Experimental Evaluation of Chlorhexidine Gluconate for Ocular Antisepsis

M. BOWES HAMIL, MICHAEL S. OSATO, AND KIRK R. WILHELMUS*
Ocular Microbiology Laboratory, Cullen Eye Institute, Baylor College of Medicine, Houston, Texas 77030

Received 19 June 1984/Accepted 19 September 1984

Chlorhexidine gluconate is a bisguanide germicide currently available with 70% isopropanol (Hibistat, Hibitan) or a detergent (Hibicelens, Hibiscrub) for preoperative skin preparation. As these solvents are toxic to the cornea, we investigated the safety and efficacy of aqueous chlorhexidine solutions for ophthalmic use. Chlorhexidine in Tris-glycine buffer was evaluated for retardation of epithelial regeneration after experimental corneal abrasion in rabbits. Irritant concentrations of 2.0 and 4.0% chlorhexidine significantly slowed the healing rate (0.546 and 0.076 mm/h, respectively) compared with saline controls (0.938 mm/h). Irritant concentrations of ≤1% did not statistically delay healing (P > 0.4). In a separate group of animals, the right conjunctiva of pigmented rabbits were inoculated with Staphylococcus epidermidis (~10^7 organisms per eye) and irrigated with 40 μl of aqueous chlorhexidine in concentrations of 0.1, 0.5, and 1.0%; the left eyes were irrigated with saline or left untreated. Quantitative conjunctival cultures were obtained, and the total number of organisms recovered per eye was calculated. All chlorhexidine-treated eyes showed significant reduction in organisms compared with either untreated or saline-irrigated control eyes (P < 0.001). In vitro antimicrobial susceptibility testing demonstrated chlorhexidine in concentrations of 0.1 to 4% to be highly active against a variety of gram-positive and gram-negative bacterial pathogens by disk diffusion and broth diffusion assays. Topical aqueous chlorhexidine may be an alternate agent for preoperative conjunctival antisepsis.

Postoperative infection is a most serious complication after ophthalmic surgery. Although the sources of contamination are multiple, the microbial population of the ocular surface is an important reservoir (8). Several different antiseptic agents are used for preoperative skin preparation in ophthalmic surgery (1), but postoperative infection may be related more commonly to residual microflora from the conjunctiva rather than from the periocular skin (8).

Topical antibiotics have been suggested to reduce the conjunctival bacterial population (7, 19), but there is no consensus regarding the optimal regimen. Unfortunately, most antiseptic agents such as povidone-iodine and hexachlorophene are toxic to the corneal epithelium (3, 14). Chlorhexidine, however, has excellent germicidal properties (12, 13, 16–18, 21) with persistent effect (16, 17, 21) and has been successfully used in contact lens solutions. Toxicological studies have shown that chlorhexidine is safe as a contact lens disinfectant (2, 10), and direct topical application of concentrations of up to 2% causes neither visible nor light microscopic corneal changes (9, 15), although superficial epithelial changes are noted by electron microscopy (4, 6). We have investigated the efficacy of various concentrations of aqueous chlorhexidine gluconate in experimental models to study its use as a potential preoperative antiseptic agent for the eye.

MATERIALS AND METHODS

Toxicity studies. The method of assessing the corneal epithelial toxicity was similar to that described by Ubels et al. (20). Pigmented rabbits weighing 2 to 3 kg were anesthetized with ketamine (2.5 mg/kg) and xylazine (5 mg/kg). A 6-mm corneal trephine marked the central corneal epithelium bilaterally that was then manually debrided with a no. 64 Beaver blade. Aqueous solutions of chlorhexidine gluconate were formulated to achieve concentrations of 0.1, 0.2, 0.4, 0.5, 1.2, and 4%, buffered with Tris-glycine to a pH of 7.2, and were stored in the dark to maintain stability (11). For each rabbit, the right eye received 40 μl of a chlorhexidine solution, and the control left eye received 40 μl of Tris-glycine buffer, instilled into the inferior conjunctival cul-de-sac.

Photographs were taken at 6- to 12-h intervals until epithelial wound closure without interruption of the normal rabbit light-dark cycle. Photographs were taken after aqueous fluorescein was applied, with Ektachrome 35-mm film and an electronic flash fitted with a Watman no. 47 gelatin filter. The photographs were projected, traced, and measured with a Zeiss videoplan to calculate the area of the epithelial defect and the rate of epithelial healing.

Antimicrobial activity studies. Various concentrations of aqueous chlorhexidine gluconate were formulated as described above, and 20 μl of each solution was placed onto individual sterile 6.35-mm filter disks and allowed to dry. Each disk was placed onto the surface of Mueller-Hinton agar that had been previously inoculated with microorganisms (0.01 ml in 10 ml of agar overlay) obtained from ocular specimens (Streptococcus pyogenes, Staphylococcus epidermidis, Staphylococcus aureus, Micrococcus sp., Escherichia coli, Psuedomonas aeruginosa, Enterobacter aerogenes, or Bacillus subtilis). The diameter of the zone of inhibition was measured after 24 h of incubation at 35°C. In addition, the chlorhexidine disks were incubated in 1 ml of Mueller-Hinton liquid nutrient media; each milliliter contained an inoculum of 10⁴ cells of the microorganism. Bacterial growth was qualitatively assessed by scoring the turbidity (3+, densely turbid; 2+, moderately turbid; 1+, minimally turbid; 0, no growth).

The in vivo antimicrobial activity of topical aqueous chlorhexidine was assessed in pigmented rabbits, using an ocular isolate of Staphylococcus epidermidis resistant to erythromycin at a concentration of >10 μg/ml. Erythromycin resistance was used as a marker to differentiate instilled

* Corresponding author.
was irrigated with 5 ml of aqueous chlorhexidine (0.1, 0.5, or 1.0%). At various time intervals after inoculation (10, 30, 60, and 120 min), the superior and inferior conjunctival fornices were gently swabbed with a calcium alginate applicator moistened with Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) nutrient media. The swabs were then placed into 3 ml of Calgon Ringer solution and dissolved with agitation (5). A sample was placed onto standard methods agar containing 8 µg of erythromycin per ml and inhibitors for chlorhexidine (1% Tween 80, 0.5% lecithin, 1% sodium thiosulfate). After incubation at 37°C for 24 h, the colonies were counted, and the total number of recovered bacteria was calculated.

The corneal epithelial toxicity data were evaluated by Student's t test. The microbial recovery results were analyzed by the Wilcoxon rank sum test. In each instance, experimental data were compared with that of matched controls.

RESULTS

Toxicity studies. Five rabbits (10 eyes) were studied, including concomitant control eyes, for each chlorhexidine concentration. Figure 1 compares the healing rates for the various concentrations of chlorhexidine. Rabbits receiving aqueous chlorhexidine (0.1, 0.2, 0.4, 0.5, and 1%) were not statistically different from saline controls (P > 0.45, P > 0.5, P > 0.5, P > 0.5, respectively). The rates of epithelial healing to complete closure were 0.076 mm²/h after receiving 4% aqueous chlorhexidine and 0.546 mm²/h after receiving 2% aqueous chlorhexidine; these rates were significantly different from the control, 0.938 mm²/h (P < 0.01 and P < 0.05, respectively). Bulbar conjunctival hyperemia and lid mattering occurred during the first 60 h after topical application of 4% and 2% aqueous chlorhexidine; application of 1% aqueous chlorhexidine resulted in mild conjunctival hyperemia that resolved within 24 h. No adverse effects were noted by penlight exam for concentrations of <1%.

Antimicrobial activity studies. All organisms tested were inhibited by chlorhexidine in both the disk diffusion (Fig. 2) and broth studies (Table 1). Growth inhibition was noted for each organism for all concentrations of aqueous chlorhexidine studied, except for moderate growth by *Enterobacter aerogenes* in the broth diffusion study at a disk concentration of 0.1% chlorhexidine (equivalent to 0.002% in the growth medium).

Figures 3 and 4 show the bacterial count of *Staphylococcus epidermidis* recovered from the rabbit conjunctival sac after treatment with wash solutions of aqueous chlorhexidine. Analyses of the 10-min recovery study (Fig. 3) showed

---

**TABLE 1. In vitro antibacterial activity of chlorhexidine solutions by the broth diffusion assay**

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Antibacterial activity* at following % chlorhexidine in broth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.08</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>0</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>0</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>0</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>0</td>
</tr>
<tr>
<td><em>Micrococcus sp.</em></td>
<td>0</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>0</td>
</tr>
<tr>
<td><em>Enterobacter aerogenes</em></td>
<td>0</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>0</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>0</td>
</tr>
</tbody>
</table>

* 3+, densely turbid; 2+, moderately turbid; 1+, minimally turbid; 0, no growth.
CHLORHEXIDINE GLUCONATE FOR OCULAR ANTISEPSIS

FIG. 2. Disk diffusion sensitivity assays of various concentrations of chlorhexidine gluconate.

FIG. 3. Recovery of *Staphylococcus epidermidis* from the conjunctiva 10 min after inoculation, after a wash with saline-0.1% chlorhexidine gluconate-1.0% chlorhexidine gluconate. One standard deviation about the mean shown. Actual mean count of organisms recovered after the wash with 1.0% chlorhexidine is 2.1.

FIG. 4. Recovery of *Staphylococcus epidermidis* from the conjunctiva after a wash with either 9.9% saline (squares) or 0.5% chlorhexidine gluconate (circles), exhibiting the persistent effect of chlorhexidine. One standard deviation about the mean shown.
DISCUSSION

Complete ocular antisepsis preceding intraocular surgery is the goal of preoperative preparatory maneuvers but with present methods is not realistically achievable. Topical antibacterial agents are effective in reducing the local microbial population but require multiple applications (7, 19). Of the antisepctic agents currently available for skin preparation, most are too toxic for direct instillation onto the ocular surface. Commercial preparations of chlorhexidine, such as Hibiclens (4% chlorhexidine gluconate in a detergent solution) and Hibitane (0.5% chlorhexidine gluconate in 70% isopropanol), are toxic to the corneal epithelium (14). Despite its poor solubility, aqueous solutions of chlorhexidine, however, may be relatively safe for topical ocular use. Chlorhexidine also has an advantage over other antisepctic agents in that it may have a persistent effect for several hours after application (16, 17, 21). We therefore have attempted to determine the effects of topically applied aqueous chlorhexidine with regard to its safety and antimicrobial properties.

Multiple concentrations of chlorhexidine ranging from 0.1 to 4% were evaluated for their toxic effects on corneal reepithelialization. Concentrations of ≥2% were clearly toxic to both the corneal epithelium and conjunctiva; a concentration of 1% produced no statistically significant delay in epithelial healing but did cause a mild conjunctivitis. Concentrations of <1% were not statistically different from the control group either in reepithelialization or visible toxic effects.

In vitro sensitivity studies with disk diffusion and broth diffusion assays verified the broad antibacterial spectrum (12) of chlorhexidine at all concentrations from 0.1 to 4%. The antimicrobial properties of aqueous chlorhexidine were also studied on the conjunctival surface. These microbial recovery studies, in which an ocular isolate of Staphylococcus epidermidis was used, showed that chlorhexidine significantly reduced the bacterial counts after a single application. Concentrations as low as 0.1% produced a nearly 100-fold decrease over saline-treated eyes after 10 min. Chlorhexidine also showed a persistent effect in suppressing bacterial recovery over a 2-h period after bacterial inoculation.

Aqueous chlorhexidine appears to be an effective topical ocular antisepctic agent that does not affect corneal reepithelialization in concentrations of <1%. With its antimicrobial spectrum against both gram-positive and gram-negative bacteria, chlorhexidine can significantly reduce and persistently suppress the conjunctival microbial population. These studies suggest that a concentration of aqueous chlorhexidine of 0.1 to 0.5% applied topically is relatively safe and effective; however, because the corneal endothelium is sensitive to extremely low levels of this drug (10), the role of topical chlorhexidine as a preoperative antisepctic agent before intraocular surgery remains to be assessed.

ACKNOWLEDGMENTS

This study was supported in part by research training grant EY-07001 from the National Eye Institute and by grants from the Sid W. Richardson Foundation and the Research to Prevent Blindness, Inc.

LITERATURE CITED