Effects of Enzymatic Sterilization Detergents on the Corneal Endothelium

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Objective: To evaluate the potential of enzymatic detergents to cause endothelial damage and anterior segment inflammation.

Methods: Paired rabbit corneas were mounted in an in vitro specular microscope. Endothelia were perfused either with the sterile irrigating solution BSS Plus (Alcon Laboratories Inc, Ft Worth, Tex) (control) or 0.1%, 0.4%, or 1.0% Medline Enzymatic Detergent (Medline Industries Inc, Mundelein, Ill) in BSS Plus. Swelling rates were determined by regression analysis. Human endothelia were perfused using 1.56% detergent. All corneas were fixed for scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Endothelial permeability was determined following perfusion of 0.78% detergent. Finally, in vivo intracameral injections with 1.56% or 3.9% detergent were performed to evaluate clinical changes and to correlate with histopathologic analysis.

Results: Dose-related corneal swelling rates were observed. Digital specular micrographs revealed greater endothelial cell damage when perfused with 1.0% detergent. The TEM of endothelia exposed to 1.0% solutions demonstrated abnormal vacuolization and dilated extracellular spaces, which manifested as an increased corneal permeability to 3 to 4 times that of controls. Human corneas swelled comparably to rabbit corneas but demonstrated increased sensitivity when evaluated by TEM and SEM. Histopathologic analysis after intracameral injection revealed thickened corneas with fewer endothelial cells and irises with increased inflammatory and fibrinous responses compared with controls.

Conclusions: Medline Enzymatic Detergent causes a dose-dependent corneal swelling, ultrastructural damage, increased corneal permeability, and increased inflammatory response in the iris after intracameral injection.

Clinical Relevance: Failure to adequately rinse the detergent from surgical instruments may result in corneal edema and intraocular inflammation.

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Postoperative endophthalmitis continues to be a complication following intraocular surgery. The differential diagnosis of acute postoperative endophthalmitis includes infection, preexisting uveitis, iris trauma, and sterile uveitis, which is usually due to retained lens fragments or foreign materials introduced intraoperatively. Sterile uveitis has been reported to occur in as many as 2% of patients following cataract extraction. This phenomenon is rarely associated with long-term visual loss, but it may manifest with worrisome acute signs, including a sterile hypopyon. Toxic endothelial cell destruction (TECD) syndrome is another important complication after intraocular surgery with an undetermined incidence, since it is a relatively new disease recognized by Breebaart et al. Several different causes have been associated with the development of TECD syndrome after cataract surgery. This syndrome is characterized by profound corneal edema less than 24 hours after surgery. Toxins implicated in TECD syndrome include topical antiseptic solutions and intraocular medications and preservatives. Kim and Breebaart et al showed for the first time that the development of acute corneal decompensation following cataract surgery could be related to a toxic product formed when detergent residue comes in contact with viscoelastic material or from the residue itself. Nuuys et al correlated laboratory data with the clinical data by Breebaart and colleagues by demonstrating that a dose-dependent corneal swelling occurred after cleaning reusable cannulas with an increasing concentration of the same detergent. They also showed that residual detergent perfused to the endothelium of iso-
**MATERIALS AND METHODS**

New Zealand white rabbits (1.5–2.5 kg) were anesthetized with an intramuscular injection of 0.6 mL of ketamine hydrochloride (100 mg/mL) and 0.6 mL of xylazine hydrochloride (20 mg/mL). They were then euthanized with an intracardiac overdose of sodium pentobarbital solution (324 mg/mL, Euthanasia-5; Henry Schein, Port Washington, NY). The eyes were enucleated and the corneas excised and mounted in a dual-chamber in vitro specular microscope for endothelial perfusion. All animals were handled according to the Association for Research in Vision and Ophthalmology statement for the use of animals in ophthalmology and vision research.

Both corneal endothelia of each pair were initially perfused with the sterile irrigating solution BSS Plus (Alcon Laboratories Inc, Ft Worth, Tex) at a rate of 0.07 mL/min for a 1-hour stabilization period. After the stabilization period, one cornea was perfused with 0.1%, 0.4%, or 1.0% solution of Enzymatic Detergent in BSS Plus for 1 hour followed by a washout phase with BSS Plus for 2 hours. The paired control cornea was continually perfused with BSS Plus for the entire experiment. Corneal thickness measurements were taken every 15 minutes, and corneal swelling rates were calculated by linear regression analysis. Serial specular photographs of the rabbit endothelium were taken with an Olympus DP10 digital camera (Olympus America Inc, Melville, NY) inserted into the eyepiece slot on the in vitro specular microscope. At the end of each experiment, the paired corneas were fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer and processed for scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

Four paired human corneas were received from the Georgia Eye Bank. The mean ± SD tissue parameters for these human eyes included a donor age of 34.8 ± 9.7 years, death-to-enucleation time of 5.3 ± 1.3 hours, and enucleation-to-experiment time of 4.2 ± 1.1 days. The corneas were transported in corneal storage media (Optisol GS; Bausch & Lomb, Irvine, Calif) and stored at 4°C. The paired human corneas were mounted in the same dual-chambered in vitro specular microscope as the rabbit corneas. After mounting, the epithelium was removed from each human cornea using a corneal Gill knife. The rest of the perfusion experiment was similar to rabbit endothelial perfusions except that we tested the effect of 1.56% detergent, which is twice the recommended concentration for instrument sterilization.

In another rabbit corneal endothelial perfusion experiment, endothelial carboxyfluorescein permeability was measured using the method of Watsky et al and Kim et al. The value of corneal endothelial permeability is expressed as centimeters per minute. In this study, one cornea served as a control and was perfused with BSS Plus for the entire experiment. Its pair was initially stabilized with BSS Plus, then exposed to 0.78% detergent in BSS Plus for another hour, and finally exposed to BSS Plus for 1 hour as a washout. After this, the permeability protocol was followed. Significance was determined using a 2-tailed t test for paired values.

In vivo experiments were conducted with another group of New Zealand white rabbits. In these experiments, the rabbits were deeply anesthetized with an intramuscular injection of 0.6 mL of ketamine hydrochloride and 0.6 mL of xylazine hydrochloride. The rabbits were positioned under an operating microscope, and a speculum was used to retract the lids. One drop of 0.3% ciprofloxacin was placed in the eye for antimicrobial prophylaxis, and one drop of 0.5% proparacaine hydrochloride was used for topical anesthesia. Using a 30-gauge needle on a 1-mL tuberculin syringe, approximately 50 to 150 mL of aqueous humor was removed from the anterior chamber. The anterior chamber of one eye was reformed through a new injection tract using either 1.56% or 3.9% detergent in BSS Plus, whereas the contralateral eye's anterior chamber was reformed with BSS Plus alone as a control. All solutions were filtered through a 0.2-μm filter before injection into the anterior chamber. A small air bubble was intentionally injected into the anterior chamber to help seal the wound and, thus, prevent extravasation of fluid during the immediate postoperative period. During the surgery, BSS Plus was used to keep the rabbit's cornea moist, and cellulose sponges were used to check wound integrity at the completion of the injection. Subsequently, each eye was examined biomicroscopically at 1, 3, and 5 hours for the 3.9% solution and additionally at 24 hours for the 1.56% solution. At the end of each experiment, the rabbits were euthanized, and the eyes were enucleated and fixed in formalin for histologic processing.

Smith et al reported another type of TECD syndrome caused by a new sterilization procedure and the use of reusable cannulas. The small lumen of these cannulas had oxidized metal residues on their internal surfaces, and on reuse, the residue was irrigated into the eye during surgery. In a related article, Dully et al described cases of TECD syndrome associated with this new sterilization method, which used acetic acid, peracetic acid, and hydrogen peroxide, that caused the reduction of hydrogen peroxide to water and the oxidation of brass to zinc and copper ions, leading to heavy metal toxicity when the residue was irrigated into the anterior chamber.

The importance of this information stems from the fact that ethylene oxide is considered an occupational carcinogen and reproductive toxin by the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention. It is currently the sterilization method of choice for ophthalmic instruments. However, as more information becomes available about the effects of this gas, there is a greater demand for better and safer alternatives. To fill this demand, enzymatic detergents, which have the ability to clean instruments of blood and tissue material, have been formulated. Medline Enzymatic Detergent (Medline Industries Inc, Mundelein, Ill), a brand of enzymatic detergent, which contains subtilisin, an exotoxin, and α-amylose, can be used for instrument sterilization by soaking in a 0.78% solution followed by an adequate rinse to remove residue and ending with an autoclave cycle. The autoclaving process does not deactivate these enzymes be-
cause they are stable until the temperature reaches and exceeds 140°C. This makes the rinsing stage crucial because the autoclaves in use today reach maximum temperatures between 120°C and 130°C.

The purpose of this study is to evaluate the direct effect on the corneal endothelium of a currently used enzymatic detergent and to determine the acute anterior chamber reaction following intracameral injection of this detergent. This study describes another potential cause of sterile uveitis and TECD syndrome that is related to the use of these new enzymatic detergents. With the current emphasis on decreasing the use of ethylene oxide, the potential for sterile uveitis and TECD to occur in the future after intraocular surgical procedures is a major concern. The possibility of such complications occurring is compounded if reusable cannulas are used because of the chance that detergent residue may build up inside the small lumens and may then be irrigated into the eye at the end of the intraocular procedure when the anterior chamber is reformed.

RESULTS

Corneal swelling rates were determined to establish that a dose-response relationship existed when various concentrations of detergent solutions were perfused to the endothelium (Figure 1). The arrows indicate the times at which the endothelium was first exposed to the detergent and the time at which the washout with BSS Plus was begun. The mean ± SD corneal swelling rate of endothelia perfused with 0.1% detergent solution is 21.3 ± 3.3 µm/h. The corneas swell at a rate of 25.5 ± 5.7 µm/h for 0.4% detergent, and 40.4 ± 21.2 µm/h for 1.0%. BSS Plus (control)–perfused corneas swell at a rate of 5.94 ± 1.23 µm/h.

Digital specular micrographs of the rabbit corneal endothelium were taken during the endothelial perfusion experiments. The specular micrographs show that the endothelial cells perfused with 0.1% detergent look similar to those perfused with BSS Plus except that they were slightly swollen. At the 1.0% concentration, there is a loss of endothelial cell structure and visualization, indicating endothelial and stromal edema (Figure 2).

Representative SEM and TEM of endothelia perfused with BSS Plus and 1.0% detergent are shown in Figure 3 and Figure 4, respectively. The SEM of a corneal endothelium perfused with BSS Plus shows confluent hexagonal endothelial cells and intact intercellular junctions (Figure 3A). The TEM illustrates normal subcellular organelles, preserved endothelial junctions, and little evidence of cellular swelling (Figure 3B). The SEM of a cornea perfused with 1.0% detergent solution shows swollen hexagonal cells and raised junctional flaps (not shown). Higher magnification of the junctional area shows swelling and suggests junctional damage (Figure 4A). The TEM reveals an irregular plasma membrane, abnormal vacuolization between and within the endothelia, disrupted intracellular organelles, and damaged intercellular junctions (Figure 4B).

Perfusion of donor human corneal endothelia with 1.56% detergent yielded an average swelling rate of 60.1 ± 14.5 µm/h, which is comparable to that of rabbits (Figure 5). Paired controls perfused with BSS Plus swell at a rate of 8.8 ± 5.1 µm/h. The SEM for the endothelia
Figure 2. Digital specular micrographs of endothelia perfused with BSS Plus (Alcon Laboratories Inc, Ft Worth, Tex) and 0.1%, 0.4%, and 1.0% Medline Enzymatic Detergent (Medline Industries Inc, Mundelein, Ill) solutions. Endothelia perfused with lower concentrations are swollen but look similar to control. Endothelia perfused with 1.0% detergent become edematous and damaged. T indicates time.

Figure 3. A, Scanning electron micrograph of endothelia perfused with BSS Plus (Alcon Laboratories Inc, Ft Worth, Tex) shows an undisturbed monolayer and intact intercellular junctions (original magnification ×1000). B, Transmission electron micrograph shows normal intracellular organelles and a regular plasma membrane (original magnification ×4350).

Figure 4. A, Scanning electron micrograph of endothelia perfused with 1.0% Medline Enzymatic Detergent (Medline Industries Inc, Mundelein, Ill) solution shows swollen intercellular regions and disrupted intercellular junctions (original magnification ×2000). B, Transmission electron micrograph shows increased intracellular vacuolization, abnormal subcellular organelles, and a slightly irregular plasma membrane (original magnification ×4350).
perfused with 1.56% detergent solution shows that many cells have detached from the basement membrane, leaving a less confluent endothelial cell monolayer (Figure 6A) than controls (Figure 7A). The TEM for the same cornea reveals cells with considerable damage. The cells have detached from the basement membrane and contracted, intracellular organelles are damaged, and cell membranes are disrupted (Figure 6B). The TEM for the control endothelia looks normal, with a regular plasma membrane and intact subcellular organelles (Figure 7B).

To assess damage to the endothelial barrier function caused by the Enzymatic Detergent, corneal permeability experiments were performed. Control rabbit corneal endothelia perfused with BSS Plus have an average permeability (mean±SEM) of $6.25 \times 10^{-4} \pm 0.58$ cm/min, whereas the corneal endothelia perfused with 0.78% detergent solution have an average permeability of $22.74 \times 10^{-4} \pm 6.83$ cm/min ($P = .04$).

Finally, in vivo experiments demonstrate that the 1.56% solutions injected into the anterior chamber of rabbits cause mild corneal edema clinically and a mild-to-moderate fibrinous exudate in the anterior chamber. Additionally, the results of the clinical examination up to 24 hours for both eyes were normal (ie, no photophobia, apparent pain, conjunctival injection, or hypopyon). Further experiments using a 3.9% detergent concentration demonstrate a marked response 1 hour after injection. The eyes become hyperemic and edematous, develop a hyphema, and are photophobic. No hypopyon develops in this model. However, rabbits were euthanized soon after development of hyphema and photophobia.

Figure 6. A, Scanning electron micrograph of human corneal endothelia perfused with 1.56% Medline Enzymatic Detergent (Medline Industries Inc, Mundelein, Ill) shows a disrupted endothelial monolayer and destroyed intercellular junctions (original magnification ×1000). B, Transmission electron micrograph of the endothelium reveals detached and contracted cells. Intracellular organelles are damaged, and vacuolization is increased (original magnification ×4350).
Histopathologic analysis reveals the average thickness for the corneas of the eyes injected with detergent solution to be 510 vs 388 µm (P = .04) for the corneas of the eyes injected with BSS Plus. The endothelial cell count is decreased to 17 cells per high-power field on the detergent-injected eye vs 20 cells per high-power field (P = .04) on the BSS Plus–injected eye. Williams et al.18 showed that there was a relationship between cell density and number of cells per high-power field. For human tissue, the formula for determining cell density is as follows:

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\text{[No. of Cells per High-Power Field} \times 145] + 668.
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Since cell densities of humans and rabbits are similar, it is likely that this relationship is comparable. Histologic analysis of the control eye provided evidence of mild inflammation in the iris with lymphocytes and polymorphonuclear leukocytes (PMNs) and a slight fibrinous exudate in the anterior chamber containing a few PMNs (Figure 8A). The iris of the detergent-treated eye is infiltrated with an increased number of lymphocytes and PMNs and demonstrates perivascular fibrinous exudates around anterior iris vessels and evidence of hemorrhage, whereas the fibrinous exudate in the anterior chamber contains numerous PMNs (Figure 8B).

Figure 7. A, Scanning electron micrograph of human corneal endothelia perfused with BSS Plus (Alcon Laboratories Inc, Ft Worth, Tex) shows a normal mosaic and intact intercellular junctions (original magnification ×10000). B, Transmission electron micrograph of the endothelium reveals normal cells with intact subcellular organelles and a regular plasma membrane (original magnification ×4350).

Figure 8. A, Histologic analysis of a rabbit eye injected with BSS Plus (Alcon Laboratories Inc, Ft Worth, Tex) demonstrates mild inflammation and a slight fibrinous exudate containing few polymorphonuclear leukocytes. B, The paired eye injected with 3.9% Medline Enzymatic Detergent (Medline Industries Inc, Mundelein, Ill) demonstrates an increased inflammatory and fibrinous response. Additionally, there is evidence of hemorrhage and perivascular fibrinous exudates around anterior iris vessels.

Since the enzymes, subtilisin, an exotoxin, and α-amylase contained in the Medline Enzymatic Detergent cannot be deactivated until temperatures reach and exceed 140°C, we did an experiment (data not shown) in which the detergent was autoclaved at 120°C and subsequently diluted to 1.0% in BSS Plus before being perfused to the rabbit corneal endothelium. The corneal swelling rate was identical to the corneal endothelial perfusions with the nonautoclaved detergent (Figure 1). This experiment confirmed that autoclaving would not deactivate the enzymes in this detergent. We continued our evaluation of this detergent by hypothesizing that inadequate rinsing of the reusable cannulas could allow residue buildup in the lumen, as described by Nuys et al.3 It was further theorized that this residue would lead to variable concentrations of this detergent being irrigated into the anterior chamber when it is reformed at the end of intraocular procedures.

By direct exposure to the rabbit corneal endothelium with multiple concentrations of the detergent in BSS Plus vs BSS Plus (control), we discovered a dose-related response (Figure 1). In the anterior chamber, subtilisin, a serine protease, has the ability to damage multiple intraocular structures. Kim et al.19 showed that subtilisin had a direct toxic effect on corneal endothelial cell struc-
ature, function, and viability. The SEM, TEM, and swelling rates support the findings of Kim and coauthors. Suzuki et al demonstrated that serine proteases will cause stratum corneum desquamation via degradation of hemidesmosomes. The carboxylfluorescein endothelial permeability experiments illustrate that these enzymes also may be involved in the breakdown of corneal endothelial tight junctions that compromise the barrier function that is crucial for the maintenance of corneal transparency. Although it is probable that higher concentrations of detergent could increase the endothelial permeability to a greater extent, it is not possible to accurately evaluate this with the current experimental procedure, since light scattering due to stromal edema occurs earlier in the perfusion experiment, causing the corneal thickness readings later in the experiment to be unreliable.

In addition to the increased permeability of rabbit corneas, we found that donor human corneas perfused with 1.56% detergent solution also displayed a marked corneal swelling rate (Figure 5) and extensive endothelial damage on SEM and TEM (Figure 6). Although the corneal endothelia perfused with BSS Plus maintained an intact endothelial monolayer (Figure 7A) with normal intracellular organelles (Figure 7B), the paired human cornea perfused with the detergent had a disrupted endothelial monolayer (Figure 6A) and detached endothelial cells (Figure 6B). This was probably caused by a breakdown of adherens-type junctions and desmosomes or hemidesmosomes that bind the endothelial cells to each other and to the Descemet membrane.

Intracameral injections using higher concentrations of detergent, 1.56% and 3.9%, caused a hyphema, corneal edema, conjunctival injection, and photophobia 1 hour after surgery. It is known that the rabbit inflammatory response in the anterior chamber is different from the human response. Although humans develop a hypopyon, rabbits will increase their aqueous humor protein and form fibrinous coagulates. We observed this fibrin coagulate at the 1.56% detergent concentration, but it was more difficult to observe at the higher concentration since the hyphema and corneal edema impeded the view of the anterior chamber and the iris.

Histopathologic examination revealed that the irises of the detergent-treated eyes had a greater number of PMNs and lymphocytes (Figure 8B) when compared with the control eyes (Figure 8A). Additionally, the fibrinous coagulate that formed in the anterior chamber of the eye injected with detergent also contained PMNs (Figure 8B). This inflammatory picture is similar to a sterile uveitis that could occur in humans if this enzymatic cleaner was introduced into the anterior chamber during intraocular surgery. At 3.9%, the highest concentration tested, there was a rapid breakdown of the blood-aqueous barrier, leading to an increase in the number of red blood cells (hyphema) in the anterior chamber (Figure 8B) when compared with controls (Figure 8A). Furthermore, the increased corneal thickness and decreased endothelial cells per high-power field could manifest in humans as a picture of TEC syndrome.

Richburg et al showed that rabbits could develop a hypopyon if the anterior chamber was injected with Kleb-}

siella endotoxin. They showed that when instruments were placed in a 3-day-old ultrasonic bath contaminated with gram-negative bacteria, endotoxins produced by these microorganisms could coat the instruments. Although the bacteria themselves were eradicated by autoclaving, the endotoxin, a heat-stable lipopolysaccharide, remained on the instruments. Introduction of endotoxin into the anterior chamber may also cause a potentially serious case of endophthalmitis. Other authors have attributed sterile hypopyons to numerous causes, including intraocular lens polish and rough edges, reaction to lens proteins, intraocular lens sterilization techniques, laser iridotomy, leukemia, iridocyclitis, and other types of uveitis. Although we are not specifically addressing the issue of endotoxin exposure, we believe it is advisable to consider endotoxins as a source of sterile hypopyons.

The clinical implication of our study is that the use of reusable cannulas can lead to unforeseeable complications that could be avoided by the use of disposable cannulas. Also, if the bath in which surgical instruments are soaked is prepared using a higher concentration of detergent, there is a possibility that the cannulas and instruments used during intraocular surgery will be coated with a thicker residue and/or higher concentration, allowing an avenue for introduction into the eye. We maintain that the rinse phase of the cycle is the most important step to prevent the consequences discussed. Use of these enzymatic detergents is increasing because the US Department of Health and Human Services has mandated the halt of ethylene oxide gas sterilization for fear of its carcinogenicity. Therefore, it is possible that these complications may become more prevalent. If any active enzymes enter the anterior chamber, they could remain for several hours during aqueous turnover, causing damage for the duration of their presence. Therefore, it is our recommendation that surgeons restrict their use of reusable cannulas and be aware of the advantages and disadvantages of the enzymatic detergents used for cleaning ophthalmic instruments.

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