Evolution of Preservatives in Topical Ophthalmic Medications

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Preservatives are an important component of ophthalmic preparations, providing antimicrobial activity in the bottle and preventing decomposition of active drug. Often under recognized, however, are the significant cytotoxic effects of preservatives associated with long-term therapy and especially use of multiple preserved drugs. The most common preservatives in ophthalmic preparations for glaucoma and surface eye disease-benzalkonium chloride (BAK), chlorobutanol, sodium perborate, and stabilized oxychloro complex (SOC)-were reviewed. Compared with other preservatives, SOC caused the least amount of damage to rabbit corneal epithelial cells. BAK has demonstrated cytotoxic effects in cell culture, as well as in animal and human studies. Physicians should consider treatment with new-generation preparations containing low-risk preservatives such as SOC, especially in patients receiving multiple ophthalmic medications.

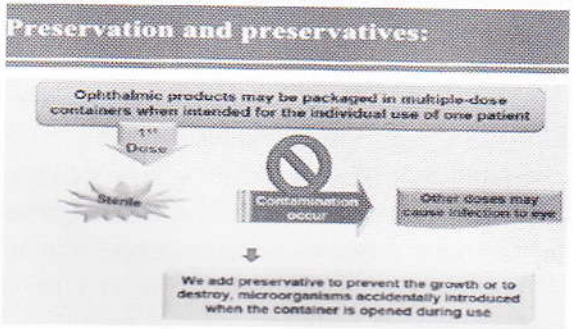

Fig. 1 Role of preservative

Modes of action of preservatives

Preservatives generally offer limited protection against viral contamination. Bactericides and fungicides may evince their effects on a variety of microbial cellular targets, for example; the cell wall, the cytoplasmic membrane or the cytoplasm. It is often difficult to assign a precise target for a specific class of preservative; the target can and does change with preservative concentration. As a consequence, preservatives can often interfere with several different microbial cellular mechanisms (Table 1).

Cell Wall	Cytoplasmic membrane	Cytoplasm
Phenols	2-Phenoxyethanol	2-Phenoxyethanol and other organic alcohols
Aryl and alkyl acids	Parabens	Aryl and alkyl acids
Organo mercurials	Organo mercurials	Halogenated preservatives
EDTA (edetic acid)	EDTA	
Chlorhexidine, cetrimide	Chlorhexidine, hexachlorophene	Chlorhexidine (high concentrations)
Glutaraldehyde	Formaldehyde donators e.g. bronopol, imidurea	Formaldehyde donators e.g. bronopol, imidurea
Anionic surfactants	Benzalkonium chloride (BKC)	

Table 1 - Site of preservative activity in microbial cell

Such cytotoxicity may also affect mammalian cells. Hence inclusion levels should be minimal, consistent with adequate preservation. There is a regulatory expectation that the reason for preservative inclusion, proof of efficacy, safety information, control methods in finished product and details of labelling in the finished product should all be addressed by the applicant [11]. Mechanisms for activity at the locations listed in Table 1.

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Performance Requirements for Preservatives	
Property	**Performance Requirement**
Antimicrobial activity	Active against bacteria (Gram +ve/Gram –ve), molds, yeasts and fungi at low inclusion levels
Aqueous solubility	Solubility exceeds minimum inhibitory concentrations (MIC) over anticipated product pH range
Partitioning Behavior	Remains essentially in the continuous aqueous phase in multi-phase products
Stability properties	Chemically and physically stable during manufacture and at end of product shelf-life.
Non-irritant properties	Non-irritant at concentration used in product, especially germane for treatment of sensitive mucosal membranes, e.g. nose, eye, etc
Organoleptic properties	Odor and taste acceptable where product is administered orally, intranasally or by inhalation (the latter two routes of administration still have a significant 'swallowed' fraction)
Compatibility properties	Does not react or reacts minimally with other product components, including the proposed container closure.

Table 3 Performance required for preservatives

Classification of Preservatives

Historically, preservatives have been classified into two categories: detergent and oxidizing preservatives. More recently, a newer system of preservation, ionic-buffered preservatives (acting as oxidizing preservatives), has been introduced; their methods of action and examples of each different type are described later.

Detergent Preservatives

Detergents are compounds that cause bacterial cell death by way of interrupting the lipid component of cell membranes. The contents of the microbial cell are extruded from the cell due to membrane instability. As described earlier, detergents have the longest running history in ophthalmic medicine. Examples of detergent-type preservatives include benzalkonium chloride (BAK) and cetrimonium.[3]

Oxidizing Preservatives

Oxidative preservatives alter the lipid membrane of microbes in a different fashion to detergent preservatives, by penetrating the membrane and altering the DNA, protein and lipid components of bacterial cells.[4] Oxidizing preservatives are considered second-generation ophthalmic preservatives and were developed because of their reduced toxicity to human ocular surface cells in comparison with detergent preservatives. Although ocular surface cells may still be injured by oxidative preservatives, the low concentrations contained in ophthalmic preparations deem these effects insignificant.[4]Noecker *et al.* reported that medications preserved with Purite® induce less corneal toxicity than those preserved with BAK.[5]Examples of oxidizing preservatives include sodium perborate and stabilized oxychloro complex (SOC).

Recently Introduced Ionic-buffered Preservatives

Ionic-buffering systems are the latest class of ophthalmic preservatives to be incorporated into topical medicines and act in a similar manner to oxidizing preservatives within multidose bottles. SofZia (Alcon, TX, USA), the most recent preservative of this kind, is a combination of boric acid, zinc, sorbitol and propylene glycol. This ionic-buffered system has been shown to have both antibacterial and antifungal qualities.[6] When exposed to cations, such as those that are normally encountered in the tear film of the eye, the substance is deemed inactive. This is thought to induce less cytotoxicity to the ocular surface compared with more conventional preservatives.

Historical & Practical Review of Benzalkonium Chloride

Benzalkonium chloride is a detergent and quaternary ammonium compound with a broad range of antimicrobial activity. It was first introduced as a germicide in the 1910s and became more widely used in the 1940s.[7] In the ophthalmic industry, BAK was first used in the 1940s as a means to preserve hard contact lens solutions. Since then, BAK has been used in nearly all classes of ophthalmic solutions, from antiglaucoma medicines to over-the-counter artificial tear solutions.

Benzalkonium chloride is the most frequently used preservative in ophthalmic solutions today, [8] and its concentration in glaucoma formulations ranges from 0.004 to 0.02%. The reasons for the frequent use of BAK as a preservative includes its extreme efficacy in combating microbial contamination of bottles, 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 familiarity among those formulating ophthalmic preparations in industry. While the efficacy of BAK is well known, there is a multitude of published studies that document the detrimental effects of BAK. [913] 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.[10] However, human ocular surface cells can also absorb this detergent, and effects on ocular surface cells are similar to those seen in bacterial cells. The effects of the detergent are cumulative and become more severe with more concentrated and frequent exposures.[10] Breakdown of the corneal epithelium and increased permeability of the cornea as a result of BAK toxicity is well documented.[11] Higher concentrations of BAK (as can be induced through repeated exposure and subsequent accumulation of BAK in ocular surface tissues) can reduce tear break-up time by causing disruption of the lipid component of the tear film and hence causes tear-film instability.[12] This is especially problematic in glaucoma patients, as they inherently have a decreased rate of basal tear turnover.[14] 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.[13]

Evolution of Preservatives sinceBenzalkonium Chloride

Cetrimonium is a detergent-type preservative. Its ophthalmic uses have included preservation of artificial tear preparations such as Civigel (Ciba Vision Ophthalmics, GA, USA) .Cetrimonium causes keratinisation and inflammatory infiltrates at the limbus and within the conjunctivalstroma and epithelium.[3] Its corneo–conjunctival cell toxicity has been deemed similar to BAK. Owing to its antiseptic and cationic surfactant qualities, cetrimonium is used mostly as a softening agent in hair treatments. It is also used as a fermentation aid, a dispersant and in preservation of antifungal creams.

Chlorobutanol

Chlorobutanol is a detergent preservative that was formerly used as an active ingredient in hypnotic and sedative agents.[9] Chlorobutanol has been used as a preservative agent in artificial tears, where it has been documented to cause significant keratitis and irritation to the ocular surface.[15] While it damages the ocular surface cells, the toxic effects take longer to manifest in human corneas than do the effects of BAK.[16] Human corneal epithelial cells exposed to chlorobutanol display a decreased amount of mitoses and deterioration of overall cell integrity.[16] Chlorobutanol does not, however, affect the stability of the lipid component of the tear film.[17]

Although the antimicrobial activity of chlorobutanol is extensive, [18] its use has been limited due to the fact that it becomes unstable when stored at room temperature for extended periods of time. Unlike BAK, chlorobutanol does not act like a surfactant.[17] The method of action of chlorobutanol is cell lysis by way of disruption of microbial cell membrane lipid configuration.[17]

Edetate Disodium

Edetate disodium (EDTA) is a chelating agent used in a variety of nonophthalmic products, including hair conditioner, facial cleansers, aftershaves and deodorants. In the recycling industry, it has been used to recover lead from used lead-acid batteries. In the medical field, uses include the treatment of acute mercury poisoning, lead poisoning and hypercalcemia. EDTA has gained use in ophthalmic solutions owing to its ability to bind metals. Therapeutically, EDTA has been used to remove calcified plaques that occur in the superficial cornea in band keratopathy.[19] EDTA has also been used in eye washes to aid in neutralization of calcium hydroxide or lime burns to the cornea.

Edetate disodium also has preservative effects based on its ability to chelate. When added to topical medicines in low concentrations, EDTA has been shown to inactivate trace amounts of heavy metals, which aids in the preservation of the solution.[18] Ophthalmic solutions that have employed EDTA include Acular® (ketorolac tromethamine ophthalmic solution) (figure 2) and Betagan® (levobunolol hydrochloride ophthalmic solution USP).

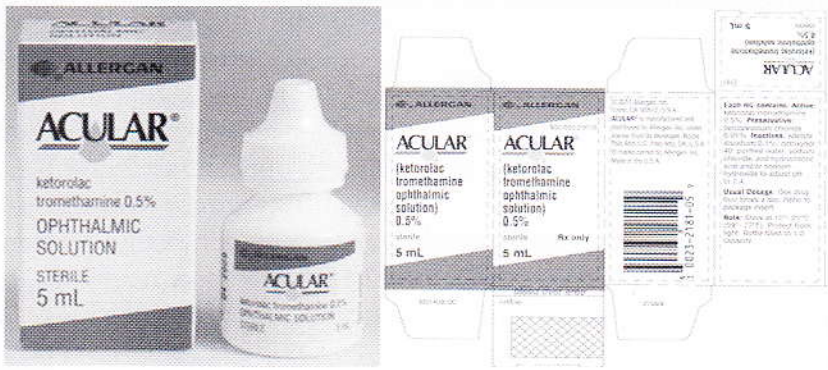

Fig. 2Acular® (ketorolac tromethamine ophthalmic solution)

Polyquaternium-1 (Polyquad®)

Polyquad® is a detergent-type preservative derived from BAK. Polyquad was formulated in the mid 1980s by Alcon as a preservative for contact lens storage solutions. It was developed because other preservatives (e.g., BAK) were known to become concentrated in contact lenses that had been stored in conventional lens solutions. When placed in an aqueous ocular environment, the contaminated contact lens can act as a reservoir of preservative that can later be released. Polyquad does not become concentrated in contact lenses.

Although it is a detergent, Polyquad has unique properties distinguishing it from BAK. Bacterial cells attract Polyquad, yet human corneal epithelial cells tend to repel the compound.[20] Polyquad is the main ingredient in Tears Naturale II (Alcon) (fig. 3) and Opti-Free Express MultiPurpose Disinfecting Solution (Alcon), as well as other storage solutions for contact lenses.

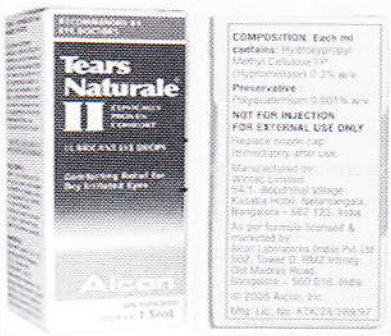

Fig. 3Tears Naturale II (Alcon)

While Polyquad has been shown to be much less toxic to the corneo–conjunctival surface than BAK, [21]it has been shown to cause superficial epithelial damage to the cornea.[22] The main detriment associated with polyquaternium-1 is its tendency to reduce the density of conjunctival goblet cells, thereby decreasing aqueous tear film production.[21]

PolyhexamethyleneBiguanide

Polyhexamethylenebiguanide (PHMB) has been used in contact lens solutions such as ReNu® (Bausch & Lomb, NY, USA) (Fig.4). The benefits of PHMB against *Acanthamoeba* and bacteria are well known.[23] PHMB has been shown to be nonirritating to human corneal cells; however, its antifungal activity is limited.[18] PHMB employs its microbial activity by integrating into bacterial cell walls, thereby disrupting its membrane and has been shown to lethally alter the transcription of bacterial DNA.[24]

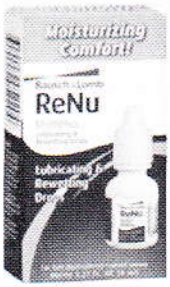

Fig. 4ReNu® (Bausch & Lomb, NY, USA)

Stabilized Oxychloro Complex

Stabilized oxychloro complex (Purite, Bio-Cide International Inc., OK, USA) is an oxidative-type preservative and was introduced into ophthalmic medicines in the mid 1990s under the trade name Purite. One of its derivatives, sodium chlorite, has been used in water purification systems since the 1940s.[25] Purite has become a component of several different types of artificial tear and antiglaucomapreparations, including brimonidine tartrate ophthalmic solution (Brimodin-P, Cipla) (fig. 5) and Refresh Tears (Allergan).

Fig. 5Brimodin-P

Stabilized oxychloro complex has been shown to be well tolerated by the ocular surface.[25] Even at very low concentrations of SOC (0.005%), the antimicrobial activity is broad.[4] This was substantiated during a trial in which SOC was administered to patients up to eight-times daily.[25] SOC has been shown to lack cytotoxicity *in vivo*; however, more studies are needed to assess ocular side effects independent of active ingredients.[26] The antimicrobial effects are broad and include antibacterial, antifungal and antiviral

effects. Chemically, SOC is a mixture of chlorine dioxide, chlorite and chlorate.[4] When exposed to light, SOC dissociates into water, oxygen, sodium and chlorine free radicals.[27] The chlorine free radicals are thought to inhibit microorganism protein synthesis within cells by way of glutathione oxidation, which causes microbe cell death.[101]

Sodium Perborate (GenAqua™)

GenAqua™ is a preservative composed of sodium perborate and is contained in Genteal lubricant eye drops (Novartis Ophthalmics, NJ, USA) (fig. 6).

Sodium perborate is an oxidative preservative that has been used in dental hygiene solutions since the 1950s. When it was introduced in ophthalmic solutions, it was one of the first of the oxidative-type preservatives used. Sodium perborate alters protein synthesis within bacterial cells by oxidizing cell membranes and altering membrane-bound enzymes, causing enzymatic inhibition. Upon exposure to an aqueous environment, it is catalyzed into hydrogen peroxide, water and oxygen. This is a property exclusive to this compound. The hydrogen peroxide formed by this reaction effectively kills microbes.[26] Furthermore, the efficacy of GenAqua has been demonstrated onAspergillusniger.[26] There are few studies documenting the ocular tolerability and side-effect profile of GenAqua.

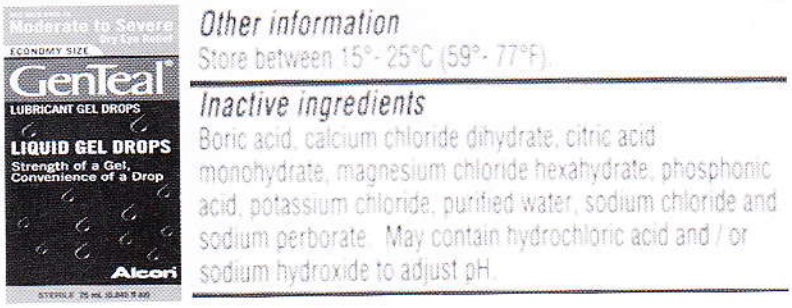

Fig. 6Genteal lubricant eye drops

SofZia™

SofZia™ is the most recent advancement in the field of ophthalmic preservatives and is the preservative system contained in one preparation of travoprost (Travatan Z®, Alcon, Texas). When exposed to cations, such as those that are normally encountered in the tear film of the eye, sofZia is deemed inactive. This is thought to induce less cytotoxicity to the ocular surface compared with more conventional preservatives.

Travatan Z (fig. 7) 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.[6] It has been demonstrated that sofZia-preserved travoprost induces corneal and conjunctival changes similar to preservative-free artificial tears. Furthermore, travoprost with sofZia also induced reduced amounts of conjunctival inflammation and corneal changes when compared with travoprost treated with BAK.[28]

Fig. 7Travatan Z

Preservative	Class	Advantages	Disadvantages	Medication examples
SofZia®	Oxidative	Modified into harmless elements upon instillation; smaller amounts of conjunctivo–corneal inflammation compared with BAK	Newer agent requiring more studies to understand ocular safety profile of the preservative independent of active ingredients	Travatan Z®
Sodium perborate (GenAqua®)	Oxidative	Catalyzed into hydrogen peroxide, water and oxygen upon instillation; activity against *Aspergillus*; less toxicity than BAK	Few studies documenting ocular tolerability and side-effect profile	Genteal®
Stabilized oxychloro complex (SOC/Purite®)	Oxidative	Dissociates into water, oxygen, sodium and chlorine free radicals	As with sofZia, more studies are needed to assess ocular side effects independent of active ingredients	Alphagan-P®, Refresh Tears®
Polyquaternium-1 (Polyquad®)	Detergent	Less toxicity to corneo–conjunctival surface than BAK	Superficial corneal epithelial damage reduces density of conjunctival goblet cells	Tears Naturale II®, Opti-Free® Express Disinfecting Solution
Chlorobutanol	Detergent	Toxic effects take longer to manifest than BAK; doesn't affect stability of lipid component of tear film; extensive antimicrobial activity	Causes keratitis and irritation to ocular surface; decreased amount of mitoses to corneal epithelial cells; unstable when stored at room temperature	TobraDex® Ointment
Cetrimonium chloride	Detergent	Excellent antiseptic qualities	Causes keratinization and inflammatory infiltrates at the limbus and within the conjunctival stroma and epithelium	Civigel®
Benzalkonium chloride	Detergent	Excellent antimicrobial efficacy; disruption of corneal cell–cell junctions allow medicinal entry to anterior chamber; well-established familiarity in industry	Breakdown of corneal epithelium; apoptosis of ocular surface cells; accumulation in surface tissues; tear-film instability	Timoptic®, Azopt, Lumigan®, Xalatan
Edetate disodium	Chelating agent	Inactivates trace amounts of heavy metals	Few studies documenting chronic side effects	Acular®, Betagan®

BAK: Benzalkonium chloride.

Table 3 Details of different kind of preservatives

Effect of preservatives on ocular surface

Ocular surface disease, OSD, (which includes dry eye syndrome) can cause redness, tearing, irritation, burning, foreign body sensation, light sensitivity and intermittent blurred vision. Although 15% of elderly patients describe some degree of OSD, up to 60% of patients with glaucoma suffer from it.

The symptoms mentioned above affect the quality of life and even the adherence to the prescribed medications to help preserve existing vision. The active ingredient of a medication, the component that is used to lower the eye pressure, can cause OSD. It is also known that inactive ingredients, such as preservatives, can contribute to OSD. Ocular surface changes, causing ocular discomfort, tear film instability, conjunctival inflammation, subconjunctival fibrosis, epithelial apoptosis, corneal surface impairment, and the potential risk of failure for further glaucoma surgery. Subclinical inflammation has also been described in patients receiving antiglaucoma treatments for long periods of time. However, the mechanisms involved, i.e., allergic, toxic, or inflammatory, as well as the respective roles of the active compound and the preservative in inducing the toxic and/or proinflammatory effects of ophthalmic solutions, is still under study.

Furthermore, the negative effects of preservatives seem to be additive. The more medications a patient takes the more likely symptoms. Preservatives were initially used to kill bacteria in the bottle and it was thought they helped the active ingredient have its desired effect. Since the active ingredient is the component of the medication that lowers the eye pressure, any negative effect it may have is thought to be a necessary evil. For patients with OSD, difficulties tolerating eye medications can possibly be improved by minimizing the effect of preservatives on the ocular surface.

Fig. 8Lissamine green (LG) staining of the conjunctiva in a patient with mild dry eye. LG is a valuable vital dye to use because it is very sensitive and highlights even early devitalisation of conjunctival epithelium

Fig. 9Fluorescein staining of a cornea in a patient with moderate dry eye. Broken tear film over the central cornea (decreased tear film break-up time) and fluorescein staining of the inferior cornea. Fluorescein stains epithelial cells in more advanced disease, as well as absent areas(erosions) on the corneal surface

Fig. 10Fibrosed bleb as result of preservatives in antiglaucoma medications as it lead to increased levels of extracellular matrix (ECM), the transforming growth factor β (TGF-β) signaling pathway-related molecules, and cyclooxygenase-2 (COX-2) in bulbar conjunctivaltissuesand results in failed filtration surgery

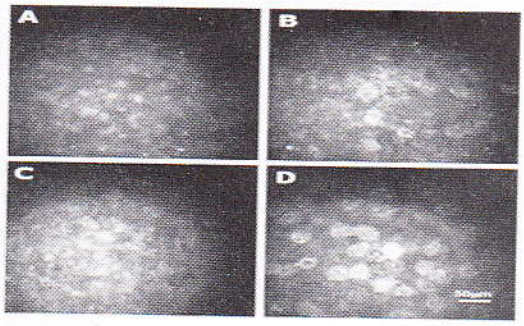

Fig. 11 Toxic effect of BAC on corneal epithelial superficial cells.Representative in vivo confocal images of the corneal epithelium in different groups. (A) Untreated control. (B) 0.01% BAC. (C) 0.05% BAC. (D) 0.1% BAC. Mean cell size at the epithelial surface was shown in (E). Note that the size of surface cells in the corneal epithelium of eyes treated with 0.1% BAC was significantly larger than that of control eyes

CONCLUSION

The most frequently used preservative, benzalkonium chloride (BAK), has consistently demonstrated its toxic effects in laboratory, experimental, and clinical studies. As a quaternary ammonium, this compound has been shown to cause tear film instability, loss of goblet cells, conjunctival squamous metaplasia and apoptosis, disruption of the corneal epithelium barrier, and damage to deeper ocular tissues. The mechanisms causing these effects have not been fully elucidated, although the involvement of immunoinflammatory reactions with the release of proinflammatory cytokines, apoptosis, oxidative stress, as well as direct interactions with the lipid components of the tear film and cell membranes have been well established. Preservative-induced adverse effects are therefore far from being restricted to only allergic reactions, and side effects are often very difficult to identify because they mostly occur in a delayed or poorly specific manner. Care should therefore be taken to avoid the long-term use of preservatives; otherwise a less toxic alternative to BAK should be developed, as this weakly allergenic but highly toxic compound exerts dose- and time-dependent effects. On the basis of all these experimental and clinical reports, it would be advisable to use benzalkonium-free solutions whenever possible, especially in patients with the greatest exposure to high doses or prolonged treatments, in those suffering from preexisting or concomitant ocular surface diseases, and those experiencing side effects related to the ocular surface. Indeed, mild symptoms should not be underestimated, neglected, or denied, because they may very well be the apparent manifestations of more severe, potentially threatening subclinical reactions that may later cause major concerns.

Preservative-free approaches are still in their infancy and much more research is required before they can be considered on an equal footing with preserved approaches. However, several preservative-free ophthalmic devices are available and do offer some promise.

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