buffered saline and studied as whole-mount preparations. The stria vascularis and organ of Corti were mounted on microscope slides in glycerol and studied as whole-mount preparations. Gross, unprocessed stria vascularis and organ of Corti were harvested. All animal procedures were approved by the UT Southwestern Institutional Animal Care and Use Committee. The middle ear mucosa was analyzed, scored, and statistically quantitated using a t test and linear regression.

### MIDDLE EAR SCORING

To determine the extent of damage, micrographs of the middle ear were analyzed and quantitated by 2 examiners (K.S.P. and C.G.W.) in a blinded manner. Each examiner used a table with values representing the specific degree of damage observed (Table 1). Scores were reported for 4 indices: mucosal thickening, hyperemia, hematomas, and mucosal adhesions. Because adhesions tend to span the middle ear space, only 1 score for adhesions was given per ear. To account for variations in response in different areas of the middle ear mucosa, each ear was divided into 4 quadrants (dorsal lateral, dorsal medial, ventral lateral, and ventral medial), and each quadrant was given a score for the other 3 indices. The mean (SD) scores for mucosal thickening, hyperemia, and hematomas were added to the score for mucosal adhesions to give each ear a cumulative score for middle ear changes. Mean (SD) cumulative scores were compared between groups to determine if there was a statistically significant difference between the conditions of the middle ears in AZ-treated ears vs controls. In addition, the extent of change with increasing azithromycin concentration was analyzed using linear regression to determine if the damage correlated with dose.

### Table 1. Definitions for Severity of Middle Ear Damage

<table>
<thead>
<tr>
<th>Score</th>
<th>Mucosal Thickening</th>
<th>Hematoma</th>
<th>Hyperemia</th>
<th>Adhesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Similar to normal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Slight amount, ie, 1 or 2 spots of thickening&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Slight amount, ie, 1 large or 2 small blood clots</td>
<td>Slight amount, ie, 1 or 2 dilated, blood-filled vessels</td>
<td>Slight amount, ie, 1 or 2 small adhesions&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>Moderate amount, ie, several small areas of thickening</td>
<td>Moderate amount, ie, several small clots, with or without large clots</td>
<td>Moderate amount, ie, several dilated, blood-filled vessels</td>
<td>Moderate amount, ie, several small adhesions</td>
</tr>
<tr>
<td>4</td>
<td>Extensive amount, ie, thickened mucosa in approximately 75% of surface area</td>
<td>Extensive amount, ie, clots fill approximately 75% of surface area</td>
<td>Extensive amount, ie, dilated, blood-filled vessels in approximately 75% of surface area</td>
<td>Extensive amount, ie, adhesions fill approximately 75% of middle ear space</td>
</tr>
<tr>
<td>5</td>
<td>Severe amount, ie, &gt;90% of surface area has thickened mucosa</td>
<td>Severe amount, ie, &gt;90% of surface area is occupied by clots</td>
<td>Severe amount, ie, &gt;90% of surface area filled by dilated, blood-filled vessels</td>
<td>Severe amount, ie, &gt;90% of middle ear space is occupied by adhesions</td>
</tr>
</tbody>
</table>

<sup>a</sup>Thickening is defined as mucosal tissue, typically dull in appearance, that has thickened so that features on bony surface of middle ear appear less distinct.

<sup>b</sup>Adhesions are defined as either thin strands of mucosa running through middle ear space (typically seen early after application) or dense, fibrous strands of mucosal tissue running through middle ear space (seen 1 to 2 weeks after initial irritation).

### Table 2. Definitions for Severity of Hair Cell Damage

<table>
<thead>
<tr>
<th>Score</th>
<th>Inner Hair Cell Region</th>
<th>Outer Hair Cell Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>90%-100% of the hair and supporting cells in the segment are present and appear normal</td>
<td>90%-100% of the hair and supporting cells in the segment are present and appear normal</td>
</tr>
<tr>
<td>2</td>
<td>75%-89% of the hair cells and all supporting cells in the segment are present and appear normal</td>
<td>75%-89% of the hair cells and all supporting cells in the segment are present and appear normal</td>
</tr>
<tr>
<td>3</td>
<td>50%-74% of the hair cells and all supporting cells in the segment are present and appear normal</td>
<td>50%-74% of the hair cells and all supporting cells in the segment are present and appear normal</td>
</tr>
<tr>
<td>4</td>
<td>25%-49% of the hair cells and all supporting cells in the segment are present and appear normal</td>
<td>25%-49% of the hair cells in the segment are present and appear normal</td>
</tr>
<tr>
<td>5</td>
<td>Less than 25% of the hair cells and all supporting cells in the segment are present and appear normal</td>
<td>No hair cells or supporting cells remain along most (75%) of the segment; the basilar membrane is covered with cuboidal shaped cells</td>
</tr>
</tbody>
</table>

The temporal bones were divided into medial and lateral portions and photographed using a surgical microscope and digital camera. The inner ear of each temporal bone was then perfused with 2.5% glutaraldehyde in phosphate-buffered saline and fixed with 2.5% glutaraldehyde in phosphate-buffered saline. Gross, unprocessed stria vascularis and organ of Corti were mounted on microscope slides in glycerol and studied as whole-mount preparations by light microscopy. In addition, selected sections of the organ of Corti were further processed by rinsing in phosphate buffer, dehydrating in a graded series of ethanol, critical point drying, and coating in gold for observation (Philips FEI XL30 environmental scanning electron microscope; FEI Company, Hillsboro, Oregon). The anatomy of the middle ear and organ of Corti was analyzed, scored, and statistically quantitated using a t test and linear regression.
ing indices used in this study, each index was analyzed independently to determine whether the individual scores reflect the overall outcome.

INNER EAR SCORING

Initial qualitative examination of the inner ear damage, performed by 2 examiners (K.S.P. and C.G.W.), suggested that there was a relationship between the extent of damage and the concentration of AZ given. Therefore, a means was devised to estimate the damage quantitatively for inner hair cells (IHCs) and outer hair cells (OHCs) from base to apex. The severity of damage to the organs of Corti was quantified in all the AZ- and saline-treated ears, plus 5 randomly selected untreated controls. For quantification of the inner ear damage, the length of the organ of Corti was measured using a camera lucida, then the organ of Corti was separated into 10 segments of equal length from base to apex, and the tissue was analyzed in a blinded manner by 1 examiner (K.S.P.) for severity of damage of IHCs and OHCs using a scoring system with values representing specific degrees of damage (Table 2). All 3 rows of OHCs were scored together, with a score of 1 indicating similar to normal (≥90% of the hair cells present, with a near-normal appearance) and a score of 5 indicating severely damaged (no hair cells remaining in most [≥75%] of the segment). IHCs were scored separately from the OHCs, with a score of 1 indicating similar to normal (≥90% normal hair cell population) and a score of 5 indicating severely damaged (only a very small portion [<25%] of the hair cells appearing normal).

Statistical analysis was performed using a t test on the cumulative scores for middle ear damage and on the scores for IHC and OHC damage, summed base to apex. Linear regression was used to determine the correlation between the degree of damage in the middle ear relative to the AZ concentration plus the degree of damage in the inner ear (sum of IHC and OHC damage, base to apex) relative to AZ concentration.

RESULTS

All but 1 animal survived the duration of the study. That animal had an unrecognized preexisting gastrointestinal condition and was euthanized the day after surgery. All other animals (n=22) recovered well from surgical anesthesia. Postsurgical monitoring of all animals revealed periodic circling and mild nystagmus the day after surgery in 2 of 3 animals receiving the 3% AZ solution. The symptoms were only evident when the animals were startled, and they resolved by the next day. None of the other animals (n=20) exhibited signs of vestibular upset at any time.

Pathological analysis performed 2 weeks after AZ application revealed extensive middle ear changes for all formulations tested (Table 3). Changes were seen in the ears treated with AZ compared with control ears (Figure 1A). The changes seen included the develop-
ment of thick, mucosal adhesions that occupied large portions of the middle ear space and were often attached to the otic capsule (Figure 1B). Hemorrhagic and hyperemic mucosal tissue was commonly seen, and the mucosa often looked thickened. The tympanic membrane typically showed minimal changes. The most common change seen in the saline-treated ears was hyperemia (Figure 2), but this was also seen in some untreated control ears. Adhesions were never seen in saline-treated or untreated controls. Mucosal thickening and hematomas were also rare in the control ears.

Changes seen were quantitated from photomicrographs of the middle ear. The mean (SD) cumulative scores for middle ear damage were 5.8 (1.4) for untreated control, 5.8 (2.4) for saline, 9.5 (0.0) for 0.5% AZ, 9.3 (1.9) for 1% AZ, 10.0 (1.4) for 2% AZ, and 9.8 (0.8) for 3% AZ. The scores for saline-treated ears were not significantly different from those of untreated controls (P = .77, df = 4). The scores for all AZ doses were significantly different from those of controls (0.05%: P < .001, df = 4; 1%: P = .01, df = 4; 2%: P = .005, df = 3; and 3%: P = .001, df = 2).

Cochlear dissection revealed changes within the organs of Corti after AZ administration. Degree of damage varied along the organ of Corti from the cochlear apex to base and between IHCs and OHCs (Figures 3, 4, 5, 6, and 7). Little nerve fiber loss was detected: it occurred in only 1 ear that was exposed to 3% AZ and was limited to areas of complete IHC and OHC loss. OHC changes ranged from scattered hair cell loss (Figure 3A) to areas where no normal OHCs remain (Figure 4B) to total loss of hair cells and supporting cells (Figure 5A) to areas of complete loss of hair cells and supporting cells (Figure 3A). There was a tendency for significant IHC damage to be present in areas of minimal damage to OHCs (Figures 3A and 5A).

The IHC damage seen in this study is uncommon compared with reports of ototoxic changes seen after administration of other antibiotics. After AZ administration, some IHCs were partially or completely extruded from the organ of Corti (Figures 3-5), with only a small portion of the cell remaining tethered at the lateral side of its cuticular plate. Free-floating or dislodged IHCs were not obvious, although the reticular lamina had depressions seen by the scanning electron microscope in positions where the IHCs had been lost. B. Scanning electron micrograph of tissue from a guinea pig that received 3% azithromycin (original magnification ×1300). Tissue was demounted from glycerol after light microscopic viewing. All OHCs are present, but IHCs have degenerated. A few partially (white arrow) or completely (black arrow) extruded IHCs are present on surface of organ of Corti.

In the ears in which the organ of Corti was microdissected, the stria vascularis was also microdissected from the cochlea and analyzed by light microscopy for any changes associated with AZ administration. Changes seen in the stria vascularis were inconsistent and similar to changes seen in controls.
Statistically significant inner ear changes were seen for all AZ formulations tested compared with controls. Cumulative IHC and OHC scores were as follows: untreated controls, 20.5 (0.6); saline, 21.4 (1.9); 0.5% AZ, 32.4 (4.8) \((P=.005, df=4)\); 1% AZ, 32.2 (6.6) \((P=.02, df=4)\); 2% AZ, 43.0 (6.1) \((P<.001, df=3)\); and 3% AZ, 53.0 (12.1) \((P=.04, df=2)\). The scores for saline-treated ears were not significantly different from those for control ears.

To determine whether the changes seen in the middle and inner ears were owing to AZ itself or were caused by other constituents of the AZ formulation, we examined the correlation of the extent of tissue damage with the concentration of AZ present within the formulations. The extent of middle ear damage correlated moderately with AZ concentration \((r^2=0.59)\). Inner ear disease was highly correlated with the concentration of AZ administered \((r^2=0.94)\).

Individual indices of middle ear damage were examined for their contribution to the overall score. Mean (SD) scores for AZ-treated ears were significantly different from those for control ears for all concentrations of AZ for mucosal thickening, hematoma, and mucosal adhesions (Table 3). Although hyperemia was slightly elevated in the 2% AZ and 3% AZ ears, it was not significantly different from the untreated or saline controls.

Administration of AZ solutions to the guinea pig middle ear caused damage to the inner and middle ear. The semi-quantitative methods developed yielded results that could be statistically analyzed. The moderate correlation found between AZ concentration and the extent of middle ear damage suggests that some of the damage seen in the middle ear after application was caused by AZ, but other factors appear to be involved, such as the other constituents of the test solutions. Middle ear changes caused by the nonmedicinal ingredients of otic agents have been reported.10 The strong correlation of AZ concentration and inner ear damage suggests that AZ applied to the guinea pig’s middle ear can cross into the inner ear and is the primary agent responsible for the ototoxicity.

The pattern of damage seen in this study, in which IHC damage is worse than OHC damage after antibiotic administration, is an unusual finding. The ototoxicity seen after administration of other antibiotics is typically greater for the OHCs or the effects for IHCs and OHCs are similar.11,12 In addition, the observation that dying IHCs tend
to be extruded onto the endolympathic surface has only rarely been reported in the literature on disease of the organ of Corti. Also, in this study, the supporting cells that surround the IHCs were often found to have disappeared in areas of extensive IHC loss. This type of damage is unexpected in view of findings from previous reports on systemically administered AZ, which suggest the target tissues for AZ ototoxicity are either the stria vascularis or the OHCs. Strial changes were not obvious in our study, although the methods used were not optimal for assessment of strial disease. It is also possible that the route of administration affects the drug target. Further study is needed to better determine the nature of cell death in the organ of Corti and possible morphologic effects on the stria vascularis after ototopical AZ administration.

Because of the development of antibiotic resistance in pathogens, there is a continuing need for discovery of effective, new antibiotics for treatment of infections. Macrolide antibiotics have been shown to be effective for treatment of otitis media. Their ability to interfere with quorum sensing in biofilms suggests that they may be effective in treating the more difficult cases of otitis media where biofilms are present. Although the present study was performed using single applications on the guinea pig model, the results suggest that AZ could be ototoxic in humans if topically applied to the ear. Therefore, AZ may not be appropriate for inclusion in formulations intended for ototopical use. In addition, there have been occasional cases of temporary and permanent hearing losses after systemic AZ treatment. That fact, along with the results of the present study showing ototoxicity of AZ occurring in a concentration-dependent manner, suggest that AZ can be ototoxic if it enters the inner ear in sufficient concentrations. Therefore, caution should be taken when administering AZ systemically.

Macrolides are an accepted and often preferred therapy for chronic rhinosinusitis. Their beneficial effect is a consequence not only of their bacteriologic and bacteriostatic effect but also because they down-regulate proinflammatory mediators. They are often used in lower dosages (250 mg/d) for longer periods of time because of the immunomodulation effect. To date,
no ototoxic effects have been reported in association with this treatment regimen. However, given that ototoxicity has occasionally occurred in patients receiving systemic AZ and the potential for some antibiotics to accumulate in perilymph over time, it may be advisable to audiometrically monitor patients who are receiving long-term therapy.

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Author Contributions: Dr Pawlowski had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Pawlowski, Si, Wright, Hosseini, and Roland. Acquisition of data: Pawlowski and Koulich. Analysis and interpretation of data: Pawlowski and Koulich. Drafting of the manuscript: Pawlowski. Critical revision of the manuscript for important intellectual content: Pawlowski, Si, Wright, Koulich, Hosseini, and Roland. Obtained funding: Si, Wright, Hosseini, and Roland. Administrative, technical, and material support: Si, Wright, Koulich, and Hosseini. Study supervision: Pawlowski.

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REFERENCES