CRITICAL REVIEW

Ocular preservatives: associated risks and newer options

Indu Pal Kaur, Shruti Lal, Cheena Rana, Shilpa Kakkar and Harinder Singh

University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India

Abstract

Presence of a preservative in an ocular medication has often been considered a culprit in damaging the epithelium. However, the inclusion of a preservative is equally necessary, especially in multiple-dose containers, in order to protect against dangerous organisms accidentally gaining access during instillation. Benzalkonium chloride (BAK), chlorobutanol, chlorhexidine acetate (CHA), and phenylmercuric nitrate or acetate are some commonly used preservatives in eye preparations. New preservatives with a wide range of activity and good safety profiles have been introduced in the market, such as stabilized oxychloro complex (SOC), sofZia, and sodium perborate. In the present review, we discuss various conventional and newly proposed and patented preservative molecules for ocular use. Reasons for discontinuing traditional preservatives and the need for less-toxic molecules are discussed at length, along with newer options coming up in this area.

Keywords: Preservative; ocular; newer; toxicity

Introduction

In chronic eye diseases, for effective therapy, ophthalmic medication is to be given regularly for longer durations than usual dosing regimens. Typical examples of such chronic pathologies are dry eye, glaucoma, and certain eye infections, especially fungal infections. Preservative is an important constituent of multiple-dose containers that helps to minimize the risk associated with accidental microbial contamination, but its frequent use has been associated with alteration in the precorneal film. In patients suffering from dry eye, preservatives tend to aggravate the already existing problem; and in glaucoma patients, the prolonged use of eye drops with preservatives has been associated with changes in ocular surface accompanied by inflammation. In fact, conjunctival biopsies in patients suffering from glaucoma have revealed an increased number of immune cells and fibroblasts (1, 2).

Ocular medications are composed of unique mixtures of the active drug, a preservative, the drug delivery system, viscosity-increasing agents, buffers and stabilizers, and a vehicle by which all the above ingredients are “carried.” Of these, the preservative has most often been considered the culprit in damaging the corneal epithelium, leading to disruption of the glycocalyx, when drops are used beyond the recommended dosing. This sequence of repeated dosing leaves the epithelium unable to keep the tear film in place and can lead to ocular surface disease. This is especially true for patients who overuse their artificial tear products, use multiple ocular medications, suffer from chronic eye diseases like dry eye or glaucoma, or require postsurgery dosing of medication. In a way, it is not incorrect to say that the preservatives are a “necessary evil.”

Today, ophthalmologists have numerous antiglaucoma medications and artificial tears from which to choose. Although topically administered medications are increasingly used with apparent safety and good tolerance, there is growing evidence that long-term use of topical drugs can induce changes in the ocular surface and may often produce damage to conjunctival and corneal epithelial cells. There have been several reports
of the toxic effects of prolonged topical treatments, partly due to the preservatives associated with the formulation of such treatments (3,4). In the eye, preservative turnover is very slow, and quaternary ammonium molecules can be retained in ocular tissues for up to 7 days (5). The lipophilic nature of some preservatives causes them to bind to the ocular tissues immediately after topical application. Previous studies by Burstein (4,5) have shown that topically applied benzalkonium chloride (BAK), the most commonly used preservative in ophthalmic solutions, can cause morphologic disruption of the corneal epithelium at high concentrations. In addition, there is evidence that clinical concentrations of BAK may change the ionic resistance of the cornea by intercalating into cellular membranes, which results in increased permeability (7).

Three types of mechanisms have been described: detergent effects causing loss of tear film stability, toxic effects to the corneal and conjunctival epithelia, and immunoallergic reactions (5,6). Furthermore, repeated doses of preserved eye drops can have a cumulative effect, because the preservatives are in prolonged contact with the epithelium. Several studies have confirmed the participation of preservatives in induction of ocular surface inflammation (7,8), allergy (8), fibrosis (9), and dry eye syndrome (10,11). Preservatives are also suspected of strongly increasing the risk of failure of trabeculectomy in glaucoma (12,13).

In vitro models have been developed to predict the cytotoxic potential of preservatives. These models were essentially based on corneal epithelial cells (14,15) or on other epithelial systems with characteristics similar to those of the superficial layer of the corneal epithelium (Madin–Darby canine kidney cells) (16). The human continuous conjunctival cell line has also been useful for ocular toxicologic studies (17,18). It has been shown that BAK is a strong proapoptotic agent in Chang’s conjunctival cells (19).

In the present review, we discuss conventional, newly proposed, and patented preservative molecules for ocular use. Reasons for discontinuing traditional preservatives and the need for less-toxic molecules are discussed at length, along with newer options coming up in this area.

**Mechanism of action of preservatives**

Preservatives can be classified into 2 main categories, on the basis of their mechanism of action:

**Detergents**—Detergent preservatives, such as BAK, act upon microorganisms by altering cell membrane permeability and lysing cytoplasmic contents (20). Ocular toxicity can occur because some detergent preservatives can affect eukaryotic cells. Mammalian cells cannot neutralize chemical preservatives, so the preservative is incorporated into the cell and causes cellular damage (21).

**Oxidants**—Oxidative preservatives are usually small molecules that penetrate cell membranes and interfere with cellular function (22). They can destabilize cell membranes, but to a lesser degree than detergent preservatives. Stabilized oxychloro complex (SOC) and sodium perborate are 2 examples of oxidative preservatives. At low levels, oxidative preservatives have an advantage over detergent preservatives because they can provide enough activity against microorganisms while having only negligible toxicity on eukaryotic cells. This is because many microorganisms do not have the ability to cope with oxidative stress, whereas mammalian cells are equipped with antioxidants, oxidases, and catalases to neutralize the effect of a low-level oxidant.

This article concentrates on some of the most commonly used detergent and oxidative preservatives, namely, BAK, phenyl mercuric nitrate or acetate, chlorhexidine acetate (CHA), sorbic acid, chlorobutanol, sodium perborate, SOC, and polyquaternium-1. Other preservatives found in ophthalmic preparations include benzododecinium bromide (BDD), cetrimonium chloride, thiomersal, methyl parahydroxybenzoate, polyquaternium ammonium chloride, polyaminopropyl biguanide, and hydrogen peroxide. Tables 1 and 2 contain a list of commonly used products and associated preservatives.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Trade name of glaucoma formulations</th>
<th>Manufacturer</th>
<th>Preservative</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alphagan&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Allergan</td>
<td>0.005% BAK</td>
</tr>
<tr>
<td>2</td>
<td>Alphagan P&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Allergan</td>
<td>0.005% SOC</td>
</tr>
<tr>
<td>3</td>
<td>Azopt&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Alcon Laboratories</td>
<td>0.01% BAK</td>
</tr>
<tr>
<td>4</td>
<td>Betagan&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Allergan</td>
<td>0.005% BAK</td>
</tr>
<tr>
<td>5</td>
<td>Betoptic S&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Alcon Laboratories</td>
<td>0.01% BAK</td>
</tr>
<tr>
<td>6</td>
<td>Cosopt&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Merck &amp; Co.</td>
<td>0.0075% BAK</td>
</tr>
<tr>
<td>7</td>
<td>Rescula&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Novartis Ophthalmics</td>
<td>0.015% BAK</td>
</tr>
<tr>
<td>8</td>
<td>Timoptic&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Merck &amp; Co.</td>
<td>0.01% BAK</td>
</tr>
<tr>
<td>9</td>
<td>Timoptic-XE&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Merck &amp; Co.</td>
<td>0.012% BDD</td>
</tr>
<tr>
<td>10</td>
<td>Trusopt&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Merck &amp; Co.</td>
<td>0.0075% BAK</td>
</tr>
<tr>
<td>11</td>
<td>Xalatan&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Pfizer</td>
<td>0.02% BAK</td>
</tr>
<tr>
<td>12</td>
<td>Humorsol&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Merck &amp; Co.</td>
<td>1:5 000 BAK</td>
</tr>
<tr>
<td>13</td>
<td>Iopidine eye drops&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Alcon Laboratories</td>
<td>0.01% BAK</td>
</tr>
<tr>
<td>14</td>
<td>Lumigan&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Allergan</td>
<td>0.005% BAK</td>
</tr>
<tr>
<td>15</td>
<td>Ocupress&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Bausch &amp; Lomb</td>
<td>0.005% BAK</td>
</tr>
<tr>
<td>16</td>
<td>Optifranolo&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Bausch &amp; Lomb</td>
<td>0.004% BAK</td>
</tr>
<tr>
<td>17</td>
<td>Propineb&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Allergan</td>
<td>0.04% BAK</td>
</tr>
</tbody>
</table>


BAK = benzalkonium chloride; BDD = benzododecinium bromide; SOC = stabilized oxychloro complex.
Table 2. Multidose, over-the-counter medications for dry eye with or without preservatives.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Trade name</th>
<th>Manufacturer</th>
<th>Active ingredients(s)</th>
<th>Preservative</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GenTeal Mild&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Novartis</td>
<td>0.2% Hydroxypropyl methylcellulose</td>
<td>GenAqua (sodium perborate)</td>
</tr>
<tr>
<td>2</td>
<td>GenTeal PF&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Novartis</td>
<td>0.3% Hydroxypropyl methylcellulose</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>Minidrops Eye Therapy&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Optics Laboratory</td>
<td>Polynvinpyrrolidone, polyvinyl alcohol</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>Preservative Free Moisture Eyes&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Bausch &amp; Lomb</td>
<td>0.95% Propylene glycol</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>Refresh Plus&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Allergan</td>
<td>0.5% Carboxymethylcellulose</td>
<td>None</td>
</tr>
<tr>
<td>6</td>
<td>Refresh Tears&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Allergan</td>
<td>0.5% Carboxymethylcellulose</td>
<td>Purite (stabilized oxychloro complex)</td>
</tr>
<tr>
<td>7</td>
<td>Refresh Celluvisc&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Allergan</td>
<td>1% Carboxymethylcellulose</td>
<td>None</td>
</tr>
<tr>
<td>8</td>
<td>Refresh Endura&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Allergan</td>
<td>1% Polysorbate 80, 1% glycerin</td>
<td>None</td>
</tr>
<tr>
<td>9</td>
<td>Refresh Liquigel&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Allergan</td>
<td>1% Carboxymethylcellulose</td>
<td>Purite (stabilized oxychloro complex)</td>
</tr>
<tr>
<td>10</td>
<td>Refresh PM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Allergan</td>
<td>White petrolatum, mineral oil</td>
<td>None</td>
</tr>
<tr>
<td>11</td>
<td>TheraTears&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Advanced Vision Research</td>
<td>0.25% Carboxymethylcellulose</td>
<td>Sodium perborate</td>
</tr>
<tr>
<td>12</td>
<td>TheraTears PF&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Advanced Vision Research</td>
<td>0.25% Carboxymethylcellulose</td>
<td>None</td>
</tr>
<tr>
<td>13</td>
<td>Systane Ultra&lt;sup&gt;d&lt;/sup&gt; (High Performance)</td>
<td>Alcon</td>
<td>0.3% Propylene glycol, 0.4% polyethylene glycol 400</td>
<td>Polyquad</td>
</tr>
<tr>
<td></td>
<td>Systane Preservative Free&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Alcon</td>
<td>0.3% Propylene glycol, 0.4% polyethylene glycol 400</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Systane&lt;sup&gt;e&lt;/sup&gt; (long lasting)</td>
<td>Alcon</td>
<td>0.3% Propylene glycol, 0.4% polyethylene glycol 400</td>
<td>Polyquad</td>
</tr>
<tr>
<td></td>
<td>Systane Nighttime Lubricant Eye Ointment&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Alcon</td>
<td>3% Mineral oil, 94% white petrolatum, 3% anhydrous liquid lanolin</td>
<td>None</td>
</tr>
<tr>
<td>14</td>
<td>Moisture Eyes PM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Bausch &amp; Lomb</td>
<td>White petrolatum, mineral oil</td>
<td>None</td>
</tr>
<tr>
<td>15</td>
<td>Lactiser&lt;sup&gt;i&lt;/sup&gt;</td>
<td>Aton Pharma</td>
<td>Hydroxypropyl cellulose 5 mg</td>
<td>None</td>
</tr>
<tr>
<td>16</td>
<td>Murine Tears Plus Lubricant Redness Reliever Eye Drops&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Prestige Brands</td>
<td>0.5% Polyvinyl alcohol, 0.6% povidone, 0.05% tetrahydrozoline hydrochloride</td>
<td>Benzalkonium chloride</td>
</tr>
<tr>
<td>17</td>
<td>Murine Tears Lubricant Eye Drops&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Medtech</td>
<td>0.5% Polyvinyl alcohol, 0.6% povidone, sodium bicarbonate</td>
<td>Benzalkonium chloride</td>
</tr>
<tr>
<td>18</td>
<td>AMO Blink Gel Tears Lubricating Eye Drops&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Amo Sales and Service Inc</td>
<td>0.25% Polyethylene glycol 400</td>
<td>None</td>
</tr>
<tr>
<td>19</td>
<td>Murocel Methylcellulose Lubricant Ophthalmic Sterile Solution USP&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Bausch &amp; Lomb</td>
<td>1% Sodium chloride, sodium borate, propylene glycol</td>
<td>Methylparaben, propylparaben</td>
</tr>
<tr>
<td>20</td>
<td>Clear Eyes for Dry Eyes Plus ACR Relief&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Prestige Brands</td>
<td>0.012% Naphazoline hydrochloride, 0.2% glycerin, 0.25% zinc sulfate</td>
<td>Benzalkonium chloride</td>
</tr>
<tr>
<td>21</td>
<td>Allergan Optive Lubricant Eye Drops&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Allergan Pharmaceutical</td>
<td>1.4% Polyvinyl alcohol, 0.6% povidone</td>
<td>None</td>
</tr>
<tr>
<td>22</td>
<td>Tears Renewed Ophthalmic Solution&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Akorn.</td>
<td>0.3% Hydroxypropyl methylcellulose, 0.1% dextran 70</td>
<td>Benzalkonium chloride</td>
</tr>
<tr>
<td>23</td>
<td>Soothe Emollient (Lubricant) Eye Drops&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Alimera</td>
<td>0.4% Polysorbate 80, Restoryl (consists of 15.1% Drakeol and 4.5% Drakeol-35)</td>
<td>Polyhexamethylene biguanide</td>
</tr>
<tr>
<td>24</td>
<td>Isopto Tears&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Alcon</td>
<td>0.5% Hydroxypropyl methylcellulose</td>
<td>Benzalkonium chloride</td>
</tr>
<tr>
<td>25</td>
<td>Puralube Ophthalmic Ointment&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Fougera</td>
<td>42.5% Mineral oil, 56.8% white petrolatum</td>
<td>Chlorobutanol</td>
</tr>
<tr>
<td>26</td>
<td>Roho V Arctic Cooling Lubricant/Redness Reliever Eye Drops&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Mentholatum Inc</td>
<td>Hypromellose, tetrahydrozoline hydrochloride</td>
<td>Benzalkonium chloride</td>
</tr>
<tr>
<td>27</td>
<td>Lacri-Lube S.O.P. Lubricant Eye Ointment&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Allergan</td>
<td>42.5% Mineral oil, 56.8% white petrolatum</td>
<td>Chlorobutanol</td>
</tr>
<tr>
<td>28</td>
<td>Allergan Optive Lubricant Eye Drops&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Allergan Pharmaceutical</td>
<td>1.4% Polyvinyl alcohol, 0.6% povidone</td>
<td>None</td>
</tr>
<tr>
<td>29</td>
<td>GenTeal Lubricant Eye Gel for Severe Dry Eye Relief&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Novartis Pharmaceutical</td>
<td>0.3% Hyromellose</td>
<td>None</td>
</tr>
</tbody>
</table>

Table 2. continued on next page.
Table 2. Continued.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Trade name</th>
<th>Manufacturer</th>
<th>Active ingredients(s)</th>
<th>Preservative</th>
</tr>
</thead>
<tbody>
<tr>
<td>30.</td>
<td>Visine Tears Lasting Relief</td>
<td>Pfizer Consumer</td>
<td>Glycerin, hypromellose, polyethylene glycol 400</td>
<td>Ascorbic acid, benzalkonium chloride</td>
</tr>
<tr>
<td></td>
<td>Lubricant Eye Drops</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31.</td>
<td>Hypo Tears Lubricant Eye Drops</td>
<td>Novartis</td>
<td>1% Polyvinyl alcohol, 1% polyethylene glycol 400</td>
<td>Benzalkonium chloride</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32.</td>
<td>Bausch &amp; Lomb Soothe Lubricant</td>
<td>Bausch &amp; Lomb Personal Product</td>
<td>0.6% Glycerin, 0.6% propylene glycol</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Free Eye Drops</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33.</td>
<td>Visine Pure Tears Lubricant</td>
<td>Pfizer Consumer</td>
<td>0.2% Glycerin, 0.2% hypromellose, 1% polyethylene glycol 400</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Eye Drops for Dry Eye Relief</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34.</td>
<td>Bion Tears</td>
<td>Alcon Laboratories</td>
<td>70.01% Dextran, 0.3% hydroxypropyl methylcellulose 2910</td>
<td>None</td>
</tr>
</tbody>
</table>


Some of the commonly used ocular preservatives

Benzalkonium chloride

BAK is a quaternary ammonium compound and is the most common antimicrobial preservative used in topical multisite ophthalmic preparations (23,24). The reasons for the frequent use of BAK as a preservative include its extreme efficacy in combating microbial contamination of bottles and its ability to break cell–cell junctions in the corneal epithelium, thus allowing for antimicrobial and antihypertensive drops to enter the anterior chamber, as well as its familiarity among those formulating ophthalmic preparations in industry. The regulatory approval from the FDA and its current widespread use in more than 70% of existing multidose bottles constitute another notable consideration. While the efficacy of BAK is well known, a multitude of published studies document the detrimental effects of BAK (23,27,30–32). Benzalkonium is known to induce necrosis (at concentrations of 0.05–0.1%) and cellular apoptosis (at concentrations of 0.01%) by way of disturbing the cellular membrane in bacterial cells (23). However, human ocular surface cells can also absorb this detergent, and its effects on ocular surface cells are similar to those seen in bacterial cells. The potential of BAK-induced damage is clinically important in patients who need eye drops several times a day for years, who use multiple eye drops, or who have compromised corneas. The effects of the detergent are cumulative and become more severe with more concentrated and frequent exposures (23). Breakdown of the corneal epithelium and increased permeability of the cornea as a result of BAK toxicity are well documented (30). Ophthalmic preparations that contain high concentrations of BAK interfere with the integrity of the superficial lipid layer of tear film and reduce its breakup time, thus affecting its stability (31–33). This is especially problematic in glaucoma patients, as they inherently have a decreased rate of basal tear turnover (34). In one study, it has been shown that ocular cells repeatedly exposed to BAK can overexpress the cell marker Apo 2.7, which has been implicated in apoptosis (32).

The epithelial cells of cornea are cemented together by the apical portions of their plasma membranes. The cells of corneal epithelium are tightly bound together with intercellular cement similar to that of desmosomes. The surfactant abilities of BAK solubilize the intercellular cement of the corneal epithelium, thereby increasing its penetration (3,26) and subsequent accumulation in the ocular tissue when used for relatively lengthy periods (3,27). Formulations containing BAK have been shown to cause the duplex tear film to become unstable. The tear film comprises an oily layer and an aqueous layer that further consists of water and mucus. It keeps the eye moist, creates a smooth surface for light to pass through the eye, nourishes the front of the eye, and provides protection from injury and infection. Tear film is compromised in patients suffering from dry eye syndrome, which makes the cornea more vulnerable to deleterious substances like preservatives. The degree of instability induced by BAK, however, may not be physiologically harmful under normal situations since the lipid layer of the tear film is re-formed every 15–30 seconds. However, BAK-induced tear film instability needs to be considered when treating patients with a compromised tear film as in the case of dry eye syndrome (32). It is also of concern in situations where the medicament needs to be instilled frequently and over a long period of time, as in conditions like glaucoma (where very frequent use is required) and dry eye (where no drop dilution occurs).

BAK is typically included in antiglaucoma medications at a concentration of 0.01%, with a range of 0.004% to 0.02% (35). BAK in antiglaucoma ophthalmic preparations does not alter the ability of the medication to lower intraocular pressure (IOP), but it can modify the ocular surface (31). This effect has been demonstrated in many
For example, timolol maleate (Timoptic) elicited cell damage along with a rapid decrease in cell number and viability in a human conjunctival cell line (32). Patients treated with timolol maleate exhibited BAK-induced ocular surface damage caused by a decrease in the aqueous layer production rate and impaired tear film mucus layer (36). Another study found that 127 patients using BAK-containing glaucoma drugs, alone or in combination, had a statistically significant degree of conjunctival metaplasia compared with patients not using topical treatment (37). Another study concluded that long-term use of antiglaucoma medications containing BAK changes the conjunctival surface and tear film function. This may increase the risk associated with future glaucoma therapy (38).

In 1990, it was reported that a significantly greater percentage of patients with aqueous tear-deficient dry eye than normal control subjects showed inflammatory cell infiltration of their conjunctival epithelium, and based on this finding, it was speculated that inflammation plays an important role in the pathogenesis of the conjunctival epithelial squamous metaplasia that develops in dry eye (39). Three-fourths of the ophthalmologists surveyed (70%) reported that the preservatives in glaucoma medications are a significant cause of the ocular surface disease they see in glaucoma patients (40). Most believe each of the following is related to the preservatives in glaucoma medications: ocular surface disease (83%), conjunctival inflammation (75%), allergic reactions (71%), and dry eye (67%). Chronic application of detergent preservatives can lead to chronic lymphocytic infiltration in conjunctival stroma in addition to deleterious changes to corneal epithelium and tear film instability (41).

BAK use may be problematic in patients with dry eye syndrome; they are often highly susceptible to its potentially harmful effects, because they lack natural tears to dilute BAK (23). With chronic, long-term exposure or in the setting of dry eye, BAK is reported to lessen the integrity of epithelial cells, increase the number of conjunctival inflammatory cells, cause a loss of goblet cells (16), reduce tear function (31), and decrease the tear film breakup time (31). Research has shown that in cell culture, animal, and human studies, BAK causes greater cytotoxic effects than some of its alternatives, such as SOC (2). Although intermittent use of preserved eye drops in healthy individuals is probably not harmful, high concentrations of some preservatives can cause damage and irritation to the ocular tissue, particularly in patients with dry eye. Frequent use of these can cause a higher incidence of epithelial damage, edema, and bullous keratopathy in patients with glaucoma, dry eye, infections, or iritis, who need to use eye drops for a long period of time (3). Therefore, patients with dry eye syndrome may be at greater risk of BAK-induced adverse effects. In these patients, the cornea is particularly susceptible to the effects of preservatives because the corneal epithelium is an exposed surface and meets the full strength of topical ophthalmic preparations (20). Moreover, these patients may not produce sufficient tears to dilute a harmful preservative (20,29). Preservatives can disrupt the precorneal tear film—which acts as a lubricant and protective layer for the epithelium—and lead to damage to the epithelial surface and worsening of the dry eye condition (29). One study showed that artificial tears containing BAK increased corneal epithelial permeability in patients with dry eye, indicating that BAK may contribute to ocular surface disease (14). Often, allergic complaints or chronic irritation of the conjunctivae and eyelids declines when patients with glaucoma or dry eye stop using preserved eye medications (3).

In a very recent study, where the authors have compared the toxicity of commonly used preservatives using immortalized human conjunctival and corneal epithelial cells, BAK (0.01%) showed a significantly higher toxicity than most of its alternatives, and the order of toxicity was as follows: thiomersal (0.01%) > BAK (0.01%) > chlorobutanol (0.5%) > methylparaben (0.01%) > sodium perborate (0.02%) = ethylenediamine tetraacetic acid (EDTA) (42).

**Phenylmercuric nitrate or acetate (0.002% w/v)**

Phenylmercuric nitrate and, to a lesser extent, phenylmercuric acetate both are extensively used in eye drops. Their solubilities are adequate (acetate: 1 in 500; nitrate: 1 in 1,500) and they are nonirritant and stable. They are effective against a wide range of bacteria and fungi but are rather slow in action, particularly against heavy inocula of *Pseudomonas aeruginosa* (43,44). Their main disadvantages are their incompatibility with halides, strong absorption by rubber, and capacity to cause mercurialentis.

**Chlorhexidine acetate (0.01% w/v)**

CHA is a nonirritant bactericide of low toxicity. It is most effective against gram-positive bacteria but inactive against spores. The susceptibility of some *Proteus* and *Pseudomonas* species is low but is increased in the presence of EDTA. CHA’s highest activity is shown in solutions with neutral or slightly alkaline pH (45).

Organic matter such as serum and, particularly, phospholipids seriously reduces its efficiency, and, like BAK, it is incompatible with anionic compounds and, because of binding, with methylcellulose and hypropomellose. Cork causes inactivation (46) and should not be a component of wads of closures.

CHA forms sparingly soluble salts with bicarbonates, borates, carbonates, chlorides, citrates, phosphates, and

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sulfates, but precipitation (which is usually delayed for about 24 hours) does not occur unless the concentration of CHA is about 0.05%, except with sulfates, which cause difficulty even when the preservative concentration is as low as 0.005%. Consequently, CHA is not used for eye drops containing the sulfates of atropine, neomycin, physostigmine, and zinc (47).

**Chlorobutanol**

Chlorobutanol is an alcohol-based preservative, with no surfactant actions (26). Although the antimicrobial activity of chlorobutanol is extensive (27), its use has been limited because it becomes unstable when stored at room temperature for extended periods of time. Unlike BAK, chlorobutanol does not act like a surfactant (48). The method of action of chlorobutanol is cell lysis by way of disruption of microbial cell membrane lipid configuration (48). Chlorobutanol has been found to be safe in rabbit corneal cells, even at 100 times the concentrations found in commercial products (8).

Although chlorobutanol seems relatively safe as an ophthalmic preservative, chlorobutanol 0.5% in artificial tears has been shown to cause irritation in more than 50% of the subjects in a double-blind crossover study (49). To this end, research has shown that in human corneal cells, 0.5% chlorobutanol caused cell retraction and cessation of normal cytokinesis, cell movement, and mitotic activity (50). It also caused degeneration of human corneal epithelial cells and the formation of conspicuous membranous blebs (48). Chlorobutanol does not, however, affect the stability of the lipid component of the tear film (48).

As compared with 0.004–0.02% BAK, 0.2–0.5% chlorobutanol is less toxic to rabbit corneal epithelial cells in vitro (51). In human corneal epithelial cells, the cytotoxic effects of chlorobutanol occur less rapidly than those of BAK, and the toxicity changes are less severe (50). Therefore, ophthalmic solutions preserved with chlorobutanol may be less damaging.

**Polyquaternium-1**

Polyquaternium-1 (Polyquad) is a polymeric quaternary ammonium antimicrobial preservative. It is used in contact lens solutions and the artificial tear product Tears Naturale II (Alcon Laboratories, Fort Worth, TX, USA). Polyquaternium-1 has been proven to have less of an effect on corneal epithelial cells than BAK. One rabbit study showed that polyquaternium-1 produces a lesser uptake of dye into the cornea than BAK and only superficial epithelial damage compared with BAK (52).

The main detriment associated with polyquaternium-1 is its tendency to reduce the density of conjunctival goblet cells, thereby decreasing aqueous tear film production (53).

**Sorbate (sorbic acid)**

Sorbate generally has limited antimicrobial activity, and it is not able to eradicate many organisms on its own. Sorbic acid molecules pass through the plasma membrane, dissociate in the cytoplasm, release protons, and inhibit growth via acidification. This may activate energetically inefficient intracellular activities, such as energy-dependent ion pumps, wasting the cell’s energy stores.

Whereas adverse reactions appear to be infrequent, a reaction consisting of punctate keratitis may rarely result from the use of sorbate. Still, sorbic acid–preserved products are commonly promoted for sensitive eyes and for contact lens wearers (54).

**Sodium perborate**

Sodium perborate (GenAquar) is a preservative contained in Genteal lubricant eye drops (Novartis Ophthalmics, East Hanover, NJ, USA). Sodium perborate was one of the first oxidative preservatives to be developed. It destroys numerous types of bacteria and can destroy Aspergillus niger, a fungus that is otherwise difficult to kill (55). Sodium perborate is typically found in lubricating drops used to combat dry eye. When sodium perborate is combined with water, it is converted to hydrogen peroxide, an effective antimicrobial agent.

Sodium perborate oxidizes cell walls or membranes, affects membrane-bound enzymes, and disrupts cellular function, such as protein synthesis. Once sodium perborate enters the eye, it is decomposed to water and oxygen by catalase and other enzymes present in the conjunctival sac (55). The hydrogen peroxide allows low levels of sodium perborate to be effective at destroying microbes while hydrogen peroxide as a byproduct also permits ocular comfort. However, hydrogen peroxide levels above 100 ppm or 3% can cause ocular stinging (56,57).

Although gentler than some other preservatives, sodium perborate may still cause ocular toxicity. Patients with dry eye may frequently instill a preserved solution, which increases the chance of aggravating the condition by disrupting the precorneal tear film (29). In limited testing, sodium perborate was found to be a direct-acting in vitro mutagen (58). It can also destabilize cell walls and membranes, but to a lesser degree than other types of preservatives because it is an oxidative preservative.

**Stabilized oxychloro complex**

SOC (Purite, Bio-Cide International Inc., Norman, OK, USA) was introduced into ophthalmic medicines in
the mid-1990s under the trade name Purite. SOC is an oxidative ophthalmic preservative that destroys many types of bacteria and can destroy the fungus Aspergillus niger (56). SOC consists of an equilibrium mixture of oxychloro species: 99.5% chloride (\(\text{ClO}_2\)), 0.5% chlorate (\(\text{ClO}_3\)), and trace amounts of chlorine dioxide (\(\text{ClO}_2\)), which have bactericidal, fungicidal, and virucidal activity (59). While the antimicrobial efficacy of SOC supports its use as a preservative agent, its mechanism of action is not fully elucidated. Most likely, the mechanism is due to the oxidation potential of chloride and possibly from the generation of chlorine dioxide in the presence of microbial acidic environments, which leads to the disruption of protein synthesis in the bacteria and ultimately its antimicrobial activity. SOC is capable of destroying microorganisms in fish, fruit, and vegetables without altering the nutritive and organoleptic properties of the food (59).

SOC dissipates by converting into components normally found in tears, such as sodium ions, chloride ions, oxygen, and water (56,59). This unique quality makes SOC an unusually gentle preservative that is ideal for chronic use. For this reason, products containing SOC may have an improved tolerability profile, reduced toxicity, and, ultimately, better patient compliance.

Although SOC is a chemical oxidant, there is no in vivo or in vitro evidence of its mutagenicity or carcinogenicity (60). Accordingly, SOC has mild cytotoxic effects and an excellent safety record. It has been given an Environmental Protection Agency Category II rating for its use as a preservative agent, its mechanism of action is not fully elucidated. Most likely, the mechanism is due to the oxidation potential of chloride and possibly from the generation of chlorine dioxide in the presence of microbial acidic environments, which leads to the disruption of protein synthesis in the bacteria and ultimately its antimicrobial activity. SOC is capable of destroying microorganisms in fish, fruit, and vegetables without altering the nutritive and organoleptic properties of the food (59).

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None of the currently available glaucoma medications contains SOC. However, the safety profile of SOC makes it likely that novel ophthalmic products containing SOC will be developed. On March 19, 2001, the FDA approved brimonidine tartrate ophthalmic solution 0.15%, preserved with SOC. This is the first glaucoma medication to contain SOC.

A number of other preservatives have also been investigated for use in eye drops and are discussed in Table 3 (46).

### Comparing safety/toxicity of preservatives

A direct comparison between preservatives can be seen via scanning electron microscopy in a 2001 study conducted by Allergan (62). To evaluate toxicity, rabbits were treated 4 times a day with products preserved with 0.001% polyquaternium-1, sodium perborate, or 0.005% SOC, and the corneas were examined after 1 week of treatment (62). The untreated rabbit corneal epithelium had extensive microvilli and tight intercellular junctions. The rabbit eye treated with the product preserved with SOC looked nearly identical to the untreated rabbit eye, and the eye treated with the perborate-preserved product also had a mostly normal epithelium with extensive microvilli and tight epithelial junctions.

### Table 3. Obsolete preservatives and reasons for their discontinuationa.

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<th>Sr. No.</th>
<th>Preservative</th>
<th>Properties</th>
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• Slow antibacterial activity, particularly against *Pseudomonas aeruginosa*.  
• Irritant to the eye. |
| 2.      | Chlorocresol (0.05% w/v) | • Replaced solution for eye drops in the 1963 British Pharmaceutical Codex, but reports of eye damage led to its deletion within a few months.  
• It is a rapidly acting bactericide and fungicide, but a 0.05% solution is no more than marginally effective against *Pseudomonas aeruginosa*.  
• Its efficiency is increased in acid solution.  
• It is more irritating than most preservatives. |
| 3.      | Thiomersal (0.01% w/v) | • Its stability is low in acid solutions, and it is not widely used in eye drops.  
• Its antibacterial activity is slow, and its absorption by rubber is very high.  
• Less stable in solution (protection from light and heavy metals is necessary) and does not cause mercurialnitis.  
• Highly toxic to corneal and conjunctival cells.‌² |
| 4.      | Phenylethyl alcohol (0.5% w/v) | • Particularly effective against gram-negative bacteria, its action on *Pseudomonas aeruginosa* is rather slow.  
• Recommended as an eye drop preservative in the U.S. Pharmacopeia.  
• Often it is combined with another bactericide, such as benzalkonium chloride, phenylmercuric nitrate, or chlorbutol, to confer enhanced activity against gram-negative organisms.  
• The solubility of chlorbutol is improved when used with phenylethyl alcohol. |

while the same workers reported that the rabbit eye treated with polyquaternium-1-preserved product had extensive superficial epithelial erosion and lack of protruding microvilli. This finding is in agreement with another study that found that 0.001% polyquaternium-1 caused a small but significant degree of epithelial damage (52). However, another study has shown that 0.001% polyquaternium-1 has a low potential for ocular irritation (63). As compared with the untreated eye, damage to the corneal epithelium was highest with polyquaternium-1, followed by the sodium perborate product, and then by the SOC-preserved product. Of the artificial tears compared in this study, the product with SOC caused the least damage to corneal epithelial cells (62).

Figure 1. Toxicity profile of benzalkonium chloride (BAK) and sodium perborate.

Sustained-release formulations

One approach is to decrease the cumulative toxic effects, hence a once-daily form of timolol maleate (Timoptic-XE) with 0.012% BDD, a preservative similar to BAK, was developed. In place of bromide, the benzododecinium cation may be used with chloride or another anion. Chemically BDD is dimethyldodecylbenzylammonium bromide and, like BAK, is a quaternary ammonium compound having a property of cationic surfactant and is primarily active against gram-positive microbes. However, the gel-forming preparation may prolong contact of the preservative with the eye, contributing to greater toxic effects to the cornea. In this regard, it may be noted that BDD, a preservative related to BAK, has been shown to induce corneal epithelial damage when incorporated into a gel (35). However, the concept of using sustained release formulations may be extended to newer and safer preservatives.

Safe, mild, and less-toxic preservatives

All of the original formulations of the glaucoma medications commonly used today contain BAK. Several agents available in the United States have been reformulated without BAK, like SOC (Purite)-preserved brimonidine tartrate (Alphagan P). These formulations appear to be benign to the ocular surface. However, the efficacy of the brimonidine class of antiglaucoma drugs is less than that of prostaglandins, which are probably the first-line monotherapy today; hence, it is important to reformulate these agents as well.

The preservative sofZia (Alcon) is a relatively new, proprietary, ionic, buffered solution consisting of zinc, borate, propylene glycol, and sorbitol—chemical entities that are themselves not significantly toxic to the ocular surface. sofZia, however, maintains an antimicrobial environment in the bottle, such that it meets the U.S. Pharmacopeia standards for antimicrobial activity. It produces more than a 3-log reduction (99.9% kill) in surviving organisms after 8 days of incubation (65). Recently, sofZia-preserved travoprost (Travatan Z, Alcon) was introduced into the American market. This formulation appears to have the same IOP-lowering efficacy as BAK-preserved travoprost in controlled clinical trials (66). Arguably, this formulation is the most potent non-BAK-containing glaucoma eye drop currently available.

Preclinical studies comparing sofZia-preserved travoprost and latanoprost, which contains the highest amount of BAK, demonstrated the relative safety to the ocular surface of the non-BAK formulation (67). Cell culture models and confocal studies of rabbit corneas have shown that BAK-containing latanoprost caused significant corneal epithelial cell death and loss with
prolonged exposure times, whereas BAK-free travoprost was fairly benign to these surface cells. Ongoing studies with more chronic dosing seem to confirm these effects (68).

Travatan Z was introduced as the first prostaglandin analogue to be preserved with a substance other than BAK. The sofZia system effectively preserves the medicine while it is being stored; however, when the drug is introduced into the eye, it is modified into harmless elements that are gentle on the ocular surface (64).

Nonpreserved formulations
Preservative-free medications may eliminate the risk of toxic side effects, which can make them attractive treatment options. For example, in a 1992 study conducted to assess the corneal epithelial toxic effects of preservative-free tear preparations, researchers used scanning electron microscopy to evaluate the corneal epithelium of rabbit eyes after the administration of 2 preservative-free ocular lubricants. The 2 preservative-free preparations were shown to be nontoxic to the corneal epithelium in both the mild- and exaggerated-use protocols. The epithelial changes observed were no different from those seen in control eyes. The study supported the belief that preservative-free preparations are safe to use in patients, especially with frequent dosing (20).

It has been reported by Pisella et al. (35) that using unpreserved timolol may be beneficial for the long-term treatment of glaucomatous patients because it increases tear film stability and decreases epithelial permeability and stromal aggression of the cornea. Baudouin and de Lunardo (31) confirmed that carteolol is well tolerated, either with or without preservative. The preservative-free group showed better stability of the tear film, without loss of effect on IOP. Berdy et al. reported that with frequent-dosage regimens, preservative-free Hypo Tears PF and various forms of Refresh formulation(s) by Allergan (Table 2) are free of the toxic effects associated with preserved solutions (29).

Nonpreserved artificial tears have an extra advantage over preserved ones: they may be the best choice for patients immediately following eye surgery. Some nonpreserved artificial tears include GenTeal, TheraTears, Refresh Tears, Moisture Eyes, and Bion Tears. These are the most highly preferred ones because they contain either no or mild and less-toxic preservatives like SOC (Purite) and sodium perborate. Table 2 lists some of the most common over-the-counter medications for dry eye along with the preservative used.

Nonpreserved drugs are available only in unit-dose vials and, as a result, the use of unit-dose bottles that do not require preservatives will increase and become more cost-effective while newer technologies will enable multidose bottles to be constructed to inhibit microbial invasion through inherent material properties independent of traditional preservatives (41). However, it may be more difficult for a patient to use a unit-dose vial correctly, affecting compliance. Poor compliance may hinder a nonpreserved drug’s effectiveness, even if it is more comfortable to use. This can be especially important when it is used concomitantly with multidose glaucoma medications, for which compliance is vital. Unit-dose vials are also more expensive than multidose containers. In addition, patients with advanced rheumatoid arthritis may find it difficult to squeeze the drops from the single-use vials. They may be tempted to use the multidose vials. While it is true that a preservative may be the cause of an allergic reaction, it is difficult to determine if the problem is caused by the preservative, the drug, the drug delivery system, the buffers, or the stabilizers. Hence, nonpreserved preparations still hold some risk.

Conclusions
At present, despite the numerous antiglaucoma medications and artificial tears in the market, none is risk-free. In the future, manufacturers may reformulate existing products with less-toxic preservatives, offer less-concentrated forms of current preservatives, or develop new ones.

Acknowledgments

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References
13. Kuppens EV, De Jong CA, Stolwijk TR, De Kreizer RJ, Van Best JA. Comparison of toxicological profiles of preserved and unpreserved for-


67. Yee RW, Norcom EG, Zhao XC. Comparison of the relative toxicity of travoprost 0.004% without BAK and latanoprost 0.005% in an immortalized human cornea epithelial cell culture system. Adv Ther 2006; 23:511–518.