Late Effects of Topical Anesthetics on the Healing of Guinea Pig Tympanic Membranes After Myringotomy

Michael M. Gnuechtel, DO; LT Lynn L. Schenk, MC, USN; Gregory N. Postma, MD

Background: The optimal local anesthetic for myringotomies or the insertion of tympanostomy tubes in adults should be easy and rapid to use, be painless during application, provide good anesthesia, be reversible, be inexpensive, and not cause any long-term damage to the tympanic membrane (TM).

Objective: To evaluate the histologic effects of topical anesthetic agents on the healing of the TM after myringotomy.

Methods: Sixty male albino guinea pigs were randomly assigned to 1 of 5 groups. Of the 5 groups, 2 were used as controls: one group underwent a myringotomy and the other group did not. The remaining 3 groups had both TMs treated with a topical anesthetic (phenol, tetracaine base, and eutectic mixture of lidocaine and prilocaine in a cream) prior to myringotomy. All TMs were inspected periodically and then harvested at 3 months or 6 months postoperatively for histologic examination.

Results: The TMs of the group treated with tetracaine appeared the most normal at 6 months ($P=0.001$). However, histologic evaluation failed to demonstrate any significant differences in the thickness of the TM or the lamina propria ($P=0.45$), the amount ($P=0.80$) and orientation ($P=0.07$) of collagen, or the number of infiltrating lymphocytes ($P=0.70$).

Conclusion: Based on the histologic findings, all 3 topical anesthetic agents appear to cause equivalent changes to the TM when used for a myringotomy.

Arch Otolaryngol Head Neck Surg. 2000;126:733-735

The optimal local anesthetic for myringotomies or the insertion of tympanostomy tubes in adults remains uncertain. Otolaryngologists have used a number of different substances and techniques over the years. The ideal agent should be easy and rapid to use, be painless during application, provide good anesthesia, be reversible, be inexpensive, and not cause any long-term damage to the tympanic membrane (TM). Local infiltration of injectable anesthetic agents is effective, but the undesirable drawbacks of painful administration and patient apprehension encouraged us to try an alternative topical method, which causes the least damage to the TM.

The first use of a topical agent for anesthesia of the TM was 10% alcohol-cocaine solution by Zaufel in 1884.1 The addition of phenol in combination with menthol and cocaine was introduced in 1898 (Bonain liquid) and was commonly used for many years.2 Currently, the most frequently used agents appear to be topical phenol alone and topical tetracaine, with the former being most commonly used.3,4 Another topical agent recently developed is an eutectic mixture of lidocaine and prilocaine in a cream (EMLA).5-7

Because of occurrence of TM damage from phenol in humans, we tested phenol, EMLA, and tetracaine in an animal model to determine which anesthetic would be the least insulting to the TM after undergoing a myringotomy. The TM morphology was studied grossly by otomicroscopic examinations and histologically by light microscopy.

RESULTS

OTOMICROSCOPIC EXAMINATION

Analysis of the otomicroscopy data (Table 1) revealed that the group treated with phenol had a TM that was significantly worse in appearance at 2 weeks ($P=0.006$). At 2 months postoperatively, the TM appearance in the group treated with tetracaine was the same as in the myringotomy control group. By the end of the

From the Guadalupe Physicians Group, Kerrville, Tex (Dr Gnuechtel); Department of Pathology, Naval Medical Center, Portsmouth, Va (Dr Schenk); and Department of Otolaryngology, Center for Voice Disorders of Wake Forest University, Wake Forest University School of Medicine, Winston-Salem, NC (Dr Postma).
MATERIALS AND METHODS

Sixty male albino Hsd:DH guinea pigs procured from Harlan Sprague Dawley Inc, Indianapolis, Ind, were used for this study according to a protocol approved by the Animal Care and Use Committee Investigational Review Board of the Clinical Investigation Division at Naval Medical Center, Portsmouth, Va. This study was performed in accordance with the “Public Health Service Policy and Government Principles Regarding the Care and Use of Animals,” the National Research Council’s Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Act; the animal use protocol was approved by the Institutional Animal Care and Use Committee of Naval Medical Center. The guinea pigs were housed in hanging rodent cages and received water and food ad libitum with additional vitamin C. Sixty animals weighing 450 to 650 g were randomized into 5 groups of 12 animals each. Two groups were controls: one without any treatment and another using topical anesthetic that underwent a myringotomy. The 3 remaining groups received 1 of 3 topical anesthetics prior to undergoing a myringotomy.

The guinea pigs were anesthetized with ketamine hydrochloride, 40 mg/kg, and xylazine, 5 mg/kg, given by intraperitoneal injection. All TMs were examined under an operative otomicroscope with a 250-mm objective lens. Both TMs of the animals in each of the 3 treatment groups were treated with one of the agents: 96% phenol, tetracaine base, or EMLA. The phenol used was a standard stock obtained from the hospital pharmacy and was applied by a rigid applicator. Tetracaine was obtained in the base form, reconstituted with 70% isopropyl alcohol, and applied to the TM using cotton for 15 minutes. A 5% EMLA cream in polyoxyethylene and carboxypolymethylene (Astra Pharmaceuticals, Westborough, Mass) was applied to the TM and then removed by suction after allowing 20 minutes of contact. Using sterile technique, a 3-mm myringotomy was made in the anterior superior quadrant of both TMs of all animals in the 3 treatment groups and 1 control group, using a 1.210 × 0.635-mm myringotomy knife (14-11000; Xomed-Trease, Jacksonville, Fla).

The animals were examined postoperatively with an otomicroscope at 2 weeks and then monthly. The examiners were blinded as to which group each animal had been randomly assigned to. The myringotomy site was graded on a 0-to-7 scale: 0, large perforation; 1, small perforation; 2, pinhole perforation; 3, moderate eschar; 4, minimal eschar; 5, moderate myringosclerosis; 6, small myringosclerosis; and 7, totally healed.

Two guinea pigs were killed from each group 3 months postoperatively using pentobarbital sodium (80 mg/kg) by intraperitoneal injection. The remaining 10 animals from each group were killed similarly 6 months postoperatively.

The histologic study was performed as follows: after the animals were killed, the tympanic bullae were opened and the temporal bones removed and immersed in a buffered 10% formalin solution, decalcified in formic acid, dehydrated in alcohol, and embedded in paraffin wax. The tissue sections were stained with hematoxylin-eosin. We recorded from the histologic specimens from each TM the maximum thickness of the TM and lamina propria, the number of lymphocytes per square millimeter in the area of highest concentration, the amount and orientation of collagen, and the presence or absence of all 3 cell layers. The 3 groups were compared both with one another and with the control groups.

Data were evaluated by analysis of variance under the general linear model followed by the Duncan mean comparison test, analysis of variance for repeated measures followed by the Duncan mean comparison test and contrast effects for time, and χ² analysis to determine the difference in healing between the groups.

6-month examination period, the TMs of the group treated with phenol appeared the worst, with the TMs of the group treated with tetracaine appearing the most normal during the time of observance (P=.001).

HISTOLOGIC EXAMINATION

Analysis of the histology data (Table 2) failed to show any statistically significant difference in all areas examined. The overall thickness of the TM and lamina propria was similar in all groups (P=.45). The number of lymphocytes per square millimeter was also similar among all groups (P=.70). Examination of the amount of collagen also failed to show any statistically significant differences (P=.80). Evaluation of the orientation of the collagen by χ² analysis was similar, with no differences found (P=.07) (Table 3). Some TMs were destroyed during histologic processing.

Table 1. Postoperative Otomicroscopic Appearance of Tympanic Membrane by Study Group

<table>
<thead>
<tr>
<th>Group</th>
<th>2 wk</th>
<th>2 mo</th>
<th>3 mo</th>
<th>4 mo</th>
<th>5 mo</th>
<th>6 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control without</td>
<td>7.00</td>
<td>6.50</td>
<td>7.00</td>
<td>7.00</td>
<td>6.80</td>
<td>7.00</td>
</tr>
<tr>
<td>myringotomy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control with</td>
<td>5.57</td>
<td>5.90</td>
<td>5.95</td>
<td>6.07</td>
<td>5.97</td>
<td>6.00</td>
</tr>
<tr>
<td>myringotomy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EMLA†</td>
<td>4.78</td>
<td>5.35</td>
<td>5.57</td>
<td>5.50</td>
<td>5.50</td>
<td>5.57</td>
</tr>
<tr>
<td>Tetracaine</td>
<td>4.43</td>
<td>5.85</td>
<td>5.92</td>
<td>6.05</td>
<td>6.12</td>
<td>6.10</td>
</tr>
<tr>
<td>Phenol</td>
<td>3.60</td>
<td>5.05</td>
<td>5.77</td>
<td>5.44</td>
<td>5.33</td>
<td>5.27</td>
</tr>
</tbody>
</table>

* The myringotomy site was graded on a 0-to-7 scale: 0, large perforation; 1, small perforation; 2, pinhole perforation; 3, moderate eschar; 4, minimal eschar; 5, moderate myringosclerosis; 6, small myringosclerosis; and 7, totally healed.

† EMLA indicates eutectic mixture of lidocaine and prilocaine.

Some TMs were destroyed during histologic processing. Using sterile technique, a 3-mm myringotomy was made in the anterior superior quadrant of both TMs of all animals in the 3 treatment groups and 1 control group, using a 1.210 × 0.635-mm myringotomy knife (14-11000; Xomed-Trease, Jacksonville, Fla).

The animals were examined postoperatively with an otomicroscope at 2 weeks and then monthly. The examiners were blinded as to which group each animal had been randomly assigned to. The myringotomy site was graded on a 0-to-7 scale: 0, large perforation; 1, small perforation; 2, pinhole perforation; 3, moderate eschar; 4, minimal eschar; 5, moderate myringosclerosis; 6, small myringosclerosis; and 7, totally healed.

Two guinea pigs were killed from each group 3 months postoperatively using pentobarbital sodium (80 mg/kg) by intraperitoneal injection. The remaining 10 animals from each group were killed similarly 6 months postoperatively.

The histologic study was performed as follows: after the animals were killed, the tympanic bullae were opened and the temporal bones removed and immersed in a buffered 10% formalin solution, decalcified in formic acid, dehydrated in alcohol, and embedded in paraffin wax. The tissue sections were stained with hematoxylin-eosin. We recorded from the histologic specimens from each TM the maximum thickness of the TM and lamina propria, the number of lymphocytes per square millimeter in the area of highest concentration, the amount and orientation of collagen, and the presence or absence of all 3 cell layers. The 3 groups were compared both with one another and with the control groups.

Data were evaluated by analysis of variance under the general linear model followed by the Duncan mean comparison test, analysis of variance for repeated measures followed by the Duncan mean comparison test and contrast effects for time, and χ² analysis to determine the difference in healing between the groups.

Table 2. Histologic Analysis of Tympanic Membrane after Myringotomy

- **Ototaryngologists have used many different methods to anesthetize the TM to safely and painlessly perform minor otologic procedures in a clinic setting.** The best re-
Topical anesthetics are an attractive alternative. Traditionally, Bonain solution, a mixture of phenol, cocaine, and menthol, was used. This eventually led to the topical use of phenol alone. Other agents have included lidocaine, tetracaine, and EMLA.

Because of the “etching” effect of phenol on the TM as well as instances of TM perforation, studies have been performed to examine the structure of intact TMs after the use of phenol and other topical agents. These studies, performed in rats and guinea pigs, have shown dramatic structural changes in the lamina propria of the TM that usually resolve in 5 months. These investigations involved the application of various agents onto an intact TM. After an extensive review of the literature using MEDLINE, CROS, BIOSIS, Excerpta Medica, AGRICOLA, MEDLARS, Dissertation Abstracts, NTIS, and Current Contents online databases, we found no reports of any experiments using topical agents when a myringotomy was subsequently performed, which is the standard clinical scenario. It was suspected that violating the middle fibrous layer of the TM after the use of phenol would result in an alteration of the healing process resulting in permanent TM changes and possibly perforations not seen in prior animal studies but occasionally seen clinically in humans. The guinea pig was chosen because its TM most closely approximates that of humans. Interestingly, in the group treated with phenol, the TM appearance was the worst, but this was not reflected in the histologic analysis. We believe the appearance is due to superficial changes to the TM that resolved with reepithelization.

Interestingly, even after the trauma during a myringotomy, there was no permanent damage to the TM regardless of the topical agent applied. Therefore, it is reasonable to conclude that concerns regarding long-term TM damage should not determine which topical agent an otolaryngologist selects for TM anesthesia.

In this study, we failed to demonstrate any significant differences in healing among the topical anesthetic agents commonly used for myringotomies. The choice of a topical anesthetic agent for myringotomy with or without tube insertion should be based on other criteria.

Table 2. Histologic Examination of Tympanic Membranes (TMs) by Study Group

<table>
<thead>
<tr>
<th>Group</th>
<th>TM Thickness, mm</th>
<th>Lamina Propria Thickness, mm</th>
<th>Lymphocytes/mm²</th>
<th>Collagen Thickness, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control without myringotomy</td>
<td>0.0355</td>
<td>0.0130</td>
<td>0.150</td>
<td>0.017</td>
</tr>
<tr>
<td>Control with myringotomy</td>
<td>0.0467</td>
<td>0.0146</td>
<td>0.041</td>
<td>0.034</td>
</tr>
<tr>
<td>EMLA*</td>
<td>0.0858</td>
<td>0.0186</td>
<td>0.117</td>
<td>0.028</td>
</tr>
<tr>
<td>Tetracaine</td>
<td>0.0647</td>
<td>0.0200</td>
<td>0.050</td>
<td>0.025</td>
</tr>
<tr>
<td>Phenol</td>
<td>0.0444</td>
<td>0.0133</td>
<td>0.100</td>
<td>0.023</td>
</tr>
</tbody>
</table>

*EMLA indicates eutectic mixture of lidocaine and prilocaine.

Table 3. Orientation of Collagen by Study Group

<table>
<thead>
<tr>
<th>Group</th>
<th>Haphazard</th>
<th>Horizontal</th>
<th>Oblique</th>
<th>Parallel</th>
<th>Whorling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control without myringotomy</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Control with myringotomy</td>
<td>0</td>
<td>1</td>
<td>19</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>EMLA*</td>
<td>1</td>
<td>0</td>
<td>14</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Tetracaine</td>
<td>0</td>
<td>0</td>
<td>17</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Phenol</td>
<td>0</td>
<td>1</td>
<td>17</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

*EMLA indicates eutectic mixture of lidocaine and prilocaine.

REFERENCES


Accepted for publication January 1, 2000.

Funding was provided by The Chief, Navy Bureau of Medicine and Surgery, Washington, DC (Clinical Investigation Program, grant P95-L-0A0000-001).

The views expressed in this article are those of the authors and do not reflect the official policy or position of the Department of the Navy, the Department of Defense, or the US government.

Corresponding author: Gregory N. Postma, MD, Center for Voice Disorders of Wake Forest University, Department of Otolaryngology, Wake Forest University School of Medicine, Winston-Salem, NC 27157-1034.