Selected Antimicrobial Activity of Topical Ophthalmic Anesthetics

Margaret M. Reynolds1, Kerry E. Greenwood-Quaintance2,3, Robin Patel2,3, and Jose S. Pulido1,4,5

1 Department of Ophthalmology, Mayo Clinic, Rochester, MN, USA
2 Department of Laboratory Medicine, Mayo Clinic, Rochester, MN, USA
3 Department of Infectious Disease, Mayo Clinic, Rochester, MN, USA
4 Department of Molecular Medicine, Mayo Clinic, Rochester, MN, USA
5 VRS Foundation Board Member, Mayo Clinic, Rochester, MN, USA

Correspondence: Jose S. Pulido, Mayo Clinic, Department of Ophthalmology and Department of Molecular Medicine, 200 First Street, SW, Rochester, MN 55905, USA. e-mail: pulido.jose@mayo.edu

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Purpose: Endophthalmitis is a rare complication of intravitreal injection (IVI). It is recommended that povidone-iodine be the last agent applied before IVI. Patients have reported povidone-iodine application to be the most bothersome part of IVIs. Topical anesthetics have been demonstrated to have antibacterial effects. This study compared the minimum inhibitory concentration (MIC) of topical anesthetic eye drops (proparacaine 0.5%, tetracaine 0.5%, lidocaine 2.0%) and the antiseptic, 5.0% povidone-iodine, against two organisms causing endophthalmitis after IVI.

Methods: Minimum inhibitory concentration values of topical anesthetics, povidone-iodine, preservative benzalkonium chloride (0.01%), and saline control were determined using five isolates of each Staphylococcus epidermidis and viridans group Streptococcus species (VGS). A broth microdilution technique was used with serial dilutions.

Results: Lidocaine (8.53 × 10^{-5} mol/mL) had MICs of 4.27 to 8.53 × 10^{-5} mol/mL, and tetracaine (1.89 × 10^{-5} mol/mL) had MICs of 9.45 × 10^{-6} mol/mL for all isolates. Proparacaine (1.7 × 10^{-5} mol/mL) had MICs of 1.32 to 5.3 × 10^{-7} and 4.25 × 10^{-6} mol/mL for S. epidermidis and VGS, respectively. Benzalkonium chloride (3.52 × 10^{-7} mol/mL) had MICs of 1.86 × 10^{-9} to 1.1 × 10^{-8} and 4.40 × 10^{-8} mol/mL for S. epidermidis and VGS, respectively. Povidone-iodine (1.37 × 10^{-3} mol/mL) had MICs of 2.14 to 4.28 × 10^{-6} and 8.56 × 10^{-6} mol/mL for S. epidermidis and VGS, respectively.

Conclusion: Proparacaine was the anesthetic with the lowest MICs, lower than that of povidone-iodine. Benzalkonium chloride had lower MICs than proparacaine. All tested anesthetics and povidone-iodine inhibited growth of S. epidermidis and VGS at commercially available concentrations.

Translational Relevance: For certain patients, it could be possible to use topical anesthetic after povidone-iodine for comfort without inhibiting and perhaps contributing additional antimicrobial benefit.

Introduction

Intravitreal injection (IVI) is one of the most common procedures performed by ophthalmologists. More than 2.3 million injections were recorded in 2012 in the United States alone. The number of IVIs is anticipated to continue to grow. Endophthalmitis is the most feared complication of intravitreal injection. To prevent endophthalmitis, antisepsis is attempted before injections using 5.0% povidone-iodine (Beta-

Topical anesthetic also is given to patients before injections for their comfort. Commonly used anesthetics include proparacaine 0.5% or tetracaine 0.5% drops, as well as subconjunctival lidocaine 2.0%. Interestingly, these anesthetics have been demonstrated to have antibacterial properties even against bacteria that cause post-IVI endophthalmitis (Table 1). The current expert guidelines for IVI stress the importance of 5.0% povidone-iodine use. Povidone-iodine should be the last agent applied before injection; therefore, it should be the first agent applied...
for antisepsis and reapplied after topical anesthetic use.2

The most common bacteria isolated in postinjection endophthalmitis have been demonstrated to be coagulase-negative staphylococci followed by Strep-

tococcus species, most commonly the viridans group streptococci (VGS) S. salivarius and S. mitis.3,4 Other bacteria, including Bacillus cereus and Staphylococcus aureus, also have been implicated.3,4 Proparacaine, tetracaine, and lidocaine have been demonstrated to have antibacterial properties against S. epidermidis and VGS.5–7 We determined the minimum inhibitory concentration (MIC) values of these three topical anesthetics, preservatives, and povidone-iodine against S. epidermidis and VGS using a broth microdilution technique. We also analyzed whether the topical anesthetics have synergistic or antagonistic effects with povidone-iodine. These results would provide information whether it could be possible to use the topical anesthetic after the povidone-iodine for patient comfort with perhaps additional antimicrobial benefit.

### Methods

Five clinical isolates of S. epidermidis and five clinical isolates of biofilm-forming VGS (four S. mitis and one S. mutans) were studied. The clinical isolates were from biofilm-associated infections. Isolates were stored at –80°C in the Mayo Clinic Infectious Diseases Research Laboratory.

Three commonly used and commercially available anesthetic eye drops were tested: tetracaine 0.5% (preservative-free; Alcon, Fort Worth, TX), proparacaine 0.5% (preservative, benzalkonium chloride 0.01%; Akorn Pharmaceuticals, Lake Forest, IL), and lidocaine 2.0% (preservative-free; Hospira, Lake Forest, IL). These anesthetics were analyzed for their effect on bacterial growth in vitro. They were tested alone and in combination with 5.0% povidone-iodine (Alcon). Also, povidone-iodine 5.0% was tested alone. The preservative, benzalkonium chloride 0.01% (Nature’s Tears; Rugby Laboratories, Livonia, MI), also was tested as a control. Finally, saline eye drops (Unisol 4; Alcon) were used as a control.

Three to five isolated bacterial colonies grown on sheep blood agar plates were used to inoculate tryptic soy broth (S. epidermidis) or Todd-Hewitt broth (VGS), which were incubated to exponential phase growth, adjusted to a 0.5 McFarland standard (1.5 x 10^7 colony-forming units [CFU]/mL), and diluted in Cation-adjusted Mueller Hinton broth (CAMHB; Becton, Dickinson France SAS, Le Pont-De-Claix, France) to generate a final concentration of 1.5 x 10^5 CFU/mL.

Stock solutions of proparacaine and tetracaine were diluted in CAMHB to obtain a working suspension of 0.5%. Lidocaine was diluted in CAMHB to obtain a working suspension of 2.0%, and benzalkonium chloride was diluted in CAMHB to a working suspension of 0.01%. Finally, povidone-iodine solution was diluted in CAMHB to obtain working suspensions of 5.0%.

Broth microdilution assays were performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.8 Briefly, serial dilutions of the stock suspensions were made in 50 μL of CAMHB with 50 μL of CAMHB alone as a growth control (with 2.5% lysed horse blood added for VGS); 50 μL of each bacterial suspension were added to the wells and incubated for 24 hours at 37°C (with 5% CO_2 for VGS). Saline eye drops were used as a negative control solvent. The MIC was determined to be the lowest concentration demonstrating no visible growth.9

Synergy versus antagonism for drug combinations was determined using a previously reported method.10,11 Briefly, MICs were used to determine the

<table>
<thead>
<tr>
<th>Coagulate-Negative Staphylococci</th>
<th>VGS</th>
<th>B. cereus</th>
<th>S. aureus</th>
<th>MRSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lidocaine</td>
<td>+ 7</td>
<td>+ 7</td>
<td>+ 25</td>
<td>+ 26, 27</td>
</tr>
<tr>
<td>Proparacaine</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+ 19</td>
</tr>
<tr>
<td>Tetracaine</td>
<td>+ 6</td>
<td>–</td>
<td>–</td>
<td>+ 19</td>
</tr>
</tbody>
</table>

+, data have been demonstrated to be effective against bacteria; –, denotes no data rather than that they are ineffective against bacteria.
fractional inhibitory concentration (FIC) as follows:

\[
FIC_{drug A} = \frac{MIC_{drug A} + MIC_{drug B}}{MIC_{drug A}}
\]

\[
FIC_{drug B} = \frac{MIC_{drug B} + MIC_{drug A}}{MIC_{drug B}}
\]

\[
FIC = FIC_A + FIC_B
\]

Fractional inhibitory concentrations were interpreted according to previously accepted criteria as follows: \(\leq 0.5\), synergy; \(0.5\) to \(1.0\), additivity; \(1.0\) to \(4.0\), indifference; and \(\geq 4\), antagonism.10–12

Results

Of the topical anesthetics, proparacaine had the lowest MICs for \(S. epidermidis\), ranging from \(1.32\) to \(5.3 \times 10^{-7}\) mol/mL, a \(1/128\) to \(1/32\) dilution of its commercially available form; MICs for VGS strains were \(4.25 \times 10^{-6}\) mol/mL, a \(1/4\) dilution of its commercially available form. Povidone-iodine had MICs for \(S. epidermidis\) ranging from \(2.14\) to \(4.28 \times 10^{-6}\) mol/mL, a \(1/64\) to \(1/32\) dilution of its commercially available form; MICs for VGS were \(8.56 \times 10^{-6}\) mol/mL, a \(1/16\) dilution of its commercially available form. The tetracaine MIC was \(9.45 \times 10^{-6}\) mol/mL for all strains (1/2 dilution of its commercially available form); MICs for VGS were \(8.53 \times 10^{-5}\) mol/mL, which is 2.0% of its commercially available concentration. The benzalkonium chloride MIC was \(1.86 \times 10^{-9}\) to \(1.1 \times 10^{-8}\) mol/mL (1/16 to 1/128 dilution of its commercially available form) for \(S. epidermidis\) and \(4.40 \times 10^{-8}\) mol/mL (1/8 dilution of its commercially available form) for all isolates of VGS studied. Normal saline did not inhibit bacterial strains at any concentration (Figs. 1, 2; Table 2).

Synergy for all study isolates was assessed using povidone-iodine at 25% and 50% of its MIC (Table 3). Lidocaine with povidone-iodine demonstrated antagonism for 60% of \(S. epidermidis\) at 50% povidone-iodine’s MIC and 80% at 25% povidone-iodine’s MIC. Lidocaine demonstrated antagonism for 80% of VGS isolates studied. Proparacaine with povidone-iodine demonstrated synergy against all VGS studied. Benzalkonium chloride with povidone-iodine demonstrated synergy against 80% of \(S. epidermidis\) isolates studied.

Discussion

Intravitreal injections are one of the most common procedures performed by ophthalmologists. As indications for IVI expand and the population with conditions treated with IVI grows, it is beneficial to know how to prevent complications, such as endophthalmitis. Antiseptic has been demonstrated to be an important part of IVI as it decreases the number of cases of endophthalmitis.13,14 Our results demonstrated that the tested topical anesthetics studied inhibited bacterial growth at their clinically used concentra-
Proparacaine and benzalkonium chloride had lower MICs than the other tested topical anesthetics as well as povidone-iodine.

The incidence of post-IVI endophthalmitis has been reported to be 0.021\% to 0.09\%.\(^3\,\(^{15}\) The most common bacteria isolated post-IVI have been reported by McCannel et al.\(^4\) to be coagulase-negative staphylococci (65\%); Streptococcus species, most commonly the VGS S. salivarius and S. mitis (31\%); and Bacillus cereus (4\%). Dossarps et al.\(^3\) found the most common bacteria isolated to be coagulase-negative staphylococci (78\%), S. aureus (9\%) and VGS (4\%).\(^3\) Because they are most commonly isolated in injection-associated endophthalmitis, coagulase-negative staphylococci and VGS were used in this study. Future studies with S. aureus would be worthwhile.

The use of povidone-iodine before IVI is supported

### Table 2. Comparison of the MIC Required to Inhibit Bacterial Growth and the Concentration of the Clinically Used Formulations

<table>
<thead>
<tr>
<th>Drug</th>
<th>MIC S. epidermidis, mol/mL</th>
<th>MIC VGS Species, mol/mL</th>
<th>Formulation, mol/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracaine</td>
<td>9.45 × 10^{-6}</td>
<td>9.45 × 10^{-6}</td>
<td>1.89 × 10^{-5}</td>
</tr>
<tr>
<td>Proparacaine</td>
<td>1.32–5.3 × 10^{-7}</td>
<td>4.25 × 10^{-6}</td>
<td>1.7 × 10^{-5}</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>4.27–8.53 × 10^{-5}</td>
<td>8.53 × 10^{-5}</td>
<td>8.53 × 10^{-5}</td>
</tr>
<tr>
<td>Povidone-iodine</td>
<td>2.14–4.28 × 10^{-6}</td>
<td>8.56 × 10^{-6}</td>
<td>1.37 × 10^{-4}</td>
</tr>
<tr>
<td>Benzalkonium chloride</td>
<td>1.86 × 10^{-9}</td>
<td>4.40 × 10^{-8}</td>
<td>3.52 × 10^{-7}</td>
</tr>
</tbody>
</table>

### Table 3. Synergy Versus Antagonism for Topical Anesthetics Combined with Povidone-Iodine for S. epidermidis and VGS as Determined by FIC Values, Where ≤0.5, Synergy; 0.5 to 1.0, Additivity; 1.0 to 4.0, Indifference; and ≥4, Antagonism

<table>
<thead>
<tr>
<th>Agent</th>
<th>MIC Range</th>
<th>Synergy, FIC ≤0.5</th>
<th>Additivity, FIC 0.5–1.0</th>
<th>Indifference, FIC 1.0–4.0</th>
<th>Antagonism, FIC ≥4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracaine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. epidermidis + 1/2 PI (n = 5)</td>
<td>0.7–1.3</td>
<td>0</td>
<td>20%</td>
<td>80%</td>
<td>0</td>
</tr>
<tr>
<td>S. epidermidis + 1/4 PI (n = 5)</td>
<td>1.3–2.1</td>
<td>0</td>
<td>0</td>
<td>100%</td>
<td>0</td>
</tr>
<tr>
<td>VGS + 1/2 MIC PI (n = 5)</td>
<td>0.45</td>
<td>100%</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VGS + 1/4 MIC PI (n = 5)</td>
<td>0.2–1.8</td>
<td>20%</td>
<td>60%</td>
<td>20%</td>
<td>0</td>
</tr>
<tr>
<td>Lidocaine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. epidermidis + 1/2 PI (n = 5)</td>
<td>3.5–7.4</td>
<td>0</td>
<td>0</td>
<td>40%</td>
<td>60%</td>
</tr>
<tr>
<td>S. epidermidis + 1/4 PI (n = 5)</td>
<td>3.5–7.4</td>
<td>0</td>
<td>0</td>
<td>20%</td>
<td>80%</td>
</tr>
<tr>
<td>VGS + 1/2 MIC PI (n = 5)</td>
<td>3.7–7.4</td>
<td>0</td>
<td>0</td>
<td>20%</td>
<td>80%</td>
</tr>
<tr>
<td>VGS + 1/4 MIC PI (n = 5)</td>
<td>3.7–7.4</td>
<td>0</td>
<td>0</td>
<td>20%</td>
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</tr>
<tr>
<td>Proparacaine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. epidermidis + 1/2 PI (n = 5)</td>
<td>0.3–1.0</td>
<td>20%</td>
<td>60%</td>
<td>20%</td>
<td>0</td>
</tr>
<tr>
<td>S. epidermidis + 1/4 PI (n = 5)</td>
<td>0.5–2.5</td>
<td>0</td>
<td>20%</td>
<td>80%</td>
<td>0</td>
</tr>
<tr>
<td>VGS + 1/2 PI (n = 5)</td>
<td>0.02–0.4</td>
<td>100%</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VGS + 1/4 MIC PI (n = 5)</td>
<td>0.02–0.2</td>
<td>100%</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Benzalkonium chloride</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. epidermidis + 1/2 PI (n = 5)</td>
<td>0.2–0.5</td>
<td>80%</td>
<td>20%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. epidermidis + 1/4 PI (n = 5)</td>
<td>0.3–1.0</td>
<td>80%</td>
<td>0</td>
<td>20%</td>
<td>0</td>
</tr>
<tr>
<td>VGS + 1/2 MIC PI (n = 5)</td>
<td>0.5</td>
<td>0</td>
<td>100%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VGS + 1/4 MIC PI (n = 5)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>100%</td>
<td>0</td>
</tr>
</tbody>
</table>

PI, povidone-iodine.
by a number of studies.\textsuperscript{13,14} Apt et al.\textsuperscript{16} demonstrated that one drop of povidone-iodine administered preoperatively reduced bacterial colonies in conjunctival cultures by 91\% versus 33\% for control eyes. Isenberg demonstrated that preoperative administration of povidone-iodine was as effective as neomycin, polymyxin B, and gramicidin (Neosporin; Johnson & Johnson, New Brunswick, NJ) ophthalmic solution given three consecutive days before surgery in reducing conjunctival cultures.\textsuperscript{13} Finally, in a study by Speaker and Menikoff,\textsuperscript{17} including 8000 patients undergoing cataract surgery, those treated with povidone-iodine had a lower rate of endophthalmitis (0.06\%) compared to controls (0.24\%, $P < 0.03$). Overall, povidone-iodine is nontoxic to the eye in the appropriate concentrations, has a broad antimicrobial spectrum (including bacteria, fungi, protozoa, and viruses), has a low propensity to select for bacterial resistance, is inexpensive, and is widely available.\textsuperscript{18,19}

The antibacterial properties of topical anesthetics reported by this study are consistent with previous results. A study on the effects of topical anesthetics on bacteria using a disk diffusion technique studied proparacaine and tetracaine at 0.5, 0.25, and 0.125\% concentrations found that tetracaine inhibited S. \textit{aureus} growth at 5000 \mu g/mL and \textit{Pseudomonas aeruginosa} at 2500 to 5000 \mu g/mL, and that proparacaine inhibited the growth of S. \textit{aureus} at 1250 \mu g/mL, and inhibited \textit{P. aeruginosa} growth at 2500 \mu g/mL.\textsuperscript{19} Notably, a 2000-disk diffusion study demonstrated conflicting results with neither proparacaine nor tetracaine inhibiting S. \textit{aureus} or \textit{P. aeruginosa}.\textsuperscript{20} Proparacaine has been shown to reduce the number of culture-positive eyes, with 4 of 40 conjunctival swabs being culture-positive after proparacaine, versus 12 of 36 eyes being culture-positive after receiving control solution, containing only preservative.\textsuperscript{3} A study using broth microdilution demonstrated that tetracaine inhibited strains of \textit{S. epidermidis} at a concentration of 625 \mu g/mL.\textsuperscript{6} Finally, 2.0\% lidocaine has been demonstrated to have rapid bactericidal effects against \textit{S. epidermidis} and VGS.\textsuperscript{7} The same study found that when patients were treated with subconjunctival injection of 2.0\% lidocaine, 0 of 6853 patients experienced endophthalmitis versus 8 of 8189 ($P = 0.03$) treated with other topical anesthetics.\textsuperscript{7} The mechanism by which topical anesthetics act as antimicrobials has been studied; they are thought to disrupt bacterial cell membranes causing permeability and lysis.\textsuperscript{21,22} Finally, benzalkonium chloride has antimicrobial effects, with an in vitro study showing that benzalkonium chloride inhibits \textit{S. aureus} growth.\textsuperscript{20} In summary, tetracaine, lidocaine, and proparacaine have been demonstrated to have antibacterial effects against organisms causing endophthalmitis. Tetracaine has been demonstrated to have effects against coagulase-negative staphylococci and \textit{S. aureus}. Lidocaine has effects against coagulase-negative staphylococci, \textit{S. aureus}, and VGS. Proparacaine has effects against \textit{S. aureus} (Table 1).

This study compared the efficacy of tetracaine, lidocaine, proparacaine, and benzalkonium chloride with the standard of care, povidone-iodine; all studied topical anesthetics inhibited bacterial growth at clinically used concentrations. Interestingly, proparacaine and benzalkonium chloride had MICs lower than those of povidone-iodine. Additionally, benzalkonium chloride had a lower MIC than did proparacaine. Proparacaine has been demonstrated to have antimicrobial effects independent of benzalkonium chloride. The effects of proparacaine with benzalkonium chloride 0.01\% versus benzalkonium chloride 0.01\% in aqueous solution have been tested on conjunctival flora,\textsuperscript{5} with findings that proparacaine reduced the number of positive conjunctival cultures compared to control (10\% vs. 33\% positive, respectively). With this in mind, it is likely that proparacaine has antimicrobial effects independent of benzalkonium chloride.

Does the observation that proparacaine has a lower MIC than povidone-iodine have clinical significance? A study comparing conjunctival cultures of patients treated with lidocaine, proparacaine, and tetracaine alone and with povidone-iodine may be informative from an antimicrobial standpoint. From the perspective of patient experience, recent studies have demonstrated great patient dissatisfaction with the application of povidone-iodine. In a study by van Asten et al.,\textsuperscript{23} more patients reported povidone-iodine than the application of povidone-iodine. In contrast, lidocaine has been demonstrated to be the most bothersome part of the intravitreal injection process. Currently, topical anesthetics before the povidone-iodine are not sufficient to prevent the discomfort of povidone-iodine; therefore, in such patients, the use of proparacaine following the povidone-iodine may be a reasonable alternative.

Fractional inhibitory concentration also was studied to determine the impact of the tested agents and povidone-iodine on each other. Lidocaine demonstrated evidence of antagonism with povidone-iodine for \textit{S. epidermidis} and VGS. It would be worthwhile to repeat the study to clarify these results. An ideal topical anesthetic would not decrease the efficacy of povidone-iodine. On the other hand, proparacaine
with povidone-iodine demonstrated synergy against all VGS studied. Benzalkonium chloride with povidone-iodine demonstrated synergy against 80% of S. epidermidis isolates studied. Overall, it was difficult to draw conclusions from these results. A study comparing the effect of 5% povidone-iodine with and without 4% lidocaine gel on bacterial colony counts on blood agar plates inoculated with S. epidermidis, S. aureus, P. aeruginosa, and H. influenzae demonstrated that when 4% lidocaine gel was applied before application with PI, there was decreased antimicrobial activity of PI. To our knowledge, our findings are the first using the FIC to compare synergy versus antagonism of PI with proparacaine, tetracaine, lidocaine, and benzalkonium chloride. It also is the first to analyze the effects of proparacaine, tetracaine, and benzalkonium chloride in combination with lidocaine.

Our study has several limitations. First, it was conducted in vitro using a broth microdilution, which is different from the situation in vivo. This limitation may be particularly significant for film-forming bacteria. For conclusions surrounding topical anesthetic use to be clinically significant, in vivo studies are necessary. Additional studies using other types of bacteria, especially those derived from IVI-associated endophthalmitis, as well as further strains of S. epidermidis and VGS, are merited. It also is important to note that it can be difficult to draw conclusions about MIC values for topical medications. Minimum inhibitory concentration values may not reflect the toxicity of the agent on the host cell. It has been postulated that topical anesthetics act on bacterial cell membranes, so it is possible that the observed effects may generalize to other bacteria, and the associated antimicrobial effects may be quickly acting. The current expert guidelines for IVI stress the importance of 5.0% povidone-iodine use. The guidelines recommend that povidone-iodine should be the last agent applied before performing injections, and physicians should be careful as the anesthetic may interfere with the contact of povidone-iodine to the conjunctival surface. Therefore, the guidelines recommend applying povidone-iodine before and after topical anesthetic. Our results suggested that, while it may not be necessary in all patients, if the patient has irritation following the application of the povidone-iodine, the reapplication of tetracaine or lidocaine afterwards will not be detrimental to the patient.

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References


