ABSTRACT

Introduction: To investigate potentially adverse effects of different topical glaucoma medications and preservatives on cultured ocular epithelial cells. Methods: Confluent cultures of human corneal (10.014 pRSV-T) and conjunctival cells (1-5c-4) were assayed with 100 µL of different glaucoma medications for 25 minutes at 37°C and 5% CO₂. We also tested the preservative sofZia® (Alcon Laboratories, Fort Worth, TX, USA), as well as a range of concentrations of the preservative benzalkonium chloride (BAK; 0.001% to 0.050%). Balanced salt solution was used as the “live” control and a solution containing 70% methanol and 0.2% saponin was used as a “dead” control. The LIVE/DEAD viability/cytotoxicity kit (Invitrogen, Carlsbad, CA, USA) was used to determine the percentage of dead and live cells via ethidium homodimer and calcein fluorescence, respectively.

Results: The toxicity of the prostaglandin analogs latanoprost, tafluprost and travoprost preserved with BAK was similar to the toxicity observed in their respective BAK concentrations. The prostaglandin analog travoprost (0.004%) preserved with the oxidizing preservative sofZia had much greater corneal and conjunctival cell survival than travoprost preserved with BAK. Travoprost (0.004%) containing polyquad also performed statistically better than its BAK-preserved formulation.

Conclusion: Ocular surface side effects have previously been demonstrated with chronic, long-term exposure to intraocular pressure-lowering medications containing the common preservative BAK. BAK alone has significant in-vitro cytotoxicity to cultured ocular epithelial cells. Substitution of BAK with polyquad or sofZia resulted in significantly higher percentages of live conjunctival and corneal cells. Further studies are needed to understand the clinical implications of these findings.

Keywords: benzalkonium chloride; conjunctival epithelium; corneal epithelium; glaucoma; latanoprost; pharmaceutical preservatives; polyquad; polyquaternium-1; tafluprost; travoprost
INTRODUCTION

Topical medications to decrease intraocular pressure are the first line of treatment for glaucoma in the United States and across the world. Since glaucoma is a chronic condition requiring long-term therapy, potential side effects can occur to the ocular surface from repeated dosing. Studies have shown that chronic topical glaucoma therapy can lead to alterations in both tear film and fluorescein staining of the corneal surface, and an increase in inflammatory cytokines among other deleterious effects.1-4 These ocular surface changes have typically been blamed on the preservative commonly used in multidose bottles of topical medication, benzalkonium chloride (BAK). However, more information is needed to better understand the relative toxicity caused by the active ingredients as well.

One of the most commonly prescribed classes of hypotensive agents are prostaglandin analogs (PGAs), used as both first-line monotherapy as well as in combination therapy with other ocular hypertensive agents. The aim of this study was to compare the in-vitro effects on both cultured human corneal and conjunctival epithelial cells of five PGA formulations preserved with either BAK (varying concentrations), the cationic polymer compound polyquad (PQ) 0.001%, or the oxidizing preservative sofZia® (Alcon Laboratories, Fort Worth, TX, USA).

MATERIALS AND METHODS

Cell Culture

The transformed human corneal epithelial cell line (10.014 pRSV-T) was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Corneal epithelial cells were cultured at 37°C and 5% CO₂ in keratinocyte serum-free media (Invitrogen, Carlsbad, CA, USA) containing 5 ng/mL human recombinant epidermal growth factor (Invitrogen), 0.05 mg/mL bovine pituitary extract (Invitrogen), 0.005 mg/mL insulin (Sigma-Aldrich, St. Louis, MO, USA), 500 ng/mL hydrocortisone (Sigma-Aldrich), and antibiotics. Flasks and plates used for culturing were previously coated with 0.01 mg/mL bovine serum albumin (Sigma-Aldrich), 0.01 mg/mL human fibronectin (BD Biosciences, San Jose, CA, USA) and 0.03 mg/mL bovine collagen type I (BD Biosciences) for 2 hours at 37°C.

A human conjunctival epithelial cell line (Wong-Kilbourne derivative of Chang conjunctiva, clone 1-5c-4) was obtained from the ATCC and cultured at 37°C and 5% CO₂ in Medium 199 (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen) and antibiotics. Flasks and plates used for culturing were previously coated with 0.1% gelatin (Sigma-Aldrich) for 2 hours at 37°C.

Assay Reagents

The LIVE/DEAD viability/cytotoxicity kit for mammalian cells (Invitrogen) contained stock solutions of 2 mM ethidium homodimer (Eth-1) and 4 mM calcein-acetoxymethyl ester (calcein-AM) dissolved in dimethyl sulfoxide (DMSO). Dulbecco’s phosphate-buffered saline without calcium or magnesium (D-PBS; Invitrogen) was used to prepare all stains prior to use. D-PBS had an approximate pH of 7.4 and contained the following: 0.2 g/L KCl, 0.2 g/L KH₂PO₄, 8 g/L sodium chloride (NaCl), 2.16 g/L Na₂HPO₄·7H₂O.

The live control solution was balanced salt solution (BSS; Alcon Laboratories) and contained the following: 6.5 g/L NaCl,