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Corneal and conjunctival toxicity of disinfectants—Assessing safety for use with ophthalmic surgical instruments ☆

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Abstract

We investigated the corneal toxicity of *ortho*-phthalaldehyde (Cidex[R]OPA, Johnson and Johnson K.K.) and its predecessor glutaraldehyde (Cidex[R], Johnson and Johnson K.K.). We made primary cultures of porcine and human corneal endothelial cells. Commercially available cell lines were also used including human, bovine, and rabbit corneal epithelium and human conjunctival cells. Following incubation for two days, cell survival was measured using a WST-1 assay for endothelia and a MTT assay for the other cells. Test solutions included 2.25% and 3.5% glutaraldehyde and 0.55% *ortho*-phthalaldehyde. Cell survival was presented as a percentage of the control value. *ortho*-phthalaldehyde displayed less toxicity than glutaraldehyde for all cell types tested. As expected 3.5% glutaraldehyde was slightly more toxic than 2.25% glutaraldehyde. When primary human corneal endothelial cultures were exposed to *ortho*-phthalaldehyde, the survival rates were 88% for 100-fold dilutions and 95% for 500-fold dilutions. The survival rates for all cells tested were greater than 90% when dilutions of 1000-fold or more were used. In conclusion, the corneal toxicity of glutaraldehyde and *ortho*-phthalaldehyde appears to be within safe levels following washing procedures and therefore the use of these disinfectants may be suitable for selected ophthalmic surgical instruments in urgent or under-equipped circumstances.

Keywords: Cornea; Disinfectant; Surgical instrument; Eye surgery; High-level disinfection; Cytotoxicity

1. Introduction

Postoperative suppurative endophthalmitis is a major concern in clinical ophthalmology. The consequences are often catastrophic and the recommended management involves intravitreal injection and early vitrectomy (Endophthalmitis Vitrectomy Study Group, 1995). The incidence is reported to be around 0.1% (Miller et al., 2005; Schmitz et al., 1999) and numerous prophylactic strategies have been suggested, including instillation of povidone iodine (Ciulla et al., 2002) and intracameral injection of antibiotics (Barry et al., 2006) as evidence-based procedures.

Sterilization of surgical instruments is a basic tenet of infection control. In ophthalmic surgery, residual chemicals on surgical instruments or irrigating tubes may result in damage to fetal corneal endothelia and other intraocular tissues (Monson et al., 1992; Nuyts et al., 1990). An extensive review was recently published by Mamalis and colleagues, addressing the clinical importance of toxic anterior segment syndrome (TASS), a postoperative aseptic endophthalmitis caused by toxic agents administered during surgery (Mamalis et al., 2006). Possible toxic agents include intraocular solutions of inappropriate chemical composition, concentration, pH, or osmolality, preservatives, denatured ophthalmic viscosurgical devices,

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enzymatic detergents, bacterial endotoxins, oxidized metal deposits and residues and factors related to intraocular lenses such as residues from polishing or sterilizing compounds (Mamalis et al., 2006). On the other hand, insufficient sterilization can increase the risk of endophthalmitis. The general recommendations for ophthalmic surgical instruments involve ultrasonic cleansing in a detergent wash, followed by ethylene oxide gas sterilization. However, not all instruments require such strict sterilization and gas sterilization is not always available, particularly in emergency situations or in rural and remote locations (Reilly et al., 2004; Fan et al., 2006).

ortho-Phthalaldehyde (Cidex^{R}OPA, Johnson and Johnson K.K., Japan) is a new aromatic dialdehyde antimicrobial agent that has been effectively used for sterilizing endoscopes (Walsh et al., 1999; Akamatsu et al., 2005). It was introduced as an alternative to the conventional agent glutaraldehyde (Cidex^{R}, Johnson and Johnson K.K., Japan). The recommended protocol for standard disinfection involves soaking for 5 min, followed by three washes. Disinfectants have been widely used in ophthalmic practice for contact lenses, examination mirrors, and tonometer tips (Cillino et al., 2006). In addition to these non-surgical devices, disinfectants are occasionally used for surgical instruments in small or under-equipped practices. There are some concerns about safety and toxicity in the case of invasive procedures. Standard hydrogen peroxide disinfection (Pandit et al., 2003), misuse of chlorhexidine (van Rij et al., 1995; Anders and Wollensak, 1997), and even the use of sterilizers such as sodium hydroxide, ethylene oxide, formaldehyde, and gamma radiation for intraocular implants (Singh et al., 1985), have been shown to be significantly toxic to ocular tissues. Recent reports of anaphylaxis (Sokol, 2004) and chemical burn injury (Venticinque et al., 2003), possibly caused by *ortho*-phthalaldehyde, have highlighted the potential problems of toxicity of disinfectants in surgical practice.

The surgical sites of cataract surgery are the ocular surface and anterior chamber, where the conjunctiva and cornea are exposed to various instruments and solutions. Corneal and conjunctival epithelia are resistant to most toxic agents, unless administered in high doses, since the epithelia are multilayered and continuously irrigated by tears to dilute and wipe away foreign materials. Even when they are damaged, corneal and conjunctival epithelia vigorously proliferate to repair the injury. The corneal endothelium is composed of hexagonal columnar cells located on the inner side of the cornea. They have little mitotic activity and excessive loss or damage leads to irreversible corneal edema requiring corneal transplantation. Intraocular surgical intervention is often evaluated by endothelial cell loss because of its clinical significance (Bourne and Kaufman, 1976). Therefore, protective or toxic factors for the corneal endothelium are very important for intraocular surgery. Although the corneal toxicity of various chemicals has been extensively investigated during the development and evaluation of intraocular irrigating solutions (Parikh and Edelhauser, 2003), nothing is known about the ocular toxicity of *ortho*-phthalaldehyde or glutaraldehyde, two commonly used disinfectants in surgical practice. We therefore investigated the corneal toxicity of *ortho*-phthalaldehyde and glutaraldehyde to explore their potential for use as disinfectants for ophthalmic surgical instruments.

2. Materials and methods

Cultures of porcine corneal endothelia and human corneal endothelia were made. Porcine samples were obtained from local slaughter houses and human tissues were retrieved from eye bank specimens, distributed by Northwest Lions Eye Bank, Seattle, USA. This research was approved by the institutional review board.

All cells were grown in Dulbecco's minimum essential medium containing 15% fetal bovine serum, 2 ng/mL epidermal growth factor, 30 mg/L L-glutamine, 2.5 mg/L fungizone, and 2.5 mg/L doxycycline. Culture dishes were coated with type IV collagen and incubated at 37 °C, in 5% CO_2. Culture media was changed every three days. Cell numbers reached sufficient levels for assays after approximately two months of culture, with three passages. Endothelial development was confirmed by typical hexagonal morphology. Approximately 10^4 cells/100 μl cells were harvested in culture well (Falcon Multiwell, 96 wells) and incubated for two days. Then 10 μl of media with various dilutions of the different solutions was added.

Commercially available cell lines were also used to test toxicity and these included SIRC (human corneal epithelium; CCL-60, distributed by American tissue and cells corporation [ATCC]), BCE C/D-1b (bovine corneal epithelium; JCRB9129, distributed by JCRB cell bank), RC1 (rabbit corneal epithelium; JCRB0246), and Chang (human conjunctival cells; CCL-20.2, ATCC). Cell survival was measured using a WST-1 (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenyl-amino)carbonyl]-2*H*-tetrazolium hydroxide, Dojindo Laboratories, Kumamoto, Japan) assay (Ishiyama et al., 1993) for cultured primary endothelia and an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, SIGMA) assay (Mosmann, 1983) for the other cell lines, following incubation for two days in media with various dilutions of the different disinfectants. These methods are quantitative colorimetric assays for cell survival and proliferation. They detect living, but not dead cells and the signal generated is dependent on the degree of activation of the cells. The results can be read on a spectrophotometer (Benchmark microplate reader, BIO-RAD). WST-1 is more sensitive than MTT and used for corneal endothelia since their growth is so slow and available cell numbers were limited. Test solutions included 2.25% and 3.5% glutaraldehyde (Cidex^{R}) and 0.55% *ortho*-phthalaldehyde (Cidex^{R}OPA). Cell survival was compared to control cells that were incubated in media with distilled water only added, and is expressed as a percentage of control. The experiments were repeated 16 times and results are presented as the average ± standard deviation. Concentrations are presented as w/v.

3. Results

Cell survival comparisons for each cell type tested with the various disinfectants are shown in Figs. 1 and 2. *ortho*-Phthalaldehyde (0.55%) was less toxic than glutaraldehyde for all cell types tested, and not surprisingly 3.5% glutaraldehyde was slightly more toxic than 2.25% glutaraldehyde. Human corneal endothelium cultured with *ortho*-phthalaldehyde showed cell survival rates of 88.2 ± 13% (average ± standard deviation), 88.1 ± 11.0%, and 95.2 ± 11.4% for 50-fold (0.011%), 100-fold (0.0055%) and 500-fold dilutions (0.0011%) respectively. Compared to controls, the survival rate was greater than 90% for all cells tested when the dilution was 1000-fold (0.00055%) or greater.