
Théa: Specialists
in Preservative-Free
Théa
let's open our eyes

For the last

150 years

we have been pioneers in ophthalmology.

By developing treatments, providing information and sharing knowledge with specialists around the world, we enable everyone to keep their eyes wide open.

Théa specialise in preservative-free treatment. Preservatives are increasingly recognised as having a negative impact on ocular structures.

This brochure summarizes the evidence and impact that preservatives can have on the eyes.

Contents

Introduction 6

Symptoms 6

Tear film & goblet cell density 6

Markers of preservative-related inflammation 6

Consequences of the inflammatory response 7

Subepithelial fibrosis 7

Ocular surface: Subjective symptoms and clinical signs 8

Complications of chronic treatment: Glaucoma surgery 10

Complications of chronic treatment: Cataract 11

Complications of chronic treatment: Conjunctival cicatrisation and pseudopemphigoid 11

Cytotoxicity in the superficial ocular tissues 12

Corneal cytotoxicity 13

Severe corneal toxicity: Clinical observations 14

Switching studies 16

Dry Eye 17

Chemical classes of preservatives 19

Preservatives: Toxicity or allergy? 19

Conclusion 23

Introduction

SYMPTOMS

The prolonged use of preserved eye drops can result in stinging upon instillation, pruritus, foreign body sensation, conjunctival hyperaemia, shortened tear film break-up time and superficial punctate keratitis.

More seriously, chronic conjunctival fibrosis *(pseudopemphigoid),* trabecular modifications, cataract, cystoid macular oedema and failed trabecular surgery have also been associated with preservatives.

TEAR FILM & GOBLET CELL DENSITY

The disruption of the tear film, caused by the detergent effect of preservatives, affects the normal supply of nutrition and protection essential for a healthy ocular surface. This in turn creates an inflammatory response in the conjunctiva and an epithelial metaplasia[1] affecting goblet cells and transmembrane mucins. A reduction of nearly 50% of the density of these cells has been observed in biopsies from glaucoma patients receiving long-term treatment;[2] the loss of goblet cells and the change in tear film quality may be associated with the development of the subconjunctival fibrosis produced by preserved eye drops.[1]

MARKERS OF PRESERVATIVE-RELATED INFLAMMATION

Patients under long-term treatment with preserved antiglaucoma medication have been found to have a three- to four-fold increase in the density of lymphocytes and macrophages in the conjunctiva and layers of Tenon's capsule in patients under long-term treatment with combinations of different antiglaucoma eyedrops.[2] The intensity of the inflammatory reaction is related to the duration and the number of antiglaucoma medications used concomitantly.[3]

CONSEQUENCES OF THE INFLAMMATORY RESPONSE

Cytology specimens of patients under long term preserved treatment show well-defined features, including:

- Disorganised conjunctival layers
- Loss of tissue cohesion
- Modification of the morphology of epithelial cells
- Keratinisation
- Loss of goblet cells

The loss of goblet cells is the first sign of squamous metaplasia and is followed by an increase in cell stratification, and then keratinisation.[1] Indeed, in healthy subjects with no ocular disorders who had received BAK instillations twice daily for three months, the degree of metaplasis was comparable to that observed in patients under long-term treatment with preserved antiglaucoma drops, and higher than that found in controls.[4]

SUBEPITHELIAL FIBROSIS

The development of progressive subepithelial fibrosis with no clinical sign of intolerance is also documented and is frequent in patients treated long-term with preserved drops.[5]

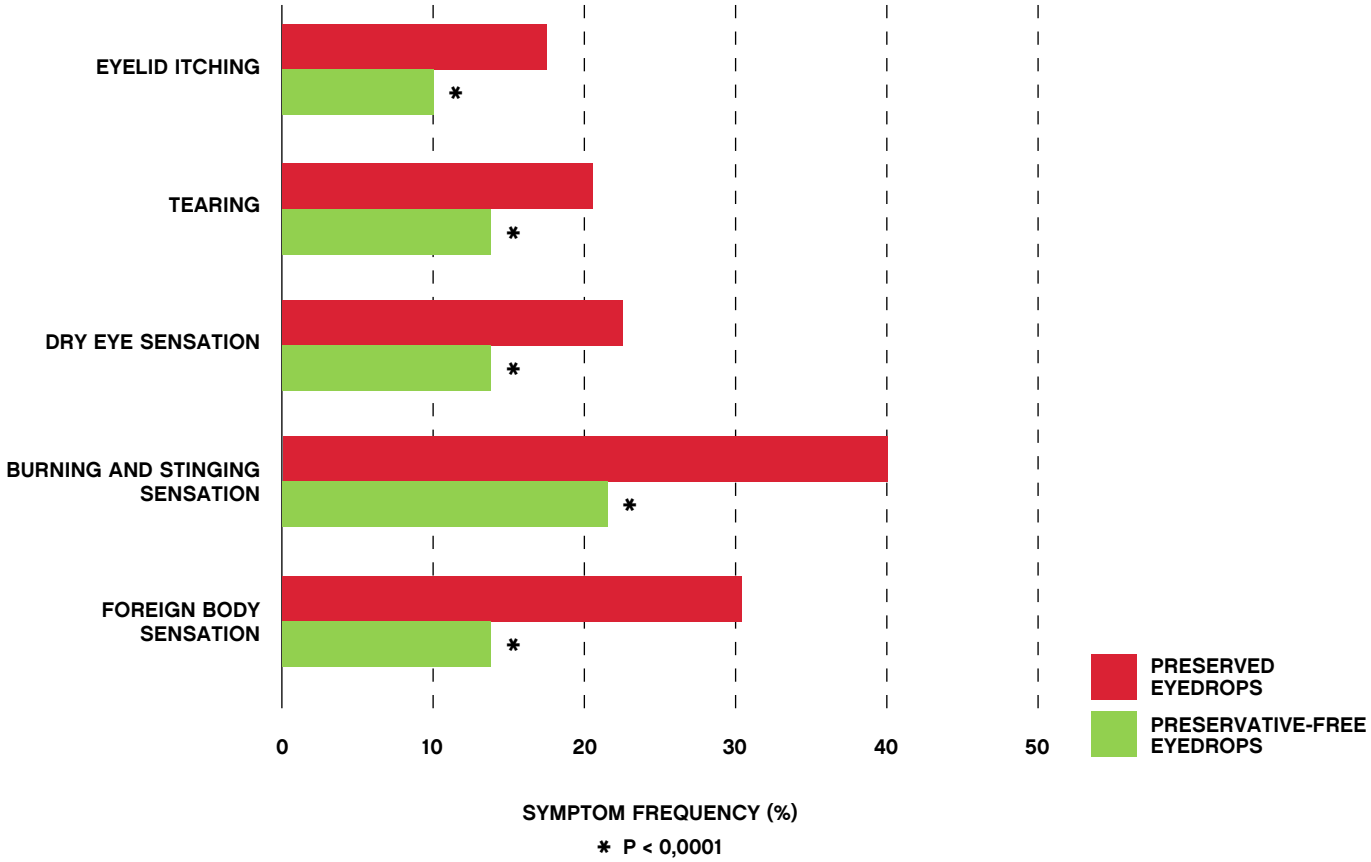
The inflammatory cell infiltration observed in these patients is generally associated with a significant increase in the fibroblast density in the subepithelial substantia propria[2,6] particularly in patients using multiple preserved treatments.[6] This suggests that the toxicity of eyedrops observed in the conjunctival epithelium may act on deeper ocular tissue.

Ocular surface: Subjective symptoms and clinical signs

Widescale studies have shown that preserved eyedrops may lead to an alteration of the ocular surface, resulting in symptoms such as stinging or burning, grittiness and foreign body sensation, as well as Dry Eye.[7,8] In comparison, these effects were reported significantly less often by patients using preservative-free drops. (See switching studies, page 16).

Figure 1

Prevalence of symptoms reported by glaucoma patients treated by preserved and preservative-free eyedrops

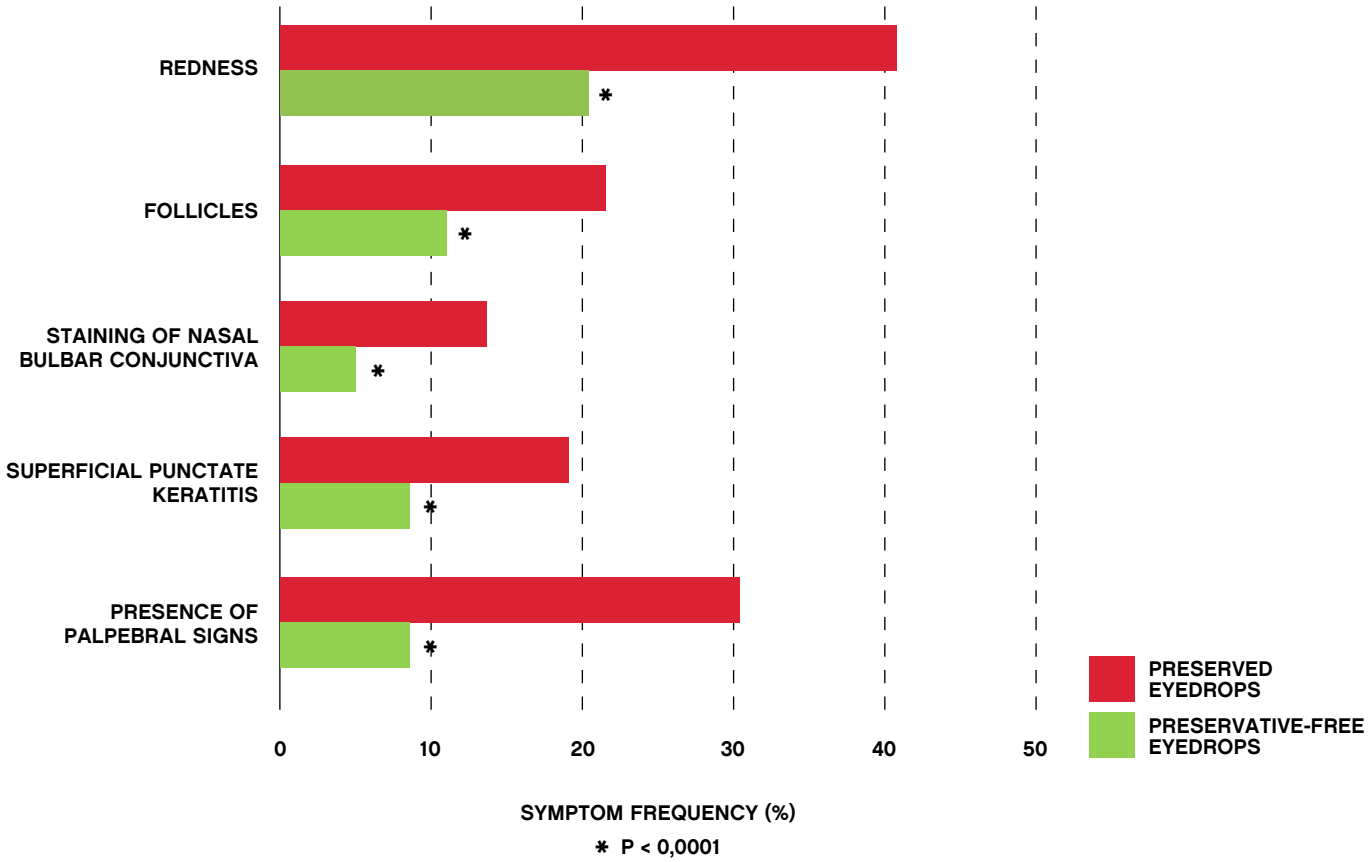


Adapted from 7, 8.

These side effects are minimised in patients treated with preservative-free eyedrops, suggesting significant involvement of the preservative in the occurrence of these functional signs and symptoms. Out of 4,107 patients receiving treatment for open-angle glaucoma, 84% were receiving one or more preserved eye drop solutions, 13% were receiving preservative-free monotherapy and 3% a combination of preserved and preservative-free drops with median treatment 3.9 years. Discomfort on instillation was reported more frequently in patients using preserved vs preservative-free drops (43% vs. 17%), particularly stinging or burning sensations (40% vs 22%) and grittiness (31% vs 14%), (Figure 1).[7]

Figure 2

Prevalence of functional signs in glaucoma patients treated by preserved and preservative-free eyedrops

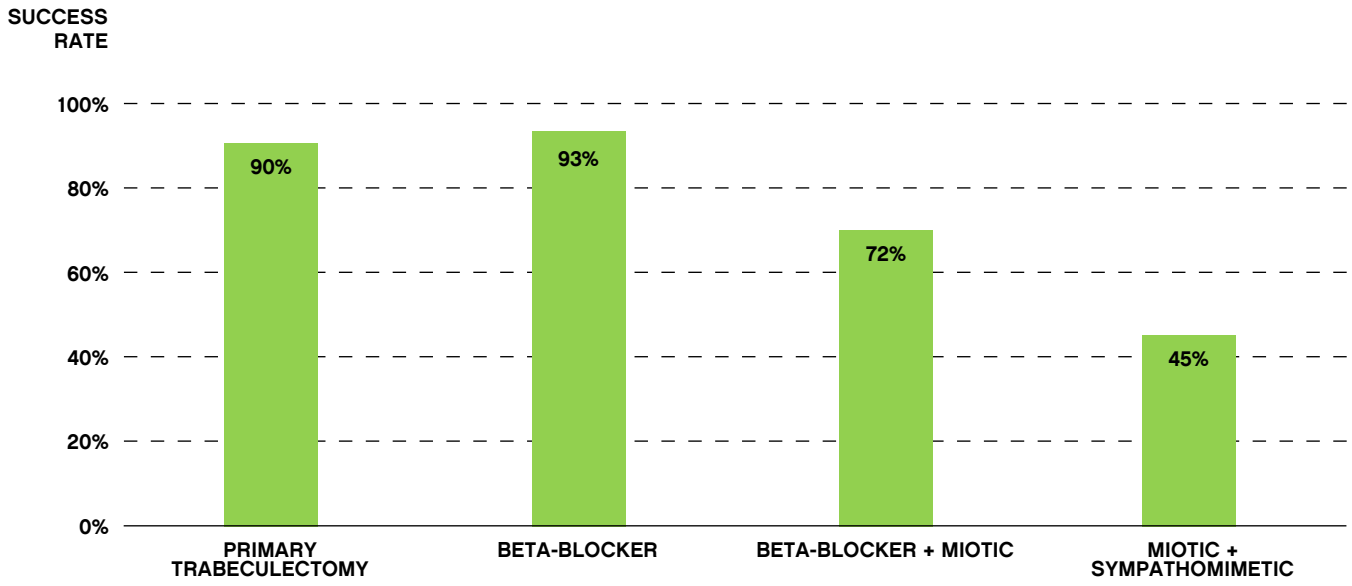


Adapted from 7, 8.

Complications of chronic treatment

It is accepted that the main cause of failed filtration surgery is the excessive development of a local fibrosis of the bleb, which hinders the flow of the aqueous humor.[9,10]; – Sherwood *et al* showed a significantly increased infiltration of inflammatory cells, fibroblasts and hyaline bodies in the substantia propria of the conjunctiva and in the layers of the Tenon's capsule in glaucoma patients undergoing long-term treatment of more than 1 year, compared to patients who had undergone a primary trabeculectomy.[2]

Success rate of filtration surgery in non-treated patients (primary surgery), in patients treated with beta-blocking agent alone, a beta-blocker plus a miotic agent, or a sympathomimetic agent plus a miotic drug.



Adapted from 9.

COMPLICATIONS OF CHRONIC TREATMENT: GLAUCOMA SURGERY

The success rate of trabeculectomy or deep non-perforating sclerectomy varies from 45-93%.[9] Broadway *et al* reported, in a series of 106 case studies, that success rates tended to decline in relation with an increased number of medications,[9] suggesting that duration and number of treatments are linked to the success of glaucoma surgery.

COMPLICATIONS OF CHRONIC TREATMENT: CATARACT

The incidence of cataract is increased significantly in patients under long-term treatment with topical antiglaucoma agents compared to non-treated patients, followed up for several years,[11] and the BISED study showed that treatments aimed at reducing intraocular pressure tripled the risk of developing nuclear opacity in the following 4 years.[12]

PRESERVATIVES AND CATARACT SURGERY

Complications post-cataract surgery are more frequent when antiglaucoma medication used pre-surgery contains BAK and anti-inflammatory medication used post-surgery contained BAK.[13] The presence of BAK appears to encourage the rupture of the blood-aqueous barrier following surgery, and also the risk of cystoid macula oedema after 5 weeks. The same study suggests that the use of a preservative-free non-steroid anti-inflammatory such as diclofenac could prevent complications and found that Fluoromethalone containing BAK did not have the same protective effect.

Similar findings were reported with patients using latanoprost.[14] Patients who received Fluoromethalone with BAK-preserved latanoprost experienced a significant increase in cystoid macular oedema compared to a group receiving a preservative-free latanoprost and preservative-free placebo. BAK would appear to lead to an increase in prostaglandin synthesis and synthesis of other substances, as well as intensify postoperative inflammation.[15]

COMPLICATIONS OF CHRONIC TREATMENT: CONJUNCTIVAL CICATRISATION AND PSEUDOPEMPHIGOID

The development of subconjunctival fibrosis during anti-glaucoma treatment is moderately frequent.[1,2] A significant diminution in the depth of the lower conjunctival fornix in glaucoma patients treated with preserved anti-glaucoma drops for at least 3 years compared to healthy subjects has been shown.[5] This effect has been shown with various types of preservative and medication. Exposure to preserved antiglaucoma eyedrops can be a risk factor in severe structural modifications such as pseudopemphigoid or chronic progressive conjunctival cicatrisation.

These changes can leave serious, irreversible sequelae; obstruction of lachrymal and Meibomium glands, changes to the tear film, trichiasis, keratopathies and possibly even blindness.[16] In addition, it is possible that exposure to preserved eyedrops may accelerate normal cicatrisation in patients at risk, presenting a pemphigoid.[17]

Cytotoxicity

The consequences of preserved eye drops on the cornea can be serious: thickening of the cornea, corneal oedema, damage of the endothelium, and opacity of the cornea.

CYTOTOXICITY IN THE SUPERFICIAL OCULAR TISSUES

CHANGES IN THE TEAR FILM

The tear film provides nutrients as well as lubrication. Detergent preservatives are able to dissolve the lipid layer of the tear film, facilitating the evaporation of water and leading to ocular dryness. The instillation of 3 drops of BAK, even at a very low concentration (0.0001%) reduces the break-up time (BUT) by more than 50%.[18] BAK prevents the lipid secretions from Meibomium glands from spreading over the aqueous phase of the tear film.[19]

CONJUNCTIVAL CYTOTOXICITY

Preservatives can have several consequences on the conjunctiva: cytotoxicity, activation of an infraclinical immune-allergic reaction and onset of a sub-conjunctival fibrosis which can lead to slow conjunctival healing.[16] The impact on the lachrymal apparatus – loss of mucous cells, dissolution of lipid tear phase, Dry Eye – can be serious and result in dry eyes, and compromise the success of filtering surgery in glaucoma patients.

LOSS OF MUCOUS CELLS

A reduction in the density of the mucous cells has been observed following the instillation of eye drops:[20]

Conjunctival imprints using confocal microscopy

The mucous cells correspond to the dark patches

A – Untreated patient: numerous mucous cells

B – Prolonged single-drug therapy: fewer mucous cells

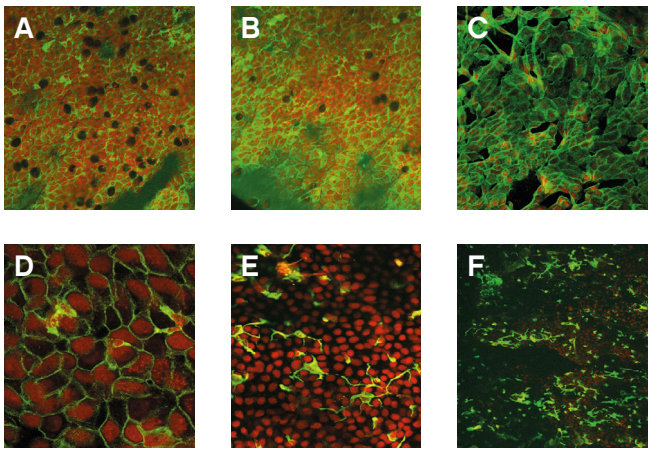
C – Multidrug therapy: metaplasia and the disappearance of the mucous cells

The cells expressing the markers of inflammation are stained green

D – Untreated patient: paucity of immune cells

E – Prolonged single-drug therapy: moderate inflammatory infiltrate

F – Multidrug therapy: very numerous immune cells



LOSS OF MUCOUS CELLS

The first consequence of this loss is a change in the composition of the tear film. Various animal studies[21,22] have demonstrated that an infiltration of fibroblasts and the onset of chronic fibrosis is induced by preservatives.

CORNEAL DISTRESS & RUPTURE OF THE EPITHELIAL BARRIER

Animal studies have demonstrated that preserved eye drops produce microlesions.[23] Repeated instillations (2 drops at 5 minute intervals for 1 hour) produces a dramatic release of dehydrogenase and albumin into the tears; correlated to corneal lesions and a sign of corneal distress.[24]

Applying various eye drops (1-2 drops for 30 days) preserved with either BAK or Purite® can result in a variable degree of the loss of the microvillosities, with puckering of the plasma membranes – a sign of cell necrosis and partial erosion of the cells in the first epithelial layer.[25] Complete destruction of the epithelial barrier with the loss of the most superficial layers of cells has been observed in rabbit eyes after contact with BAK 0.01%.[26] Damaged corneas could be susceptible to invasion by pathogens.

CORNEAL REPAIR

Repeated applications of BAK can lead to damage in the lower levels of the epithelium and can retard, or even inhibit, the repair of the epithelial barrier. BAK inhibits the extra-cellular matrix which facilities re-epithelialisation[27] even at concentrations 200 times lower than those used in commercial preparations. In some circumstances, particularly when the corneo-conjunctival surface is severely affected, the penetration of the eye drops and therefore of the preservative may be increased, and the deep tissues of the eye may be affected.

SEVERE CORNEAL TOXICITY: CLINICAL OBSERVATIONS

Long term exposure to preservatives can interfere with cell metabolism, produce toxic effects leading to cell death, premature desquamation of the epithelial cells, rupture of stromal keratocytes and possibly degeneration of endothelial cells, and lead to marked ulcerative keratopathies.[28]

Gasset[29] reports a corneal ulcer in a patient who had undergone extracapsular cataract extraction, who wore a protective soft contact lens cleaned with a solution containing BAK. It is likely that the prolonged contact with BAK produced toxic effects on the conjunctiva and core, resulting in the ulcer. Kilp *et al*[30] report a case of the development of superficial keratitis with vortex-like arrangement of the hyperplastic epithelial areas following the two-hourly, then every 30 minute instillation of an artificial tear solution containing BAK for Dry Eye Disease. And there have been multiple reports of symptoms of toxicity following the use of preserved ocular lubricants during general anaesthesia including keratopathies, axial fibrosis, and even permanent corneal oedemas.[5,31,32]

TRABECULUM

The failure of trabeculectomies in patients using preserved eye drops long-term can be attributed to preservatives.[35]
It has been shown that BAK can induce apoptosis in trabecular cells after a brief exposure, 15mins, to a low concentration 0.0001%.[33,34,35]

LENS

Patients receiving long-term antiglaucoma treatment tend to develop macular cystoid oedema more easily after cataract surgery.[15] The causes of this are not yet clear, but a possible link with inflammatory mechanisms has been suggested.[36]

RETINA

In pigmented rabbits, the subconjunctival injection of BAK-preserved timolol 0.5% of befunolol 1% produced retinal lesions that can be detected on the electroretinogram as a 50% reduction in the a and b waves after exposure for one week.[37]

This is followed by the detachment of the retina, a loss of visual acuity and the atrophy of the pigmented epithelium of the retina and the choroid. Preservative-free eye drops displayed only non-significant effects, indicating that the cause of these lesions and related effects is specific to BAK.

IMPROVING CLINICAL SIGNS AND SYMPTOMS

Cross-sectional study observations have been confirmed by switching studies. In patients treated by poorly-tolerated preserved eye drops, presenting with impairment of the ocular surface (functional symptoms, conjunctival, corneal or palpebral signs) or Dry Eye, changing to preservative free eye drops leads to a rapid improvement in the ocular symptoms.[7,8,38,39,40] and / or the tear film.[39,41,42,43] Such improvement however does not come at the expense of efficacy.[38]

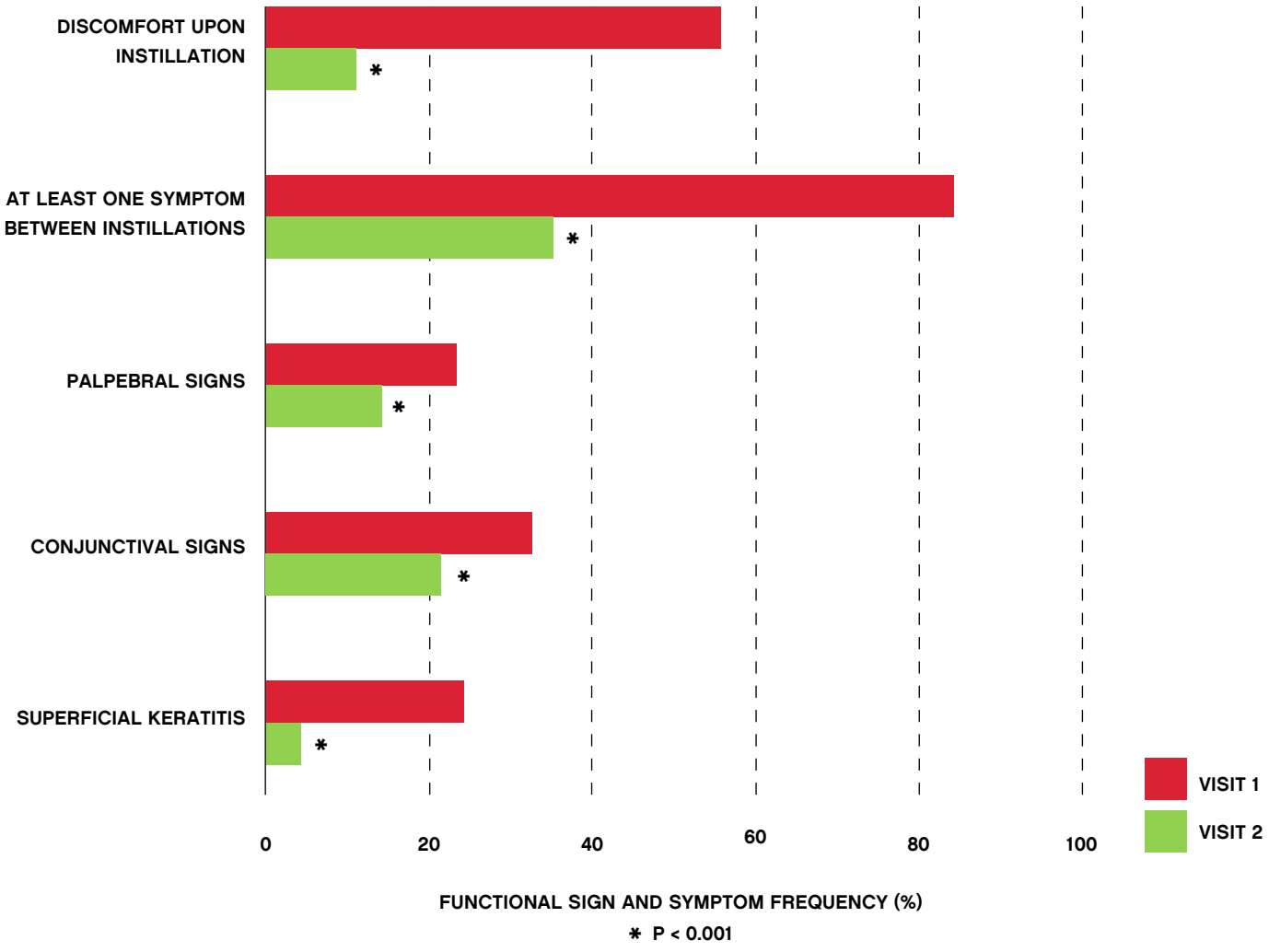
SWITCHING STUDIES

Switching studies, where preserved treatment is replaced by preservative-free treatment, illustrate a sizeable and significant ($p<0.001$) decrease in signs and symptoms of ocular impairment.[7]

Switching to a preservative-free solution leads to a significant increase in mucous cells and to a significant improvement in conjunctival epithelial cell impairment[7] as well as reduction in symptoms of stinging and foreign body sensation.

Switching to preservative-free also leads to a significant improvement in lachrymal secretion.[44] In the same study, Campagna[39] *et al* also found that lachrymal secretion, evaluated by fluorophotometry increased by an average of 28%, as well as significant improvement in tear film break-up time from 7.9sec prior to switching drops to 9.1 and then 9.3sec respectively at 2 months and 3 months.

Prevalence of functional signs and symptoms upon enrolment (visit 1) and 3 months after switching to preservative-free eye drops (visit 2) in glaucomatous patients.[7]



Adapted from 16

DRY EYE

In the treatment of Dry Eye, changing to preservative-free artificial tears improves symptoms and leads notably to a significant decrease in burning and irritation sensations, a reduction in the degree of keratitis, an improvement in the quality of the mucous and a better patient acceptance.[46,47] Preservative-free eye drops also preserve the integrity of the corneal epithelium more effectively than preserved drops.

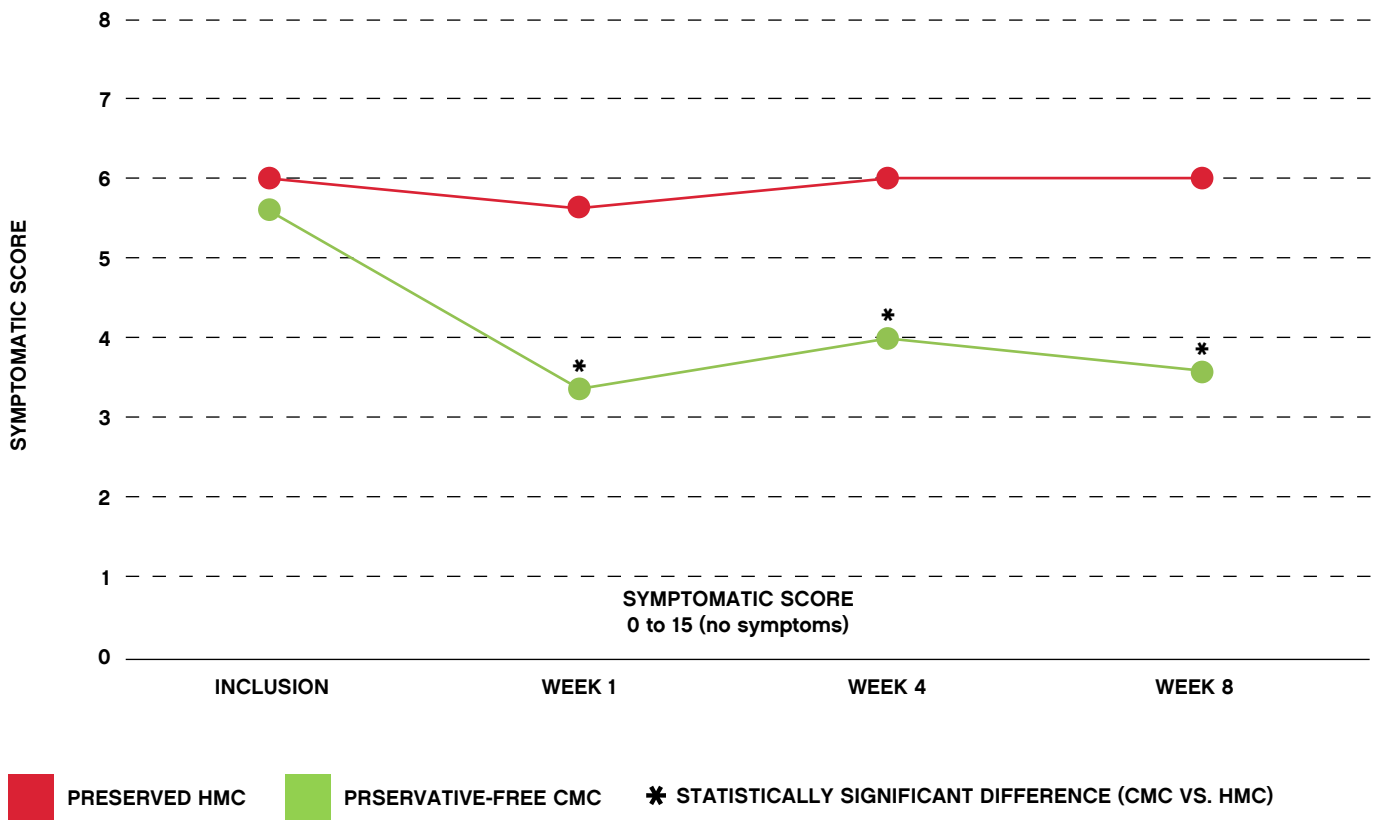
In a study[45] of 40 patients complaining of at least 2 severe symptoms (burning, pruritus or foreign body sensation) presenting with moderate metaplasia of the conjunctival epithelium and for the following 6 weeks they received a 2% polyvinylpyrrolidone (PVP) solution with 0.005% BAK, or a preservative-free solution of PVP in 6-9 instillations per eye, in both eyes. The study showed a significant improvement in the corneal surface of patients treated with preservative-free solution – epithelial permeability was reduced by 37% ($p<0.001$) in this group. In the group using preserved drops, corneal permeability deteriorated – it increased by 21% ($p=0.05$).

In another study by Grene *et al*[46] on 56 patients presenting with keratoconjunctivitis sicca, instillation of preservative-free carboxymethylcellulose (CMC) based artificial tears of 8 instillations per day for 8 weeks, led to a significant improvement in functional symptoms, superficial punctate keratitis and squamous metaplasia compared to patients treated with preserved drops.

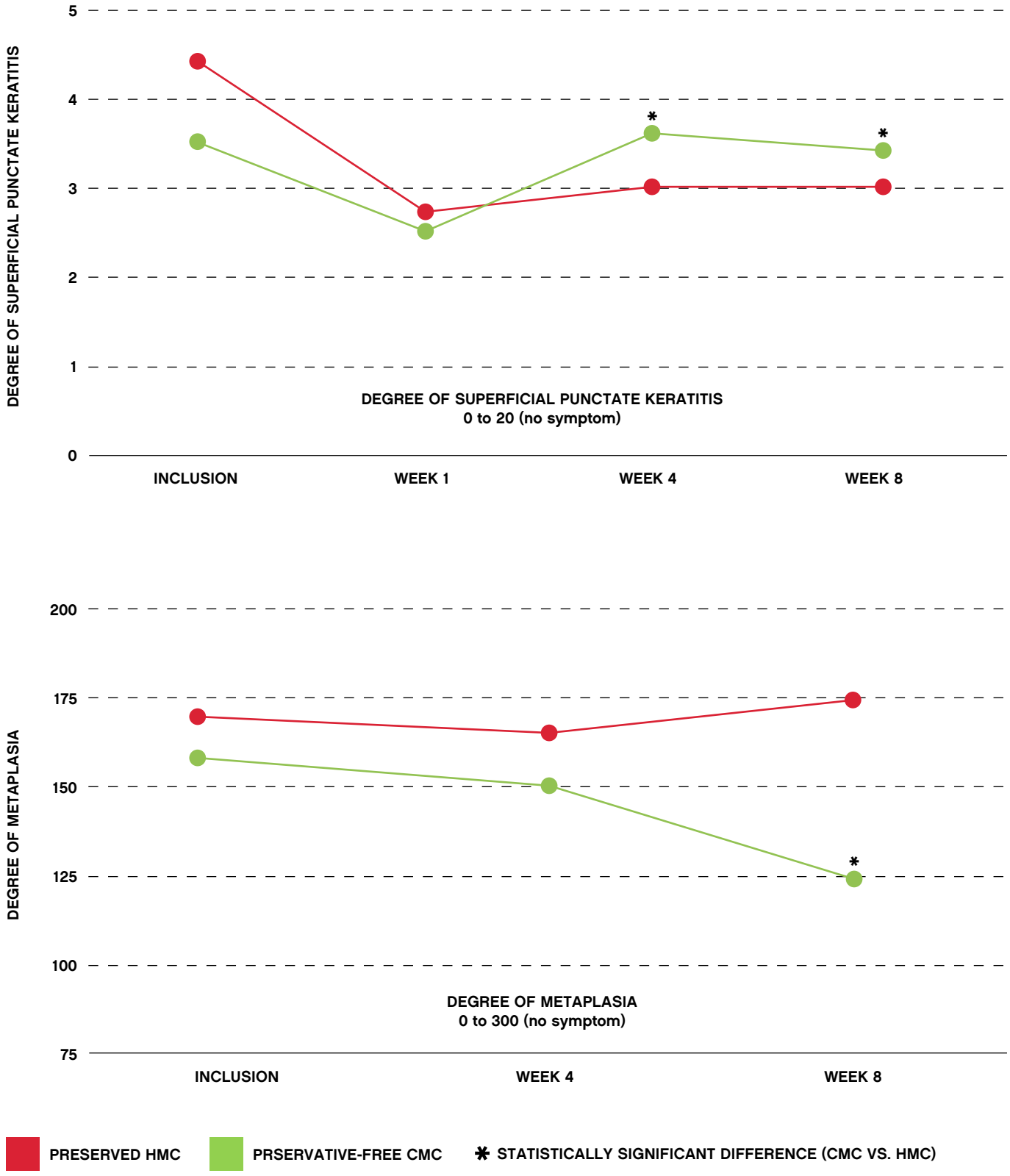
A randomised, open-label, controlled, intra-individual study by Smith *et al*[47] on 30 Dry Eye patients who had been ineffectively managed with preserved drops, when switched to preservative-free hydroxyethylcellulose (HMC) drops in one their two eyes whilst continuing preserved treatment in the other eye.

After 2 weeks of treatment, 63% of patients preferred the preservative-free artificial tears. Symptoms of sensations of grittiness, Dry Eye and rose Bengal staining were significantly reduced in the preservative-free eye with no change in the preserved eye:

Effects of preserved lubricant (HMC) and a preservative-free lubricant (CMC) on functional symptoms, superficial punctate keratitis and epithelial metaplasia in patients with keratoconjunctivitis sicca. Double-blind, randomised clinical study.[47]



Effects of preserved lubricant (HMC) and a preservative-free lubricant (CMC) on functional symptoms, superficial punctate keratitis and epithelial metaplasia in patients with keratoconjunctivitis sicca. Double-blind, randomised clinical study.[47]



Chemical classes of preservatives

Several different classes of preservatives are available. They have differing bactericidal potentials. Most have a non-specific detergent effect and as a result can also act against, and damage eukaryote cells.

Different classes are:

– Quarternary ammoniums e.g. benzalkonium chloride
– Organo-mercurial deriratives e.g. thimerosal
– Amidines e.g. chlorhexadine
– Alcohols e.g. chloributanol and phenylethanol
– Parabens
– Oxychlorinated complexes e.g. stabilized oxychlorinated complexes (Purite®)

PRESERVATIVES: TOXICITY OR ALLERGY?

Repeated and prolonged exposure to preservatives can induce sensitisation. Sensitisation is increasing, not only because preservatives are commonly used in eye drops but also because they are used in soap, cosmetics, disinfectants and other commonly used products.[49]

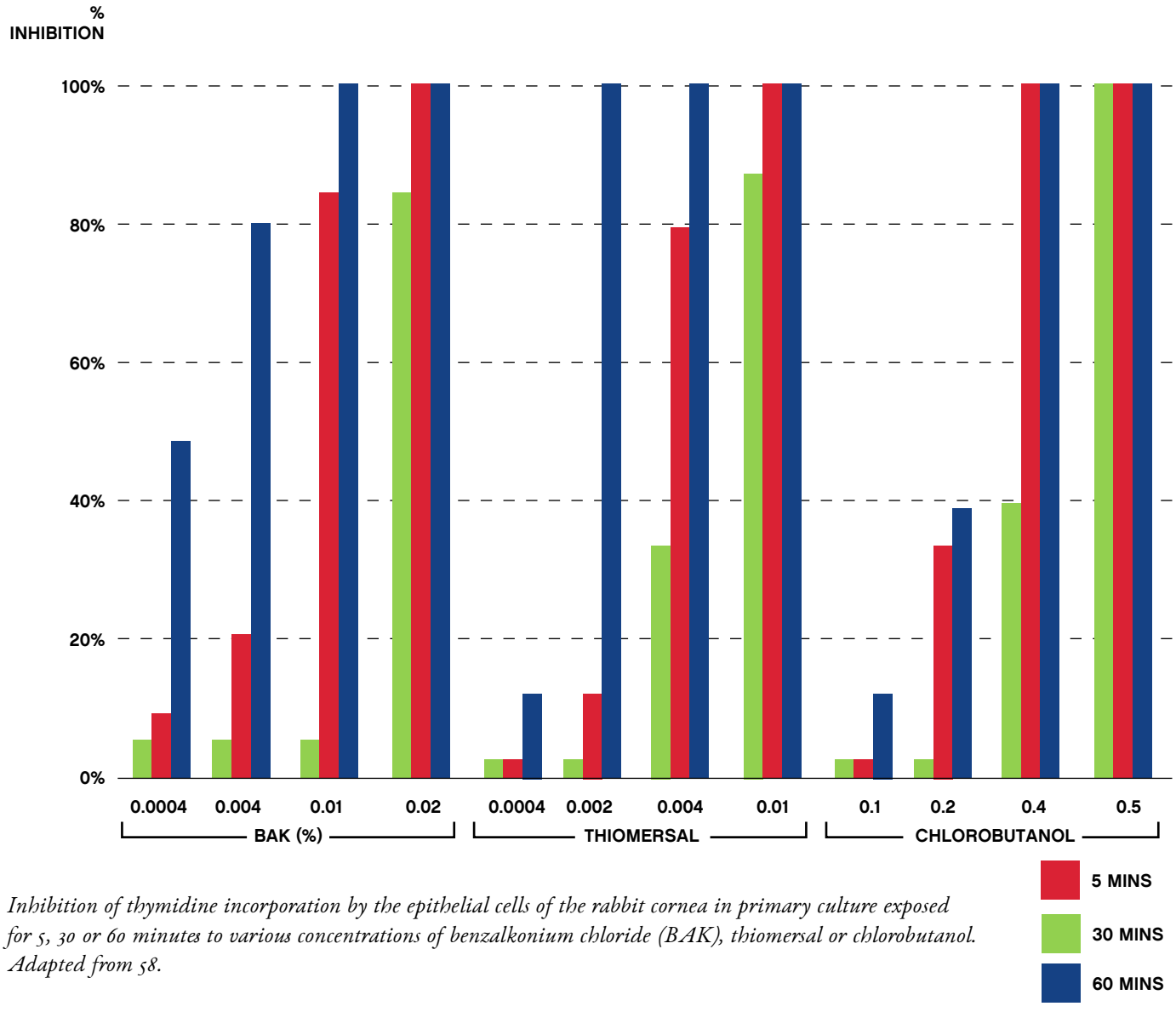
The inflammatory reaction is characterised by the infiltration of polymorphonuclear and mononuclear cells into the corneo-conjunctival tissues. Allergy towards preservatives usually presents as a conjunctivitis-like condition; this may consist of simple hyperaemia of the conjunctiva, or of papillary conjunctivitis with or without eczema of the eyelids.[50]

TOXICITY OF PRESERVATIVES

In vitro cytotoxicity has been well demonstrated in epithelial cells of the cornea and conjunctiva and keratocytes,[51,52,53] endothelial cells of the cornea,[53] fibroblasts from Tenon's capsule,[54] trabecular cells[34,53] and the epithelial cells of the lens.[36] These cytotoxic effects increase with the concentration of the preservative, and the duration of exposure.

Some cellular changes are irreversible, and eliminating the preservative may not always be enough to allow the cells to recover.[55,56,57]

Inhibition of cell proliferation by preservatives



Inhibition of thymidine incorporation by the epithelial cells of the rabbit cornea in primary culture exposed for 5, 30 or 60 minutes to various concentrations of benzalkonium chloride (BAK), thiomersal or chlorobutanol. Adapted from 58.

MORPHOLOGICAL CHANGES

The morphological changes produced by preservatives or preserved eye drops are widely documented by ex-vivo observations using electronic scanning microscopy[57-59] or in-vivo observations using confocal microscopy.[59]

Corneal changes have been demonstrated under extreme experimental conditions e.g. prolonged exposure or high concentrations. The following changes have been observed:

- Loss of microvillosities on the surface of epithelial cells
- Loss of contact with the adjacent cells
- Marginalisation of the cells and cell death characterized by puckering of the plasma membrane
- Desquamation of the surface layers exposing the cells in the other layers of the cornea.[57,59]

Dormans[57] *et al* reported that the first effects of instilling one drop of BAK 0.01% appeared within less than 10 minutes. The first symptom was swelling of epithelial cells and loss of microvillosities. After exposure for 30 minutes, the cornea is covered in swollen cells and the first 2 layers of the epithelium are severely affected. There is complete loss of the microvillosities, degenerative changes in the membrane, cell death and desquamation of the first two surface layers after exposure for 3 hours.

MECHANISMS RESPONSIBLE FOR THE TOXICITY OF PRESERVATIVES

The physical and chemical properties of preservatives account for their toxicity. At high concentrations they cause cell lysis by dissolving the membranes, and at lower concentrations they prevent intercellular interactions essential for cell survival. By being intercalated in the cell membranes, some preservatives (BAK in particular) can induce secondary degeneration as a result of a biological cascade leading to apoptosis. Indirectly, the cell damage, the denaturing of proteins and the metabolic changes, can trigger and maintain an immune-inflammatory reaction with the risk of scarring.

Two possible mechanisms for cell death have been suggested, depending on the concentration of the preservative. At high concentrations 0.01-0.05%, the mechanism is necrosis:[61] cells are lysed and membrane debris are visible in the cultures, the cells are very small and irregular in volume, the pattern of DNA migration on agar gel is characteristic.

At low concentrations, the quaternary ammoniums stop cell growth and trigger a process of programmed cell death. Cell death occurs after some delay, with the morphological and metabolic changes characteristic of apoptosis (cell retraction, chromatin condensation, DNA fragmentation and the expression of apoptosis markers).[62]

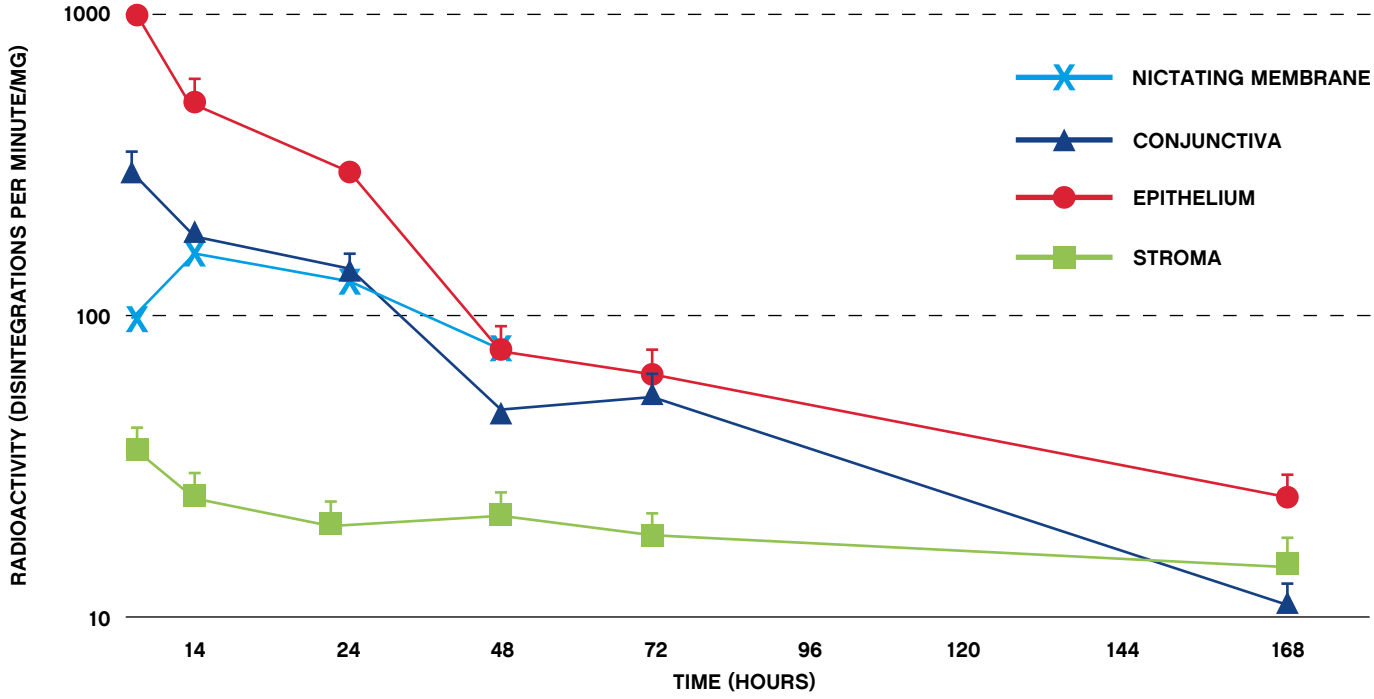
Eye drops containing quaternary ammoniums (0.01%) generate significantly more superoxide anions than preservative-free drops.[61] The superoxide anion O_2^- has a cytotoxic effect on cultured cells: it can break down polysaccharides and DNA; alter structure of membranes by lipid peroxidation, impair vascular permeability and potentiate inflammatory reactions.[62] It is probable that preservatives stimulate immunocompetent cells, starting the immuno-inflammatory reaction and onset of sub-conjunctival fibrosis. Baudoin *et al*[6] demonstrated the infiltration of inflammatory cells in the conjunctiva and trabeculum following instillation of preserved drops. The infiltration was absent in non-preserved drops in a rat model, suggesting that preservatives are responsible for the inflammatory response.

PENETRATION OF BAK

Champeau *et al*[49] and Green,[63] in rabbits, show that there is a considerable accumulation of BAK in the corneo-conjunctival epithelium and in the stroma.

BAK is also detected in the deepest structures: the lens, iris, vitreous, choroid and retina. It is broken down slowly and has a long half life.[49] The conjunctival and corneal epithelium acts as a reservoir; it very rapidly saturates and can then gradually release the prescriptive and redistribute it to the tear film or other ocular tissues.

Incorporation and elimination of benzalkonium chloride



A drop of carbon-labelled benzalkonium chloride (BAK) was instilled into the rabbit eye. Benzalkonium chloride (BAK) is rapidly incoporated by the superficial ocular tissures. Elimination is slow. Mean ± standard deviation of 8 to 10 experiments. Adapted from 49.

Conclusion

In conclusion, it should be kept in mind that preservatives in ocular medications are toxic for the ocular surface as well as deeper tissues.
These effects are dose-and time-dependant and the risk in developing ocular surface disease is increased, particularly in patients who have required multi therapy for some time.

Beyond ocular discomfort and a subsequent decreased quality of life, chronic inflammation of the ocular surface may produce severe sight-threatening side effects and is an important risk factor in filtration surgery.

Ophthalmologists should consider the risks and benefits of ophthalmic medications before initiating therapy, identify the minimum dose necessary to achieve a therapeutic benefit, and monitor patients.

Patient preference should also be considered; when offered a comparison of a preserved drop in one eye and preservative-free drop in the other eye, patients preferred the preservative-free treatment.[50]

1 Liesegang TJ. Conjunctival changes associated with glaucoma therapy: implications for the external disease consultant and the treatment of glaucoma. Cornea 1998; 17(6): 574-83
2 Sherwood MB *et al.* Long-term morphologic effects of antiglaucoma drugs on the conjunctiva and Tenon's capsule in glaucomatous patients. Ophthalmology 1989; 96(3):327-35
3 Ariturk N *et al.* The effects of antiglaucomatous agents on the conjunctiva use for various durations. Int. Ophthalmol. 1996; 20 (1-3): 57-62
4 Nuzzi R *et al.* Adverse effects of antiglaucomatous medications on the conjunctiva and the lachrymal response.
5 Schwab *et al.* Foreshortening of the inferior conjunctival fornix associated with chronic glaucoma medications. Ophthalmology 1992; 99(2): 197-202
6 Baudoin C *et al.* Ocular Surface inflammatory changes induced by topical antiglaucoma drugs. Human and animal studies. Ophthalmology 1999; 106: 556-63
7 Pisella PJ *et al.* Prevalence of ocular symptoms and signs with preserved and preservative free medication. Br J Ophthalmol 2002; 86: 418-23.
8 Levrat F *et al.* Clinical tolerance of antiglaucoma eye-drops with and without a preservative. Results of an unpublished survey in Europe. J Fr Ophthalmol 1999; 22: 186-91.
9 Broadway D *et al.* Adverse effects of topical antiglaucoma medication. II. The outcome of filtration surgery. Arch Ophthalmol. 1994; 112(11): 1446-54
10 Baudoin, C. Mechanisms of failure in glaucoma filtering surgery: a consequence of antiglaucoma drugs? Int J Clin Pharm Res 1996; 16(1):29-41
11 Brandt JD. Does benzalkonium chloride cause cataract. Arch ophthalmol. 2003; 121 (6): 892-3
12 Leske MC et al, Barbados eye studies group. Risk factors for incident nuclear opacities. Ophthalmol. 202 Jul; 109(7): 1303-8
13 Heijil A et al; Early Manifest Glaucoma Trial Group. Reduction of intraocular pressure and glaucoma progression: results from the Early Manifest Glaucoma Trial. Arch Ophthalmol. 2002; 120(10): 1268-79
14 Miyake K, Ibarai N. Prostaglandins and cystoid macular edema. Surv Ophthalmol 2002; 47 Suppl 1:S203-18
15 Miyake K *et al.* ESCRA Binkhorst lecture 2002: Pseudophakic preservative maculopathy. J Cataract Refract Surg. 2003: 1800-10.
16 Bernauer W *et al.* Chronic progressive cicatrisation. Eye 1993; 7 (Pt 3): 371-8
17 Butt Z *et al.* Drug-induced ocular cicatricial pemphogoidL a series of clinic-pathological reports. Eye 1998; 12 (Pt 2): 285-90
18 Wilson WS *et al.* Effects of benzalkonium chloride on the stability of the precorneal tear film in rabbit and man. Br J Ophthalmol 1975; 59:667-9
19 Kaercher T *et al.* How the most common preservatives affects the Meibomian lipid layer. Orbit 1999; 18:89-97
20 Yalvac , IS *et al.* Effects of antiglaucoma drugs on ocular surface. Acta ophthalmol Scand 1995; 73:246-8.
21 Mietz *et al.* The effect of preservatives and anti laucoma medication on the histopathology of the conjunctiva. Graefe's Arch Clin Exp Ophthalmol 1994; 232: 561-5
22 Mietz *et al.* Early conjunctival changes following treatment with metipranolol and preservatives are not reversible with dexamethasone. Graefe's Arch Clin Exp Opthamol 1997; 235:452-9
23 Furrer P *et al.* Ocular tolerance of preservatives on the murine cornea. Eur J Pharm Biopharm 1999; 47: 105-12
24 Imayasu M *et al.* A quantitative method of LDH, MDH and albumin levels in tears with ocular surface toxicity scored by Draize criteria in rabbit eyes. CLAO J 1992; 18:260-6.
25 Noecker RJ *et al.* Corneal and conjunctival changes caused by commonly used glaucoma medications. Cornea 2004; 23: 490-6
26 Lopez BD *et al.* Quantitative evaluation of the corneal epithelial barrier: effect of artificial tears and preservatives. Curr Eye Res 1991; 10: 645-56
27 Salonen EM *et al.* Toxicity of ingredients in artificial tears and ophthalmic drugs in a cell detachment and spreading test. J Toxicol Cutan and Ocul Toxicol 1991; 10:157-66
28 Lemp MA *et al.* Toxic endothelial degeneration in ocular surface disease treated with topical medications containing benzalkonium chloride. AM J Ophthalmol 1988; 105 (6): 670-3.
29 Gasset AR. Benzalkonium toxicity to the human cornea. Am J Ophthalmol. 1977; 84(2): 169-71
30 Kilp *et al.* Acute and chronic influence of benzalkonium chloride as a preservative. Concepts Toxicol 1987; 4: 59-63
31 Liu H *et al.* Toxic endothelial cell destruction from intraocular benzalkonium chloride. J Cataract Refract Surg. 2001; 27 (11): 1746-50
32 Zabel RW *et al.* Corneal toxic changes after cataract extraction. Can J Ophthalmol 1989; 24(7): 311-6
33 Lavin MJ et al. The influence of prior therapy on the success of trabeculectomy. Arch Ophthalmol 1990; 108 (11): 1543-8
34 Hamard P *et al.* Apoptose et cellules trabeculaires humaines: evaluation in vitor de ;'effet du betatoxolol avec ou sans conservateur. J Fr Ophthalmol 2002; 25: 777-84.
35 Hamard P *et al.* in vitro effects of preserved and unpreserved antiglaucoma drugs on apoptotic marker expression by human trabecular cells. Garefes Arch Clin Exp Ophthalmol 2003; 241: 1037-43.
36 Goto, Y *et al.* Human lens epithelial cell damage and stimulation of their secretion of chemical mediators by BAK rather than latanoprost and timolol. Arch ophthalmol 2003; 121:835-9
37 Chou A *et al.* Ocular toxicity of beta-blockers and BAK in pigmented rabbits: electrophysiological and morphological studies. Jpn J Ophthalmol 1985; 29:13-23.
38 Bron A *et al.* Interet de la substitution d'un traitement journalier de 2 instillations de timolol par 1 instillation quotidienne de betabloquant non conserve chez des patients presentant un glaucoma chronique ou une hypertonie oculaire. J Fr Ophthalmol 2003l 26: 668-74.
39 Campagna P *et al.* Chronic topical eye preservative-free beta-blocker therapy effect on the ocular surface on glaucomatous patients. Acta Ophthalmol Scand Suppl 1997; 224: 53.
40 De Jong *et al.* Topical timolol with and without benzalkonium chloride: epithelial permeability and autofluorescence of the cornea in glaucoma. Graefe's Arch Clin Exp Ophthalmol 1994; 232:221-4
41 Brewitt H *et al.* Studie zur Wirksamkeit eines nicht konservierten Tranenersatzmittels. Klin Mbl Augenheilk 1991; 199:160-4
42 Laflamme MY Swieca R. A comparative study of two preservative-free tear substitutes in the management of severe dry eye. Can J Ophthalmol 1988; 23:174-6
43 Mayer H *et al.* KlinischeStudie zur Wirksamkeit enies naeuen unkonservierten Polyvidon-Praparats zur Therapie von Benetzungsstorungen. Klin Montatsbl Augenheilkd 1994; 205:138-42
44 Kuppens EVMJ *et al.* Effect of timolol with and without preservative on the basal tear turnover in glaucoma.
45 Gobbels M, Spitznas M. Corneal epithelial permeability of dry eyes before and after treatment with artificial tears. Ophthalmology 1992; 99: 873-8.
46 Grene RB *et al.* Unpreserved carboxymethylcellulose artificial tears evaluated in patients with keratoconjunctivitis sicca. Cornea 1992; 11: 294-301
47 Smith GH *et al.* Open evaluation of a new non-preserved artificial tear. Aust NZ J Ophthalmol 1993; 21: 105-9
48 Fisher AA. Allergic contact dermatitis and conjunctivitis from benzalkonium chloride. Cutis 19871 39: 381-83.
49 Champeau EJ, Edelhauser HF. Effect of ophthalmic preservatives on the ocular surface conjunctival and corneal uptake and distribution of benzalkonium chloride and chlorhexidine digluconate. In: Holly FJ *et al.* The preocular tear film in health, disease and contact lens wear. Dry Eye Institute Lubbok, Texas 1986; 292-302.
50 Lapalus P *et al.* Cytotoxicity studies in ophthalmology. Lens Eye Tox Res 1990; 7: 231-42
51 Parnigotto PP. Bovine Corneal stroma and epithelium reconstructed in vitro: characterisation and response to surfactants. Eye 1998; 12: 304-10
52 Samples JR et al . The effect of epinephrine and benzalkonium chloride on cultured corneal endothelial and trabecula meshwork cells. Exp Eye Res 1989; 49:1-12.
53 Williams DE *et al.* Effects of timolol, betaxolol and levobunolol on human tenon's fibroblasts in tissue culture. Invest Ophthalmol Vis Sci 1992; 33:2233-41
54 De Saint Jean M *et al.* Toxicity of preserved and unpreserved antiglaucoma topical drugs in an in vitro model of conjunctival cells. Curr Eye Res 2000; 20:85-94
55 Tripathi BJ *et al.* Cytotoxic effects of benzalkonium chloride on human corneal epithelium. Lens Eye Toxic Res 1992; 9:361-75 XXV Conc . Ophthalmol. Ed. Kugler & Ghedini, Amsterdam 1987, 1, 564-69
56 Imperia PS *et al.* An in vitro method for measuring ophthalmic preservative toxicity. J Toxicol Cut Ocular Toxicol 1986; 5: 309-17
57 Takahashi N, Mukai Y. Cytotoxicity of benzalkonium chloride in cell culture. In Blodi R *et al.* Proceedings of the XXVth International Congress of Ophthalmology. Rome. May 4-10, 1986; Acta
58 Dormans JA, van Logten MJ. The effect of ophthalmic preservatives on corneal epithelium of the rabbit a scanning electron microscopial study. Toxicol Appl Pharmacol 1982; 62: 251-61
59 Mehta MR *et al.* Epitheliotoxicity of contact lens solutions: an experimental study on rabbit cornea using scanning electron microscopy. Proceedings of the XXVth International Congress of Ophthalmology. Rome. May 4-10, 1986; Acta XXV Conc. Ophthalmol. Ed. Kugler & Ghedini, Amsterdam 1987, 1, 564-69
60 Pfister RR, Burstein N. The effects of ophthalmic drugs, vehicles and preservatives on corneal epithelium: a scanning electron microscope study. Invest. Ophthalmol 1976; 15:246-59
61 Ichijama H *et al.* Confocal microscopic studies of living rabbit cornea treated with benzalkonium chloride. Cornea 1992; 11: 221-5. Erratum in: Cornea 1992; 11:368.
62 Debbasch C *et al.* Quaternary ammoniums and other preservatives' contribution in oxidative stress and apoptosis on Chang conjunctival cells. Invest Ophthalmol Vis Sci 2001; 42: 642-52.
63 Champeau EJ *et al.* Effect of ophthalmic preservatives on the ocular surface: conjunctival and corneal uptake and distribution of benxalkonium chloride and chlorhexidine digluconate. In: Holly FJ, Flamberts DW, MacKeen DL, Esquivel ED. The preocular tear film in health, disease and contact lens wear. Dry Eye Institute Lubbok, Texas 1986; 292-302
64 Green , K *et al.* Detergent penetration into young and adult rabbit eyes: comparative pharmacokinetics.J Toxicol 1987; 6: 89-107

Thea Pharmaceuticals Limited, IC5 Innovation Way, Keele University Science and Business Park, Keele, Newcastle-under-Lyme, ST5 5NT

Head Office: 0345 521 1290 Medical Information: 0345 521 1290

Email: theasupport@theapharma.co.uk www.thea-pharmaceuticals.co.uk

PF006 SEPT2019 ©Thea Pharmaceuticals 2019

Théa
let's open our eyes