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Comparative Toxicity of Preservatives on Immortalized Corneal and Conjunctival Epithelial Cells

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Abstract

Purpose: Nearly all eye drops contain preservatives to decrease contamination. Nonpreservatives such as disodium-ethylene diamine tetra-acetate (EDTA) and phosphate-buffered saline are also regularly added as buffering agents. These components can add to the toxicity of eye drops and cause ocular surface disease. To evaluate the potential toxicity of these common components and their comparative effects on the ocular surface, a tissue culture model utilizing immortalized corneal and conjunctival epithelial cells was utilized.

Methods: Immortalized human conjunctival and corneal epithelial cells were grown. At confluency, medium was replaced with 100 µL of varying concentrations of preservatives: benzalkonium chloride (BAK), methyl paraben (MP), sodium perborate (SP), chlorobutanol (Cbl), and stabilized thimerosal (Thi); varying concentrations of buffer: EDTA; media (viable control); and formalin (dead control). After 1 h, solutions were replaced with 150 µL of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazonium bromide). After 4 h, solutions decanted, 100 µL of acid isopropanol added, and the optical density determined at 572 nm to evaluate cell viability.

Results: Conjunctival and corneal cell toxicity was seen with all preservatives. Depending upon concentration, BAK exhibited from 56% to 89% toxicity. In comparison, Cbl exhibited from 50% to 86%, MP from 30% to 76%, SP from 23% to 59%, and Thi from 70% to 95%. EDTA with minimal toxicity (from 6% to 59%) was indistinguishable from SP.

Conclusions: Generally, the order of decreasing toxicity at the most commonly used concentrations: Thi (0.0025%) > BAK (0.025%) > Cbl (0.25%) > MP (0.01%) > SP (0.0025%) ≈ EDTA (0.01%). Even at low concentration, these agents will cause some degree of ocular tissue damage.

Introduction

Most eye drops contain preservatives to provide a level of antimicrobial activity in the bottle, limiting secondary bacterial, mycotic, and amoebal ocular infections caused by contaminated solutions and prolong the half-life of the drug by preventing biodegradation and maintaining drug potency.[1] Preservatives can be classified into four main categories: detergents, oxidants, chelating agents, and metabolic inhibitors (pentavalent antimonials [Sb^V], quaternary ammoniums, and organomercurials).[2,3] Examples of such preservatives include: benzalkonium chloride (BAK; detergent), chlorobutanol (Cbl; detergent), methyl paraben (MP; chelating agent), sodium perborate (SP; oxidative agent), and stabilized thimerosal (Thi; organomercurial); although by far, the most common of the topical ophthalmic medication preservatives is BAK, typically used in concentrations varying from 0.015% to 0.05%. Disodium-ethylene diamine tetra-acetate (EDTA) and phosphate-buffered saline, while not preservatives, are added to most ophthalmic formulations as buffering agents. While stabilizing agents such as buffers are generally thought of as nontoxic, the potential for toxicity still exists. In fact any chemical added to eye drops, such as the preservative and buffering agents just mentioned, have the potential to harm the eye.[1] Toxicity from pharmaceutical agents can result in decreased visual acuity and/or patient comfort that can lead to decreased compliance.

Benzalkonium chloride (BAK) stabilizes drugs in solution and prevents spoilage by microbial growth; but it can also initiate ocular surface damage and subconjunctival inflammation.[1,4] BAK is a detergent preservative that can affect cell membrane permeability, interrupt the metabolic processes of the cell, cause lysis of cell contents, and allow

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