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Expert Opinion

Ophthalmic preservatives: focus on polyquaternium-1

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Introduction: Ophthalmic preservatives, such as polyquaternium-1 (PQ-1), are critical for the inhibition of growth of microbial contaminants in multi-dose bottles of topical medications. These antimicrobial agents must have a high efficacy against pathogenic organisms, while maintaining a favorable tolerability and safety profile.

Areas covered: This review focuses on the ophthalmic preservative PQ-1. For comparison purposes, the most commonly used preservative, benzalkonium chloride (BAK), is also discussed. This survey focuses primarily on data collected during the past 10 years.

Expert opinion: Effective drug delivery requires more than just an active ingredient that achieves its desired biological effect on end-target tissues. In addition, drugs must be stable in the containers that they are stored in, and must possess minimal undesired local and systemic side effects that can cause patients to decrease their adherence. In addressing these concerns, specifically in topical ophthalmic drops, one must take into account the active ingredients, vehicle components and preservatives. Medications with fewer adverse effects may lead to enhanced adherence to therapy; therefore, the induction of such adverse outcomes must be considered by physicians when treating patients with chronic ocular disease. Although BAK will continue to be used in ophthalmic medications, due to its familiarity and compatibility with a broad range of topical ocular formulations, PQ-1 is certainly a viable alternative in the preservative formulary armamentarium.

Keywords: benzalkonium chloride, cytotoxicity, dry eye, glaucoma, multi-purpose disinfecting solution, polyquaternium-1, preservative

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1. Introduction

Preservatives contained in ophthalmic formulations are used to inhibit the growth of microorganisms that may be introduced inadvertently into multiuse containers [1,2]. These agents must possess antimicrobial activity and also be safe for use on the ocular surface. The main purpose of this article is to provide a survey of the current data on the preservative polyquaternium-1 (Polyquad®, PQ-1, Alcon Laboratories, Inc., Fort Worth, TX, USA). Benzalkonium chloride (BAK), the most commonly used ophthalmic preservative [3,] is also be discussed as providing a basis for comparison in terms of antimicrobial efficacy, mechanism of action, toxicity and tolerability (i.e., ocular adverse effect profile). Some other ocular preservatives, comparators in studies with PQ-1 and BAK, are also included in this survey.

Over the past several decades, a wide array of ocular preservatives has been used for maintaining the sterility of multi-dose bottles [4]. These compounds have differing efficacy profiles against microorganisms and degrees of toxic effects on ocular cells and tissues [2,3,5]. Ophthalmic preservatives have historically been classified based on their surfactant or oxidizing properties [1,6]. The earlier generation preservatives

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Article highlights.

- Benzalkonium chloride (BAK) is a quaternary ammonium compound that is the most commonly used preservative in topical ophthalmic preparations.
- This compound has a broad spectrum of activity against bacteria and fungi; however, BAK may be more likely to have toxic effects on the ocular surface compared to newer preservatives.
- BAK has detergent properties which cause damage to bacterial and fungal cell walls and membranes. The compound is known to disrupt intercellular tight junctions in the corneal epithelial layer.
- Chronic dosing of topical medications in diseases such as dry eye and glaucoma can exacerbate the deleterious effects of BAK on the surface of the eye. This long-term use of BAK can have a negative effect on the ocular tolerability of a topical regimen in some patients.
- The induction of adverse effects must be considered when improving drug delivery and the physicians' ability to treat patients who suffer from chronic ocular disease.
- Polyquaternium-1 (PQ-1, Polyquad), a polycationic preservative, has been developed as an alternative to BAK in an attempt to enhance the tolerability of topical ophthalmic drops.
- PQ-1 acts by disrupting the bacterial cell membrane, which causes leakage of the cytoplasmic contents. The compound is thought to be too large to enter mammalian cells.
- Nonclinical studies have shown that PQ-1 possesses excellent antimicrobial efficacy while maintaining a low toxicity profile compared to BAK.
- PQ-1 is compatible with a variety of buffering systems and active ingredients across several classes of therapeutic agents such as contact lens disinfecting solutions, dry eye products, antibiotics and intraocular pressure-lowering medications.
- Although BAK will continue to be used in ophthalmic medications, due to its familiarity and compatibility with a broad range of topical ocular formulations, PQ-1 is certainly a viable alternative in the preservative formulary armamentarium.

This box summarizes key points contained in the article.

included BAK, which has been in use in the ophthalmic products since the 1940s, and the mercury-containing compounds, phenylmercuric salts (nitrate/acetate/borate) and thiomersal (THI). BAK is widely used as an ophthalmic preservative due to its broad spectrum of antimicrobial efficacy, its ease of use and compatibility with most formulations, and its familiarity within the pharmaceutical industry [4]. THI is no longer used as a preservative due to the potential toxicity of mercury and also due to environmental considerations. Other agents that have been used as preservatives of ophthalmic drops and multipurpose contact lens solutions include alexidine hydrochloride, chlorobutanol, sorbic acid, sodium perborate, methyl paraben, benzododecinium bromide (BDD), cetrimide, EDTA, phenylmercuric nitrate, methyl parahydroxybenzoate, chlorhexidine acetate, chlorhexidine digluconate, myristamidopropyl dimethylamine, phenoxyethanol, phenyethyl alcohol and polyhexamethylene biguanide (PHMB). Although all of these preservatives have shown efficacy against microorganisms, they may induce toxic effects on the cornea and ocular tissues [2,3]. These deleterious effects tend to occur particularly after long-term topical dosing for the treatment of chronic disease states such as dry eye and glaucoma.

Only a small number of preservatives are currently included in topical intraocular pressure (IOP)-lowering formulations. The most common preservative contained in these medications is BAK [3,6], which is known to cause toxic effects on chronic dosing [7]. In 2001, Novack and Evans published a report in which 89% of the ocular hypotensive drops marketed in the US contained BAK as their primary preservative [8]. At that time, the authors reported that the only other preservative included in these topical IOP-lowering formulations was BDD. There has been a recent trend toward the development of alternative preservatives that are more gentle to the ocular surface [2,3]. The advancement in preservative technology is especially important for products that are used on a routine basis over an extended period of time. These newer preservatives have been formulated in such products as multipurpose contact lens solutions, artificial tears and, more recently, topical IOP-lowering formulations. Purite® (chlorine dioxide, Allergan, Inc., Irvine, CA, USA) is a stabilized oxychloro complex (SOC) that is used in formulations of the IOP-lowering agent, brimonidine [2]. SofZia® (Alcon Laboratories), used in a formulation of the IOP-lowering agent travoprost, is a preservative system that comprises sorbitol, boric acid, propylene glycol and zinc chloride [6,9]. By 2007, Yee noted that SofZia and Purite had been added to the list of available antimicrobial agents included in IOP-lowering medications [3]. The antimicrobial agent PQ-1 is a polycationic polymer that was first introduced in the 1980s for use in contact lens solutions and later in artificial tear products [6,10]. More recently, PQ-1 has also been included in formulations of three ocular hypotensive agents: travoprost, travoprost–timolol fixed combination outside the US and brimonidine in the US [6,10-12]. PQ-1 was first developed because other preservatives were found to be concentrated in contact lenses on storage in conventional solutions [1]. In addition, contact lens solutions containing hydrogen peroxide generally required neutralization before the lenses could be placed in the eye, which made these solutions less convenient for the patient. PQ-1 possesses sufficient activity against microorganisms to serve as a disinfectant in contact lens storage solutions.

2. Mechanisms of action

The mechanisms of action for the antimicrobial effects of BAK are well known. The compound is a quaternary ammonium that has surfactant properties (**Figure 1**) [1,3,6]. These detergent characteristics, the result of hydrophobic domains within the molecule, convey the ability to disrupt bacterial cell walls. The antibacterial activity of BAK is

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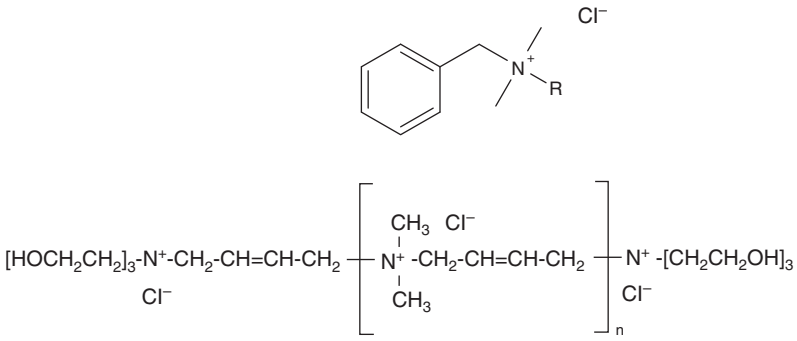

Figure 1. The molecular structures of BAK and PQ-1. R is predominantly C12H25 and C14H24. Mean value of n is typically 90.
BAK: Benzalkonium chloride; PQ-1: Polyquaternium-1.

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greatest at ~ 37°C, at pH values ranging from 6 to 8, and the compound is effective against both bacteria and fungi [6,13].

PQ-1 is classified as a polycationic preservative [10]. The compound is much larger than BAK (**Figure 1**). Unlike BAK, PQ-1 is considered to be too large a molecule to enter mammalian cells [10,14]. While PQ-1 could be classified as a quaternary ammonium, another distinction from BAK is that PQ-1 lacks a large hydrophobic domain (compared to the extended hydrophobic domains in BAK) and does not act as a detergent. In experiments with various species of bacteria, fungi and protozoans, Codling *et al.* found that PQ-1 caused blebbing, K^+ leakage and eventual cellular destruction [15]. For example, PQ-1 has been reported to induce K^+ leakage in *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus* and *Candida albicans*. PQ-1 exhibits biocide activity against the cytoplasmic membrane and speroplasts of *S. marcescens* [16,17].

SofZia and Purite are both classified as oxidizing preservatives [12,18]. The oxidative damage and subsequent cell death caused by these agents act to target organisms that do not have catalase or cytochrome oxidase, such as most bacterial species [10].

3. Drug delivery

BAK is widely used as a preservative in topical formulations partially due to its chemical compatibility with many different classes of therapeutic agents and excipients of topical ocular formulations [4,8]. BAK is typically formulated at concentrations ranging from 0.004 to 0.02% in ophthalmic medications [6,19-20]. This agent is also thought to enhance penetration of some drugs through the cornea by disruption of the hydrophobic barrier, which consists of tight junctions in the epithelial layer [6]. Corneal epithelial cells are tightly bound together by an intercellular cement-like substance, made up of proteins including occludins and claudins [4,21-22]. BAK acts to solubilize this biological glue, thus increasing penetration of some therapeutic agents through the cornea [23]. A toxic consequence of this action has been demonstrated *in vivo* where BAK-containing solutions have been shown to cause the breakdown of corneal epithelial tight junctions and loss of superficial epithelial cells [24,25].

The results have been mixed regarding the actual drug concentrations at the target ocular tissues and the efficacy of BAK-containing formulations. Okabe *et al.* conducted a rabbit study examining the effect of BAK on drug penetration through the sclera [26]. Betamethasone 21-phosphate concentrations were increased by BAK in the vitreous and retina-choroid compared with the control. Another study evaluated the effect of BAK on the permeation of acyclovir through excised rabbit corneas (at 34°C) [27]. Permeability of acyclovir was increased ~ 3-fold with 0.005% BAK and > 10-fold with 0.01% BAK. Another rabbit study showed that incorporating 0.025% BAK into the formulation increased the ocular absorption of timolol [28]. However, this increase in penetration does carry a liability in terms of damage to the ocular surface, which is discussed later in more detail.

Modern topical formulations, containing alternative preservatives, have been shown to effectively penetrate the cornea in the absence of BAK. This has been observed in numerous clinical studies with topical IOP-lowering medications. Baudouin and de Lunardo observed similar IOP readings for patients taking an ocular hypotensive agent, carteolol, with and without 0.005% BAK [29]. Another study reported mean IOP reductions from baseline of 24% for patients on either unpreserved or BAK-preserved timolol gel [30]. No statistically significant differences were found in systemic pharmacokinetic parameters or IOP (at 1 and 8 days of treatment) for patients taking either 0.0015% tafluprost unpreserved or preserved with BAK [31,32]. Similarly, patients dosing with the fixed combination of timolol and dorzolamide experienced equivalent IOP control (defined as having a difference in IOP < 0.5 mmHg) with the preservative-free and BAK-preserved formulations [33]. Katz reported similar IOP-lowering efficacy for patients receiving three different brimonidine regimens (either 0.15 or 0.2% preserved with Purite or 0.2% preserved with BAK) [18]. The results from this study and others have led to the development of additional alternative preservative systems.

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Recent studies have compared the efficacy of travoprost preserved with either BAK or with the preservative system, SofZia. Lewis *et al.* conducted a study of these two formulations that measured IOP at 08.00, 10.00 and 16.00 h at study visits on week 2, week 6 and month 3 [34]. The mean difference in IOP for all nine of these time points was ≤ 0.3 mmHg, which met the criteria for statistical equivalence. Gross *et al.* also evaluated these two travoprost formulations in a 2-week clinical study [35]. On receiving their last once-daily dose on day 13, patients had their IOP measured every 12 h for 60 h. No significant differences in mean IOP values were observed between the travoprost formulation with and without BAK at any of the time points. Both clinical studies suggest that BAK is not necessary for adequate drug delivery of this prostaglandin analog (travoprost) to the target ocular tissues.

Despite PQ-1 use since the mid-1980s, little has been published regarding its effect on drug delivery. The concentration of PQ-1 used in multipurpose contact lens solutions, dry eye products and IOP-lowering medications is 0.001%, which is considerably lower than the concentration of BAK in ophthalmic formulations (0.004 – 0.04%) [6]. Much of the available information on PQ-1 comes from the patent literature. PQ-1 is compatible with cationic surfactants, nonionic surfactants, polyvinyl alcohols, sodium chloride, polyvinylpyrrolidone, hydroxymethyl cellulose, salts of ethylenediamine tetraacetic acid and other agents used in contact lens solutions [36]. In addition, PQ-1 is compatible with borate, carbonate, citrate and phosphate buffered systems at pH values ranging from 3 to 11 (BAK optimal pH range is 6 – 8) [13]. However, preservative effectiveness is greatest at pH values of ≥ 6 in buffers other than phosphate or citrate.

PQ-1 is compatible with ophthalmically acceptable salts, amides, esters and prodrugs of the following types of drugs (containing an acidic functionality such as SO_2NH_2 or SO_2NHR groups): IOP-lowering agents, such as carbonic anhydrase inhibitors, prostaglandins and prostaglandin derivatives; antibacterials and anti-infectives, including sulfa drugs, such as sulfacetamide sodium, and β-lactams such as penicillins and cephalosporins; and diagnostic agents such as sodium fluorescein [37]. PQ-1 has been included as a preservative in commercial formulations of contact lens rewetting drops and storage solutions, artificial tear products, non-steroidal anti-inflammatory drops and IOP-lowering medications [4,10] due to its favorable tolerability profile.

Gandolfi *et al.* recently conducted a 3-month study comparing the efficacy and safety of travoprost preserved with PQ-1 with travoprost preserved with BAK [38]. At the primary efficacy end points (month 3, at 09.00, 11.00 and 16.00 h time points), travoprost with PQ-1 was found to be non-inferior to the formulation preserved with BAK. These results suggest that travoprost preserved with PQ-1 is similar in efficacy but may confer a lower probability for adverse tolerability effects compared to the formulation containing BAK.

4. Preservative efficacy

The US Pharmacopeia, a nonprofit organization that sets standards that are adhered to by pharmaceutical manufacturers, requires evaluations of the efficacy of preservatives in formulations with the preservative effectiveness test (PET) [6,39-40]. This test is performed by inoculating the test medium with between 1×10^5 and 1×10^6 colony forming units (cfu)/ml of various microorganisms (bacteria: *S. aureus*, *P. aeruginosa*, *Escherichia coli*; fungi: *Aspergillus niger* and *C. albicans*). The regulatory requirements for the PET are: 1 log unit reduction in cfu by day 7, 3 log unit reduction in cfu by day 14, no increase in survivors at day 14 or from days 14 to 28, and no increase in survivors for yeast and mold species on day 7, 14 or 28. The European Pharmacopoeia requirements for log reductions for bacteria and fungi, which are similar but slightly more stringent than US guidelines, are as follows: reduction in bacteria at 6 h: 2 log units, at 24 h: 3 log units, at 28 days: no recovery of the microorganisms; and reduction in fungi at 7 days: 2 log units and at 28 days: no increase in survivors [41].

It is well known that BAK exhibits a broad spectrum of antimicrobial activity. The compound has excellent activity against Gram-positive bacteria such as *Staphylococcus* as well as Gram-negative organisms such as *P. aeruginosa* [6]. BAK also possesses antifungal efficacy against such species as *C. albicans* and *Aspergillus fumigatus*.

Numerous studies with multipurpose disinfecting solutions (MPDSs) for contact lenses have measured the preservative efficacy of PQ-1. PQ-1 complies with both the US and European pharmacopeia guidelines for preservative efficacy. Rosenthal *et al.* reported that Opti-Free® Express® MPDS (Alcon Laboratories, Inc.) containing PQ-1 and myristamidopropyl dimethylamine met the United States Pharmacopeia and ISO primary stand-alone test criteria for the disinfection of contact lenses [42,43]. A later study showed this same multipurpose solution (MPS) to be effective against *Fusarium solani*, *C. albicans*, *S. marcescens*, *P. aeruginosa* and *S. aureus* even under non-compliant conditions (e.g., no rubbing or rinsing of the lenses) [44]. Zhu *et al.* reported broad spectrum of antibacterial activity for solutions containing PQ-1 [45]. They examined species that were beyond the scope of the PET guidelines. These investigators found a > 3 log reduction (mean, 4.2 ± 1.4) in bacterial counts for 8 of 10 Gram-positive bacteria and a > 4 log reduction (mean, 5.3 ± 0.5) in all 8 Gram-negative bacteria at 6 h; a > 4 log reduction for Gram-positive bacteria and a > 5 log reduction for all Gram-negative species at 24 h; and a > 3 log reduction for all fungal species tested at 24 h.

Preservative efficacy of contact lens solutions with PQ-1 has been evaluated over extended periods of time. In a comparison study, contact lens solutions with PQ-1 retained nearly 100% of their biocidal activity over a 6-h period [46]. Conversely, about 30 – 60% of PHMB and Alexidine had been depleted by 6 h. Another study showed that contact lens solutions preserved with PQ-1 maintained their bactericidal efficacy overnight and during prolonged storage [47]. Another study showed that unlike

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hydrogen peroxide, Opti-free Express MPDS with PQ-1 prevented regrowth of microorganisms on extended storage [48]. Antibacterial efficacy was maintained during the entire course of the 7-day study. This sustained efficacy is particularly important in light of the fact that some contact lens disinfecting solution formulations without PQ-1 were implicated in a previous outbreak of *Fusarium* keratitis [49].

5. Cytotoxicity and tolerability: preclinical studies

Studies have examined the potential of preservatives to cause irritation or an allergic response. One published review examined the hypersensitivity reactions to preservatives in topical ophthalmic formulations [50]. Quaternary ammoniums (e.g., BAK) were most commonly (8% of reported cases in OVID and PubMed based searches) associated with irritant toxic effects while the organomercurials (e.g., thimerosal) and the alcohols (e.g., chlorobutanol) have the highest association (19% of OVID and 14% of PubMed based searches and 20% of OVID and 11% of PubMed searches), respectively, with allergic responses. Skin patch tests have demonstrated that BAK can induce an allergic response in some subjects [51]. Overall, the incidence of true allergic reactions to BAK is low.

The importance of minimizing the toxic effects of ophthalmic preservatives was highlighted in a recent public statement from the European Medicines Agency (EMA) [52]. The Agency recommended: 'When preservatives are required, the concentration should be at the minimum level consistent with satisfactory antimicrobial function in each individual preparation and a thorough justification for the choice of the preservative should be provided.' The EMA also advocated against the use of 'mercury-containing preservatives' such as THI. However, the agency stopped short of making a general recommendation in favor of preservative-free formulations. This may be due to the fact that these medications are considerably more expensive than multi-dose bottles that contain preservatives. Also, as a single-dose vial can contain 10 or more drops, patients may continue to use the medication on a second day, which can pose a risk for microbial contamination [53]. Some physicians may prefer unit dose medications but the limiting factors of cost, decreased stability, difficulty in patient handling and reduced overall shelf life lead most to prescribe multi-dose bottles that contain preservatives.

BAK is known to produce toxic effects in mammalian cells and tissues. The compound binds to cellular membrane proteins and may affect corneal ionic resistance by incorporating into cell membranes [54-59]. BAK is also hypothesized to accumulate in ocular cell pigments and can remain in ocular tissues for long periods of time [60]. One study by Champeau and Edelhauser showed that the half-life for elimination of BAK from the conjunctival and corneal epithelium was ~ 20 h [56]. The preservative could be detected in the cornea and conjunctiva for up to 1 week following the instillation of a single 30 µl drop of 0.01% BAK. This accumulation is an important risk factor for ocular surface damage, especially in patients with glaucoma, many of whom instill one or more preserved drops each day for years. There may also be a significant risk of damage for dry eye patients who experience a decreased tear clearance from the surface, which together with the typical increase in tear evaporation of these eyes can build up higher, more toxic concentrations of the preservative on the ocular surface, even when BAK is instilled at relatively low dosages.

In vitro studies have demonstrated that BAK can induce toxicity or cell death in a dose-dependent manner [7,55,57,61-62]. For example, at low concentrations, BAK was shown to cause apoptosis in human conjunctival cells, while at higher concentrations necrosis was observed [55]. BAK, at concentrations of 0.005% and higher, caused a significant decrease of membrane integrity with chromatin condensation. Superoxide anions appear to play a role in tissue damage induced by preservatives, such as BAK, in ocular surface disease (OSD). BAK also induces the release of inflammatory mediators such as IL-1 and TNF and to a lesser extent C-reactive protein, IL-10 and IL-12 [63]. Studies with cultured human corneal epithelial cells showed that concentrations of BAK (0.001 – 0.05%) increased expressions of Ki67, a marker of proliferation, and inter-cellular adhesion molecule 1, an adhesion molecule involved with inflammatory responses, as well as by increasing occludin mRNA expression as a possible compensation for the disruption of tight junctions [23].

One way of assessing corneal damage is to measure the amount of hydration in the tissue [64]. The barrier function of the epithelium is one of the main factors that control corneal hydration. When the epithelial layer is damaged, excess fluid penetrates into the stroma, which leads to increased hydration and corneal edema. Monti *et al.* found that 0.001, 0.002 and 0.01% BAK significantly increased the percent of corneal hydration above control values [64]. The authors suggested that long-term use of an ocular formulation containing BAK 'should be considered with caution'.

Yee *et al.* reported the viabilities of human corneal epithelial cells exposed to 0.005% latanoprost preserved with 0.02% BAK to be ~ 10-fold lower than for those cells exposed to 0.004% travoprost with the SofZia preservative system [19]. A rabbit corneal model showed that greater epithelial toxicity was associated with the long-term use of BAK-preserved IOP-lowering agents compared with those formulations containing SofZia [65]. A significant increase in corneal epithelial cell permeability was accompanied by a loss of tight cell junctions in cells exposed to latanoprost preserved with 0.02% BAK. After a 48-h exposure to 10-fold dilutions of ocular hypotensive medications, Ayaki and Iwasawa reported that the corneal endothelial cell viabilities were 47% for 1% dorzolamide (0.005% BAK), 48.5% for 0.5% timolol maleate (0.005% BAK), 52.5% for latanoprost (0.02% BAK), 55.5% for travoprost (0.015% BAK), 71.7% for preservative-free 1% dorzolamide, 80.9% for preservative-free 0.5% timolol maleate and 88.5% for travoprost preserved with SofZia [66].

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Kahook and Noecker conducted a rabbit study to evaluate the effects of latanoprost (with 0.02% BAK), travoprost (with SofZia) and preservative-free artificial tears on goblet cell density [67]. Each treatment group received a once-daily topical application of the medications for 30 days. Immunohistochemical analysis found the number of goblet cells (per high-power field) were 7.03 ± 1.33 in the preservative-free artificial tear group, 2.21 ± 0.40 in the latanoprost with BAK group and 6.02 ± 1.2 in the travoprost with SofZia preservative group. The reductions in the number of goblet cells between the latanoprost group and either the artificial tear group or the travoprost group were significant ($p = 0.0001$). However, there was not a significant difference between the travoprost and artificial tear groups in terms of goblet cell numbers ($p = 0.24$).

Another rabbit study measured the changes to the corneal and conjunctival epithelium following a 30-day exposure to one of the following: two solutions that were preserved with SOC (artificial tears and brimonidine) or four IOP-lowering agents preserved with BAK (bimatoprost, latanoprost, dorzolamide and timolol) [68]. The 1-month regimen of IOP-lowering medications containing higher levels of BAK produced higher levels of corneal damage and conjunctival cell infiltration than a regimen based on medication preserved with SOC or with lower concentrations of BAK (bimatoprost).

Comparison studies have shown a more favorable tolerability profile for products preserved with PQ-1 than for those containing BAK. Unlike BAK, the PQ-1 molecule is thought to be too large to enter mammalian cells [10]. PQ-1 (0.001%) caused no discernible effects on mitotic activity or cytokinetic movement in human corneal epithelial cells [14]. In contrast, 0.01% BAK caused immediate cell retraction, cessation of movement and mitosis within 2 h of exposure. Opti-Free MPDS preserved with PQ-1 produced less cytotoxicity in bovine corneal epithelial cells than other MPDSs preserved with either disodium edetate or polyaminopropyl biguanide [69]. Meloni *et al.* reported human corneal epithelial cell viability was only moderately reduced by sodium perborate- and PQ-1-preserved artificial tears but drastically reduced by those tears preserved with BAK, THI and OxyD-8® (Fresh Start, LLC, Brunswick, GA, USA) [70]. A rabbit study on the effects of preservatives on the corneal epithelial barrier function showed a 9- to 99-fold increase (compared with unpreserved controls) in carboxyfluorescein uptake on 1.5 – 3 h of exposure to artificial tears containing 0.01% BAK [71]. Uptake increased only two to threefold over control values in corneas exposed to tears containing 0.001% PQ-1.

Labbe *et al.* conducted an *in vivo* study in rats using BAK and PQ-1 at concentrations several fold higher than those found in commercial eye drop Formulations (0.1 and 0.5%) [72]. Slit-lamp examination, fluorescein tests, impression cytology, *in vivo* confocal microscopy and histology showed that BAK consistently altered the corneoconjunctival surface. Goblet cells densities (per 0.01 mm^2) were as follows: control group (21.6 ± 1.6), 0.1% BAK (6 ± 6; $p < 0.0001$), 0.5% BAK (0.5 ± 0.8; $p < 0.0001$), 0.1% PQ-1 (18.8 ± 2.8; $p = 0.14$) and 0.5% PQ-1 (14.6 ± 1.1; $p < 0.0001$; all p values are compared to controls). PQ-1 was not statistically significantly different from the control group in terms of the tear production test, slit-lamp and fluorescein evaluation, and overall histology.

Ubels *et al.* demonstrated that an artificial tear (Systane®, Alcon Laboratories, Inc.) preserved with PQ-1 enhanced corneal epithelial recovery in rabbits following a 5-min exposure to 0.01% BAK and desiccating conditions [73]. The artificial tear normalized carboxyfluroescein uptake to levels that were not significantly different from untreated controls. Follow-up studies with corneal epithelial and Chang conjunctival cells showed significantly greater viability after experimentally-induced desiccation in the PQ-1 preserved tear product compared to those products containing BAK or Purite.

A study by Ammar *et al.* measured the adverse effects of different preservatives and IOP-lowering medications on cultured human corneal and conjunctival epithelial cells [10]. The medications tested included 0.0015% tafluprost with 0.010% BAK (Taflotan®; Santen Pharmaceutical, Tampere, Finland), 0.004% travoprost with 0.015% BAK (Travatan®; Alcon Laboratories), 0.004% travoprost with 0.001% PQ-1 (Travatan; Alcon Laboratories, UK Ltd.), 0.004% travoprost with SofZia (Travatan Z®; Alcon), and 0.005% latanoprost with 0.020% BAK (Xalatan®; Pfizer, New York, NY, USA). Cells exposed to BAK for 25 min exhibited a concentration-dependent reduction in viability (**Figure 2**). There was no statistical difference in corneal cell viability between travoprost with SofZia (72%) and travoprost preserved with PQ-1 (80%, $p = 0.18$). A significantly lower percentage of cells exposed to prostaglandins formulated with BAK were viable compared with those incubated in medications preserved with PQ-1 and SofZia ($p < 0.05$). Although corneal cells were more sensitive to the toxic effects of BAK, similar trends in terms of viability were observed with the conjunctival cells (**Figure 3**).

Brignole-Baudouin *et al.* conducted a series of *in vitro* and *in vivo* experiments that examined the toxicological profiles of PBS, PQ-1 (0.001%), BAK (0.015 and 0.020%), 0.004% travoprost (0.001% PQ-1 or 0.015% BAK) or 0.005% latanoprost (0.02% BAK) [74,75]. In assays that assessed cell viability apoptosis and oxidative stress, responses from cells exposed to 0.001% PQ-1 and travoprost with 0.001% PQ-1 were not statistically different from the PBS controls [74]. Cells incubated in solutions containing BAK exhibited more effects of cytotoxicity than those incubated in PQ-1 preserved solutions. Ocular surface reactions were evaluated in rabbits using slit-lamp examination, *in vivo* confocal microscopy, conjunctival impression cytology and standard immunohistology [75].The formulations that contained BAK caused epithelial cell damage, inflammatory cell infiltration, decreases in goblet cell density, conjunctival hyperemia, chemosis, abnormal changes in the ocular surface microstructure and increases in total ocular surface toxicity scores. In contrast to BAK and

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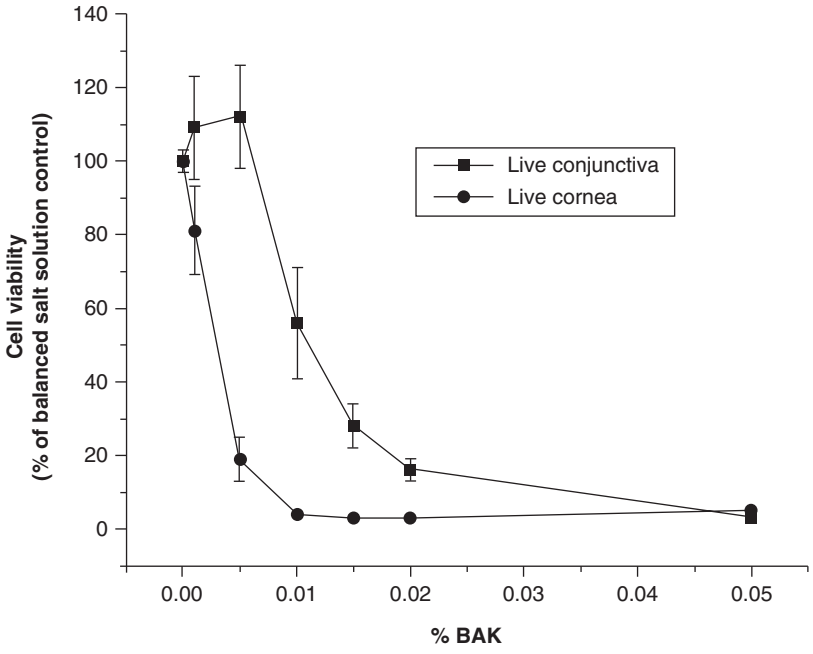

Figure 2. The effects of increasing concentrations of BAK on cell viability.
Adapted from Ammar and Kahook 2010 [10].
BAK: Benzalkonium chloride.

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BAK-preserved products, PBS, 0.001% PQ-1 and travoprost with 0.001% PQ-1 did not induce obvious cell damage or ocular irritation as measured by these assay systems.

Another set of experiments evaluated the toxicological profiles of PBS, 0.015% BAK, 0.020% BAK, 0.001% PQ-1, travoprost–timolol fixed combination preserved with 0.001% PQ-1 or 0.015% BAK, and latanoprost–timolol preserved with 0.020% BAK [76,77]. Human conjunctival cells incubated in travoprost–timolol with 0.001% PQ-1 responded more favorably in terms of cell viability, apoptosis, and oxidative stress than did cells exposed to solutions containing BAK [76]. Cells exposed to tavoprost–timolol PQ-1 retained significantly greater viability that those incubated with formulations containing BAK ($p < 0.0001$). Significant reductions ($p < 0.0001$) in favor of travoprost–timolol 0.001% PQ-1 were reported for apoptosis, reactive oxygen species and superoxide anions. *In vivo* (rabbit) studies demonstrated that travoprost–timolol preserved with 0.001% PQ-1 was better tolerated than either of the prostaglandin analogs preserved with BAK [77].

6. Tolerability and safety: clinical studies

Clinical trials of ophthalmic formulations with alternative preservative systems have demonstrated their favorable tolerability and safety profiles. Henry *et al.* evaluated the effects of changing from BAK-preserved prostaglandin analog therapy (bimatoprost or latanoprost) to a prostaglandin analogue (PGA) without BAK (travoprost with SofZia) [78]. All patients who entered the study with OSD symptoms reported significant improvement after transitioning to the PGA without BAK ($p < 0.0001$). Ocular surface disease index (OSDI) scores were reduced by ≥ 1 level of severity in 70.2% of these patients. Another study measured the symptoms of OSD in patients who had been treated with a BAK-containing IOP-lowering agent (latanoprost) [79]. One group of patients remained on latanoprost while the other was transitioned to travoprost preserved without BAK (SofZia). Patients with mild OSD had significantly lower OSDI scores at 12 weeks on travoprost (11.6 ± 10.8) therapy than those on latanoprost (14.4 ± 11.9; $p = 0.04$). Horsley and Kahook reported significant decreases in mean OSDI scores (26.31 – 16.56), reductions in mean inferior corneal staining scores (2.40 – 1.38) and increases in mean TFBUT (2.02 – 6.34 s) for patients who transitioned from latanoprost with BAK to travoprost with SofZia preservative ($p < 0.001$) [80]. However, Whitson *et al.* reported that there were no significant differences among bimatoprost (0.005% BAK), latanoprost (0.02% BAK) and travoprost (SofZia) for corneal staining, hyperemia and TFBUT following 3 months of treatment [81].

Numerous studies have shown that PQ-1-based MPDSs are gentle on the ocular surface. In a pilot study, Hall *et al.* showed that a PQ-1-based MPDS maintained epithelial barrier function in contact lens patients [82]. In a follow-up study, Webb *et al.* showed that an MPDS preserved with PQ-1 provided better maintenance of the corneal epithelial barrier than an MPDS preserved with PHMB [83]. Another study found that contact lenses soaked in a PQ-1-based solution showed minimal uptake and corneal staining was low [84]. Epstein found that patients habitually using an MPDS with PQ-1 (Opti-Free Express

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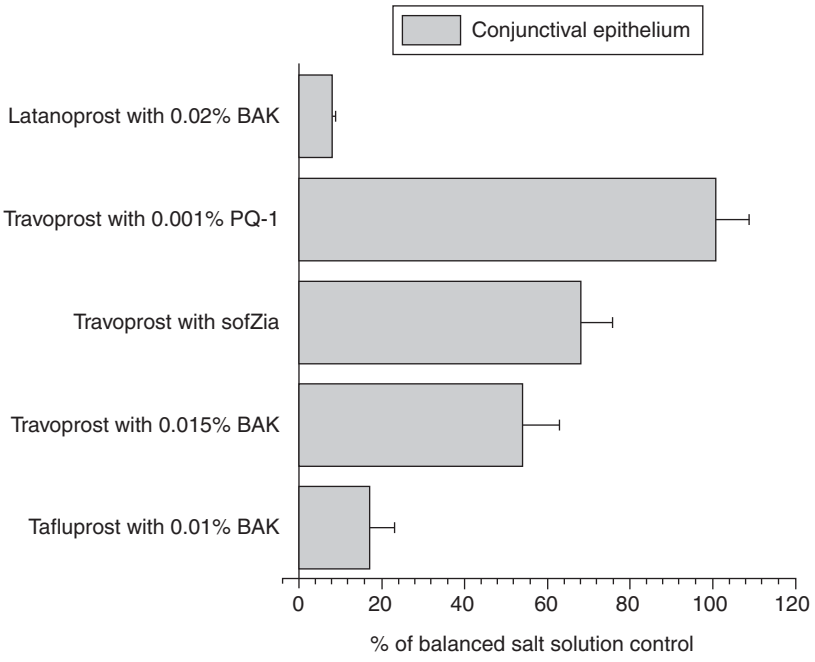

Figure 3. Conjunctival cell viability of various prostaglandin analogs preserved with BAK, SofZia or PQ-1.
Adapted from Ammar and Kahook 2010 [10].
BAK: Benzalkonium chloride; PQ-1: Polyquaternium-1.

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MPDS) had higher comfort ratings than did those using a solution preserved with PHMB (ReNu MultiPlus®, Bausch & Lomb, Inc., Rochester, NY, USA) [85]. Esthesiometry showed significant differences in average corneal sensitivity that favored the MPDS with PQ-1 ($p = 0.0041$).

Gibbs *et al.* published the 6-month safety data for Opti-Free MPDS preserved with PQ-1 [86]. The product was found to be safe and effective under both normal and exaggerated conditions (lenses soaked for 16 h each day). A Phase I clinical study evaluated a 0.005% concentration (5× the marketed concentration) of PQ-1 in a contact lens solution [87]. Nine (out of twenty-five in the PQ-1 group) patients discontinued the study due to mild intolerance that was readily reversible. The patients who continued the study found the lens regime to be 'comfortable' or 'very comfortable' 99% of the time.

Comparison studies have shown that MPDSs preserved with PQ-1 have tolerability advantages over those using PHMB. Lipener found that the total corneal staining score of area and type was statistically significantly less in the regime with an MPS preserved with PQ-1 (Opti-Free Express MPDS) than in the regimen with an MPS preserved with PHMB (ReNu MultiPlus; $p < 0.001$) [88]. The area of conjunctival staining was also statistically significantly less in the regimen preserved with PQ-1 ($p = 0.03$). Other studies have reported that PQ-1-preserved solutions had an advantage over those preserved with PHMB in terms of corneal and conjunctival staining, wettability, grittiness, scratchiness and palpebral roughness [89-93].

Clinical studies with dry eye patients have demonstrated that artificial tears preserved with PQ-1 exhibit a favorable tolerability profile. This is of particular importance as a majority of the patients who use this type of therapy have a compromised ocular surface [94]. Patients using artificial tears preserved with PQ-1 reported significantly less morning dryness, end of day dryness and foreign body sensation than those using the control drop. Another study comparing Systane to Optive Lubricant Eye Drops (Allergan, Inc.) found that patients receiving the HP-guar drop, preserved with PQ-1, experienced decreased corneal staining at each visit [95]. At day 42, the percent change from baseline was 47.1% ($p < 0.0001$). Mocanu *et al.* conducted a study to evaluate patients before and after dry eye therapy with Systane [96]. Patients experienced increased tear film break-up time, reduced cornea/conjunctival staining and less conjunctival hyperemia. Following treatment, the proliferation of conjunctival and goblet cells with normal morphology was observed.

Hartstein *et al.* evaluated the efficacy of Systane (preserved with PQ-1) in reducing the signs and symptoms of moderate to severe dry eye in patients who had previously been administering their usual artificial tear product [97]. The patients were placed on a run-in regimen of Opti-Free Express Rewetting Drops (four times a day for 7 days). A statistically significant reduction in corneal staining ($p < 0.0001$) was observed after 28 days of dry eye therapy. Most patients (94%) reported improvements from baseline; mean reductions in total corneal staining were 4.1 units (on a total scale from 0 to 15 representing a 62% improvement). Conjunctival

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Table 1. Tear function test (Schirmer test, fluorescein staining, TFBUT) values, computerized perimetry parameters and the number of significantly depressed points in pattern deviation plots measured before and after lubricating eye drop (preserved with PQ-1) treatment for 8 weeks.

Tear function test	Before artificial tear treatment	After artificial tear treatment	p Value
Schirmer test	3.32 ± 1.27	6.33 ± 0.74	< 0.0001
Fluorescein staining	4.93 ± 1.52	3.27 ± 1.18	< 0.001
TFBUT, s	6.74 ± 1.46	8.92 ± 1.81	< 0.01
FASTPAC perimetry test parameter			
Mean deviation	7.14 ± 3.64	5.35 ± 2.82	< 0.001
Pattern s.d.	5.17 ± 3.07	3.86 ± 2.58	< 0.001
Corrected pattern s.d.	2.92 ± 1.69	2.43 ± 1.47	< 0.05
Short-term fluctuation	2.66 ± 1.54	2.14 ± 1.17	< 0.01
Test duration, min	10.11 ± 3.42	8.73 ± 1.76	< 0.01
Probability level			
p < 2%	6.89 ± 4.32	4.27 ± 3.66	< 0.001
p < 0.5%	6.06 ± 3.84	3.82 ± 2.63	< 0.001

Means ± s.d. are presented.
Adapted from Guzey *et al.* 2010 [105].
PQ-1: Polyquaternium-1; TFBUT: Tear film break-up time.

staining also improved significantly ($p < 0.0001$) with a mean total decrease of 3.1 units (59%). Patients experienced statistically significant symptomatic relief from days 0 to 28 for all six ocular discomfort severity questions ($p < 0.0001$).

Rolando *et al.* conducted a 28-day study to evaluate the efficacy of an artificial tear (Systane, preserved with PQ-1) for reducing the signs and symptoms of dry eye [98]. Patients with moderate to severe dry eye experienced significant improvements compared to baseline in dry eye symptoms ($p < 0.0001$ on days 7, 14 and 28); ocular surface staining ($p < 0.0001$ at days 7, 14, and 28); and in the Ocular Protection Index ($p = 0.0025$ at day 14 and $p = 0.0067$ at day 28).

Glaucoma and ocular hypertensive patients often have a compromised ocular surface due to the chronic use of one or more BAK-containing ocular hypotensive drops. The effects of BAK tend to be cumulative and increasingly problematic as concentrations and dosing frequency increase [1,99]. Not surprisingly, OCD is prevalent among glaucoma patients [100]. Another study (n = 101) that used the Ocular Surface Disease Questionnaire (OSDI, Allergan, Inc.) found that 59% of the patients surveyed suffered from symptoms of dry eye [100]. Rossi *et al.* reported that 39 and 40% of the glaucoma patients in their study who took 2 or 3 drops/day, respectively, suffered from dry eye symptoms [101]. In a clinical trial involving > 4000 patients, Pisella *et al.* found that 84% of these patients used ocular hypotensive medications that contained preservatives (BAK in most instances) [102]. Ocular symptoms (discomfort, burning/stinging, foreign body sensation, dry eye sensation, tearing, itching) and signs (redness, corneal staining, superficial punctate keratitis, blepharitis) were more prevalent for patients using the preserved eye drops than for those using preservative-free formulations ($p < 0.001$). These symptoms are known to play a role in reduced adherence with respect to topical IOP-lowering regimens [103]. It is thought that long-term use of topical IOP-lowering therapies containing preservatives is a risk factor for glaucoma filtration surgery failure [104].

Clinical studies have evaluated the effect of topical eye drop formulations preserved with PQ-1 in glaucoma and ocular hypertensive patients. Guzey *et al.* evaluated the effects of Systane with PQ-1 on tear function and computerized perimetry in open-angle glaucoma patients with trachomatous dry eye [105]. All patients had been treated with the same ocular hypotensive eye drop that contained 2% dorzolamide hydrochloride/0.5% timolol maleate with 0.0075% BAK as a preservative (Cosopt®, Merck and Co., Inc., Whitehouse Station, NJ, USA) twice daily for 4 months to 5 years. Significant improvements were observed in tear function tests, perimetry indices, perimetry test duration and the number of depressed points on pattern deviation plots following 8 weeks of treatment with the lubricant eye drop (**Table 1**). Whitson *et al.* found that the incidence of treatment related adverse events (ocular hyperemia, conjunctivitis, allergic reaction, conjunctival follicles, ocular pruritus, ocular discomfort) was similar between brimonidine preserved with PQ-1 versus brimonidine preserved with chlorine dioxide (Purite) [11].

7. Conclusions

A wide variety of preservatives have been used to inhibit the growth of microbial contaminants in ophthalmic multidose preparations. BAK, still the most common agent used for this purpose, is a quaternary ammonium compound with surfactant properties. This agent has broad spectrum activity against microorganisms but may carry a liability of toxicity to mammalian cells and tissues both *in vitro* and *in vivo*. In an attempt to alleviate the toxic ocular effects observed with BAK, other preservatives have been developed (including PQ-1, Purite and SofZia).

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PQ-1 is a polycationic preservative that, unlike BAK, does not have detergent properties. This agent complies with both the US and European Pharmacopeia guidelines for preservative efficacy. PQ-1 is a large molecule that preferentially targets microorganisms and produces minimal toxicity towards mammalian cells. PQ-1 compares favorably to other preservatives in terms of efficacy. Published studies demonstrate that this preservative, added to contact lens solutions, artificial tears and ocular hypotensive drops, may offer benefits in terms of ocular tolerability compared to BAK in some patients. PQ-1 is comparable in tolerability to other alternative preservatives such as sodium perborate, SofZia and Purite. Therefore, PQ-1 may be considered as a viable alternative to BAK for many topical ophthalmic formulations.

8. Expert opinion

Although we have observed that numerous preservatives are included in ophthalmic formulations, BAK is still the most prevalent. This compound displays efficacy against microorganisms and its main caveat relates to the toxic effects it has on the eye. Studies have shown that ocular surface symptoms induced by ophthalmic preservatives, and BAK in particular, can affect patient compliance to their therapeutic regimens [106,107]. In the past few years, alternative preservatives have been developed that provide improved adverse effect profiles. BAK may serve as a penetration enhancer for β-blockers that penetrate poorly across the cornea. While BAK was thought to be required in the past due to the more hydrophilic nature of ophthalmic preparations (i.e., topical β-blockers), many modern topical medications do not require a disruption in the tight cell junctions of the corneal epithelial layer in order to achieve efficacy at the target tissues [6,34].

PQ-1, one example of an alternative preservative, does not exhibit detergent properties. The compound appears to be compatible with a wide variety of buffering systems and the active ingredients in contact lens solutions, artificial tears and lipophilic agents such as prostaglandin analog formulations [37]. PQ-1 is a large molecule that preferentially targets microorganisms. This agent has maintained a proven record of efficacy and tolerability in ophthalmic formulations for > 20 years. Recent studies demonstrate that PQ-1 is as gentle as other newer generation preservatives such as SofZia and Purite.

Effective drug delivery requires more than just an active ingredient that achieves its desired biological effect on end-target tissues. In addition, drugs must be stable in the containers that they are stored in and must minimize undesired local and systemic side effects that cause patients to decrease their adherence to prescribed therapies. In addressing this concern, specifically as it relates to topical ophthalmic drops, one must take into account the active ingredient as well as any vehicle components or preservatives that can lead to deleterious effects. With all else equal, medications with fewer adverse effects may lead to enhanced adherence to therapy. Therefore, the induction of adverse outcomes must be considered when attempting to enhance drug delivery and improve physicians' ability to treat patients with chronic ocular disease.

Declaration of interest

M Rolando is on the advisory board for Alcon, Allergan, Pfizer, Farmigia, Sooft, Sifi, Thea, and Bausch & Lomb. M Kahook has received research support from Alcon, Allergan and Merck. J Crider has received consulting support from Alcon, Regeneron and Occular Therapeutix, and has also provided medical writing assistance that was funded by Alcon Laboratories, Inc.

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