Allergy to ophthalmic preservatives

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Purpose of review

The purpose of the present review is to examine the hypersensitivity reactions to preservatives in topical ophthalmic therapies.

Recent findings

Ocular hypersensitivity reactions to different types of preservatives in different chemical classes of topical ophthalmic treatments reviewed in the literature include IgE-mast cell mediated, cell mediated and toxic. Quaternary ammoniums (benzalkonium chloride) are most commonly (8% reported cases in *OVID* and *PubMED* based searches) associated with irritant toxic reactions whereas the organomercurials (thimerosal) and the alcohols (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 although the term allergy for the 'alcohols' appears to be actually an irritant effect whereas the organomercurials appear to truly interact with the immune system as neoantigens.

Summary

A large number of clinical and experimental studies reveal that preservatives in topical ophthalmic medications have been demonstrated to produce effects from inflammation/hypersensitivity to permanent cytotoxic effects involving all structures of the eye.

Keywords

eyedrops, hypersensitivity, ophthalmic, preservatives, topical, toxic

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Introduction

The use of preservatives in topical ophthalmic treatments is ubiquitous as they allow their use in compromised eyes with a poor defense against infection. However, although providing effective biocidal properties with well tolerated short-term use at low concentrations, preservatives can cause serious inflammatory effects on the eye with long-term use in chronic conditions, such as glaucoma or potentially ocular allergies. This study reviews the reactions associated with the most commonly used ophthalmic preservatives in animal and human participants.

Preservatives and hypersensitivity reactions in the eye

There are many adverse reactions associated with topical ophthalmic medications. Most of these reactions are toxic and result from chemical irritation. Only about 10% of all adverse reactions to topical ophthalmic drugs are truly allergic. Furthermore, allergies (IgE and cell mediated) are more commonly caused by the active pharmaceutical agents, such as neomycin or sulfa-based agents and rarely by preservatives or other additives [1,2]. As the incorporation of preservatives in topical ophthalmic solutions becomes more common, sensitization toward preservatives is increasing. The salts of benzalkonium have been classified as being moderately allergenic (4–11% skin test positive) whereas mercurial products are strongly allergenic (13–37% of skin tests are positive). True allergic sensitization by other preservatives (chlorhexidine and chlorobutanol) is unusual.

The different types of hypersensitivity reactions can be separated into the following categories: allergic reactions (IgE-mast cell mediated hypersensitivity), cicatrizing allergic conjunctivitis (type II and III hypersensitivities) due to antibody localizing to ocular tissue or immune complexes deposition and allergic contact conjunctivitis, a type IV hypersensitivity reaction (Table 1) [3–5]. The term allergy in the ophthalmological literature is commonly used interchangeably with immunological responses of any type and does not necessarily denote an IgE-mast cell mediated process.

Preservatives

Nature and properties of the various preservatives: the different chemical classes (Table 2).

Benzalkonium chloride

Benzalkonium chloride, also known as BAC, is a quaternary ammonium, which is a highly hydrosoluble

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Table 1 Hypersensitivity reactions in the eye and the associated preservatives

Type of reaction	Description	Ocular manifestations	Preservative association
Type I hypersensitivity	Triggered by the classical activation of the IgE-Mast Cell axis and its associated early phase and late phase responses with an inflammatory reaction characterized by infiltration of PMNs, eosinophils and mononuclear cells into the corneo-conjunctival tissues and are also known as anaphylactoid reactions.	Characterized by acute itching, conjunctival hyperemia and chemosis and by edema of the eyelids either as urticaria (hives or wheals) in the superficial layers of the skin (epidermis and dermis) or angioedema (in the deeper subcutaneous tissues) or both, as well as production of significant quantities of mucus, edema and neovascularization of the cornea, and inflammation of the iris and infiltration of the anterior chamber [1]. Histopathologically, these reactions show edema of the eyelids and conjunctiva, dilation of the venules and capillaries and infiltration of lymphocytes, eosinophils and neutrophils.	Chlorhexidine: a 58-year-old male patient developed anaphylactic shock, possibly due to the use of chlorhexidine as an ophthalmic wash solution. He was successfully resuscitated without any sequelae. The patient had increased levels of both histamine and tryptase. The skin test for allergy resulted in strong positive to chlorhexidine. There have been many reports regarding severe adverse reactions associated with use of chlorhexidine [3].
Type II–III hypersensitivity	Antibody-mediated hypersensitivity reactions are also known as localized antibody-specific disease or immune complex mediated reactions.	Cicatrizing allergic conjunctivitis (pseudopemphigoid) reaction is a response to topical medication that results in cicatrizing conjunctivitis resembling ocular cicatricial pemphigoid (OCP). It is characterized by scarring in the bulbar, forniceal, and palpebral conjunctiva that is worse inferiorly along with conjunctival keratinization and punctual occlusion. The progression of symptoms cease once the offending medication is discontinued [1].	BAC, Kilp [4] report a case of a woman instilling artificial tear solution containing benzalkonium for treatment of dry eye syndrome, who developed a superficial keratitis which regressed after substitution with a preservative-free treatment [5].
Type IV hypersensitivity	Drug induced ocular allergies are most often the result of type IV hypersensitivity. Type IV hypersensitivity is cell mediated and also known as delayed-hypersensitivity reactions. (contact conjunctivitis)	Many of the type IV hypersensitivity reactions occur at the eyelid level that often makes it difficult to differentiate from other causes of eyelid inflammation or contact dermatitis. These types of allergic reactions can be detected by skin tests.	Thimerosal: the manifestations of the ocular delayed hypersensitivity reactions include conjunctival hyperemia, corneal infiltrates, and intolerance to lens wear with the use of soft contact lens solutions or other topical ophthalmic medications containing thimerosal. Delayed hypersensitivity to thimerosal can be demonstrated by an occlusive patch test or intradermal injection [5].
	Allergic contact lens keratoconjunctivitis (CLK) reaction is a type IV delayed hypersensitivity reaction secondary to use of contact lens solution.	The patient must be exposed to the preservative for several years before sensitization occurs. It is characterized by progressively increasing intolerance of contact lenses, punctuate staining along the limbus for 360 degrees and above the superior limbus, and a whorl-like staining over much of the cornea. There is also an associated fine papillary conjunctival reaction [1].	The classic cause of CLK is thimerosal, although it can also be attributable to chlorhexidine gluconate or EDTA.

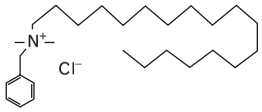
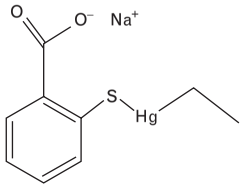
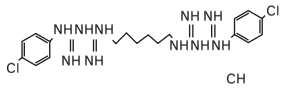
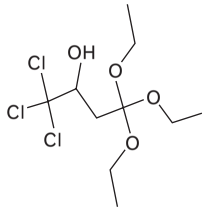
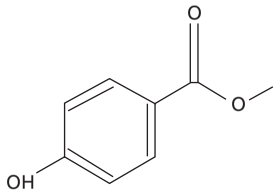
PMNs, polymorphonuclear leukocytes.

bipolar compound with surfactant properties. Their mechanism of action primarily involves its intrinsic detergent activities depending on its concentration (ranges from between 0.004 and 0.02% in most topical products) leading to dissolution of bacterial cell walls and membranes. The spectrum of activity is mainly focused on Gram-positive bacteria. BAC is used in a wide range of commonly used products, such as soaps, cosmetics, cleaning products, ophthalmic preparations, disinfectants, and spermicides. BAC is known to cause damage/toxicity in almost all ocular structures.

Animal studies

Although the use of BAC does not appear to interfere with the absorption of the therapeutic agent in animal models [6•], Becquet *et al.* [7] performed a study using rats to demonstrate the toxic and immunoallergic reactions that take place in the corneo-conjunctival surface after subjecting the eyes to the application of various preservatives. They found that even at low concentrations of a single instillation of BAC, toxic effects on the corneo-conjunctival surface were noted most likely due to its intrinsic detergent properties that can alter tear fluid stability, particularly in its lipid phase. In rats treated with various other preservative solutions (BAC 0.01%, methyl parahydroxybenzoate 0.05%, and thiomersal 0.004%) there was an infiltration of immunocompetent cells into the limbus and bulbar conjunctiva that expressed class II and CD11b membrane HLA antigens (leukocyte integrin). Similar results were reported later by Baudouin [8] in rats treated by timolol 0.5% containing BAC (0.01%) with abnormal expression

Table 2 Preservatives commonly used in topical ophthalmic agents

Chemical class	Most commonly used preservative	Structure	Properties of class	OVID search (and 'preservatives' and 'ophthalmology')[a]	'Irritant', 'inflammatory', 'allergic'[b] %	PubMED search (and 'preservatives' and 'ophthalmology')	'Irritant', 'inflammatory', 'allergic' %
Quaternary ammonium	BAC		Act mainly via detergent activities by dissolving the bacterial walls and membranes and destroys the semi-permeable cytoplasmic layer	593 (25)	Irritant 40 (8%); inflammatory 114 (21%); allergic 99 (19%)	438 (27)	Irritant 37 (8%); inflammatory 32 (7%); allergic-31 (7%)
Organo-mercurial derivative	Thimerosal		Act as a result of the sulfur-removing properties of the mercuric ion by combining with the sulfhydril groups of proteins to precipitate bacterial proteins by forming proteinates of mercury	500 (7)	Irritant 19 (4%); inflammatory 43 (9%); allergic 96 (19%)	309 (1)	Irritant 10 (3%); inflammatory 9 (3%); allergic 42 (14%)
Amidine	Chlorhexidine		Acts by destroying the semi-permeable layer of the cytoplasmic membrane and has antimicrobial activity mainly against cocci and gram positive bacteria and some gram negative; Also has fungistatic activity	2685 (3)	Irritant 65 (2%); inflammatory 305 (11%); allergic 124 (5%)	1499 (0)	Irritant 3 (0.2%); inflammatory 70 (5%); allergic 23 (2%)
Alcohol	Chlorobutanol and phenylethanol		Chlorobutanol: increases lipid solubility and can cross the bacterial lipid layer; Phenylethanol: exhibits synergistic activity when combined with other preservatives (chlorobutanol, BAC, chlorhexidine)	Chlorobutanol 46 (3); phenylethanol 221 (1)	Irritant 3 (7%); inflammatory 8 (17%); allergic 9 (20%); irritant 0; inflammatory 3 (1%); allergic 1 (0.5%)	Chlorobutanol 27 (3); phenylethanol 640 (0)	Irritant 0; inflammatory 2 (7%); allergic 3 (11%); irritant 2 (0.3%); inflammatory 59 (9%); allergic 4 (0.6%)
Parabens	Esters of parahydroxy-benzoic acid		Activity targets mold and fungi rather than bacteria	381 (0)	Irritant 9 (2%); inflammatory 17 (4%); allergic 40 (10%)	339 (0)	Irritant 3 (0.9%); inflammatory 5 (1%); allergic 23 (7%)

All search terms in the year range 2004 to current.

[a] The number of results from an OVID or PubMED search for the specific type of preservative (e.g., BAC, chlorhexidine, chlorobutanol, etc.) is shown in the table. The number in parenthesis represents the number of search items that result from combining the specific preservative with the terms 'ophthalmology' and 'preservative'. (e.g. chlorhexidine and preservative and ophthalmology).

[b] The number of results when combining the preservative with the search terms 'irritant', 'inflammatory', or 'allergic' is shown in the table. The number of parenthesis is the percentage found by dividing the number of results in this new search category with the number of total search results for that preservative. (e.g., searching chlorobutanol and irritant yields 3 results, 3 is then divided by 46, the total number of results for the term chlorobutanol, 3/46 is 7%).

of antigens human leukocyte antigen-DR and clusters of designation 23.

Human studies

Human in-vitro studies performed by Becquet *et al.* [7] showed that unpreserved β-blockers showed no toxic effects on cultured human Tenon's capsule fibroblasts, whereas preserved β-blockers showed toxicity and inhibition of fibroblast proliferation. Mietz *et al.* [9] also demonstrated that instillation of another β-blocker, metipranolol 0.3% preserved in BAC, produced deterioration of the composition of the extracellular matrix and the organization of the conjunctival stroma, combined with an increase in the number of activated subepithelial fibroblasts, in the deposits of collagen and the thickening of the basal membrane of the endothelium. In a tissue culture model utilizing immortalized corneal and conjunctival epithelial cells, toxicity was observed with all preservatives, but dependent upon concentration with the order of decreasing toxicity observed for thimerosal (0.0025%) more than BAC (0.025%) more than chlorobutanol (0.25%) more than methylparaben (0.01%) more than sodium perborate (0.0025%) [10•]. Goto *et al.* [11] performed a study in which human lens epithelial cells were cultured in medium containing different dilutions of latanoprost, timolol maleate, and BAC and then assessed using phase-contrast microscopy after 7 days' culture to determine the morphological changes that take place. The experiment showed that there is a dose-dependent toxic effect of BAC induced by the expression of prostaglandin E2 (PGE2), IL-1α and IL-6, resulting in the inhibition of the proliferation and elongation of the human lens cell and then to cell death.

The effects of preservatives on the eye are sometimes obscured by the chronic disease process, for which topical ophthalmic medications are used. A study performed by Hamard *et al.* [12] showed that BAC played a role in trabecular cell death in glaucoma patients from the chronic use of topical ophthalmic medications containing BAC. In the study, normal and glaucomatous trabecular cell lines were treated for 15 min with antiglaucoma drugs (1/100 and 1/10 dilutions): timolol BAC+ or BAC−, betaxolol BAC+ or BAC−, latanoprost BAC+ or pure BAC. Apoptotic marker (Apo2.7) expression, annexin V binding and DNA content were evaluated by flow cytometry and confocal microscopy. They found that benzalkonium-containing β-blockers and prostaglandin analogue triggered mild expression of one out of three apoptotic markers, whereas the proapoptotic effect observed with BAC appeared to be largely hindered by active compounds in the preserved eyedrops. The use of BAC may be worse in patients with more chronic ocular disorders as patients with atopic dermatitis had an increased sensitivity to preservatives, such as thimerosal, parabens, and BAC [5]. However, when actually trying to assess the impact of BAC in cell-mediated responses, a recent study [13] tested 42 898 patients with BAC 0.1% in petrolatum (topical drugs, ophthalmics, and disinfectants; http://www.ivdk.org) between 1996 and 2006 demonstrated 0.6–1.5% reactions with a total of 41 stronger positive reactions.

Although human in-vivo studies have generated rare reports of BAC induced IgE-mast cell type reactions, a recent study [14] demonstrated bronchoconstriction in asthmatics when challenged with BAC suggesting a non-specific trigger. Specifically relating to the eye, a study by Ishibashi *et al.* [15] evaluated preserved and nonpreserved topical timolol and noted that the NIBUT (non-invasive breakup time) of the precorneal tear film was significantly shortened. They evaluated precorneal tear film stability without fluorescein instillation that facilitates the in-vivo noninvasive observation of precorneal tear film breakup and found that eye exposure to preserved timolol resulted in significant instability in the precorneal tear film at 30 min after instillation, whereas the preservative-free timolol had no such effect suggesting that even a single exposure to 0.005% BAC may produce precornealtear film instability.

Thimerosal

Thimerosal, in its usual concentrations range from 0.001 to 0.004%, is an organomercurial derivative that acts as a result of the sulfur-removing properties of the mercuric ion. They act by combining with the sulfhydryl groups of proteins to precipitate bacterial proteins by forming proteinates of mercury. The proteinates act as a neoantigen that causes the highest frequency of cell-mediated responses of the ophthalmic preservatives [10•]. It is most commonly found in soft contact lens solutions and may cause ocular delayed hypersensitivity.

Animal studies

In 1991, a study [16] on ocular hypersensitivity to thimerosal in rabbits documented that the signs and symptoms observed included corneal edema, corneal infiltration and erosion, infiltration of the anterior chamber, iritis, conjunctival edema and hyperemia, and a significant increase in mucous production. They found that the IgG tear antibodies increased as a result of increased vascular permeability with the tear IgA titers increasing to a lesser extent than IgG during the ocular challenge. The major class of serum antibodies consisted of IgG, with IgA compromising approximately 5% of serum antibodies. Histologic analysis showed that the ocular inflammatory response was accompanied by both polymorphonuclear (PMN) and mononuclear cell infiltrates into the cornea and conjunctiva. Both serum and tear antibodies correlate with the severity of the ocular inflammatory response and support an

immune complex mediated or Arthus type of ocular hypersensitivity to foreign antigens. In another animal study utilizing rat model performed by Becquet *et al.* [7], thimerosal application to the eye resulted in hyperplastic changes to the corneo-conjunctival surface with increasing expression of Limbal class II antibody. In this study, anticlass II (RT1b) antibody was found to be the most reliable marker to locate and count inflammatory cells.

Human studies

Thimerosal has demonstrated in a concentration-dependent manner on human dendritic cells, inhibition of lipopolysaccharide (LPS)-induced proinflammatory cytokines including TNFα, IL-6, and IL-12p70 while having no effect on IL-10. These thimerosal-exposed dendritic cells induced increased TH2 (IL-5 and IL-13) and decreased TH1 (IFNγ) cytokine secretion from the T cells in the absence of additional thimerosal added to the coculture [17]. In addition, there is a potential impact of thimerosal on limbal stem cells as documented in a recent case report [18].

Tosti and Tosti [3] provides a case report of 36 patients with follicular allergic contact conjunctivitis induced by thimerosal. All of these patients report using eye drops containing thimerosal. Furthermore, 13 patients were soft contact lens wearers who became sensitized to their contact lens solution containing thimerosal. In the majority of these cases, the eyelids were spared. But in five patients, they also developed an allergic contact dermatitis of the eyelids. All of the 36 patients had a positive patch test reaction to thimerosal.

Chlorhexidine

Chlorhexidine is a cationic agent that belongs to the family of the bis-diguanides. It is used in the digluconate form, and acts by destroying the semi-permeable layer of the cytoplasmic membrane and produces its antimicrobial activity mainly against cocci and Gram-positive bacteria, Gram-negative bacteria as well as fungistatic activity.

Human studies

Although chlorhexidine has been associated with IgE-mast cell mediated reactions, such as anaphylaxis, the evidence for localized ocular allergy is lacking [19–22]. Vaahtoranta-Lehtonen *et al.* [23] performed an experiment comparing ethyl-6-*O*-decanoyl-glucoside 0.005% (EDG) combined with 0.00025% chlorhexidine acetate (EDGC) to a commercial polyaminpropylbiguanide (PAPB) used daily as a cleaning and disinfectant agent for both ionic and nonionic contact lenses in 59 patients. The following symptoms were compared for each solution; blurred vision, dryness, foreign body sensation, redness, and dirty lenses. The following signs were also compared for each solution; conjunctival hyperemia, papillary hypertrophy, corneal deposits, purulence, limbal vascularization, subepithelial scarring, visual acuity, bulbar hyperemia, and tear breakup time. After 8 weeks, 52% of the participants in the EDGC group showed no evidence of corneal or conjunctival abnormalities. In contrast, only 19% of the participants in the PAPB group showed no abnormalities of the conjunctiva or cornea. After 8 weeks, 25% of the EDGC group showed evidence of papillary hypertrophy, whereas 50% of the PAPB group showed similar findings [23].

In three consecutive cataract operations, chlorhexidine was inadvertently used as an intraocular irrigating solution as a result of inattentiveness of an assistant. In two of the three patients, corneal endothelium damage was so severe that penetrating keratoplasty had to be performed. Further effects included pronounced iris atrophy, anterior chamber applanation, and a retrocorneal membrane. In one case, an increase in intraocular pressure developed. No effects were observed in the retina or optic nerve [24].

Chlorobutanol and phenylethanol

Chlorobutanol is an alcohol that acts by increasing lipid solubility, and its antimicrobial activity is based on its ability to cross the bacterial lipid layer. Chlorobutanol is a widely used, very effective preservative in many pharmaceuticals and cosmetic products, for example, injections, ointments, products for eyes, ears and nose, dental preparations, etc. It has antibacterial and antifungal properties. Chlorobutanol is typically used at a concentration of 0.5% where it lends long-term stability to multi-ingredient formulations.

Phenylethanol is an antimicrobial, antiseptic, and disinfectant, which is used also as an aromatic essence and preservative in pharmaceutics and perfumery.

Animal studies

Two drops of a chlorobutanol-containing or BAC-containing artificial tear were instilled into the right eye of six rabbits. At the same time six control animals received no eyedrops. The central region of the corneal epithelium was quantitatively assessed using a computer system. There were up to 5% exfoliating cells evident at the ocular surface in treated rabbits but with no difference between the two products. Controls had no cell exfoliation (<0.5%). The distribution of surface areas of the squamous cells in the treated eyes was shifted to slightly larger values than in the controls after use of the chlorobutanol-containing product but the number of epithelial cell craters/cell was unchanged from that of the controls. Cell surface areas were shifted to significantly smaller values than controls after use of the BAC-containing product and there were much fewer epithelial cell craters/cell. The results reveal differences in the effects of preservative-containing

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