Cytotoxicity of cannulas for ophthalmic surgery after cleaning and sterilization: Evaluation of the use of enzymatic detergent to remove residual ophthalmic viscosurgical device material

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PURPOSE: To evaluate the cytotoxicity of reusable cannulas for ophthalmic surgery after the cannulas were filled with an ophthalmic viscosurgical device (OVD) and cleaned with an enzymatic detergent.

SETTING: Microbiological Testing Laboratory, Department of Medical-Surgical Nursing, University of São Paulo School of Nursing, and Cell Culture Section, Adolfo Lutz Institute, São Paulo, Brazil.

DESIGN: Experimental study.

METHODS: The sample consisted of 30 reusable 25-gauge injection cannulas, 20.0 mm in length, whose lumens were filled with an OVD solution for 50 minutes. The following steps were used to process the cannulas: (1) presoaking, (2) washing the lumen using a high-pressure water jet, (3) backwashing with enzymatic detergent in ultrasonic cleaner, (4) preliminary rinsing with tap water, (5) final rinsing with sterile distilled water, (6) drying with compressed filtered air, (7) wrapping in surgical-grade paper, and (8) steam sterilization at 134°C for 4 minutes. The cannulas were then tested for cytotoxicity according to the United States Pharmacopeia 32.

RESULTS: The cleaning protocol used in this study removed residues of OVD solution and enzymatic detergent as shown by the lack of cytotoxicity of all sample extracts.

CONCLUSION: This cleaning protocol has the potential to minimize the occurrence of toxic anterior segment syndrome associated with residues of OVD solutions and enzymatic detergents.

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Toxic anterior segment syndrome (TASS) is an acute and rare postoperative inflammatory reaction caused by noninfectious substances introduced into the anterior chamber of the eye during surgical procedures. Toxic anterior segment syndrome may damage intraocular structures, including the corneal endothelium and trabecular meshwork.[1] The major signs and symptoms of TASS are anterior chamber reaction, diffuse corneal edema, hypopyon, visual impairment, fibrin formation in the anterior chamber, pupil dilation, and increased intraocular pressure. The onset of symptoms usually occurs on the first postoperative day; however, in some cases symptoms are observed up to 2 weeks after surgery.[2,3] Possible causes of TASS include irrigating solutions with inappropriate pH and osmolarity; high levels of endotoxin[4]; wrong doses of ophthalmic drugs[5]; ointments that enter the eye[3]; and contamination of ophthalmic instruments with residual detergent, endotoxins, metal residues, or denatured ophthalmic viscosurgical device (OVD) substances.[6–12]

The Central Sterile Supply Department (CSSD) is responsible for ensuring the complete removal of residual enzymatic detergents, endotoxins, metal residues, and residual OVD substances from instruments. However, in practice, problems may occur during the processing of ophthalmic instruments at the CSSD, resulting in improperly cleaned instruments

contaminated with residues, which can be potential causative agents of TASS. To improve cleaning and sterilization practices at the CSSD and prevent TASS, the American Society of Cataract and Refractive Surgery (ASCRS) and the American Society of Ophthalmic Registered Nurses (ASORN) published guidelines in 2007 entitled "Recommended Practices for Cleaning and Sterilizing Intraocular Surgical Instruments."[13] However, it is necessary to determine whether these recommendations are effective in removing contaminants and residues that can cause TASS from ophthalmic instruments. The purpose of this study was to evaluate the cytotoxicity of reusable injection cannulas after the cannulas were filled with an OVD solution and cleaned with an enzymatic detergent according to the ASCRS–ASORN recommendations.

MATERIALS AND METHODS

This experimental laboratory study was performed at the Microbiological Testing Laboratory, Department of Medical-Surgical Nursing, University of São Paulo School of Nursing, and at the Cell Culture Section, Adolfo Lutz Institute, São Paulo, Brazil. The sample consisted of 30 reusable 25-gauge cannulas, 20.0 mm in length, for the injection of OVD substances in ophthalmic surgery. This type of cannula was selected for the study because of its narrow lumen and the difficulty cleaning it.

The lumen of the cannulas was completely filled with an OVD solution (methylcellulose 2%). The solution remained in the lumen for 50 minutes to simulate a worst-case scenario in health care delivery regarding the processing of instruments that had been in contact with OVD substances during presoaking. After this period, the cannulas were processed according to the ASCRS–ASORN recommendations.[13] The cleaning and sterilization of the cannulas consisted of the following steps: (1) presoaking in tap water for approximately 5 minutes, (2) washing the lumen using a high-pressure water jet for 5 seconds, (3) backwashing with an enzymatic detergent composed of 4 enzymes (Riozyme IV) in an ultrasonic cleaner (Medisafe SI Digital, Medisafe UK Ltd.) for 15 minutes, (4) preliminary rinsing of the external surfaces with tap water and washing of the lumen using a high-pressure water jet, (5) final rinsing of each cannula with 10 mL of sterile distilled water for the external surface and 20 mL for the lumen, (6) drying with compressed filtered air, (7) individual wrapping in surgical-grade paper, and (8) steam sterilization at 134°C for 4 minutes.

After sterilization, the cannulas were tested for cytotoxicity using the extraction method described in the United States Pharmacopeia 32.[14] For every 0.2 g of the sample, 1 mL of Eagle minimal essential medium (MEM) containing 5% fetal bovine serum (FBS) was used as extraction fluid. The lumen of each cannula was flushed with 2 mL of culture medium and remained in contact with extraction fluid at 37°C ± 1°C (SD) for 24 hours. To meet the required weight:volume ratio, an extract was prepared by processing 3 cannulas. The extracts were tested for cytotoxicity; National Collection of Type Cultures (NCTC) clone 929 cells were suspended in Eagle MEM containing 10% FBS at a concentration of approximately 1.5×10^5 cells/mL, seeded into 12–well plates, and incubated at 37 ± 1°C for 24 hours. After a cell monolayer was formed, the culture medium was shifted to the test medium containing extract at 100% concentration. The plates were incubated once more at 37 ± 1°C for 24 hours. Next, the plates were examined by inverted microscopy.[15]

The negative control consisted of 6 cannulas ready to use and as supplied by the manufacturer. The positive control consisted of 6 cannulas whose lumen was completely filled with an OVD solution (methylcellulose 2%) for 50 minutes. The cannulas were then immersed in enzymatic detergent, according to the manufacturer's directions regarding dilution and immersion time, but not rinsed. Next, they were transferred to a beaker using tweezers and dried in air for 12 hours.

The same wrapping and sterilization procedures and cytotoxicity testing were performed for the positive and negative controls and test group. Cytotoxicity was determined by qualitative evaluation of morphologic changes in cell monolayers, as described in the International Organization for Standardization 10993-5:2009 (Table 1).[16] Validation of the experimental model was performed using latex extract, a well-known cytotoxic substance, and culture medium as a noncytotoxic substance.[16] The latex extract exhibited severe reactivity (grade 4) and the culture medium exhibited no reactivity (grade 0), validating the model used in the study.

RESULTS

The cleaning protocol was followed completely; however, more time was necessary to remove residual OVD material. Most samples required 7 seconds of washing instead of the 5 seconds originally recommended in the step 2 of the cleaning protocol, and therefore a continuous high-pressure water jet was used for up to 7 seconds to flush the lumen of the cannulas. No reactivity (grade 0) was observed for all sample extracts and extracts from negative controls tested against NCTC clone 929 cells; therefore, the extracts were considered noncytotoxic (Figure 1).

In the positive control group, it was possible to see with the naked eye a white layer of OVD material coating the cannulas (Figure 2), even after immersion in

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