The Effect of Povidone Iodine on the Corneal Endothelium

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Purpose. Povidone iodine has been proven to be a valuable antiseptic solution in preparing the eye for surgery and is an alternative to postoperative topical antibiotics. No study has addressed the intraocular toxicity of povidone iodine after injection into the anterior chamber. We investigated the potential toxicity of povidone iodine on the corneal endothelium after injections into the anterior chamber in a rabbit model. Methods. In this study we used 24 eyes of 12 albino rabbits. The eyes were divided into the following three groups according to the drugs tested: group A, 5% povidone iodine; group B, 10% povidone iodine; group C, balanced salt solution. The injected eyes were evaluated by biomicroscopy, specular microscopy, corneal pachymetry, and transmission and scanning electron microscopy. Results. Corneal edema was observed in all eyes of groups A and B. In groups A and C, the endothelial cell morphology was not significantly changed and the mean endothelial cell count of the eyes did not change significantly (p = 0.5054). There was no significant difference in corneal thickness between groups A and C (p = 0.3823), but there was a significant difference between groups B and C (p = 0.0002). Transmission and scanning electron microscopy results were normal in group C but not in groups A and B. Conclusion. Povidone iodine in both 5% and 10% concentrations demonstrates severe toxicity when one drop of either concentration is placed directly in the anterior chamber. When povidone iodine is used in preparing the eye for intraocular surgery and as an alternative to postoperative antibiotics, the inadvertent leakage of povidone iodine into the anterior chamber must definitely be prevented.


The use of topical povidone iodine preparations has recently been advocated as part of the preoperative preparation of the eye. Povidone iodine has low ocular toxicity and a long history of safety and causes no demonstrable damage to the corneal endothelium in a single application as an ophthalmic surgery preparatory agent. It is reported that for postoperative use, 5% povidone iodine ophthalmic solution is more effective during the first postoperative day than the antibiotic applied at the conclusion of ophthalmic surgery. One drop of povidone iodine solution given three times daily in the first postoperative week controls the increase in conjunctival bacterial colony-forming units as well as a broad-spectrum antibiotic solution does and is superior to the antibiotics in retarding the increase in bacterial species counts.

It has been reported that povidone iodine placed in the conjunctival sac before intraocular surgery caused no significant effect on endothelial thickness or cell counts. However, it is indicated that corneal endothelial damage occurs after donor corneas are soaked in even small concentrations of povidone iodine. Although retinal toxicity has been demonstrated after intravitreal injection of povidone iodine in a rabbit model, no studies have addressed intraocular toxicity after injection into the anterior chamber. We investigated the potential toxicity of povidone iodine on the corneal endothelium after injections into the anterior chamber in a rabbit model.

The objectives of this study, therefore, were to characterize the physiologic and ultrastructural effects of povidone iodine on the corneal endothelium. Assuming that one drop of povidone iodine solution is inevitably introduced into the anterior chamber, we used povidone iodine solutions in concentrations of 10% (undiluted povidone iodine solution) and 5%.

METHODS

Twenty-four eyes of 12 albino rabbits weighing 2.0–3.0 kg were used. The eyes were randomly and equally divided into the following three groups according to the drugs tested: group A, 5% povidone iodine; group B, 10% povidone iodine; group C, balanced salt solution (BSS) as the control group. All procedures were performed in compliance with the Association for Research in Vision and Ophthalmology Statement for Use of Animals in Ophthalmic and Vision Research. A complete slit-lamp examination was performed to determine the lack of corneal and anterior segment pathologic appearance. The rabbits were anesthetized with intramuscular injections of ketamine hydrochloride (30 mg/kg).
and xylazine hydrochloride (5 mg/kg). A wire lid speculum was used to separate the eyelids. Using an operating microscope for visualization, the eye was stabilized by grasping the superior and inferior rectus muscles with a toothed forceps.

A 30-gauge needle was used to perform paracentesis at the corneoscleral limbus, and 0.05 mL of aqueous humor was removed using a 1.0-mL syringe. After paracentesis, a 30-gauge needle was positioned with the tip at the center of the anterior chamber with the needle bevel facing the lens. Then 0.05 mL of 5% or 10% povidone iodine solution or 0.05 mL of a commercial BSS (Alcon, Fort Worth, TX, U.S.A.) was injected into the anterior chamber. A 5% povidone iodine solution was prepared by diluting 10% povidone iodine (Betadine solution; Purdue Frederick Co., Norwalk, CT, U.S.A.) with an equal volume of BSS.

All rabbits were reexamined 48 hours after the injection. The eyes injected with 5% and 10% povidone iodine solutions had edematous corneas, which were scored from 0 to 4, as explained in Table 1. Contact specular microscopy was performed, with a total of three photographs of the central corneal endothelium taken of each eye (TOMEY EM-1000; Tomey Co., Napoya, Japan), and endothelial cell morphology and counts were obtained. Corneal thickness measurements were taken with an ultrasonic pachymeter (UP-1000 Nidek; Nidek Co., Tokyo, Japan) after the topical application of proparacaine HCl 0.5%.

All rabbits were killed by anesthetizing them with a fatal dose of pentobarbital sodium 1 week after injection. After enucleation, corneal buttons were excised atraumatically. For transmission electron microscopy, the specimens were fixed in 2.5% glutaraldehyde for 24 hours, washed in phosphate buffer (pH 7.4), postfixed in 1% osmium tetroxide in phosphate buffer (pH 7.4), and dehydrated in increasing concentrations of alcohol. The tissues fixed in 1% osmium tetroxide in phosphate buffer (pH 7.4), and postfixed in 1% osmium tetroxide in phosphate buffer (pH 7.4), and dehydrated in increasing concentrations of alcohol. The tissues were then washed with propylene oxide and embedded in epoxy resin embedding media. Ultrathin sections ~60 nm in thickness were cut with a glass knife on a LKB-Nova microtome (Bromma, Sweden) ultramicrotome. These sections were collected on copper grids, stained with uranyl acetate and lead citrate, and examined with a JEOL JEM 1200 EX (Jeol Jem Co., Tokyo, Japan) transmission electron microscope. For scanning electron microscopy, fixed samples were dehydrated in increasing concentrations of acetone. They were critical-point dried, mounted on metal stubs with conductive silver paint, and then sputtered with a 10-nm thick layer of gold in a BIO-RAD sputter apparatus (London, England). The tissue samples were examined with a Jeol scanning electron microscope (SEM ASID-10) at an acceleration voltage of 80 kV.

### TABLE 1. Clinical scoring of corneal edema

<table>
<thead>
<tr>
<th>Score</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (n = 8)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group B (n = 8)</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Group C (n = 8)</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

### TABLE 2. Clinical severity of corneal edema

<table>
<thead>
<tr>
<th>Score</th>
<th>Perfectly clear cornea</th>
<th>Slightly edematous cornea; iris, pupil and lens can be observed in detail</th>
<th>Mild edematous cornea; iris, pupil and lens can be observed</th>
<th>Severe edematous cornea; lens cannot be observed, cornea has keratic striae</th>
<th>Diffuse edematous cornea, iris and lens cannot be observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (n = 8)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group B (n = 8)</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Group C (n = 8)</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</table>

### TABLE 3. Specular microscopic data of corneal endothelium

<table>
<thead>
<tr>
<th>Mean endothelial cell count ± SD (cells/mm²)</th>
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<tbody>
<tr>
<td>Group A n = 8 eyes</td>
</tr>
<tr>
<td>Group C n = 8 eyes</td>
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U = 25.5; p = 0.5054.

### RESULTS

Corneal edema was observed in both groups A and B. In group C, all the eyes had clear corneas (Table 2). Specular microscopy of the corneas in groups A and C demonstrated normal endothelium. Specular microphotographs could not be obtained from all the eyes in group B because of the mild, severe, or diffuse corneal edema, and this group could not be included in the statistical analysis. In group A, the mean endothelial cell count was 2,777.62 ± 177.26 cells/mm²; in group C, the mean endothelial cell count was 2,872.37 ± 210.66 cells/mm². There was no significant change in these two groups (Table 3). In group A, the mean corneal thickness was 386.5 ± 43.62 μm; in group B, the mean corneal thickness was 1,023.87 ± 591.18 μm; in group C, the mean corneal thickness was 360.62 ± 23.04 μm. Between groups A and C, there was a significant difference (p = 0.3823); between groups B and C, there was a significant difference (p = 0.0047); and between groups A and B, there was a significant difference (p = 0.0002) (Table 4).

On transmission electron microscopic examination of group A (Fig. 1), edema and a few vacuoles were observed in some parts of the corneal endothelium. The height of the corneal endothelium was normal when compared with the control group (Fig. 2), and no irregularities were observed in the corneal endothelium. However, in this group, microvilli were not seen in the corneal endothelium. Intercellular spaces were normal. On scanning electron microscopic examination of this group (Fig. 3), the cells of the corneal endothelium appeared swollen when compared with the control group (Fig. 4). The endothelium was normal in appearance, and a normal mosaic-like cellular pattern of the corneal endothelium was observed.

On transmission electron microscopic examination of group B (Fig. 5), edema of the cytoplasm and a few vacuoles were present in the corneal endothelium. A marked decrease was observed in the height of the corneal endothelium, and the height of the corneal endothelium was irregular in appearance. Intercellular spaces were normal, but microvilli of the corneal endothelium were lost. On the scanning electron microscopic examination of this group (Fig. 6), an irregular appearance of the corneal endothelium was observed, and the mosaic-like cellular pattern of the corneal endothelium was not preserved. In addition, in some parts of the tissue specimen, the corneal endothelium was completely absent and the underlying Descemet’s membrane was seen. The cells of the corneal endothelium appeared swollen when compared with the controls.

### TABLE 4. Corneal thickness measurements

<table>
<thead>
<tr>
<th>Mean corneal thickness ± SD (μm)</th>
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<tbody>
<tr>
<td>Group A n = 8 eyes</td>
</tr>
<tr>
<td>Group B n = 8 eyes</td>
</tr>
<tr>
<td>Group C n = 8 eyes</td>
</tr>
</tbody>
</table>

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FIG. 1. Transmission electron micrograph of corneal endothelia exposed to 5% povidone iodine. Original magnification ×20,000.

FIG. 2. Transmission electron micrograph of corneal endothelia exposed to BSS. Original magnification ×20,000.

FIG. 3. Scanning electron micrograph of corneal endothelia exposed to 5% povidone iodine. Original magnification ×3,000.

FIG. 4. Scanning electron micrograph of corneal endothelia exposed to BSS. Original magnification ×3,000.

FIG. 5. Transmission electron micrograph of corneal endothelia exposed to 10% povidone iodine. Original magnification ×20,000.

FIG. 6. Scanning electron micrograph of corneal endothelia exposed to 10% povidone iodine. Original magnification ×3,000.
POVIDONE IODINE ON THE CORNEAL ENDOTHELIUM

DISCUSSION

Povidone iodine is a complex of polyvinylpyrrolidone and iodine. The chemistry of this complex is only partially understood and has been the subject of several reviews. Polyvinylpyrrolidone has been used in the anterior chamber in animal and human eyes and is not believed to be toxic intraocularly. The bactericidal action of povidone iodine is the result of free aqueous iodine released by the polyvinylpyrrolidone-iodine complex.

Povidone iodine has been used for many years in ophthalmic surgery and is an effective broad-spectrum disinfectant. The solution form is inexpensive and widely available and has not been associated with corneal or ocular toxicity when applied in a single dose to the intact ocular surface. It has been suggested that the small amount that might be washed from the conjunctival sac into the eye during an intraocular procedure does not damage the corneal endothelium.

At the outset of this study, we were concerned whether the use of povidone iodine as a preparatory agent for intraocular surgery and for surgery of injured eyes without massive tissue loss and as an alternative to the postoperative use of antibiotics was safe. Therefore, we investigated the administration of one drop of (50 μL) povidone iodine at concentrations of 5% and 10% into the anterior chamber and potential toxicity on the corneal endothelium.

Assuming a rabbit anterior chamber volume of 0.30 mL and a uniform distribution of the injected solution in the anterior chamber, the injection of 0.05 mL 5% povidone iodine solution would produce an intracameral concentration of 0.8%. An injection of 10% solution would produce a concentration of 1.6%. These are the cytotoxic concentrations established by in vitro and in vivo testing of various tissues. Although the toxicity of intravitreal povidone iodine has been studied and no corneal changes were observed, no studies have addressed the effect of povidone iodine after injection into the anterior chamber. However, in human donor corneas, endothelial toxicity has been shown at levels as low as 0.1% after a 5-day exposure.

The results of our laboratory study on povidone iodine toxicity after anterior chamber injection suggested that the concentrations of both 5% and 10% povidone iodine were toxic to the corneal endothelium in rabbit eyes. With 5% povidone iodine, specular microscopy and corneal pachymetry values were within normal range. However, slight corneal edema and ultrastructural damage were found in all rabbit eyes. With 10% povidone iodine, all the parameters that we had evaluated showed severe damage in all rabbit eyes. There are better models of the human eye for studying endothelial toxicity than the rabbit model. Unlike its human counterpart, rabbit corneal endothelium maintains mitotic activity and regenerates after thermal and chemical insult. Cat and monkey eyes are better models because mitotic activity is rare. Recovery after insult in the the human eye and in the better models occurs by growth in size of endothelial cells and migration to cover the denuded surface of Descemet’s membrane. If the observation period is limited to 1 week, however, the long-term reparative effects of the mitotic endothelium can be overlooked. The reasons for choosing the rabbit model included the lower cost associated with rabbits, the ease of obtaining pachymetry measurements and corneal photographs in alert animals, and the general familiarity of the medical community with the rabbit model.

In conclusion, these experiments indicate that exposure of the rabbit corneal endothelium to povidone iodine at concentrations of 0.8% and 1.6% is toxic, and these concentrations might be achieved when one drop of 5% and 10% povidone iodine (stock solution) with inadvertent entry into the anterior chamber during preparation of the intact or injured eye for surgery or instillations of postoperative periods. Therefore, the inadvertent leakage of povidone iodine into the anterior chamber must be prevented.

REFERENCES