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Cytotoxic and inflammatory effects of contact lens solutions on human corneal epithelial cells in vitro

Sarah Oh, David J. McCanna*, Lakshman N. Subbaraman, Lyndon W. Jones

Centre for Contact Lens Research, School of Optometry & Vision Science, University of Waterloo, 200 University Avenue West, Waterloo Ontario, N2L 3G1, Canada

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ABSTRACT

Purpose: To ascertain the effect that four contact lens (CL) multipurpose solutions (MPS) have on the viability and release of pro-inflammatory cytokines from human corneal epithelial cells (HCEC).

Methods: HCEC were exposed to four different MPS at various concentrations for 18 hours. The cells were also exposed to phosphate buffer, borate buffer, and PHMB. The cell viability was evaluated using the alamarBlue assay. The release of pro-inflammatory cytokines was measured using a Multiplex electrochemiluminescent assay.

Results: MPS-A, MPS-B and MPS-C all reduced cell metabolic activity $p < 0.05$ from control with MPS-A showing the greatest cytotoxic effect (maximum reduction, 90.6%). In contrast, MPS-D showed no significant reductions in cytotoxicity except at the highest concentration tested (19% reduction at 20% MPS concentration). Of the four cytokines evaluated MPS-C showed a substantial increase in the release of IL-1β, IL-6, IL-8, and TNF-α at higher concentrations when compared to control $p < 0.05$. At the 20% concentration of MPS-A and MPS-B the release of IL-1 β increased $p < 0.05$ but the release of IL-6, IL-8, and TNF-α decreased. MPS-D did not cause a change in the release of cytokines IL-1β, IL-6, IL-8 and TNF-α $p > 0.05$. Exposing the cells to borate buffer and PHMB caused an increase in the release of TNF-α $p < 0.05$.

Conclusions: This investigation demonstrates that at different concentration levels, several of the MPS tested showed a decrease in viability and an increase in the release of inflammatory cytokines from HCEC. The borate buffer component as well as PHMB appears to contribute to this pro-inflammatory reaction.

1. Introduction

Multipurpose solutions (MPS) are widely used by contact lens (CL) wearers who wear reusable lenses, primarily due to their convenience [1]. However, the anti-microbial preservatives contained in the MPS may exhibit negative effects on the corneal epithelium. Quaternary ammoniums and polyhexamethylene biguanides (PHMB) are the most commonly used preservatives and both have demonstrated negative effects on corneal epithelial cells. Several studies have demonstrated that MPS containing polyquaternium-1 are relatively more cytotoxic, induce greater oxidative stress, and disrupt epithelial tight junctions [2–8]. Conversely, studies have shown that certain MPS containing PHMB and borate show increased corneal staining and reduced comfort, particularly with specific lens and solution combinations [4,9–13]. Patient comfort while wearing CL may be affected not only by the CL material properties and design, but also by the MPS that is used concurrently [14,15]. Contact lens materials can adsorb and absorb components of the MPS and release them onto the corneal surface during lens wear [16–18], potentially eliciting a cytotoxic and inflammatory response. The majority of the currently used MPS have been shown to induce some degree of damage and inflammation in corneal epithelial cells, and these effects may contribute to patient discomfort and subsequent intolerance to CL wear [3,8,19,20].

In CL wearers, use of MPS is associated with an increased risk of corneal infiltrative and inflammatory events [21,22]. Some studies have shown that MPS may impair corneal epithelial tight junctions, weakening its barrier function as a result [6,23–25]. A weakened epithelial barrier may lead to infiltration by microbes or environmental toxins, and consequently may lead to an inflammatory event [24,26]. Additionally, cytokines released by the ocular surface in response to MPS may induce cellular infiltrates in the cornea [27]. Corneal infiltrative events are three times more likely to occur in eyes in which solution-induced corneal staining is detected [22], a characteristic linked with certain PHMB-containing MPS [12,28,29]. Previous studies have demonstrated that MPS containing PHMB can induce an increase in the number of dendritic immune cells in the cornea compared to MPS

* Corresponding author.
E-mail address: djmccann@uwaterloo.ca (D.J. McCanna).

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containing polyquaternium [30]. An inflammatory response to the lens solution (potentially at a sub-clinical level) could be an underlying cause of lens discomfort, which is the main cause of CL drop out [31]. However, the preservative itself is not the only contributing factor, as different MPS formulations that contain equal concentrations of PHMB do not produce the same effects [10,32]. Other components of the MPS, such as the buffer solution, may be responsible for the observed adverse effects. Borate buffer is found in many commercial MPS, and is itself an antimicrobial. Compared to phosphate buffer, borate buffer has significantly greater antimicrobial efficacy, particularly against Gram-negative bacteria [33]. It has also been shown that contact lenses packaged in borate buffered solutions can cause a reduction in human corneal epithelial cell (HCEC) viability [34].

This study investigated the cytotoxicity and pro-inflammatory response of HCEC cells with various multipurpose solutions, and sought to determine whether PHMB or borate buffer is primarily responsible for the effects observed.

2. Materials and methods

2.1. Multipurpose solutions

Four common MPS were evaluated: OPTI-FREE PureMoist, ReNu fresh, Biotrue, and Complete. All of the MPS were obtained commercially and used within their expiration dates. The compositions of the MPS are described in Table 1.

2.2. In vitro cell culture conditions: primary human corneal epithelial cells

Primary HCEC (Millipore, Temecula, CA) were obtained and cultured in serum-free EpiGRO™ Human Ocular Epithelia Media (Millipore, Temecula, CA), supplemented with manufacturer's kit components: 6 mM L-Glutamine, 0.002% EpiFactor O, 1.0 µM Epinephrine, 0.4% EpiFactor P, 5 µg/mL rh Insulin, 5 µg/mL Apo-Transferrin, and 100 ng/mL Hydrocortisone. The cells were propagated in Collagen 1-coated BioCoat™ culture flasks (Corning, Corning, NY) in a 37 °C incubator with 5% CO_2, and media was replaced every 2-3 days. Adherent cells were dissociated from the culture flask using TrypLE Express (Invitrogen, Carlsbad, CA) without phenol red. Cells were routinely observed under a microscope for any morphological changes. All steps were performed under sterile conditions.

2.3. In vitro test conditions

HCEC were seeded onto a 24-well Collagen 1 BioCoat™ coated culture plate (Corning, Corning, NY) at 10^5 cells per well with 1 mL of EpiGRO media. Cells were incubated at 37 °C and 5% CO_2 for 24 h to allow for adherence and formation of a monolayer on the bottom of the well.

After adherence, old media was removed from the wells. The cells were then exposed to MPS diluted in EpiGRO media in a total volume of 1 mL to achieve concentrations of 1%, 5%, 10% and 20%. Cells were also exposed to 20% sterile phosphate buffered saline (Lonza BioWhittaker, Walkersville, MD), 20% sterile borate saline buffer (Teknova, Hollister, CA), and 0.0001% PHMB solution diluted in EpiGRO media. A media control was included containing EpiGRO alone. The cells were incubated with the solutions at 37 °C and 5% CO_2 for 18 h. All steps were performed under sterile conditions.

The exposure time of 18 h was chosen to cover the upper end of wearing time for soft contact lenses. During lens wear, MPS components are sorbed into and onto the lens and released into the stagnant tear film between the back surface of the lens and the cornea. Release rates of various solution components in CLs will differ, depending on the water content and pore size of the contact lens material, as well as the ionic charge, hydrophobicity and hydrophilicity of the chemical and material [18]. A study that evaluated the PHMB release from five

Table 1
Composition of Multi-Purpose Solutions used in the study.

Manufacturer	Brand Name	Disinfecting Agent	Buffer	Other Ingredients
Alcon	OPTI-FREE PureMoist (MPS-A)	Polyquaternium-1 (0.001%), myristamidopropyl dimethylamine (ALDOX; 0.0006%)	Borate	aminomethylpropanol, boric acid, disodium EDTA, Tetronic 1304, -polyoxyethylene-polyoxybutylene-(EOBO-41), sodium citrate, sodium chloride, sorbitol, water
Bausch & Lomb	ReNu fresh (MPS-B)	Polyaminopropyl biguanide (PHMB; 0.0001%)	Borate	edetate sodium (0.1%), boric acid, hydroxyalkylphosphonate, poloxamine 1107, purified water, sodium borate, sodium chloride
Bausch & Lomb	Biotrue (MPS-C)	Polyquaternium-1 (0.0001%), polyaminopropyl biguanide (PHMB; 0.00013%)	Borate	edetate sodium, boric acid, sodium borate, poloxamine, sulfobetaine, hyaluronan, sodium chloride, water
Abbott Medical Optics	Complete Easy Rub Formula (MPS-D)	Polyhexamethylene biguanide HCl (PHMB; 0.0001%)	Phosphate	edetate disodium, potassium chloride, poloxamer 237, sodium chloride, sodium phosphate, water