Toxicity of 0.2% chlorhexidine gluconate on the cornea and adjacent structures

ABSTRACT

Objective
To determine the toxicity of the potential fungicide chlorhexidine gluconate (as a pure 0.2% solution and as a commercially available skin antiseptic diluted to contain 0.2% chlorhexidine gluconate) to the cornea and adjacent ocular structures.

Methods
An experimental study was performed at the Animal Facility of the Massachusetts Eye and Ear Infirmary. Pure chlorhexidine gluconate 0.2% was applied to the right eye of 10 female albino rabbits 4 times daily for 14 days. Prior to treatment, all eyes underwent debridement of the corneal epithelium and were examined on days 1, 3, 7, and 14. The toxicity of the following was also determined: a commercial skin antiseptic solution diluted to contain 0.2% chlorhexidine gluconate and 0.2% isopropyl alcohol as active ingredients (4), 0.2% isopropyl alcohol (3), and sterile distilled water (2). The acute toxicity of the diluted solution was determined by application every hour for 6 hours. Histopathological examination was done.

Results
Complete reepithelialization was noted by the seventh day in all eyes. At day 14, all corneal epithelia remained intact, but mild superficial punctate keratopathy was observed in some eyes treated with pure 0.2% chlorhexidine, in all eyes treated with the diluted solution and distilled water, but none in alcohol-treated eyes. Histopathological examination revealed no signs of corneal inflammation in 6 of 10 eyes treated with pure chlorhexidine. Very mild inflammation was noted in the remaining 4 eyes, and in all eyes treated with the diluted solution, 0.2% isopropyl alcohol, and sterile distilled water. Acute toxicity studies using the diluted solution applied hourly showed mild inflammation not only of the anterior corneal stroma (2 of 3 eyes) but also of the sclera (1 of 3 eyes).

Conclusion
Multiple applications of 0.2% chlorhexidine gluconate as a pure solution and as a diluted skin-antiseptic solution did not produce severe inflammation and structural alterations in the deep layers of the cornea in rabbit eyes. This suggests that the solutions may be safe for alternative use in the prolonged and intensive treatment of filamentous keratomycosis.

Key words: Chlorhexidine, Toxicity, Cornea, Keratomycosis, Keratitis
KERATOMYCOSIS is an important cause of ocular morbidity in developing tropical countries, often leading to blindness.\(^1\) Farmers are particularly at risk because of their constant exposure to soil and plant, both rich sources of fungi. Keratomycosis due to filamentous fungi is difficult to cure even with optimal agents such as topical amphotericin and natamycin. Moreover, in developing countries, these agents are often unavailable or unaffordable for most patients. This has triggered a search for less expensive antimicrobials that would be stable in tropical climates and effective in the treatment of filamentous corneal ulcers.\(^2\)

In two clinical studies in humans, Rahman et al. found that 0.2% chlorhexidine gluconate as well as 0.2% natamycin in treating keratomycosis.\(^3,5\) Although they found no significant corneal toxicity, previous studies have.\(^3,5,6\) Furthermore, they stressed that the chlorhexidine preparation used should not contain alcohol or detergent. However, pure chlorhexidine may be difficult to obtain in the Philippines and in some areas of the world, while the combination of chlorhexidine plus isopropyl alcohol is readily available as a skin antiseptic. This study evaluated the toxicity of pure 0.2% chlorhexidine, and a diluted commercial skin antiseptic containing 0.2% chlorhexidine and 0.2% isopropyl alcohol as its active ingredients.

**METHODOLOGY**

This experimental study was performed at the Animal Facility of the Massachusetts Eye and Ear Infirmary using 25 female albino rabbits randomly assigned to receive treatment of the right eye under phase 1 or phase 2.

**Treatment of rabbit eyes**

**Phase 1**

Nineteen female albino rabbits (1.5 to 2.5 kg) were randomly allocated to receive pure 0.2% chlorhexidine gluconate (10 rabbits), dilute scrub solution (4 rabbits), 0.2% isopropyl alcohol (3 rabbits), or sterile distilled water as control (2 rabbits). All the rabbits underwent debridement of the corneal epithelium of the right eye by marking a 7-mm disc with a disposable trephine and then gently removing the epithelium with a number 15 Bard-Parker blade. Prior to the procedure, the rabbits were anesthetized with intramuscular ketamine (40 mg/kg body weight) and xylazine (5 mg/kg body weight), and one drop of proparacaine HCl was placed on the eye.

The right eyes of the rabbits were then treated with their respective test solutions four times a day for 14 days. The left eye served as a control and received sterile distilled water at the same frequency as the fellow eye. Slit-lamp biomicroscopy was performed on all eyes prior to treatment (day 0) and prior to enucleation (day 15). Examination and fluorescein staining was also done on days 1, 3, 7, and 14.

At the end of each treatment interval, the rabbits were sacrificed by intravenous injection of pentobarbital 75–100 mg/kg followed by enucleation of both eyes. Histopathological examination was performed using hematoxylin and eosin (H and E) by one masked reader (CSF).

**Phase 2**

The acute toxicity of the dilute chlorhexidine antiseptic solution was determined by applying the solution to the right eye of 6 rabbits every hour for 6 hours. Debridement of the epithelium was done on half of the rabbit corneas (3 eyes) prior to the treatment (Group A), while no debridement was performed on the other half (Group B) (3 eyes). The left eye of each rabbit served as control and received sterile distilled water one drop every hour. Slit-lamp biomicroscopy with fluorescein staining was performed at the end of the treatment period. The rabbits were sacrificed as described in Phase 1 and histopathological examination was performed.

This animal protocol was designed and conducted in accordance with the ARVO Resolution on the Use of Animals in Research and was approved by the Animal Care Committee of the Massachusetts Eye and Ear Infirmary.

**Preparation of 0.2% chlorhexidine gluconate**

100 ml of sterile water for injection was added to 1 ml of chlorhexidine gluconate 20% to create a solution of 0.2% concentration.

**Preparation of dilute scrub solution containing 0.2% chlorhexidine gluconate and 0.2% isopropyl alcohol**

50 ml of skin-antiseptic solution containing 4% chlorhexidine gluconate and 4% isopropyl alcohol plus inactive ingredients (Biocyde, Biomed Systems, Norwalk, CT, USA) was added to 450 ml of a 0.05M buffer solution containing potassium phosphate monobasic and sodium hydroxate (pH 7.00) to produce 500 ml of 0.4% chlorhexidine gluconate stock solution with a pH of 7.05. 250 ml of the stock solution was then diluted with 250 ml sterile distilled water to produce 500 ml of 0.2% chlorhexidine gluconate with 0.2% isopropyl alcohol test solution with a pH of 6.98.

**RESULTS**

**Phase 1**

**Slit-lamp examination on days 1, 3, 7, and 14**

Results of fluorescein examination at various time points are summarized in Table I. On day 1, healing of 10 to 30% of the de epithelialized cornea was noted in all rabbits, except for one rabbit in the pure chlorhexidine group, which had 90% healing. The latter was completely healed by day 3. By day 3 there was at least 70% healing in all eyes. By day 7, the epithelium had healed in all corneas.
On day 14, all corneal epithelia remained intact, but mild superficial punctate keratopathy (SPK) was observed in some eyes treated with 0.2% chlorhexidine, all eyes treated with the diluted solution and distilled water, but none in alcohol-treated eyes. No lid swelling, conjunctival injection, chemosis, or discharge was noted.

**Phase 1**

**Histopathology of rabbit corneas**

Histopathological findings are summarized in Table 2. Complete epithelialization was noted in areas debrided in all four groups. Histological examination of the pure chlorhexidine-treated eyes revealed no signs of corneal toxicity in 6 eyes, and mild anterior stromal edema in 3 eyes (Figure 1A). One rabbit cornea was normal except for two very small areas of degenerating epithelial cells (one area with infiltration of two neutrophils). Aside from this, no inflammatory cells were noted in the cornea of the rest of the eyes.

Two of the 4 eyes in the diluted-solution-treated group showed a small (2 mm) central linear scar in the new epithelium. Mild stromal edema was noted in all eyes treated with the diluted solution, 0.2% isopropyl alcohol, and sterile distilled water (Figures 1B, 1C, 1D). Neither scleritis nor limbitis was observed on pathologic studies. All control eyes were normal on histopathologic study.

**Phase 2**

**Slit-lamp examination for acute toxicity of diluted antiseptic solution**

All 3 eyes with debrided corneal epithelium (Group A) showed 2+ conjunctival injection. This was not evident in eyes with intact corneal epithelium (Group B) or in the control eyes. There was no lid swelling, conjunctival chemosis, or discharge noted in all eyes.

**Phase 2**

**Histopathology of rabbit corneas**

Histopathological findings are summarized in Table 2.

**DISCUSSION**

The choice of chlorhexidine gluconate as a promising alternative treatment for keratomycosis was made because of its ability to penetrate the deeper layers of the cornea where fungal elements are known to be located. It is believed to bind avidly and rapidly to the corneal epithelium, with subsequent intercalation to the bilaminar membrane. Disruption of tight junctional complexes then

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**Table 1.** Fluorescein examination of rabbit eyes on days 1, 3, 7, and 14 (Trial 1): Estimated percentage healing and other staining abnormalities.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure chlorhexidine 0.2% (n = 10 eyes)</td>
<td>90% healed (1 eye)</td>
<td>100% healed (5 eyes)</td>
<td>100% healed (10 eyes)</td>
<td>100% healed (10 eyes)</td>
</tr>
<tr>
<td></td>
<td>10-30% healed (9 eyes)</td>
<td>with 2+ SPK in 4 eyes</td>
<td>with 1/2+ SPK in 3 eyes</td>
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<td></td>
<td></td>
<td>85-90% healed (2 eyes)</td>
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<td></td>
<td></td>
<td>70-80% healed (3 eyes)</td>
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<tr>
<td></td>
<td></td>
<td>70-90% healed (4 eyes)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diluted antiseptic solution (n = 4 eyes)</td>
<td>10-30% healed (4 eyes)</td>
<td>70-90% healed (3 eyes)</td>
<td>100% healed (4 eyes)</td>
<td>100% healed (4 eyes)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>70-90% healed (2 eyes)</td>
<td></td>
<td>with 1+ SPK in all eyes</td>
</tr>
<tr>
<td>0.2% isopropyl alcohol (n = 3 eyes)</td>
<td>10-30% healed (3 eyes)</td>
<td>70-90% healed (3 eyes)</td>
<td>100% healed (3 eyes)</td>
<td>100% healed (3 eyes)</td>
</tr>
<tr>
<td>Sterile distilled water (n = 2 eyes)</td>
<td>10-30% healed (2 eyes)</td>
<td>70-90% healed (2 eyes)</td>
<td>100% healed (2 eyes)</td>
<td>100% healed (2 eyes)</td>
</tr>
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</table>

*aPercentage healing is based on fluorescein dye uptake after a 7-mm disc diameter debridement of the corneal epithelium. There is no other fluorescein pattern abnormality unless otherwise stated.

*bSuperficial punctate keratopathy

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**Table 2.** Histopathological findings.

<table>
<thead>
<tr>
<th>Phase 1</th>
<th>Pure chlorhexidine 0.2% (N = 10 eyes)</th>
<th>Diluted antiseptic solution (N = 4 eyes)</th>
<th>Isopropyl alcohol (N = 3 eyes)</th>
<th>Sterile distilled water (N = 2 eyes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment Group</td>
<td>No histological changes (6 eyes)</td>
<td>&lt;1/2+ anterior corneal stromal edema (3 eyes)</td>
<td>&lt;1/2+ anterior corneal stromal edema (3 eyes)</td>
<td>&lt;1/2+ anterior corneal stromal edema (2 eyes)</td>
</tr>
<tr>
<td></td>
<td>Normal cornea except for two small areas of degenerating epithelial cells (1 eye)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Isopropyl alcohol (N = 3 eyes)</td>
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<td></td>
<td></td>
<td></td>
<td>&lt;1/2+ anterior corneal stromal edema (3 eyes)</td>
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<td></td>
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<td></td>
<td>Sterile distilled water (N = 2 eyes)</td>
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<td></td>
<td></td>
<td></td>
<td>&lt;1/2+ anterior corneal stromal edema (2 eyes)</td>
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</tbody>
</table>

* Conjunctiva, sclera, and iris are normal on histopathology unless specific abnormalities are listed.

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In Group A, inflammatory cells were seen in the sclera (1 of 5 eyes) and anterior stromal layer of the cornea (2 of 3). In Group B, there was mild inflammation of the limbus (2 of 3) and of the anterior stromal layer of the cornea (1 of 3) (Figure 2). All control eyes were normal on histopathologic study.
allows tear-film access to the intercellular spaces of the corneal epithelium, thereby creating a low-resistance pathway across stroma and the endothelial layer. This allows chlorhexidine gluconate to reach pathogens even in the deeper layers of the cornea. The cationic antiseptic is believed to interact with the anions on the bacterial-cell wall with subsequent damage to the latter, permitting the agent to enter the cytoplasm and to precipitate its contents or to allow the entry of a synergistic drug. Whether this is also the manner by which chlorhexidine gluconate acts as an antimycotic is still unclear.

Four-percent chlorhexidine gluconate is a widely used skin antiseptic known to have bactericidal activity against some Gram-negative and Gram-positive organisms. It is employed for irrigation of the bladder and the pleural cavity, as well as for application to burnt skin. It is currently used as a mainstay treatment for *Acanthamoeba keratitis* at
a much lower concentration (0.02%), proving successful even against the resilient cyst stage of the amoeba.\textsuperscript{10}

Previous studies have shown that the cytotoxic activity of chlorhexidine gluconate is not specific for microorganisms. It has been observed to damage ocular structures most notably the cornea, at concentrations greater than 0.1%.\textsuperscript{4, 5, 6, 11} On the other hand, Rahman et al. found that 0.2% chlorhexidine gluconate solution was effective against filamentous fungi with no toxic effects to the cornea.\textsuperscript{2, 3}

In our study, a very mild inflammation of the superficial corneal layers resulted from the application of chlorhexidine gluconate 0.2% four times a day for 14 days, as a pure solution (4 of 10 eyes), or as a diluted commercial skin antiseptic (4 of 4 eyes). More frequent applications of the diluted skin antiseptic at hourly administration for 6 hours showed mild inflammation of the superficial corneal layers and the adjacent ocular structures. We do not know whether this is due to a short-term toxicity of the solution, or represents a reaction to the introduction of a foreign substance to the eye. The edema could be part of the recovery process of the cornea and not due to the toxic effects of the test solutions since even the corneas given sterile distilled water manifested mild stromal edema. Furthermore, the adequate healing of the corneal epithelial defect, the absence of severe inflammation and structural alteration in the deeper layers of the cornea by the end of the 14-day trial is reassuring, particularly since prolonged treatment with chlorhexidine gluconate would be required for an effective antimicrobial effect.

The results of this study should be used with caution since there are some differences between the anatomy and biochemistry of rabbit and human corneas.\textsuperscript{12} Moreover, the corneas used in this study did not have any active keratitis, thus the response to the chlorhexidine solution may be different in corneas with active keratitis. It also remains to be established if more frequent and prolonged applications of chlorhexidine gluconate at 0.2% concentration, as may be required for fungal corneal infections, would produce more severe toxicity than that observed in the study. Although the number of rabbits used in each treatment group was small, our findings are reassuring and suggest that using 0.2% chlorhexidine gluconate to treat keratomycosis may be safe. Future studies should evaluate the safety of hourly applications for prolonged periods of time.

References