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Dry Eye and Ocular Surface Disease

Balancing antimicrobial efficacy and toxicity of currently available topical ophthalmic preservatives

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Abstract

Medical treatment of ophthalmic diseases relies primarily on the use of multidose drugs. Short term use is highly effective usually with little local toxicity. However, chronic use of these preparations not only increases the likelihood of microbial contamination and secondary ocular infection, but also of toxicity from the drug formulation itself. Increasing awareness of the toxicity of ophthalmic preservatives has led to an increasing variety of preservative schemes ranging from ''self-preservation'' to ionic buffer systems. Beyond outdated testing methods, the anti-microbial efficacy of most of these systems is poorly defined, potentially placing these preparations at an unknown risk of contamination by unmonitored, untested organisms. No uniformity in toxicity testing exists which further complicates the clinician's judgment of the risk–benefit of using a particular drug formulation. In this manuscript we examine in detail each of the current employed ophthalmic preservative regimens with respect to their known antimicrobial activity and potential toxicity, where known. We also survey the most popular ophthalmic preparations, detailing their preservation schemes as well as concentrations to help the clinician in choosing an appropriate formulation for the treatment of various ophthalmic diseases.

Keywords: Antimicrobial, Preservatives, Toxicity, Ophthalmic, Eye infection, Benzalkonium chloride, Purite, Glaucoma, Keratitis

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Introduction

Topical ophthalmic medications are the mainstay of therapy for almost all ocular disorders of the anterior segment including dry eye, glaucoma, ocular surface infections and tumors. Topical dosing provides direct access to higher concentrations of a drug at its therapeutic target while minimizing or avoiding systemic side effects which might otherwise limit their use. Several medications deemed too toxic for systemic use can be used safely on the eye, e.g. neomycin. Short term use of commercially available topical ophthalmic preparations in an otherwise healthy eye is very safe and is associated with few local complications. However, many drugs require extended or chronic, repeated applications for control or maintenance of disease. In this instance, side effects or toxicity from the active drug can increase in frequency. For example, with extended use, prostaglandin analogs may cause complications ranging from simple hyperemia or cosmetically troublesome pigmentation to severe prostaglandin associated periorbitopathy.[1,2]

Long term, repeated dosing of topical medications presents several other challenges that are common to all medications. Cost and compliance for chronic use often necessitates the use of multidose preparations. Although the healthy ocular surface is relatively resistant to microbial challenge, direct applications of a high load of microorganisms may overwhelm the normal protective mechanisms leading to vision-threatening infection. Further, many of these

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patients have a compromised ocular surface from ocular disease which leaves them more susceptible to infection. Recently, a number of severe infections have been associated with pharmacy-compounded ophthalmic preparations, which are normally not preserved, because of contamination during compounding,[3,4] but for commercially prepared topical ophthalmic preparations, contamination usually occurs as a result of poor patient compliance.[5–8] Prior to adequately preserved ophthalmic drugs, several severe, blinding infections were traced to in-use contamination of multidose preparations.[9,10] For these reasons, manufactured topical ophthalmic preparations are required to be sterilely created and to be preserved against secondary contamination. Unfortunately, the additives required for preservation may themselves be toxic to the eye requiring the physician to have a thorough understanding of the potential risks of toxicity, therapeutic efficacy and risk of infection for each individual patient in deciding on appropriate medical management.

Antimicrobial efficacy testing (AET)

All manufactured multidose ophthalmic products are required to resist contamination both in the U.S., Japan and the European Union. Their tests differ slightly, but significantly in some cases. Since it would be difficult to test preservative efficacy against every possible contaminating pathogen, each involves the testing of solutions against a small number of bacteria and fungi chosen with the results proxied as a general measure of contamination prevention. The same general principle is applied to other ophthalmic products, such as contact lens disinfection systems. These tests are clearly not perfect and, combined with minimal post-market surveillance, are not validated as reflective of real world efficacy. Failures result from a number of factors including an inadequate representation of poor patient compliance and an inadequate reflection of potentially pathogenic organisms. A recent example was the recent outbreak of Fusarium keratitis as a result of the failure of Renu with Moistureloc to adequately disinfect contact lenses despite excellent antifungal activity on AET specific to contact lens solutions.[11–14]

Current United States Pharmacopoeia (USP) Antimicrobial Effectiveness Testing involves 3 stock bacteria (*Pseudomonas aeruginosa*, *Staphylococcus Aureus* and *Escherichia coli*) and 2 fungi (*Candida albicans and Aspergillus niger*). Ophthalmic compounds are Category 1 and the methodology is similar to that specified in the Japan Pharmacopoeia. These organisms are cultured and added to the solution to be tested with a final concentration of between 1×10^5 and 1×10^6 colony forming units (CFU). The solutions are then sampled at 7, 14 and 28 days with a requirement of a 1 log unit reduction at 7 days, a 3 log unit reduction at 14 days and no increase in CFUs from Day 14 to 28 for bacterial pathogens. For fungi, the requirement is simply no increase in CFUs from the initial inoculum at any of the three time points. European Pharmacopoeia standards are significantly more stringent. Ophthalmic products for topical use are considered category EP-A where 2 bacteria (*P. aeruginosa and S. Aureus)* and 2 fungi (*C. albicans and Aspergillus brasiliensis*) are inoculated into the solution to be tested. Samples for bacteria are taken at 6 and 24 h and then at 28 days with passing criteria of reduction in CFUs by 2 log units at 6 h, 3 log units at 24 h and no subsequent increase at 28 days. Fungi are tested at 7 and 28 days with a required reduction in CFUs of 2 log units at 7 days without an increase from this point at 28 days.[15]

Efficacy and toxicity of current ophthalmic preservatives

The number of ophthalmic solution preservatives in current use is relatively small and can be classified into two or three categories (Table 1). Surfactants, or detergents, directly damage the cell wall of microbes by disrupting their lipid component causing cell lysis. Because of their non-specific nature, these agents are both generally more effective with a broad spectrum of activity across different classes of pathogens but are more toxic to human cells. Oxidizing preservatives are small molecules that can pass the microbial cell wall to enter the cell and disrupt internal enzymatic function. These tend to be less toxic to human ocular surface cells, but its efficacy against pathogens and pathogen classes outside of those included in the testing regimen are either unknown or unreported. Recently, an ionic buffer system was introduced which acts similarly as an oxidizer while in solution. Because these compounds are highly reactive, they will rapidly be consumed when in contact with the ocular surface.

The simplest alternative to preservation of multidose preparations is sealed, unpreserved, single dose aliquots. These not only eliminate the need for preservative additives, but also the cycle of contamination, incubation and proliferation which would increase the concentration of microbes to an infectious level. Unfortunately, the cost of production is significantly higher resulting in higher costs to the patient. Although they are labeled for single use, studies have demonstrated that repeated use of a single dose applicator over a 10 h period results in a relatively low rate (2.4%) of contamination, dependent on the type of drug and patient compliance.[16,17] Another study suggested a relatively low rate of contamination even if used for a longer period of time.[16] The contamination risk of a particular compound is dependent multiple factors including not only patient handling and compliance, but also on the compound itself. Unless there is gross contamination, growth and proliferation of the pathogen after inoculation of the preparation is likely required to reach an infectious threshold concentration. Some microbes have demonstrated the ability to use the base drug, such as corticosteroids, in these preparations as a carbon source (food) to support this growth.[18] There is evidence that corticosteroids themselves further influence microbial growth in both an inhibitory and supportive role through hormonal action. Consequentially, corticosteroid and corticosteroid combination medications have been shown to have a much higher rate of contamination than other medications.[19]

Detergents

Benzalkonium chloride is a quaternary ammonium cationic surfactant which has been the dominant ophthalmic preservative over the past several decades. Found in over 70% of topical ophthalmic drugs, it combines the advantage of highly efficacious antimicrobial action with limited ocular penetration and excellent solution stability. The compound has been demonstrated to have a very broad spectrum of

Table 1. Common ophthalmic compounds and their primary preservative additive.*

Drug class	Drug name	Primary preservative	Concentration
Ocular hypotensives			
Miotics	Echothiophate	Chlorobutanol	0.55%
	Pilocarpine	BAK	0.01%
	Pilocarpine gel	BAK	0.008%
	Carbachol	BAK	0.005%
Beta blockers	Timolol maleate (PFU)	BAK	0.01%
	Timolol XE	Benzododecinium bromide	0.012%
	Cartelol	BAK	0.005%
	Betaxalol	BAK	0.01%
	Levabunolol	BAK	0.005%
	Metipranolol	BAK	0.004%
	Apraclonidine (PFU)	BAK	0.01%
	Brimonidine	BAK	0.005%
	Alphagan	BAK	0.005%
	Alphagan-P	Purite	0.005%
	Brinzolamide	BAK	0.01%
	Dorzolamide	BAK	0.0075%
Prostaglandin analogs	Latanoprost	BAK	0.02%
	Bimatoprost 0.01%	BAK	0.02%
	Bimatoprost 0.03%	BAK	0.005%
	Travoprost	BAK	0.015%
	Travoprost Z	Sofzia	Ionic buffer system (Sofzia)
	Tafluprost (PFU)	N/A	N/A
	Rescula	BAK	0.015%
Combination drugs	Brimonidine/Timolol	BAK	0.005%
	Timolol/Dorzolamide	BAK	0.0075%
	Brinzolamide/Brimonidine	BAK	0.003%
Anti-infectives			
Antibacterials	Gentamicin	BAK	0.02%
	Gentamicin ointment	Methylparaben/ Propylparaben	
	Tobramycin	BAK	0.02%
	Sulfacetamide	BAK	0.01%
	Polysporin/trimethoprim sulfa	BAK	0.004%
	Gramicidin/Neosporin/polymyxin b (solution)	Thimerosal	0.001%
	Bacitracin/Neosporin/polymyxin b (ointment)	PF	None
	Bacitracin/ Polymyxin b (ointment)	PF	N/A
	Ciprofloxacin	BAK	0.006%
	Ofloxacin	BAK	0.005%
	Norfloxacin	BAK	0.0025%
	Levofloxacin 0.5%	BAK	0.005%
	Levofloxacin 1.5%	PF	N/A
	Gatifloxacin	BAK	0.005%
	Moxifloxacin	PF	N/A
	Besifloxacin	BAK	0.01%
	Azithromycin	BAK	0.003%
	Chloramphenicol	Phenylmercuric nitrate	0.002%
	Erythromycin (ointment)	PF	N/A
	Oxytetracycline/polymyxin b		
Antifungal	Natamycin	BAK	0.02%
Corticosteroid/ Antibiotic Combinations	Neomycin and Polymyxin B Sulfates, Bacitracin Zinc and Hydrocortisone (ointment)	PF	N/A
	Loteprednol/tobramycin	BAK	0.01%
	Sulfacetamide 10%/Prednisolone acetate (drops)	BAK	0.004%
	Sulfacetamide 10%/ Prednisolone acetate (ointment)	Phenylmercuric acetate	0.0008%
	Tobramycin/Dexamethasone (ointment)	Chlorobutanol	0.5%
	Tobramycin/Dexamethasone (drops)	BAK	0.01%
	Sulfacetamide/Prednisolone phosphate	Thimerosal	0.01%
Antivirals	Vidarabine	PF	N/A
	Idoxuridine	BAK	0.01%
	Trifluridine	Thimerosal	0.001%
	Ganciclovir	BAK	0.0075%
Anti-Inflammatories/anti-infectives			
NSAIDs	Cromolyn Sodium	BAK	0.01%
	Diclofenac	Sorbic Acid	0.2%
	Ketorolac 0.4%	BAK	0.006%
	Ketorolac 0.5%	BAK	0.01%
	Nepafenac	BAK	0.005%
Anti-allergics	Ketotifen	BAK	0.01%

Table 1 (*continued*)

Drug class	Drug name	Primary preservative	Concentration
	Naphazoline	BAK	0.01%
	Olopatadine	BAK	0.01%
	Necrodomil	BAK	0.01%
	Epinastine	BAK	0.01%
	Lodoxamide	BAK	0.01%
	Alcaftadine	BAK	0.005%
Corticosteroids	Loteprednol	BAK	0.007%
	Fluorometholone	BAK	0.005%
	Prednisolone acetate	BAK	0.01%
	Prednisolone phosphate	BAK	0.01%
	Dexamethasone Phosphate (drops)	BAK	0.02%
	Rimexolone	BAK	0.01%
	Difluprednate	Sorbic acid	0.1%
Artificial Tears			
	Bion Tears (PFU)	PF	N/A
	Genteal family (PFU)	Sodium Perborate	
	Hypotears	BAK	0.01%
	Systane lubricant eye gel	Sodium perborate	
	Systane (ointment)	PF	N/A
	Tears Naturale II	Polyquaternium-1	0.001%
	Tears Naturale Forte	Polyquaternium-1	0.001%
	Systane Ultra	Polyquaternium-1	0.001%
	Systane Balance	Polyquaternium-1	0.001%
	Refresh family (PFU)	Purite	
	Soothe Hydration	Sorbic Acid	0.1%
	Soothe Long-lasting	BAK	0.005%
	Soothe Tired	BAK	0.01%
	Theratears	Sodium perborate	
	Visine	BAK	

BAK - Benzalkonium chloride, PFU - Preservative -Free Unit Dose, PF- Preservative Free.

* Many preparations include other components which may have antimicrobial effect, e.g. EDTA, boric acid, etc.... which are proportionally less toxic.

activity in vitro against all pathogen classes including *acanthamoebae*.[20] Since its introduction, it has been known to cause significant ocular surface toxicity where the drug is preferentially absorbed, the conjunctiva and corneal epithelium.[21,22] Radioisotope studies indicate virtually no penetration into the aqueous humor, iris, ciliary body or vitreous with minimal penetration into the corneal stroma with a single drop on short term application.[23–25] Although a single study suggested that its resident time in tears is very short,[26] this is probably due to its rapid uptake by the surface epithelium. Several studies have demonstrated the significant ocular surface toxicity especially in compromised ocular surfaces or with extended and/or frequent use.[19,27–30] BAK has been demonstrated in in vitro studies to induce apoptosis. Recently, however, further concerns have been raised about the potential for intraocular penetration having been found in animal studies in the area of the trabecular meshwork, choroid and even the optic nerve.[31–33] This raises the possibility of direct toxic effects on the nerve as well as advancing trabecular meshwork changes which may lead to further reduction in outflow facility. It should be noted that this was observed in at 5 months of normal dosing at 0.01% and primarily anterior segment penetration was seen at a the excessive concentration of 0.2% once a day for 1 month.

Polyquaternium-1 is a quaternary ammonium compound with a spectrum of activity against primarily bacteria with some antifungal activity against yeasts presumed to act on cell membranes.[34] Although similar to BAK, it has limited activity against other fungi and *acanthamoeba*. While in vivo and in vitro studies demonstrate less toxicity in comparison to BAK, toxicity is still seen in the concentrations used in ophthalmic compounds- primarily artificial tears (Table 1) and contact lens solutions.[35–38] Benzododecinium bromide is another quaternary ammonium compound which has a similar mechanism of action.

Organomercurial compounds are primarily used in legacy medications and consists of thimerosal as well as phenylmercuric acetate and nitrate (Table 1). Thimerosal is an organic mercurial compound that was very commonly used in contact lens disinfection systems. Reports of severe ocular surface toxicity caused these solutions to be phased out.[39] These compounds bind to the cell wall, increasing permeability and cell death.[40–42] Sorbic acid has minimal primary antimicrobial activity, but may acidify the cell environment resulting in inhibition of microbial growth.[41]

Chlorobutanol formed by the combination of chloroform and acetone is sparingly used as an ophthalmic preservative (Table 1), limited by its lability when exposed to heat or even room temperature. Like benzalkonium chloride, it acts as a detergent to non-specifically disrupt cell membranes and has a broad spectrum of anti-microbial activity but is less effective.[41,43] Studies have shown that it is less toxic than benzalkonium chloride both in vitro and in vivo, but still remains more toxic than most oxidative preservatives at the concentrations used for ophthalmic compounds.[21,29]

Oxidative preservatives

Stabilized Oxychloro Complex (SOC) (Purite) is a combination of chlorine dioxide, chlorite and chlorate which causes oxidation of intracellular lipids and glutathione, interrupting vital enzymes for cell function and maintenance.[41,44] It has demonstrated antimicrobial activity against bacteria, viruses and some fungi. Because of its propensity to generate free radicals, it is an effective oxidizer and also breaks down

rapidly when exposed to the ocular surface. Although the compound passes the USP AET, studies have suggested that the slower microbial killing rates in artificial tear preparations would not pass EP-A criteria for ophthalmic preservation.[43]

Sodium perborate (GenAqua) is another oxidative preservative which is used as a bleaching agent in dentistry albeit at higher concentrations. It acts by forming hydrogen peroxide, a powerful oxidizing agent and antimicrobial, when combined with water leading to a similar mechanism of action as SOC.[41,44] This also leads to its rapid degradation when exposed to tears. It conforms to the USP AET test and hydrogen peroxide does have anti-acanthamoebal activity, but data are limited beyond the required testing.[45] Hydrogen peroxide can cause significant ocular toxicity at higher levels, but the concentration in the artificial tear products is reasonably well tolerated.[46]

Alternative systems

SofZia is a unique preservative system that is composed of boric acid, propylene glycol, sorbitol, and zinc chloride which creates an ionic buffer system which has antibacterial and antifungal activities. Like other oxidative preservatives, it degrades quickly when exposed to the ocular toxicity. It is very well tolerated in vivo and in vitro and significantly less toxic than BAK.[47,48] However, the antimicrobial action is slow to a microbial challenge causing it to fail EP-A criteria. Interestingly, a related preservation system which also passed USP AET testing was introduced for a multidose form of Systane Free demonstrating adequate or superior antimicrobial activity when compared to other artificial tears.[45] After its commercial introduction, however, several reports of mold growth in Systane Free Liquid Gel led to its recall and elimination from the market in 2006. Although Travatan Z does not contain the aminomethylpropanol component pointed to by the manufacturer as the promoter of the contamination, it does suggest when considered in the context of its failure to meet EP-A criteria that it is not a comparatively robust preservative system.

Conclusion

Toxicity from topical compounds may be a result of either the active drug, one of the other components in the preparation or both. The toxicity of benzalkonium chloride to the ocular surface is well known and may result in both short term and permanent alterations to the ocular surface including limbal stem cell deficiency. Of equal concern are the potential side effects in patients requiring BAK-containing compounds under chronic therapy even at low doses. It is important to understand, however, that BAK remains the gold standard for preservative efficacy in ophthalmic solutions and, despite this, ''in-use'' solution studies continue to show high levels of contamination and can lead to severe ocular surface infections.[7–10] Although controversial, there is some evidence that the epithelial toxic effects may aid in penetration of certain medications and that BAK may have therapeutic effects in patients being treated for different forms of infectious keratitis where the short term use of a BAK-containing drug is likely relatively safe.[20,33]

Unfortunately, the level of antimicrobial activity of all of the current ophthalmic preservatives is, at this point in time, largely inversely proportional to its compatibility with the ocular surface. Specifically, compounds that are highly effective antimicrobials are toxic to the ocular surface and those which have less robust antimicrobial activity sufficient to pass some AETs are relatively non-toxic. Given current knowledge, for short term indications where the risk of contamination or secondary infection is high, choosing a preparation with the best preservation efficacy is reasonable. These indications would include infectious keratitis, prophylaxis for epithelial defects, surgical prophylaxis, etc. Patients with ocular surface disease would likely demand the use of unpreserved, unit dose medications for long or short term use. While a patient with a healthy ocular surface which, on its own, would confer some resistance to infection, a less efficacious but also less toxic preservative would suffice. Further, the concentration of preservative may not be entirely indicative of a preparation's toxicity as other factors including frequency of dosing and resident time on the ocular surface will affect the amount of exposure. The ophthalmologist is then left to individualize medication choices based on risk of infection and contamination balancing the risks of both ocular surface toxicity and the potential for other adverse effects.

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Conflict of interest

The authors declared that there is no conflict of interest.

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