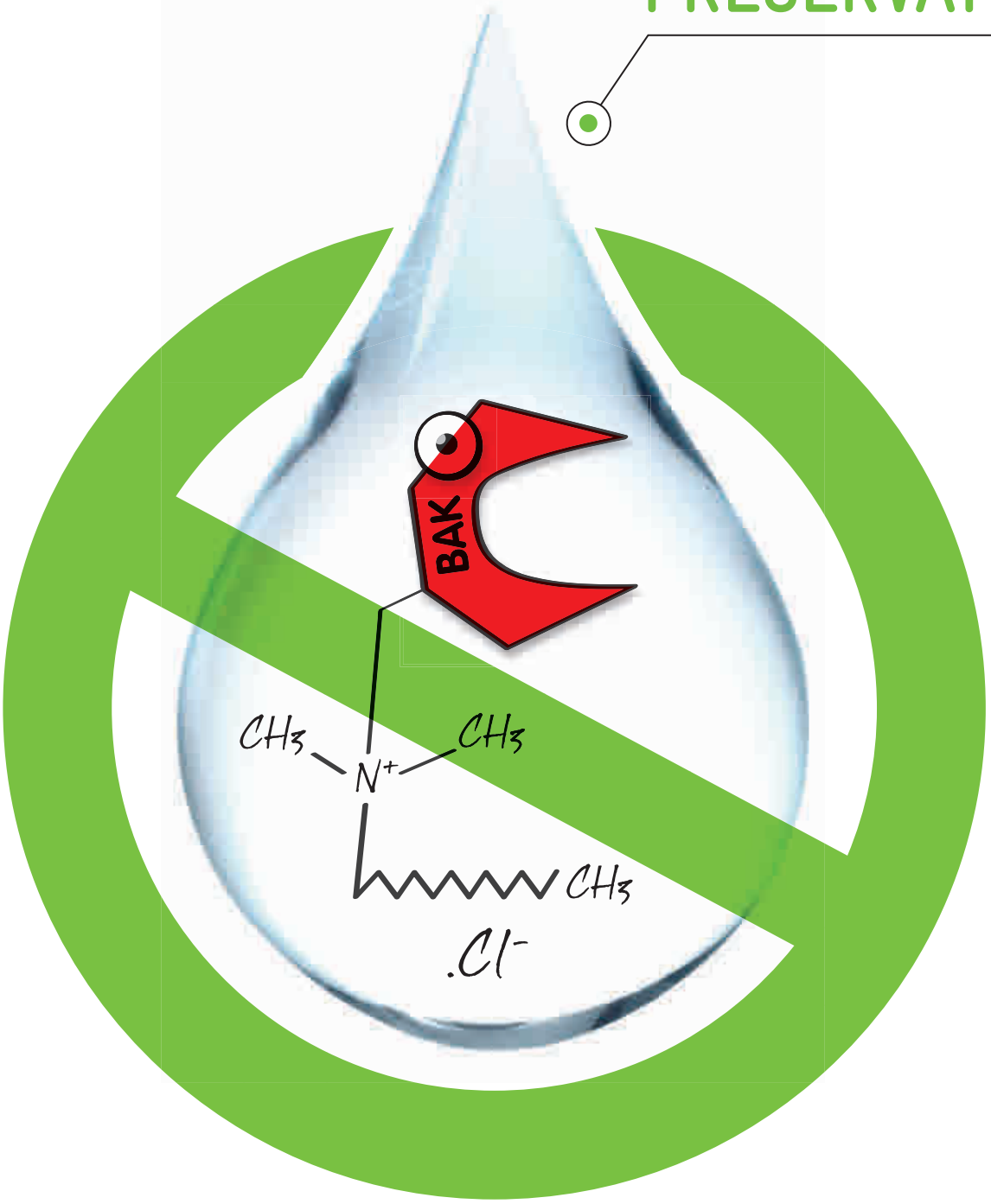
0% PRESERVATIVES



Volume 4

25 YEARS OF PRESERVATIVE-FREE EYE DROP

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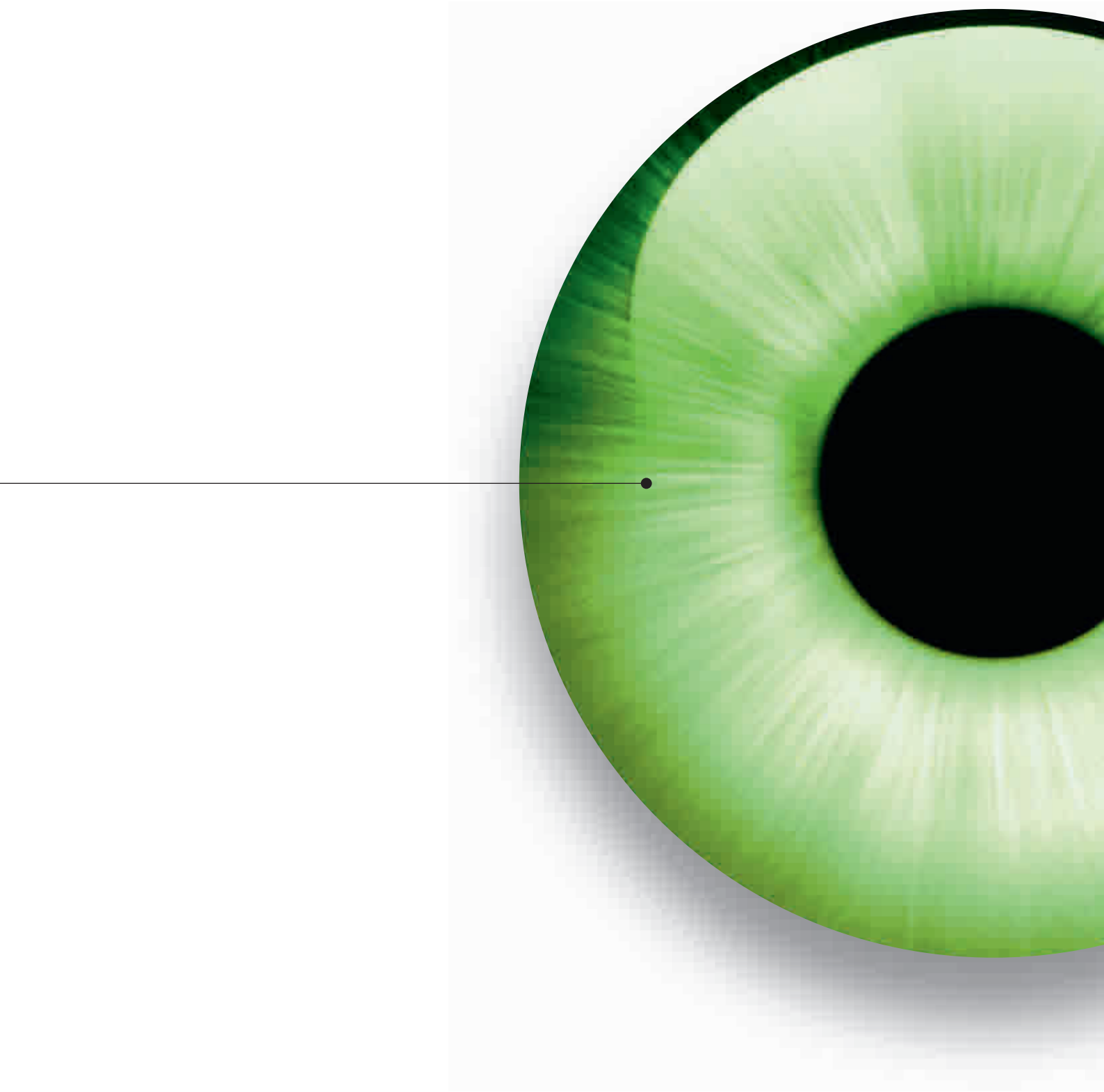
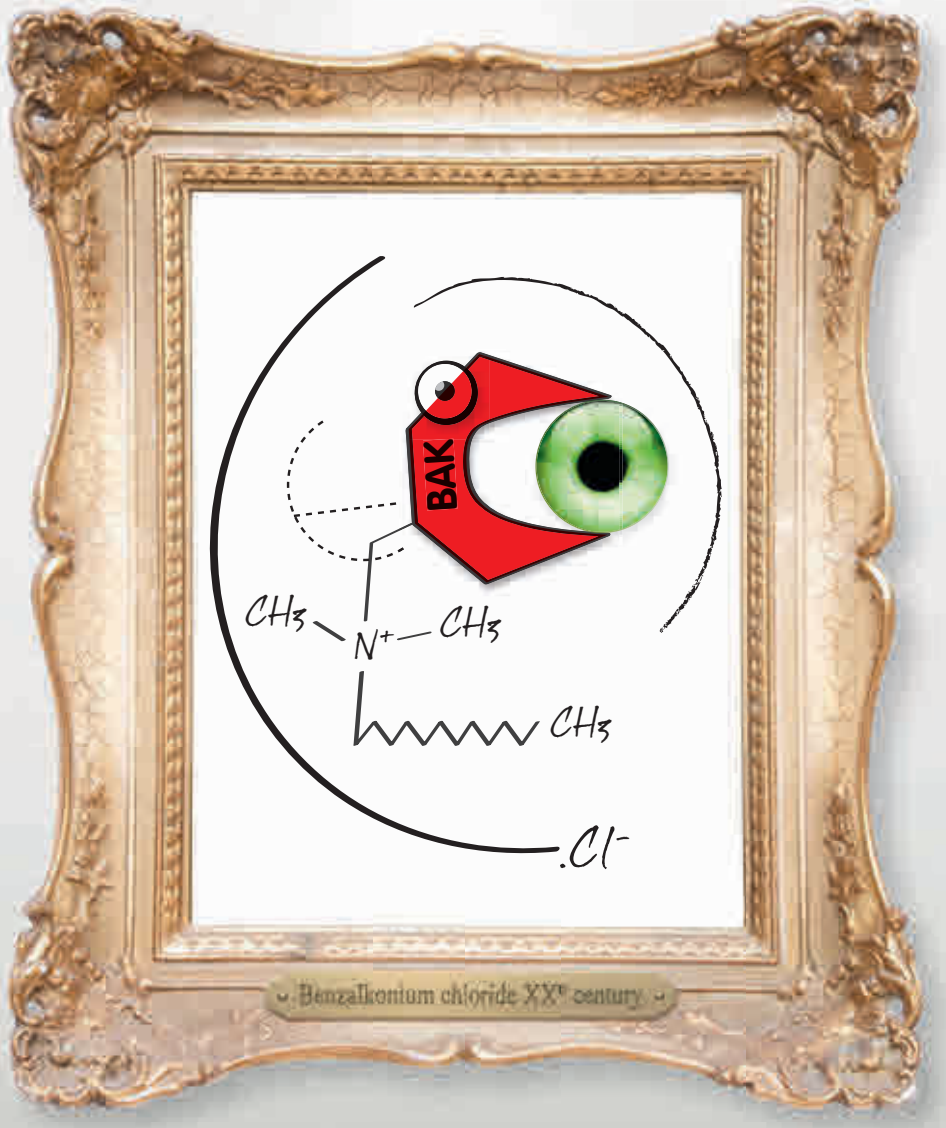
Théa





Preservatives were developed at the end of the Second World War to solve the problem of contamination of ophthalmic solutions. The best known and most widely used was Benzalkonium chloride.

Once upon a time
There were preservatives...

The use of preservatives allowed considerable progress to be made in the food, cosmetics and pharmaceutical industries. The industrial manufacture of eye drops, which are more easily contaminated than ointments, was transformed by the introduction of preservatives.

The pioneer was my father, Jean CHIBRET, who was always concerned about the serious problem of microbial contamination, and consequently was the first to add a mercurial derivate to eye drops, followed a decade later by benzalkonium chloride, a more powerful but less allergenic compound. He also imposed the use of an after-opening use-by date. These two apparently simple ideas were adopted by all the health administration authorities.

However, the repeated use of all these preservatives has not only had the desired effects, but has also turned out to be harmful to the ocular surface over the years.

In the nineties, Professor Christophe BAUDOUIN, Head of Department at the National XV-XX Eye Hospital in Paris, established the link between the use of preservatives and certain inflammatory reactions of the ocular surface. He quickly gained recognition among the international ophthalmic community.

Since then, his experimental and clinical work, rapidly confirmed by other research teams around the world, has allowed further data to be collected which clearly highlights the determining, if not exclusive role of preservatives in certain irritative and inflammatory conditions linked to the treatment of eye diseases.

0%

PRESERVATIVES

These past years have raised awareness and led to the following conclusion, based on a large amount of scientific evidence following the "evidence based medicine" concept: we should reduce the quantity of preservatives used in eye drops, or even eliminate them completely.

This is why, ironically enough, whilst still pursuing the CHIBRET family tradition, I have decided to eliminate the use of preservatives that my father had pioneered, by developing new eye drop packaging forms. Accordingly, in 1995 we launched the first preservative-free multi-dose bottle, Abak, which preserves the sterility of the bottle contents through a filter membrane for up to 3 months after opening.

Therefore, 25 years later, we thought it would be of interest to review the latest findings and advances on the subject of 0% preservatives.

Ophthalmology has entered into a new era, creating
"a preservative-free generation of patients".

I wish you a pleasant read.

Henri Chibret
Founder of Transphyto and Laboratoires Théa
Chairman of the Board of Théa Holding

Table of Content


BAK

1

Introduction

Preservatives in topical ocular medications are known to exert toxicity on the ocular surface. The effect on the precorneal tear film of the most commonly used preservative, benzalkonium chloride (BAK) was described several decades ago [1]. Preservatives were then suspected to induce subclinical ocular surface inflammation especially when repeated administrations are used over the long term [2]. Nowadays, it is no doubt that preservatives play a crucial role in most side effects induced by preserved ocular medications.

PRESERVATIVES PLAY A CRUCIAL ROLE IN MOST SIDE EFFECTS INDUCED BY PRESERVED OCULAR MEDICATIONS.

Preservatives are known to produce side effects both in superficial and deep internal ocular structures. This included damages on:

- Ocular surface components: conjunctiva, cornea, tear film
- Internal structures: trabeculum, lens, retina.

In most patients, preservatives in ophthalmic medications produce mild to moderate transient ocular reactions. However, repeated administrations for a long period, as in the treatment of ocular hypertension or dry eye syndrome, may also cause a chronic disease, leading in some cases to serious complications [3] such as:

- toxic endothelial degeneration,
- chronic subconjonctival fibrosis,
- cataract,
- cystoid macular oedema,
- failure of glaucoma filtering surgery.

Patients at risk included primarily those having already an ocular surface disease (dry eye, meibomian gland disease, blepharitis…), and those treated with multiple preserved-medications.

PATIENTS AT RISK INCLUDE THOSE HAVING ALREADY AN OCULAR SURFACE DISEASE (DRY EYE, MEIBOMIAN GLAND DISEASE, BLEPHARITIS…), AND PATIENTS TREATED WITH MULTIPLE PRESERVED-MEDICATIONS.

For long, preservative toxicity in ophthalmic medications has been neglected or ignored mainly because pivotal studies required by Regulatory Authorities for licensing ophthalmic medications are short-term clinical trials conducted in selected populations of patients with the objective on efficacy, and thus not intended to detect long-term safety issues. So preservative-induced ocular toxicity was until recently largely underestimated or even not suspected by ophthalmologists. Since, the severe ocular adverse reactions usually occur after a slow and delayed process involving subclinical inflammation and chronic fibrosis, the role of the preserved-medication, so far well tolerated, is not suspected in most cases. There are nowadays a number of studies suggesting that preservatives exert their effects through a cumulative, dose- and time-dependent mechanism.

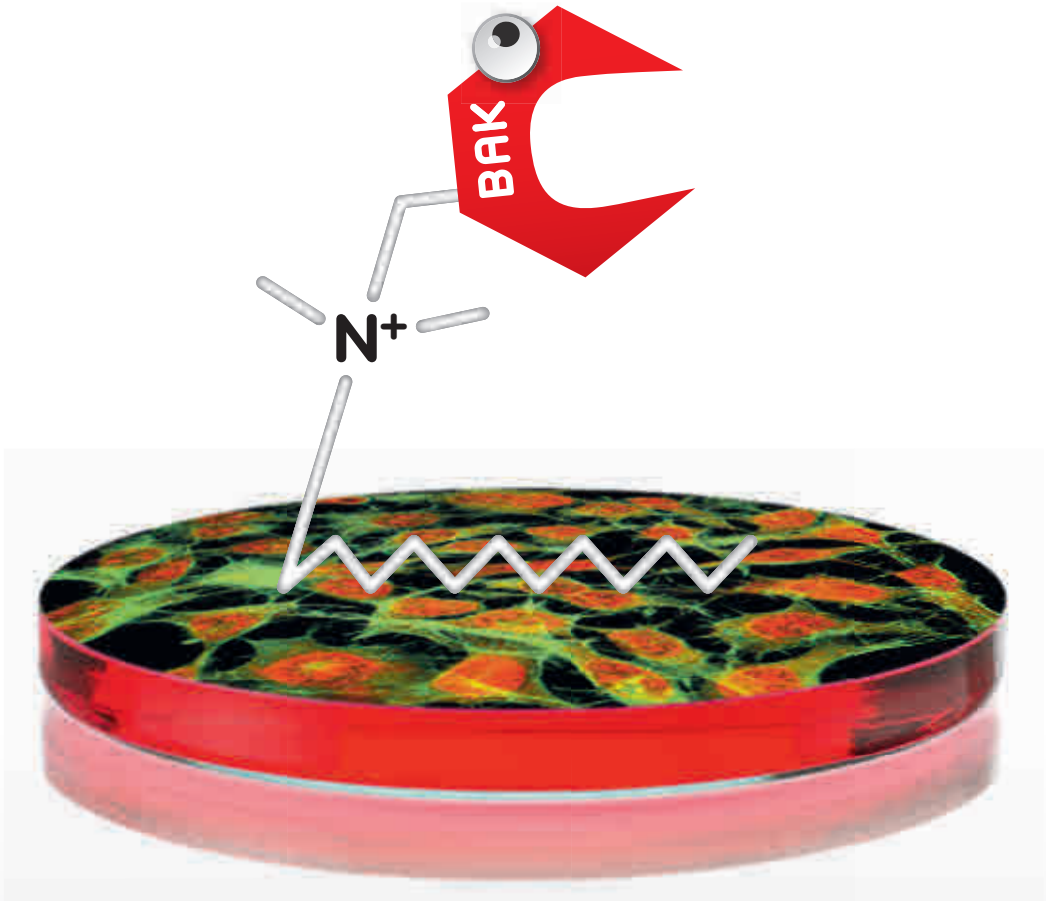
AN APPARENTLY MILD TOXICITY OF THE OCULAR SURFACE IN SHORT-TERM SHOULD NOT BE NEGLECTED IN ORDER TO AVOID A SEVERE REACTION IN LONG-TERM.

However, the toxicity of preservative in topical ocular medications is still debated among ophthalmologists. Most of them continue to consider the preservative adverse effects as negligible ocular reactions, in comparison with the efficacy on the treated disease such as ocular hypertension or glaucoma that can induce blindness. This is the price to pay for preventing disease progression and potentially visual impairment or loss. However, unpreserved treatments were shown in the recent years to be equivalent or non-inferior in efficacy in most pathologies [4, 5, 5 bis]. Thus switch can be done easily from preserved to preservative-free treatment.

PRESERVATIVE-INDUCED OCULAR TOXICITY IS LARGELY UNDERESTIMATED OR EVEN NOT SUSPECTED BY OPHTHALMOLOGISTS.

Since the last issue of our series on preservative toxicity in 2004, scientific researches worldwide have confirmed their deleterious effects on surface and deep ocular tissues. This was consequently followed by a growing awareness of the toxicity among ophthalmologists and the scientific communities. New alternatives to manage the ocular surface of patients treated with repeated doses of ocular medications have been proposed by pharmaceutical industry. This includes the development of new preservative-free formulations.

The purpose of this new brochure was to give an overview of most recent progress in the knowledge of preservative toxicity and the alternative treatment options.


BAK
N+

2

Recent progress in the mechanisms of toxic reaction of preservatives

The mechanisms of preservative toxicity are still not fully elucidated, but significant progress has been performed since two decades of research. It is now well established that benzalkonium chloride (BAK) exerts significant toxic, pro-oxidative, pro-apoptotic, and pro-inflammatory activity on exposed cells or tissues.

Three mechanisms of BAK toxicity have been described [6]:

- detergent effect, causing loss of tear film stability;
- direct damages to the corneal and conjunctival epithelium;
- immunoallergic reactions.

As summarised in Table 1, BAK in case of glaucoma medication can cause tear film instability, goblet cell loss, conjunctival squamous metaplasia and apoptosis, disruption of the corneal epithelial barriers, and damages to deeper ocular tissues [3].

TABLE **1**

Histopathologic modifications produced by preserved (BAK) glaucoma medications

Reduction of goblet cells
Epithelial keratinisation
Squamous metaplasia
Loss of microvilli
Increased number of desmosomes
Epithelial bullous dystrophy
Increased number of sub-epithelial fibroblasts
Sub-epithelial fibrosis
Reduction of intravascular spaces
Increased number of sub-epithelial lymphocytes and plasmocytes
Thickening of basal membrane
Immunoglobulins on basal membrane

Adapted from Vaede et al. [7]

One of most important progress in preservative research was the confirmation using sensitive and non invasive technics (including in vivo confocal microscopy) that preservatives may exert their toxicity at low concentrations and at a subclinical level. It was evidenced that not only the ocular surface but also deep ocular structures, including the trabeculum, may be affected (Table 2). Other data from numerous studies suggest that adverse effects of preserved ocular medication may occur following a cumulative process involving a long, dose-dependent and time-dependent exposure.

TABLE **2**

Dose-dependent toxicity of benzalkonium chloride on the ocular surface

BAK concentration	Ocular effects
0.004%	Significant reduction of the Break Up Time (BUT)
0.005%	Direct toxicity on superficial cells with epithelial erosion
0.007%	90 to 100 sec to induce lysis of 50% of conjunctival epithelial cells in culture
0.01 %	Important epithelium alteration, stimulation of lymbal and conjunctival infiltration of inflammatory cells
0.02%	Corneal wound healing delay
0.1%	Destruction of the endothelium and irreversible corneal oedema in case of intracameral injection or instillation in patients with corneal ulcer
0.1 to 0.5%	Major toxic keratitis, epithelial metaplasia, corneal infiltration of inflammatory cells, and neovascularisation induced by repeated administration in rat
1 to 2% (in animals)	Total destruction of the anterior segment (conjunctiva and cornea) in less than one week

Adapted from Vaede et al. [7]

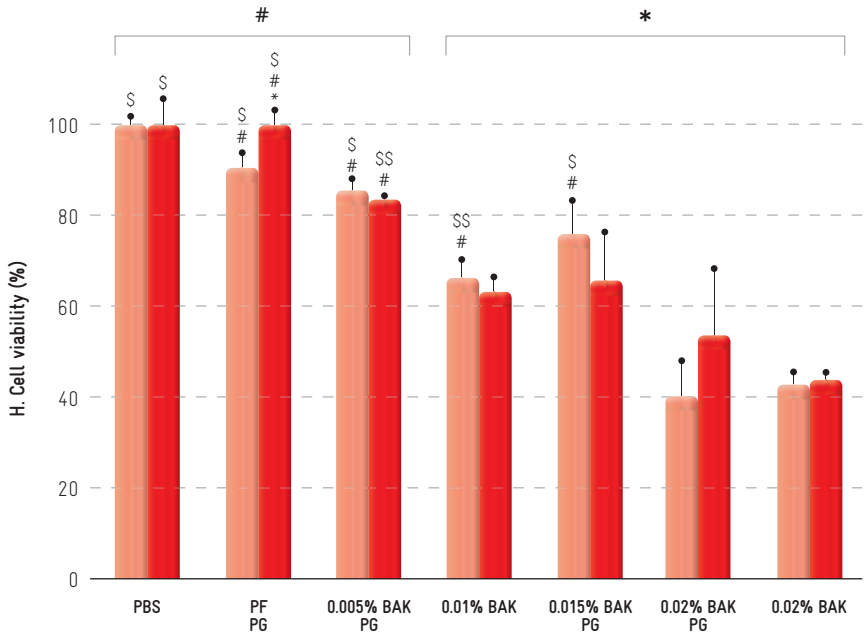
Conjunctival and corneal toxicity

The toxicological model of 3D-reconstructed cornea epithelial (3D-HCE) confirmed the cytotoxicity of BAK-containing solutions with a better approach than previous in-vivo or in-vitro studies. The presence of cell apoptosis, activation/inflammation, proliferation/turnover and cellular tight junctions after application of different preserved antiglaucoma eye drops was detected (Figure 1) [8 Bis].

FIG.**1**

Dose-dependent BAK-induced toxicity on human corneal cells

Adapted from Liang et al. [8 Bis]



Glaucoma treatments induced a dose-dependent loss in cell viability

* p<0.01 compared with PBS at the same time point

\# p<0.03 compared to 0.01% BAK at the same time point

$ p<0.002 or p<0.03 ($$) compared with 0.02% BAK at the same time point

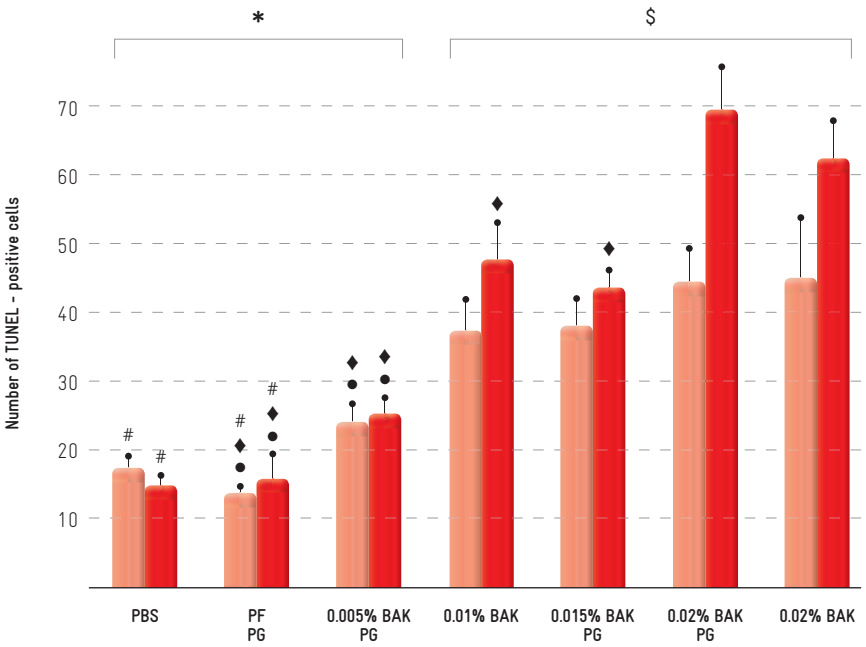
♦ p< 0.05 compared with 0.02% BAK at the same time point

● p< 0.02 compared with 0.015% BAK at the same time point

24h

24h + 24h recovery

PG: Prostaglandin



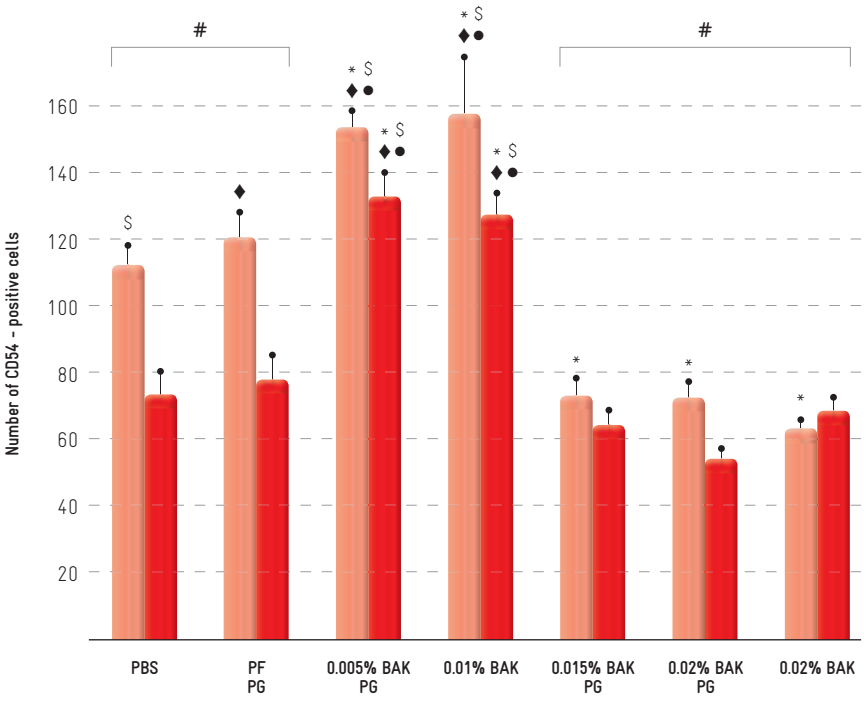
Glaucoma treatments induced a dose-dependent increase in apoptotic cell number

- * p<0.02 for 0.010% BAK and p<0.01 for other solutions compared with PBS at the same time point
- # p<0.008 compared to 0.01% BAK at the same time point
- $ p<0.001 compared with 0.02% BAK at the same time point
- ♦ p< 0.003 compared with 0.02% BAK at the same time point
- ● p< 0.03 compared with 0.015% BAK at the same time point

24h

24h + 24h recovery

PG: Prostaglandin



Glaucoma treatments induced a dose-dependent increase in ICAM-1 (CD54) expression

- * p<0.001 compared with PBS at the same time point
- # p<0.005 compared to 0.01% BAK at the same time point
- $ p<0.0001 compared with 0.02% BAK at the same time point
- ♦ p< 0.002 compared with 0.02% BAK at the same time point
- ● p< 0.008 compared with 0.015% BAK at the same time point

24h

24h + 24h recovery

PG: Prostaglandin

Human corneal epithelial cells (HCE) were exposed to different preserved eye drops containing benzalkonium chloride (BAK), preservative-free (PF) eye drops or phosphate buffer (PBS). The human corneal epithelial cells were exposed for 24 hours with or without a 24 hour recovery period before assessments for cell viability, Tunnel positive cells, or CD54 (ICAM-1) positive cells.

In human corneal epithelial cells exposed with various concentrations of BAK for 6 to 24 hours, a dose-dependent response of BAK with significant toxic effects for concentrations as low as 0.005% was evidenced using fluorescence confocal microscopy. Increasing BAK concentrations induced:

- increased apoptotic cells from the superficial to the deeper layers
- large TUNEL cell positivity, consistent with apoptosis and cell death, from the most superficial cell layer (i.e. the layer most exposed to the toxic effect)
- activation of caspase-3 consistent with the early stage of apoptosis from the deepest layers (less exposed) [3, 9].

BAK dose-dependently also induced:

- the expression of Ki67 (a marker of cell proliferation)
- the expression of ICAM-1 (an adhesion molecule related to inflammation and cell recruitment)
- reduced level of occludin (a tight junction protein)mRNA in the superficial layers while increasing its gene expression up to the 0.02% BAK concentration probably in response to BAK-induced corneal cell injury [3, 9].

In another experimental study using primary culture of human corneal-limbal epithelial cells, the expression of two mucin proteins (MUC1 and MUC16) was significantly reduced after brief exposure of BAK. Transmission electron microscopy of the anterior corneal surface revealed fixation of the mucus layer after exposure to 0.01% BAK for 5 or 15 min, whereas more prolonged exposure (60 min) to 0.01% BAK destroyed the mucus layer [10].

IN VITRO, STUDIES SHOW THAT :

- BAK INDUCES A DOSE-DEPENDENT TOXICITY ON HUMAN CORNEAL EPITHELIAL CELLS.
- BAK INCREASES EARLY STAGE OF APOPTOSIS AND CELL DEATH.
- BAK INCREASES INFLAMMATORY CELL ADHESION AND PROLIFERATION.
- BAK DECREASES TIGHT JUNCTION, AND PRECORNEAL MUCIN.

2.2

Immuno inflammatory reactions

Research conducted since a decade have confirmed the increased expression of immuno inflammatory markers by the conjunctival epithelium in glaucoma patients treated with preserved medications over the long term.

A significantly increased expression of immuno inflammatory markers and mediators was found in the conjunctival epithelium of glaucoma patients compared with normal eyes. HLA-DR was significantly higher in the patients receiving preserved eye drops compared to patients treated with unpreserved-eye drops. The IL-6, IL-8 and IL-10 were similarly overexpressed in all glaucoma groups, with no significant between-group differences except for the expression level of IL-8, which was significantly higher in patients treated with preservative eye drops [11].

There is now evidence of immune cells infiltration in the different stratum of the conjunctiva (epithelium, superficial and deep stroma) as demonstrated in rabbit treated for 1 month with BAK-containing eye drops [12]. A recent experiment in rabbits [8] also showed that antiglaucoma eye drops stimulated inflammatory cell infiltration in the conjunctiva-associated lymphoid tissue (CALT). This effect was shown to be primarily related to the concentration of BAK in glaucoma medication. In this study, the CALT reaction after instillation of BAK-containing eye drops was characterized by:

- Strong CD45 expression after instillation within 4h following BAK-challenge.
- Inflammatory cell infiltration in the most superficial and intrafollicular layers.
- Cell circulation inside the lymph vessels.
- Dramactic reduction of mucus cell (MUC-5AC+ cells).

This study showed for the first time the in-vivo aspect of CALT after toxic stimuli, confirming the concentration-dependent toxic effects of BAK [8].

In patients with glaucoma therapy, the expression of HLA-DR (as the hallmark of inflammation on conjunctival cells) was correlated with the duration of treatment and the number of preserved-glaucomatous medications. In addition, it was found that the ocular surface of patients receiving long-term treatment expresses inflammatory markers related to both T-helper 1 (Th1) and T-helper 2 (Th2) pathways [13].

In a randomized double-blind placebo controlled study in healthy subjects [84], administration of 0.01% BAK solution for 12 weeks induces a significant increase in Languerans cells in the peripheral and central cornea without signs of dry eye. This is consistent with the development of a subclinical inflammatory reaction induced by BAK.

Implication of macrophages to the inflammatory reaction was suggested by experiments showing that low concentration of BAK (10(-5)%) increased the

activation of THP-1 cells in-vitro [14]. Stimulation of human macrophages (THP-1) with a low concentration of BAK:

- Increased expression of cell adhesion molecules (integrin, CD11b and CD11c)
- Increased cell differentiation as shown by the decreased expression of CD33.
- Activation of phagocytosis and migration.

Cytokines in supernatants of macrophages exposed to BAK also revealed an increased release of pro-inflammatory mediators including CCL1, CCL4/MIP-1β, TNF-α, soluble CD54/ICAM-1 and IL-1β.

IN CONCLUSION:

INFLAMMATION OF THE OCULAR SURFACE IN PATIENTS TREATED WITH PRESERVED-EYE DROPS WAS CLEARLY EVIDENCED BY THE EXPRESSION OF INFLAMMATORY MARKERS (HLA-DR, IL-8, ...) ON THE OCULAR SURFACE.

BAK-CONTAINING EYE DROPS STIMULATE THE OCULAR SURFACE IMMUNITY AFTER AN ACUTE CHALLENGE.

LONG-TERM USE OF TOPICAL TREATMENT CONTAINING BAK STIMULATES BOTH LYMPHOCYTE T-HELPER IMMUNOLOGIC PATHWAYS.

LONG-TERM EXPOSURE TO LOW CONCENTRATIONS OF BAK MAY BE RESPONSIBLE FOR INFLAMMATION THROUGH T LYMPHOCYTES AND MACROPHAGE ACTIVATION.

2.3

Damages in deep ocular structures

Recent studies suggest that preservative may accumulate and damage deep ocular structures implying new safety concerns in long-term use of preserved-eye drops.

BAK was shown to penetrate rabbit healthy eyes even after a short exposure and was not only detected on the ocular surface structures, but also in deeper tissues, especially in sensitive areas involved in glaucoma pathophysiology, such as the trabecular meshwork and the optic nerve areas, as confirmed by images with histological stainings [15].

Potential deleterious effects on trabecular cells.

There is cumulative evidence that BAK could affect trabecular cells. BAK can exert significant toxic, pro-oxidative, and/or proinflammatory effect on the trabecular meshwork (TM) [3,17]. A brief exposure of cultured human TM cells with BAK at low concentration, increased apoptotic cell markers and significantly decreased cell growth [16]. In-vivo experiments, in rabbits confirmed that topical application of BAK (0.01%), one drop administered twice a day for 5 months or one drop once a day for 1 month at high concentration (BAK 0.2%) can exert toxic, pro-oxidative, and/or proinflammatory effect on the trabecular meshwork (TM) [15]. Interestingly, the expression of inflammatory markers seems to be higher in eyes exposed to a low-dose/long-term treatment, than eyes exposed to high-dose/short-term treatment, suggesting that the duration of exposure is a key element in BAK toxicity.

Other research suggests that trabecular cell damages may have deleterious effect on the intraocular pressure. Using a rat model, it was found that one subconjunctival injection of BAK 0.01% produced a significant increase in intraocular pressure sustained for 7 days compared to vehicle-treated eyes. Outflow facility was significantly reduced in BAK-treated eyes compared to control eyes.

Histological analysis by TUNEL labelling showed an increased density of apoptotic cells in the trabecular meshwork and iris root. These data suggest that BAK could affect intraocular pressure and aqueous outflow facility and thus compromise the treatment efficacy [17].

In patients previously treated with preservative-containing compounds, trabecular specimens obtained during surgical nonpenetrating trabeculectomy, showed extremely low densities of trabecular cells and presence of cells expressing fractalkine (CX3CL1, a cytokine with chemoattractant activity) and fractalkine receptor, as well as their respective mRNAs [17]. Consistent results were shown in human TM-derived cell lines HTM3 exposed to BAK induced apoptosis, oxidative stress, and fractalkine expression and inhibited the expression of antiprotective chemokines (SDF-1 and Bcl2) (Figure 2).

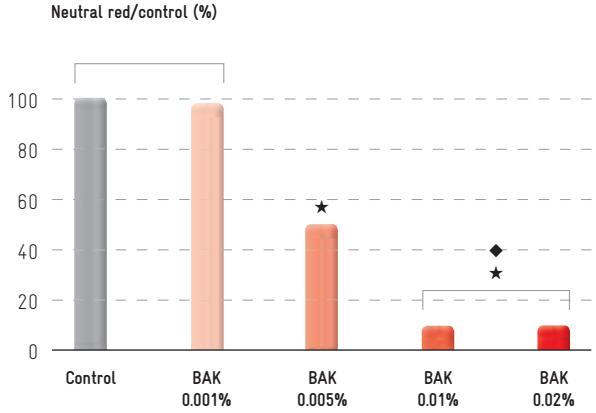
These findings support the hypothesis that antiglaucoma medications, through toxicity of their preservatives, may cause further long-term degeneration enhancing outflow resistance, and reducing the efficacy of IOP-lowering agents with a risk to threaten visual function over the long term [15].

IN VIVO, STUDIES SHOW THAT:

- BAK CAN EXERT SIGNIFICANT TOXIC, PRO-OXIDATIVE, AND/OR PROINFLAMMATORY EFFECT ON THE TRABECULAR MESHWORK.
- PRESERVATIVE MAY CAUSE LONG-TERM OCULAR STRUCTURE DEGENERATION ENHANCING OUTFLOW RESISTANCE, AND REDUCING THE EFFICACY OF IOP-LOWERING AGENTS WITH A RISK TO THREATEN VISUAL FUNCTION OVER THE LONG TERM.
- THESE EFFECTS ARE PROBABLY EXERTED THROUGH A CUMULATIVE LONG-TERM DOSE EFFECT.

FIG.**2**

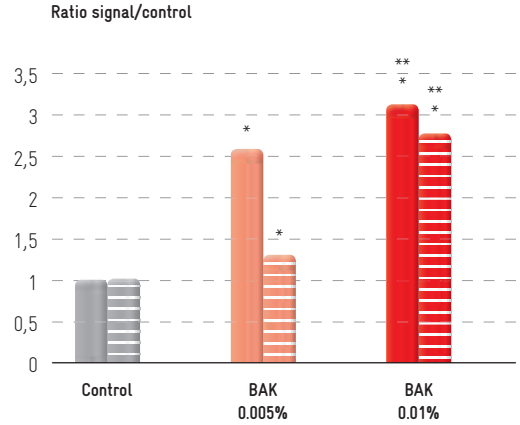
Effect of BAK on trabecular cells (HTM3) in patients treated with BAK-preserved ocular medications
Cell viability and expression of apoptosis-related markers



Evaluation of cellular viability of benzalkonium (BAK)-treated HTM3 cells using the neutral red test. Results are expressed as the percentage of positive cells reported to the control.

★ p<0.001 vs control
◆ p<0.001 vs BAK 0.005% (ANOVA)

Decreased trabecular cell viability

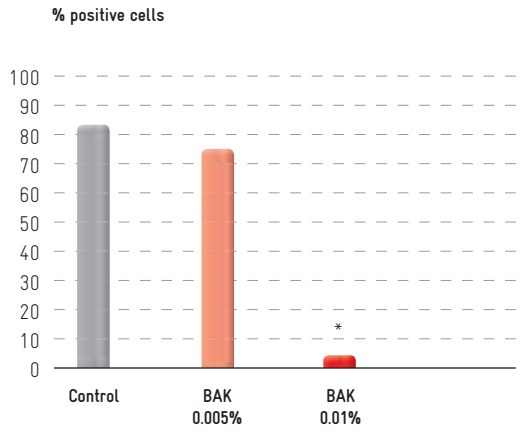


Evaluation of apoptosis in BAK-treated HTM3 cells using YO-PRO-1 and Hoechst 33342 tests. Results are expressed as the ratio of signal over the control.

YOPRO - 1 test
Hoechst 33342 test

* p<0.001 vs control
** p<0.01 vs BAK 0.005% (ANOVA)

Increased trabecular cells apoptosis



Flow cytometry measurement of the anti-apoptosis molecule Bcl2 in control or BAK-stimulated HTM3 cells. Results are expressed as the percentage of positive cells.

* p<0.001 vs control and BAK 0.005% (ANOVA)

Decreased expression of the anti-apoptosis molecule (Bcl2) in trabecular cells

Adapted from Baudouin et al. [17]


BAK

3

Preservative toxicity and clinical implications

For most of patients and some ophthalmologists, the local tolerance of the ocular medication concerned primarily the conjunctival allergy [19] and the ocular symptoms mainly burning, stinging sensations observed at treatment initiation. These symptoms frequently occurred upon eye drop instillation, and are generally not specific as they can be due to the preservative, the active substance or another ingredient in the formulation. These adverse effects are often not pronounced and disappear in a few minutes.

Considering the tolerance of ocular medications based on these sole short-term adverse events is quite a restrictive approach. More chronic ocular reactions can develop several months or years after the treatment initiation, even though the treatment was initially well tolerated. In this case, symptoms may occur at distance of instillation at any time during the day. Delayed ocular reactions are difficult to assess in relation with the ocular medication [3]. They are explained by a cumulative effect due to the long-term treatment with multi preserved ocular medications and to the individual ocular susceptibility.

When not diagnosed and treated, severe adverse events, sight-threatening in some cases, may develop. In addition, as part of adverse reactions, preservatives in ocular medication may have significant other significant clinical implications. They may negatively impact the quality of life, leading in some cases to treatment interruption, which may compromise the treatment efficacy.

CHRONIC OCULAR REACTION CAN DEVELOP SEVERAL MONTHS OR YEARS AFTER THE TREATMENT INITIATION, EVEN THOUGH THE TREATMENT WAS INITIALLY WELL TOLERATED.

3.1 Ocular surface disease

Medical therapy for chronic ocular diseases such as glaucoma can lead to ocular surface disease (OSD), with various disorders affecting the eyelids, conjunctiva, and/or the multi-layered corneal surface. Symptoms include burning, redness, irritation, fatigue, fluctuating visual acuity, infection, and potential loss of vision [20]. Conjunctival hyperaemia, decreased tear production and function, and superficial punctate keratitis are among the most common signs seen on routine clinical examination [21].

Recent cross-sectional studies conducted in Europe and US have shown consistent prevalence of about 50% (ranging from 40% to 60%) of ocular surface disease (OSD) among patients treated with topical glaucoma medications [22-24].

In a prospective observational multicentre study of 630 patients treated with topical IOP-lowering eye drops, 48.5% of patients had symptoms of ocular surface disease including 13.8% with severe OSD [24]. In another cross-sectional study [23], in 60 patients (59%), the prevalence of severe OSD was estimated to 27% (Figure 3).

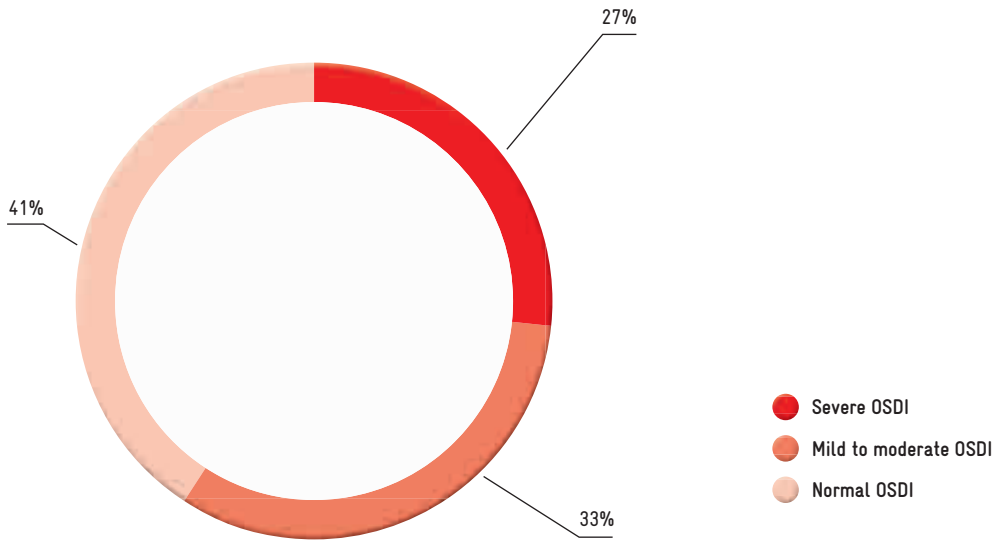


FIG.3 Prevalence of ocular surface disease in patients treated with preserved ocular medications

Adapted from Leung et al. [23]

These symptoms are mainly due to the presence of preservative in the ocular medication. In a study of 4107 glaucoma patients treated with preserved or preservative-free eye drops, symptoms such as discomfort on instillation, foreign body sensation, dry eye and stinging were significantly more prevalent with preserved eye drops than with preservative-free eye drops. Similarly clinical signs observed at ophthalmological examination were more common with preserved eye drops than preservative-free eye drops [25].

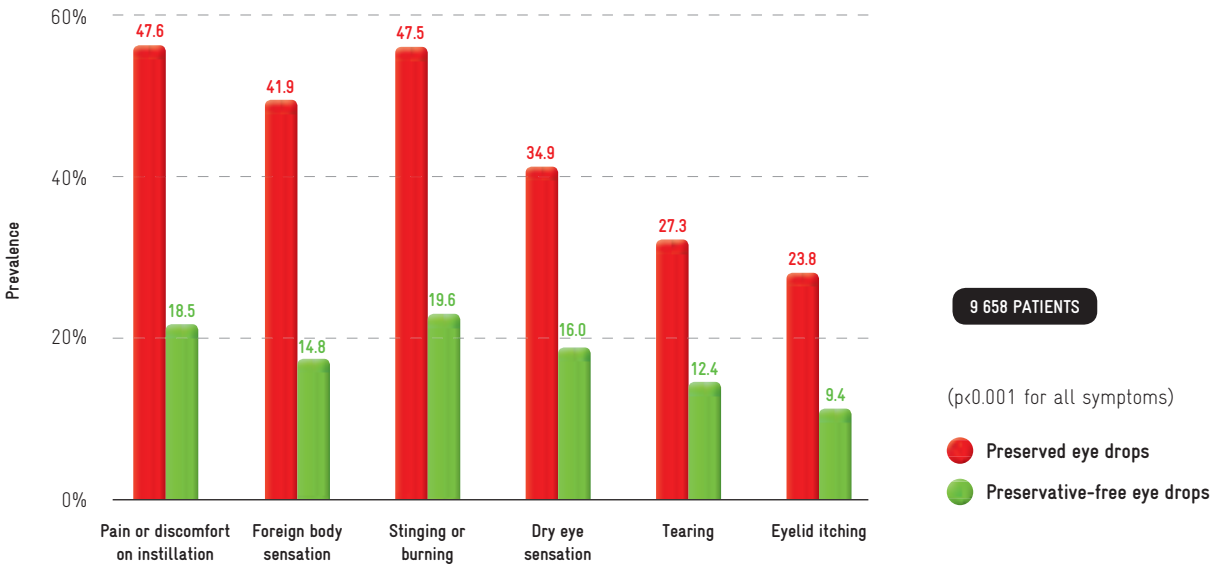
Another multinational epidemiologic survey examined patient-reported symptoms as well as palpebral, conjunctival and corneal signs in 9658 patients using beta-blocker eye drops. Overall, 74% of patients used preservative containing drops and 12% used preservative-free drops.

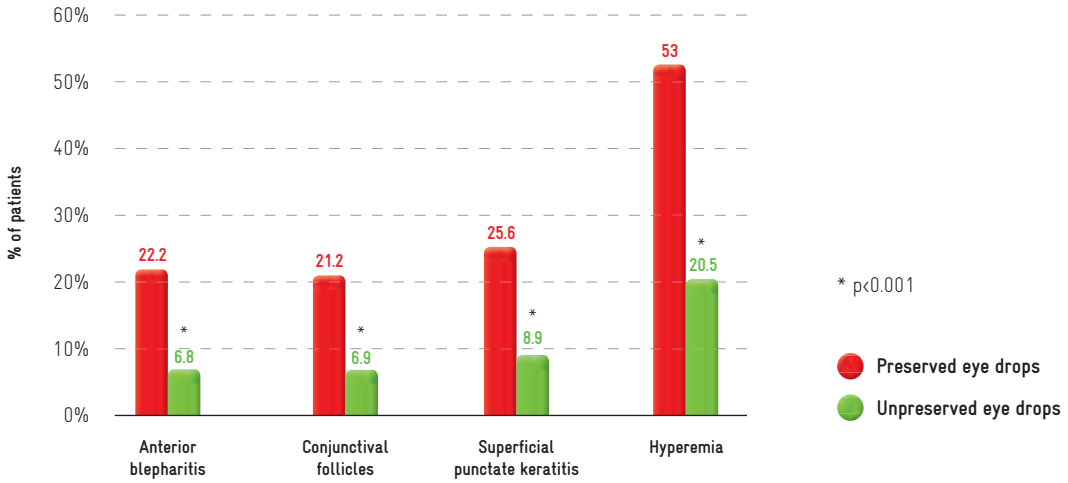
Reported symptoms as well as all palpebral, conjunctival, and corneal signs were significantly more frequent in patients using preservative containing drops than those using preservative-free drops (Figures 4 and 5). Patients who reduced their dosage or switched to preservative-free drops experienced a significant amelioration of their symptoms as well as clinical signs. The most frequent symptoms in patients treated with preserved eye drops compared to unpreserved eye drops were pain and incomfort (48% versus 19%), a foreign-body sensation (42% versus 15%), a burning sensation (48% versus 20%), and a dryness sensation (35% versus 16%) [26]. In conclusion, compared to preserved eye drops, preservative free eye drops are significantly less associated with ocular symptoms and signs of irritation.

FIG.**4**

Frequency of ocular symptoms during or after instillations in patients treated with preserved or preservative-free glaucoma medication

Adapted from Jaenen et al. (26)





Frequency of ocular signs in patients with preserved or preservative-free glaucoma medication

Adapted from Jaenen et al. (26)

FIG.**5**

More severe ocular reactions may develop in preservative-exposed eyes. The use of long-term antiglaucoma medications has been shown to cause conjunctival foreshortening and shrinkage, which may be associated with an ocular pemphigoid-like condition or evolve into severe scarring conjunctivitis with definitive corneal opacities [27]. Toxic endothelial degeneration in ocular surface disease treated with topical medications containing BAK was also described [28]. In a series of 145 patients presenting a pseudopemphigoid, Thorne et al. showed that exposure to antiglaucoma eye drops was the primary cause of pseudopemphigoid. Almost all the cases reported (97.4%) involved an association of antiglaucoma medications [29].

SEVERE OCULAR REACTIONS CAUSED BY PRESERVATIVES MAY INCLUDE:

- CONJUNCTIVAL FORESHORTENING AND SHRINKAGE
- TOXIC ENDOTHELIAL DEGENERATION
- OCULAR PEMPHIGOID-LIKE CONDITION

THESE STUDIES CLEARLY SUPPORT THAT PRESERVATIVE-FREE EYE DROPS ARE SIGNIFICANTLY LESS ASSOCIATED WITH OCULAR SIGNS AND SYMPTOMS IN PATIENTS TREATED WITH GLAUCOMA MEDICATIONS.

3.2 Impaired corneal sensitivity

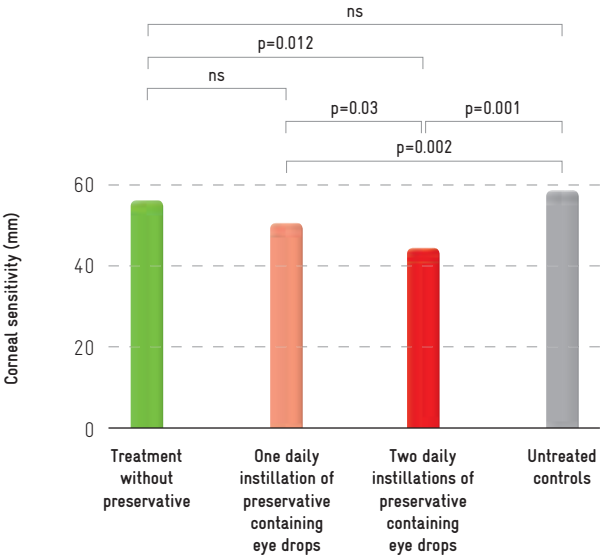
Although most research has focused on the ocular surface there are also studies suggesting that BAK might also reduce corneal sensitivity. This raises the worrying possibility that symptoms of serious, possibly sight-threatening, ocular surface disease might be masked by reduced corneal sensitivity [30, 31].

Several studies have shown that the corneal sensitivity of patients treated with glaucoma medications was reduced compared to untreated patients. Using in-vivo confocal microscopy, Martone et al. [30] found a reduced density of epithelial cells in glaucomatous patients treated with preserved eye drops for at least 12 months compared to patients treated with unpreserved eye drops. On the contrary, the density of basal epithelial cells was increased and the stromal keratocyte activation and the number of beads were significantly higher in glaucoma preservative groups. They also found a lower number of sub-basal nerves. This study are consistent with a significantly lower corneal sensitivity in patients treated with preserved glaucoma eye drops compared to untreated patients or patients treated with preservative-free eye drops. The reduced density of superficial epithelial cells in all groups of glaucoma patients, except the preservative-free group, could be related to the toxic effect of BAK, according to the following proposed mechanism:

- Increased density of epithelial cells, attributable to a proliferate stimulus from the superficial layer.
- Stromal changes due to epithelial cell modification.
- Inflammatory process at the ocular surface and induction of apoptosis of stromal cells and increased stromal proteolytic activity.
- Stimulation of cell proliferation leading to keratocyte activation and secretion of neural growth factors contributing to changes in nerve number and shape.

Indeed, patients on glaucoma medication had fewer sub-basal corneal nerves than control. Nerve fibers are important for corneal trophism and help to maintain a healthy corneal surface. The lower number and density of nerves in the sub-basal level may explain the lower corneal sensitivity observed in the glaucoma therapy group.

In another study, van Went et al. [31] compared the corneal sensitivity in patients treated with IOP-lowering medications (N=35) and untreated patients (N=9). Corneal sensitivity was assessed using the Cochet-Bonnet esthesiometer. Treated patients were divided into three groups according to the daily number of preserved eye drops (0, 1 and ≥2). As shown in Figure 6, corneal sensitivity was 58.8±2.8mm in untreated patients, and 56.2±5.2mm, 50.3±12.5mm and 44.3±13.6mm, in patients treated with none, one and two or more instillations of preserved eye drops, respectively. Corneal sensitivity in patients treated with preserved eye drops was significantly lower as compared to untreated patients ($p<0.001$) and patients treated with preservative-free eye drops ($p=0.012$). Interestingly, corneal sensitivity of patients treated with IOP-lowering medications was negatively correlated to the number of instillations of preserved eye drops ($r=-0.390$; $p<0.001$) as well as to the duration of treatment ($r=-0.357$; $p=0.001$) consistent with a cumulative effect of BAK-induced toxicity. Consistent results were also reported by Labbé et al. [32].



In this study, 35 patients with glaucoma or ocular hypertension and 9 untreated patients were analysed for corneal sensitivity. Corneal sensitivity was reduced by eye drops containing BAK compared to preservative-free eye drops or untreated eyes controls.

FIG.**6**

Reduced corneal sensitivity in glaucomatous patients treated with preserved eye drops compared with untreated patients or patients treated with preservative-free eye drops

Adapted from Van Went et al. [31]

IN CONCLUSION, BAK DECREASES CORNEAL SENSITIVITY IN A DOSE AND TIME DEPENDENT MANNER, CONSISTENT WITH A CUMULATIVE EFFECT OF BAK-INDUCED TOXICITY.

3.3

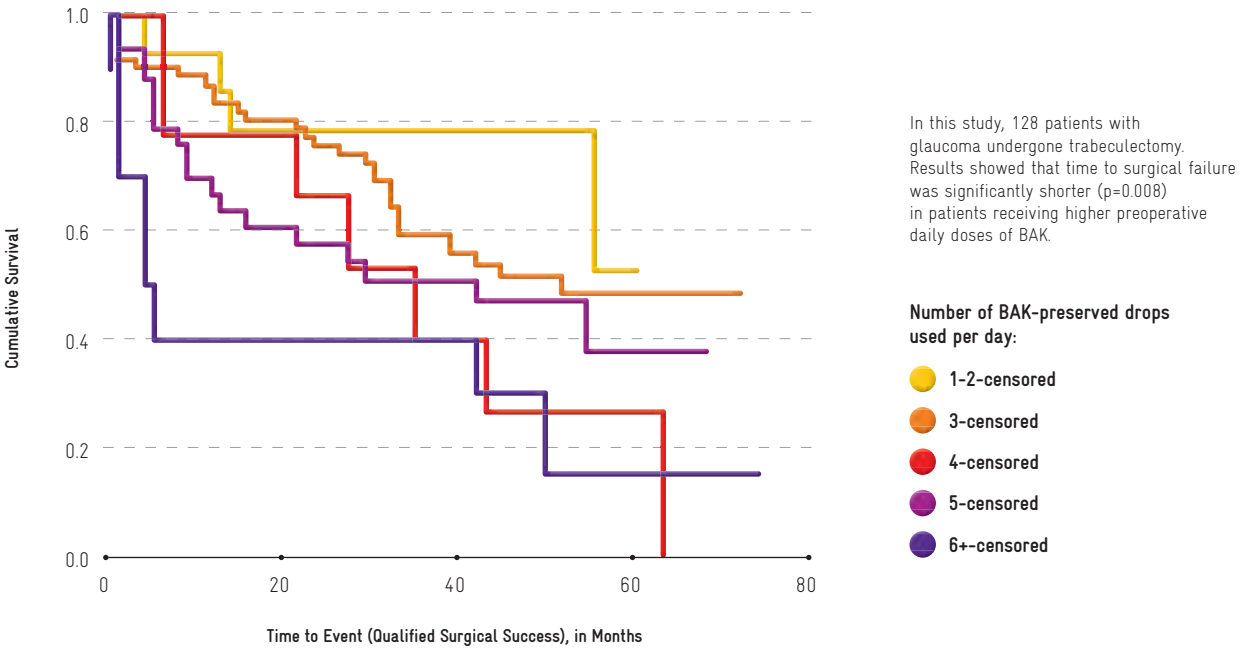
Outcome of filtering surgery

Ophthalmologists may propose filtration surgery in case of uncontrolled IOP or when chronic therapy with eye drops is not well tolerated. The critical role of the conjunctiva in glaucoma filtration surgery is known. It is recognised that a healthy conjunctiva allows drainage channel to form and less opportunity for inflammation and scar tissue formation which are frequent cause of failure in glaucoma filtration.

Previous work in the 90th, suggested that prolonged treatment with antiglaucoma medications increases the risk of future filtration surgery failure [34].
BAK has been suspected as the most likely candidate for filtering surgery failure [3]. A recent study showed a dose-response curve for the amount of preoperative BAK exposure and trabeculectomy failure. The study was based on the review of retrospective charts of 128 glaucoma patients who had previously undergone a trabeculectomy between 2004 and 2006. Surgical failure criteria included inadequate pressure lowering or need for post-operative ocular hypertensive laser trabeculoplasty, 5-fluorouracil needling, or repeated surgery. The average length (±SD) of time with glaucoma was 8.2±5.5 years, ranging from 4 months to 34.8 years. The mean post-operative follow-up time was 4.3±1.0 years ranging from 2.0 to 6.3 years.

BAK concentration in the medications used in this study ranged from 0.005% to 0.02%. Patients received between 1 and 8 BAK-containing eye drops daily with a median of 3. The analysis showed that complete surgical success was achieved in 48% of patients. Using multivariate survival models, it was shown that the time to surgical failure receiving higher preoperative daily doses of BAK was shorter than in patients who had less BAK exposure (p=0.008) (Figure 7). For each additional drop containing BAK, the risk of early failure increases by a factor of 1.21. This study suggests that an increased amount of preserved drops used per day increased the risk of surgical failure. Although the mechanisms are not clear, it is possible that inflammation and fibrosis increase the risk of outflow blockage and therefore early surgery failure [35].

Recently, using optical coherence tomography (OCT), Meziani et al. showed that success filtering surgery was associated with a higher density of intraepithelial microcysts. They also found an inverse relationship between the duration of preserved eyedrops used before surgery and the density of intraepithelial microcysts (r=-0.5436, p=0.006), thus suggesting further the negative impact of preservative eye drops when used for years on the filtering surgery outcome [83].



Kaplan-Meier survival analysis for glaucoma surgery outcome stratified by exposure to benzalkonium chloride (BAK)

Adapted from Boimer et al. [35]

FIG.**7**

- PROLONGED TREATMENT WITH PRESERVED ANTIGLAUCOMA MEDICATION IS LINKED WITH AN INCREASED RISK OF FILTERING SURGERY FAILURE
- THE GLAUCOMA SURGERY OUTCOME DEPENDS ON THE NUMBER OF BAK-PRESERVED EYE DROPS USED: FOR EACH ADDITIONAL DROP CONTAINING BAK, THE RISK OF EARLY FAILURE INCREASED BY 21%

3.4 Development of cataract

Although the effects of BAK are generally manifest as ocular surface disease, there is also some experience that it may be involved in the development of cataract. Although not yet definitive, the evidence that glaucoma medication in general (rather than any particular active substance) is associated with an increased risk of cataract (odds ratio 1.56) is suggestive of such an association (even if no evidence was found for a general effect of topical ocular hypotensive medication on lens opacification or visual fonction) [36].

3.5 Anterior chamber inflammation and cystoid macular oedema

Miyake et al. previously suggested that benzalkonium chloride was the causative factor in the disruption of the blood-aqueous barrier in early post-operative pseudophakia and increased the incidence of angiographic cystoid macular oedema (CME) [37, 38].

A recent prospective, randomised, investigator-masked, comparative study confirmed that a short-term exposure to BAK can cause disruption of the blood-aqueous barriers in pseudophakic eyes [39]. When one drop of artificial tears containing benzalkonium chloride (BAK, 0.006%) was instilled 4 times daily for 30 days, a statistically significant ($p=0.017$) increase in laser flare measurements was shown after 15 days (from 8.4±2.7 to 11.4±5.1 ph/ms) compared to eyes of patients treated with unpreserved eye drops (from 9.3±2.6 to 8.4±2.8 ph/ms) (Figure 8). After 30 days, the BAK-preserved group maintained significantly higher mean flare values (11.9±5.9 ph/ms) compared with baseline ($p=0.043$). This study suggests that BAK can cause disruption of the blood-aqueous barriers, and thus caution should be taken when using BAK-preserved eye drops in pseudophakic eyes.

FIG.**8**

Effect of BAK on the blood-aqueous barrier in pseudophakic eyes. Mean laser flare values before treatment exposure and after preserved or preservative-free eye drops exposure

(Adapted from Abe et al. [39]

One drop of preservative-free artificial tears or BAK (0.006%) preserved artificial tears was instilled 4 times a day for 30 days in healthy eyes of 44 pseudophakic volunteers. Results showed increase in laser flare measurements after 15 days in eyes compared to eyes of patients treated with unpreserved eye drops.

Another randomised prospective single-masked clinical study showed that short-term BAK administration in patients with ocular hypertension produces inflammations in the anterior segment of previously untreated patients whose blood-aqueous barriers was not affected by recent intraocular surgery [40]. Patients (N=28) were treated twice daily for 1 month with either preserved-Beta blocker containing 0.01% BAK in one eye or unpreserved-Beta blocker in the fellow eye. After treatment, mean flare values were significantly increased from baseline ($p<0.001$) in eyes treated with preserved-Beta blocker eye drops and the difference between group was statistically significant ($p=0.003$).

Thus, BAK may cause a rapid disruption of the blood-aqueous barrier and in this case it may promote the generation of inflammatory mediators in the anterior chamber and vitreous. In turns, the generation of inflammatory mediators could disrupt the blood-retinal barrier leading with time to an increased incidence of postoperative CME.

IN CONCLUSION, BAK CAN CAUSE DISRUPTION OF THE BLOOD-AQUEOUS BARRIER IN PSEUDOPHAKIC EYES. THUS, CAUTION SHOULD BE TAKEN WHEN USING BAK-PRESERVED EYE DROPS IN PSEUDOPHAKIC EYES.

3.6 Other implications

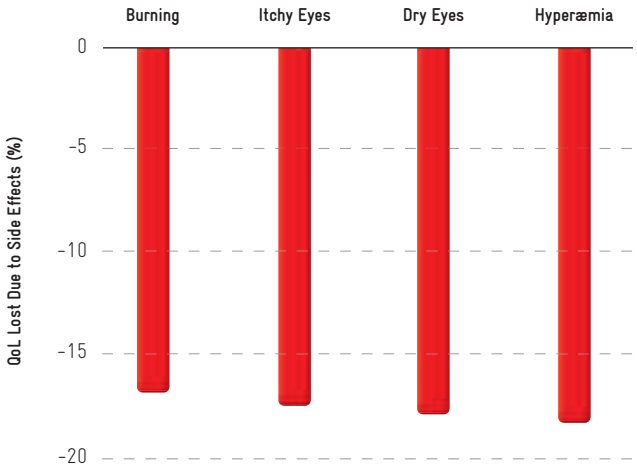
3.6.1 Impact on patient's quality of life

Although in most cases ocular symptoms such as burning, redness, irritation, fatigue, and fluctuating visual acuity are mild to moderate, they often negatively impact the quality of life (QoL) in patients treated with preserved eye drops. It was reported that 62.4% (Fig 9) of patients treated with IOP-lowering eye drops complained of undesirable ocular effects including burning (25.4%), blurred vision (20.8%), and tearing (20.2%) [41]. In this study, poor vision related QoL was associated with topical drug side effects (Figure 9). In another study in patients with glaucoma or ocular hypertension, a statistically significant relationship was shown between the OSDI score and QoL measured using the Glaucoma Quality of Life -15 questionnaire. OSD was more common in patients with increasing glaucoma severity and is associated with poorer glaucoma-related QoL and higher BAK exposure [42].

Using the Glaucoma Symptom Scale, a statistically significant ($p<0.01$) improvement in quality of life was achieved by the switch from a preserved to a preservative-free therapy in glaucoma patients. Scores for symptoms and functioning improved significantly from baseline (+21.2% and +10.3%, respectively) 8 weeks after the switch [43].

Effect of main ocular symptoms and hyperemia on quality of Life (QoL) FIG.**9**

Adapted from Nordmann et al. [41]



Quality of Life score difference on the ocular pain domain of the NEI-VFQ-25 quality of Life questionnaire between patients reporting an adverse event and those not reporting adverse events (Least square means after adjustment on gender).

$p<0.001$ for burning, itchy eye, and hyperæmia.

OCULAR SURFACE DISEASE IS MORE COMMON IN PATIENTS WITH INCREASING GLAUCOMA SEVERITY AND IS ASSOCIATED WITH POORER GLAUCOMA-RELATED QUALITY-OF-LIFE AND HIGHER BAK EXPOSURE.

3.6.2 Impact on treatment adherence

Poor adherence to treatment with glaucoma medications have been consistently shown in several studies [44 - 47].

As shown in Figure 10, adverse events accounted for 12% of non compliance. According to a medical chart review, 67% of patients remained persistent (i.e. no discontinuation) 12 months after start of therapy [46]. Adverse events in patients treated with IOP-lowering eye drops is the second most common reasons for switching medication after lack of efficacy [48]. In a recent cross-sectional study, 40% of patients had previously stopped treatment due to ocular surface disease and move to alternative eye drops, laser, or filtration surgery, or additive treatment [49]. Preservatives are responsible for at least some of these local adverse events and removing preservative from the patient's medication is supposed to improve both, quality of life and adherence to treatment [50].

In a case series of glaucoma patients refractive to treatment and presenting with severe ocular surface disease, replacement of the preserved-glaucoma medication with unpreserved eye drops and the management of ocular surface (with lid hygiene measures, topical antibiotics and preservative-free lubricants) led to a sustained control of the IOP and stabilisation of the visual field. This suggests that a healthy ocular surface helps in the medical control of glaucoma in the longer term, in part due to improved treatment compliance. This procedure probably helped to avoid filtering surgery which outcome may be compromised in these patients presenting with scarring and inflammation of the conjunctiva [71].

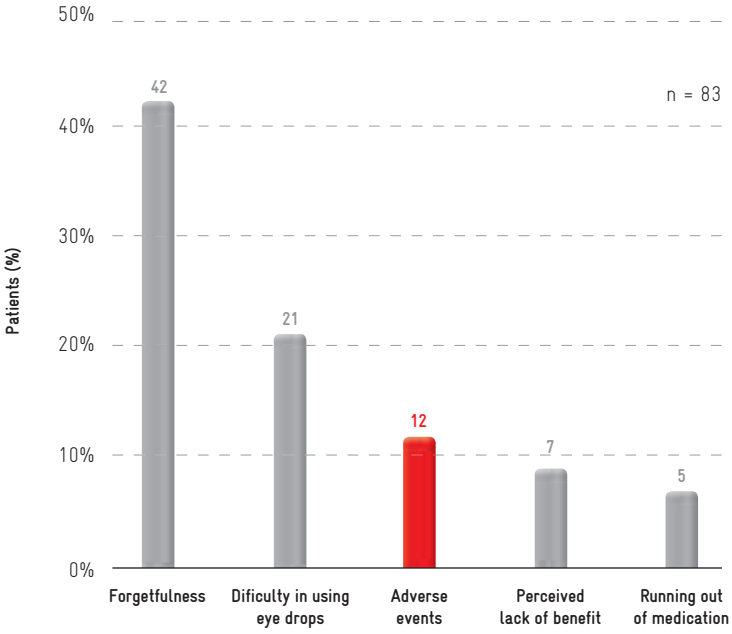


FIG.**10**

Reasons for treatment non adherence in patients treated with glaucoma ocular medications

Adapted from Chawla et al. [45]

In allergic conjunctivitis, treatment compliance measured by the number of instillations per day was significantly lower ($p<0.001$) in patients treated with preserved eye drops which was consistent with a significant reduction in the number of instillations missed ($p=0.01$) and the proportion of patients reporting adverse drug reactions ($p<0.001$) (Figure 11).

FIG.**11**

Adverse reactions and compliance in patients taking preserved and preservative-free medication for allergic conjunctivitis

Adapted from Beden et al. [51]

* $p<0.001$
** $p=0.01$

	Preservative (n=121)	Preservative-free (n=2712)
Patients reporting at least one adverse drug reaction	89%	24%*
Instillations per day	2.9	3.5*
Proportion of patients who take the treatment every day	74.8%	82%
Number of instillations missed	4.2	3.6**

IN CONCLUSION, REMOVING PRESERVATIVE FROM THE PATIENT'S MEDICATION MAY IMPROVE BOTH QUALITY OF LIFE AND ADHERENCE TO TREATMENT.

3.6.3 Impact on disease progression

Some scientists consider that chronic medical therapy with drugs containing BAK could make glaucomatous outflow tract pathology worse and itself damages the trabeculum meshwork, decreasing outflow facility and possibly contributing to elevated IOP [17,20].

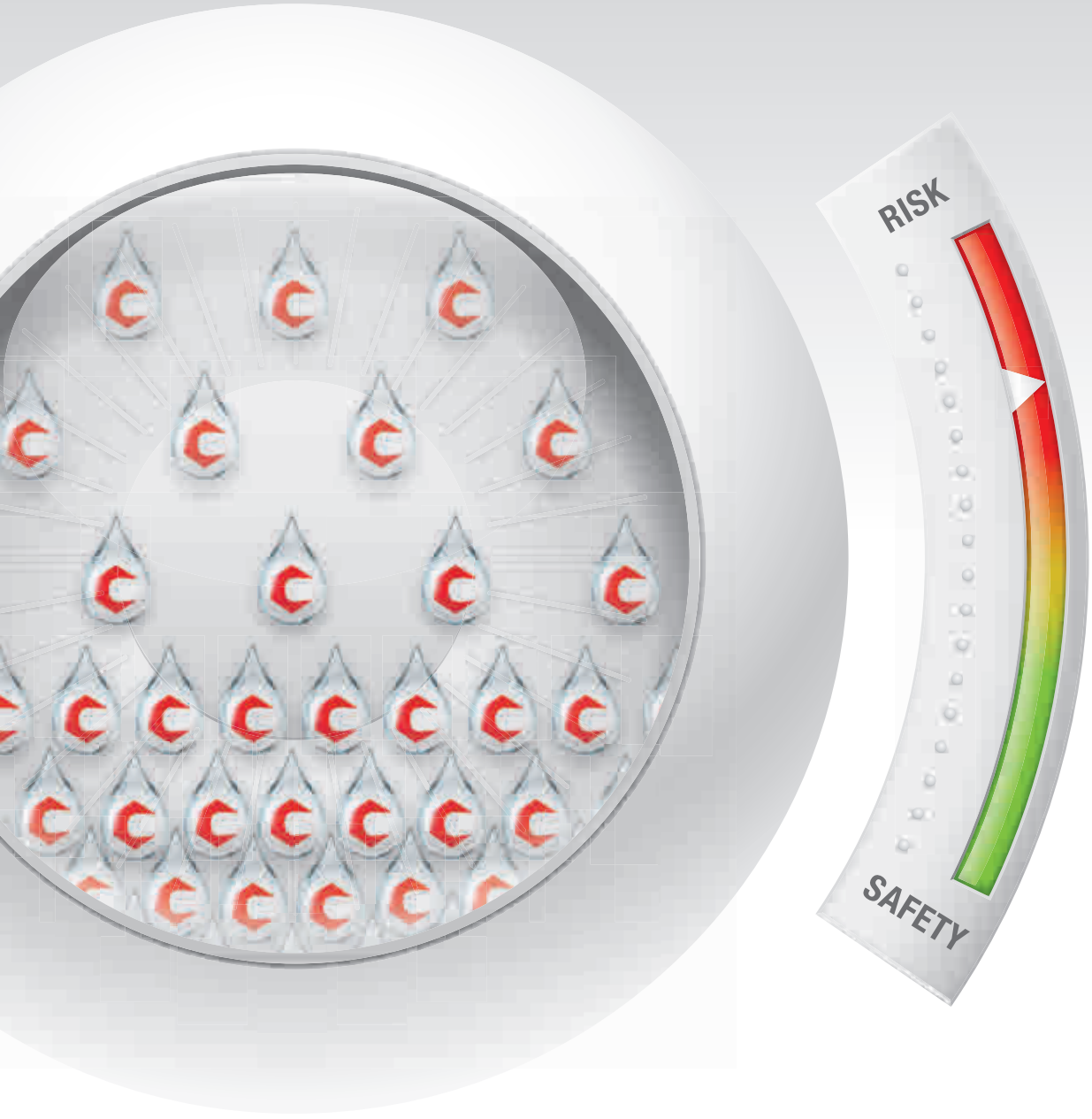
In a recent study, the severity of the ocular surface disease in patients with ocular hypertension or glaucoma was significantly correlated with the intraocular pressure [49]. The severity of the OSD could be such that it required discontinuation of the glaucoma medication. In some cases, the discontinuation was shown to produce an improvement of the IOP value. The severity of the ocular surface inflammation may impact the evolution of intraocular pressure, and thus the evolution of glaucoma.

In another prospective observational study, Van Went et al. [52] demonstrated that ocular surface disease influenced no only the quality of life, but also the therapeutic management in numerous patients treated with glaucoma medications. It was shown that 38% of patients had at least one therapeutic modification related to their ocular surface disease. For 6 patients (6.8%), a filtering surgery was performed and for 1 patient a selective laser trabeculoplasty was necessary due to a severe ocular surface disease preventing the tolerability of ocular medications.

THE SEVERITY OF THE OCULAR SURFACE DISEASE MAY BE SUCH THAT IT REQUIRES DISCONTINUATION OF THE GLAUCOMA MEDICATION.

3.6.4 Consequences on diagnostic procedures

As reported recently in glaucoma patients, ocular surface disease may affect diagnostic procedures. In dry eye patients, measurements using new perimetry procedures, such as frequency doubling technology, flicker-defined form perimetry, and pulsar perimetry may be affected as a result of stray light and reduced contrast sensitivity. This may lead to over estimation of non-existent glaucoma progression. The authors recommended to use lubricating eye drops or to switch therapy to preservative-free IOP-lowering eye drops [53].



- The number of medications
- The prolonged use of preserved medications
- The total BAK exposure

THESE ARE SIGNIFICANT PREDICTORS
OF OCULAR DISEASE

4

Risk factors and susceptibility to preservative toxicity

Ocular surface disease in patients treated with preserved ocular medication is a long and delayed process. A number of patients, especially those using a monotherapy may probably not complain of significant ocular side effects. Since in most cases, ocular side effects are mild, they are often initially underestimated. However, a more severe ocular surface disease may progressively develop with the duration of treatment and the number of ocular medications used.

As detailed below, recent studies confirmed the cumulative effect of preservative with a strong correlation between the toxicity of preserved ocular medication, the treatment duration, and the number of eye drops instilled (multitherapy). Several risk levels must be mentioned:

- a cumulative effect over time and duration of treatment in patients with chronic ocular disease treated for years or even requiring lifelong therapy;
- a cumulative effect due to the instillation of multiple eye drops daily in patients with a more severe disease who need multiple therapy for IOP control,
- the individual patients susceptibility, keeping in mind that this susceptibility may increase over time with aging.

4.1

The cumulative effect of preservative toxicity

The pharmacokinetic of BAK in human ocular tissue is not known, but experimental studies in rabbits showed a persistence in ocular tissues up to 7 days with half-lives of approximately 20 hours in corneal and conjunctival tissue and 11 hours in deeper conjunctival structures including in the corneo-conjunctival epithelium and stroma and to a lesser extent in the iris, lens, choroid and retina [54]. It is believed that the epithelium acts as a reservoir and gradually releases the preservative agent into the eye [55].

THE ACCUMULATION OF BAK MAY PRODUCE DELAYED CYTOTOXIC DOSE-DEPENDENT EFFECTS IN RELATION WITH DURATION OF THE EXPOSURE.

Baudouin et al. were the first to demonstrate that the conjunctival inflammation increased with the number of therapies used. Using conjunctival impression cytology, they found that patients who received 2 or more antiglaucoma eye drops for at least one year had greater expression of inflammatory markers compared with those treated with just a beta-blocker for 1 year [56].

Then, in a large observational study (4107 patients followed by 249 ophthalmologists), it was shown that the frequency of signs and symptoms was increased with the number of preserved medications used [25].

The cumulative effect of preservative toxicity has been repeatedly suggested in a number of observational studies. There is clinical evidence that the number of medications, their prolonged use, and the total BAK exposure are risk factors to develop OSD in patients with glaucoma [22-24, 26, 49, 57-60].

Epidemiological data from a German register of more than 20 000 glaucomatous patients in 900 centres across Germany showed that dry eye occurred more frequently when 3 or more antiglaucoma drugs were used and increased with the duration of glaucoma disease [22]. This was also reported by Fechtner et al. [24] in a prospective observational study in 630 US patients currently treated with topical IOP-lowering eye drops. Patients using 1 single medication had an OSDI score of 12.9±13.1 which was significantly lower (better) compared with patients using 2 medications (16.7±17.0, p=0.007) or 3 medications (19.4±18.1, p=0.0001).

In a cross-sectional study, it was shown that 59% of patients with OAG or OHT reported ocular symptoms in at least one eye. Severe symptoms were reported in 27% of patients, decreased tear production in 61% of patients and severe tear deficiency in 35%. Corneal and conjunctival lissamine green staining showed positive results in 22% of patients. Abnormal tear quality assessed by the tear-break up time (TBUT) was shown in 78% of patients and was severe in 65%. Using multivariate regression analysis, it was shown that each additional BAK-containing eye drop was associated with an approximately two times higher odds of showing abnormal results on the lissamine green staining test [23]. Thus patients with a more severe glaucoma treated with multiple-preservative containing eye drops have a higher risk of OSD.

Rossi et al. showed abnormal TBUT and punctate keratitis which was more frequent with increasing number of eye drops and number of instillations per day [59]. In this observational, cross-sectional study of 233 patients topically treated with glaucoma medications, TBUT was abnormal in 30.5% eyes, punctate keratitis in 31.7% and ocular surface disease was evidenced in 41.6%. Keratitis was more frequent with increasing number of eye drops (p=0.008), and the number of instillations per day (p=0.009). Using multivariate analysis, it was shown that the number of medications used, the prolonged used of preserved medications, and the total BAK exposure were significant predictors of ocular surface disease (Table 3).

TABLE **3**

Risk factors to develop ocular surface disease in patients treated for glaucoma or ocular hypertension patients

Univariate analysis	p-value
Age	p=0.04
Low IOP	p=0.03
More time treatment	p<0.0001
More BAK exposure	p<0.0001
Worst quality of life	p<0.01

Multivariate analysis	p-value
Number of medication used	p=0.002
Prolonged use of preserved medications	p=0.005
Total BAK exposure	p<0.001

Adapted from Rossi et al. [59]

The significant increase in the prevalence of ocular surface disease signs observed in patients with glaucoma was confirmed by Ghosh et al. [57]. Signs and symptoms of ocular surface disease were compared between a glaucoma population treated with eye drop medications (N=300) and control untreated patients (N=100). The logistic regression analysis showed that the number of anti-glaucoma medications and duration of therapy were key predictors of significant ocular surface disease signs.

In a recent study in 40 patients treated with preserved-glaucoma medications, 24 patients (60%) reported ocular surface disease symptoms [58]. Nineteen patients (47.5%) had a tear osmolarity ≤308 mOsms/L, 11 (27.5%) between 309 and 328 mOsms/L, and 10 (25%) >328 mOsms/L. A tear deficiency was observed in 20 patients (50%). Twenty-seven patients (67.5%) had an abnormal tear quality analysed with TBUT, and 16 patients (40%) showed positive staining using the Oxford scheme. Tear osmolarity was significantly correlated to OSDI (p=0.002) and TBUT (p=0.009). There was a statistically significant correlation between tear osmolarity and the number of drugs (p=0.009), the number of instillations (p=0.01), and the number of instillations of preserved eye drops (p < 0.0001). Using a multiple regression method, tear osmolarity remained significantly correlated to the number of instillations of preserved eye drops (p=0.004) [58]. Thus tear film osmolarity is increased in patients treated with IOP-lowering medications. This study showed a clear relationship between BAK and ocular surface tear osmolarity.

A recent study conducted by Baudouin et al. [49] in patients with glaucoma showed significant OSD in 51% of patients, including mild to moderate OSD in 30% of patients, and severe OSD in 21%. The factors significantly related with the severity of the OSD was the patient age, the number of eye drops used daily, the past topical treatment changes due to ocular intolerance, and the IOP, which was significantly higher in case of more severe ocular surface disease. It was found that 57% of patients treated with ocular medications for glaucoma or OHT since at least 10 years had ocular surface disease. The prevalence of OSD was 71% in patients treated with 3 or more medications, 54% in patients treated with 2 medications, and 38% in patients treated with monotherapy.

Similarly, according to the number of eye drops used daily, the prevalence of OSD (regardless of severity) was 63% in patients treated with more than 2 drops daily, 41% in those treated with 2 drops daily, and 46% in those treated with one drop daily. The prevalence of OSD was increased with the number of BAK-preserved eye drops and with the glaucoma severity (Figure 12).

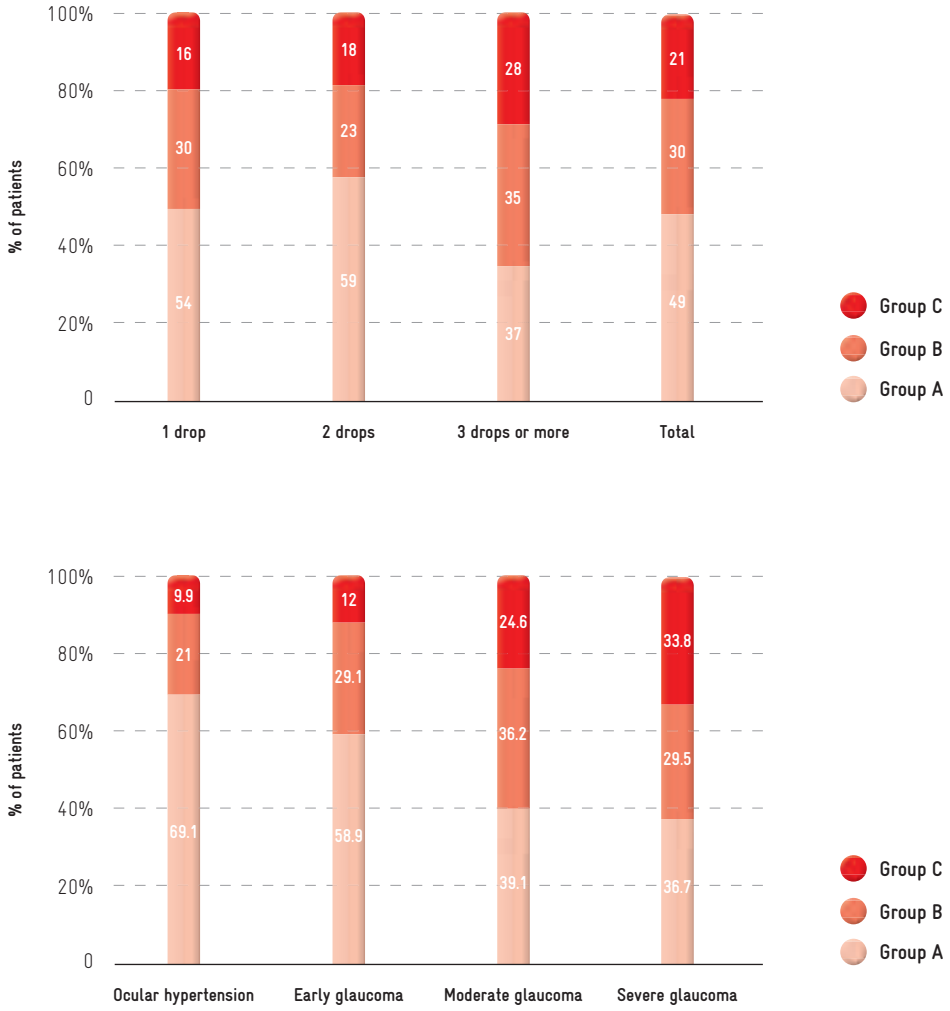


FIG.**12**

Increased prevalence of OSD with the number of BAK-preserved eye drops and with disease severity

Group assignment was based on the ocular surface symptom scores, rated from 0 to 3, and the sign scores rated from 1 to 3. The totals for combined symptom and sign severity ranged between 1 and 30, and the patients were then classified into 3 groups, according to their total scores:

Group A: score from 1 to 4 (N=254 patients);

Group B: score from 5 to 10 (N=154 patients);

Group C: score from 11 to 30 (N=108 patients).

Adapted from Baudouin et al. [49]

In addition, 40% of patients reported a modification of treatment in the past due to ocular surface intolerance and treatment persistence was also related to the severity of OSD (Figure 13).

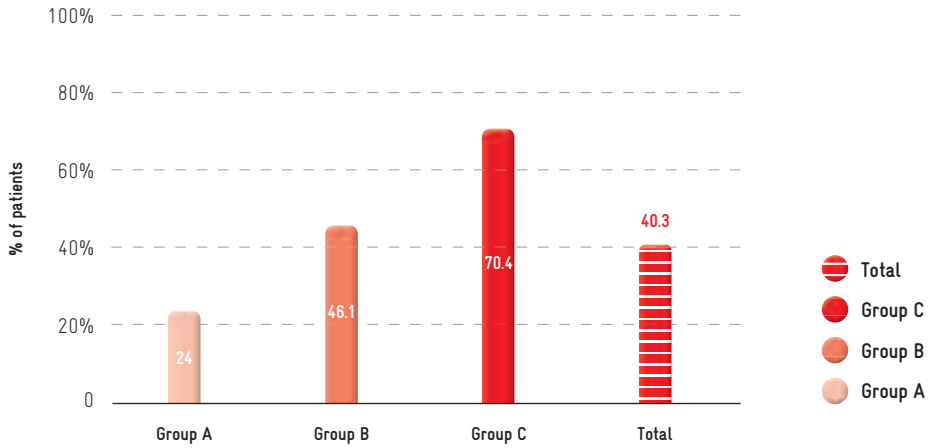


FIG.**13**

Cumulative effects of BAK toxicity: Decreased persistence of treatment with OSD severity

Group assignment was based on the ocular surface symptom scores, rated from 0 to 3, and the sign scores rated from 1 to 3. The totals for combined symptom and sign severity ranged between 1 and 30, and the patients (516) were then classified into 3 groups, according to their total scores:

Group A: score from 1 to 4 (N=254 patients);
Group B: score from 5 to 10 (N=154 patients);
Group C: score from 11 to 30 (N=108 patients).

Adapted from Baudouin et al. [49]

In a recent cross-sectional epidemiological survey among glaucoma patients (N=164 patients with a mean disease duration of about 9 years) receiving therapy with prostaglandin analogs, 44% of patients showed evident OSDs on ophthalmological examination and 38% used artificial tear substitutes. Although 89% of patients were satisfied or very satisfied with their anti-glaucoma medication, the main reason for dissatisfaction was significantly associated with the OSD ($p<0.001$) [85]. In addition, it is striking to note that more than 50% of cases, the tear substitutes contained a preservative.

IN CONCLUSION, THE PREVALENCE OF THE OSD CLEARLY INCREASED WITH THE NUMBER OF DAILY INSTILLATIONS AND THE DURATION OF TREATMENT SUPPORTING THE CUMULATIVE EFFECT OF BAK TOXICITY.

- THE NUMBER OF DAILY INSTILLATIONS OF BAK-PRESERVED EYE DROPS INCREASES PREVALENCE OF OSD.
- THE PERSISTENCE OF TREATMENT IS DECREASED IN PATIENTS TREATED WITH BAK-PRESERVED EYE DROPS.
- CUMULATIVE EXPOSURE OF BAK-PRESERVED EYE DROPS INCREASES TEAR OSMOLARITY.
- EACH ADDITIONAL BAK-CONTAINING EYE DROPS WAS SHOWN TO BE ASSOCIATED WITH AN ABOUT TWO TIMES HIGHER ODDS OF SHOWING ABNORMAL RESULTS ON THE LISSAMINE GREEN STAINING TEST.

4.2 Individual susceptibility to preservative toxicity

There are cases where the intolerance to preservative is a more important issue [3]. This includes pre-existing diseases involving ocular surface (dry eye, allergy...).

4.2.1 Patients with dry eye

Patients with dry eye are at particular risk because the low volume of their tear secretion allows higher concentrations of BAK to remain in contact with the cornea for longer periods of time [61]. Long-term use of preservative-containing artificial tears is associated with an increased risk of adverse events and epithelial surface damage and diminished compliance due to ocular irritation [3]. Experimental studies in cultured conjunctival cells have shown increased cytotoxic effects of BAK in hyperosmolarity conditions with characteristic cell death process, including caspase-dependent and independent apoptosis and oxidative stress [62]. This suggests that BAK administered in an eye already submitted to hyperosmolar conditions would be more toxic than in a healthy normal ocular surface. This also highlights the importance of avoiding preservatives like BAK even at low concentrations in a dry eye patient because cytotoxic effects of BAK will act synergistically with hyperosmolarity. The extensive use of BAK over the long term, as in glaucoma, may progressively cause tear-film hyperosmolarity and instability. This could explain the high prevalence of ocular surface disease and dry eye observed in patients with glaucoma [22, 24].

SINCE THE EFFECTS OF PRESERVATIVE RESEMBLE THOSE OF DRY EYE, THEY ARE EASILY MISTAKEN FOR AN EXACERBATION OF THE UNDERLYING DISEASE, RATHER THAN A SYNDROME OF TOXICITY.

4.2.2 Patients with ocular allergy

The effects of preservative toxicity also affect patients with allergic conditions. A prospective cohort study examined the occurrence of adverse effects among 3090 patients taking preserved or preservative-free eye drops for allergic conjunctivitis. Adverse reactions were more frequent and compliance was lower in the patients using preserved eye drops [51]. All symptoms were reported significantly less frequently by patients using preservative-free than those using preserved medication.

4.2.3 Patients with meibomian gland disease

Patients with meibomian gland dysfunction (MGD) are likely to be at particular risk from the toxicity of preservatives eye drops, since the composition of tears in such patients is already impaired and symptoms of eye irritation, inflammation, and ocular surface disorders are exacerbated and mimicked by preservative toxicity [63]. Guidelines from the International Workshop on Meibomian Gland Dysfunction published in 2011 suggest that patients with symptomatic meibomian gland dysfunction should receive artificial lubricants and where they are used frequently preservative-free formulations are to be preferred [64].

4.2.4 Patients with ocular surgery

Cataract surgery is the most common surgical procedure undertaken by ophthalmic surgeons and is increasing in frequency as the population ages. Several topical preparations are used during the course of cataract surgery including cleansing agents (particularly in patients with blepharitis), mydriatics, anaesthetics, antibiotics and anti-inflammatories. Interactions appear between ocular surface disease and cataract surgery: on one hand cataract surgery worsens ocular surface disease, at least in the short term and on the other hand more severe ocular surface disease is a risk for post-operative complications. Clearly the use of preserved medications that may worsen ocular surface disease is undesirable in this situation [65].

IT IS IMPORTANT TO AVOID PRESERVATIVES LIKE BAK EVEN AT LOW CONCENTRATIONS IN PATIENTS WITH CHRONIC OCULAR SURFACE DISEASE BECAUSE OF CYTOTOXIC EFFECTS PARTICULARLY IN PATIENTS WITH:

- DRY EYE DISEASE
- OCULAR ALLERGY
- MEIBOMIAN GLAND DISEASE
- OCULAR SURGERY (CATARACT, REFRACTIVE, GLAUCOMA SURGERIES).

How to manage the ocular surface?

As described above, ocular surface disease is a common problem with preserved ocular medications especially when used in long-term. There is strong evidence for a cumulative effect of preservative toxicity with delayed adverse reactions. Side effects may be only ocular discomfort of more and less severity, but it should be kept in mind that chronic ocular surface inflammation, even subclinical, may have major consequences, in particular on glaucoma filtering surgery success.

Thus, the ophthalmologists should remain vigilant to ocular surface disease among their medically treated patients and should manage signs and symptoms appropriately as part of the comprehensive management of patients with glaucoma. In any case, it is clear that the treatment of the glaucoma pathology remains the priority, but assessing ocular surface should become a routine exam also. The lack of time is not a valuable reason for not assessing the ocular surface in glaucomatous patients. The assessment of the ocular surface is rapid, easy and does not need sophisticated or time-consuming measurements. Questioning the patients on current ocular discomfort, use of artificial tears or lachrymal substitute for ocular dryness, a rapid ocular and eyelid examination, and the instillation of one drop of fluorescein to assess the conjunctiva, cornea, and lacrimal tear film stability is achievable in one minute. Uncontrolled intraocular pressure should also suspect treatment non adherence due to ocular surface disease.

Early recognition of the deleterious effects of preservatives on the ocular surface should allow the treating physician to intervene prior to disease progression. Two strategies are currently adopted [2]:

- to treat ocular surface disease early and aggressively (addition strategy)
- to attempt to minimize the exposure to detergent preservative when possible (subtraction strategy). The treatment should consist of the discontinuation of unnecessary medications, attempting to limit the number of medicines containing BAK, and possibly changing medications with less toxic eye drops or unpreserved eye drops when available.

EARLY RECOGNITION OF THE DELETERIOUS EFFECTS OF PRESERVATIVES ON THE OCULAR SURFACE SHOULD ALLOW THE TREATING PHYSICIAN TO INTERVENE PRIOR TO DISEASE PROGRESSION.

5.1 The addition strategy

In addition to the glaucoma, it is also important to focus on aggressively treating the underlying ocular surface disease (dry eye syndrome, blepharitis, rosacea,..). Therapeutic options to treat dry eye syndrome and meibomian gland diseases included lid hygiene measures, tear substitutes without preservatives, anti-allergic eye drops, anti-inflammatory eye drops, immunomodulators, antibiotics, corticosteroids [64, 66-69].

It is possible that aggressive treatment of ocular surface disease may improve the patient's tolerability to glaucoma medication [70]. However, this may be not convenient for the patients to use two or three drops of glaucoma medication plus artificial tears four to six time daily. However, this strategy is not successful because it is not intended to target the origin of the surface pathology [2].

Obviously, if additional tear substitutes are used to treat the ocular surface disorder, it is recommended to choose an unpreserved preparation [86, 87, 88]. However, it's still not always evident for some ophthalmologists as suggested recently in a cross sectional study in the Netherlands. Lemij et al. reported that 38% of patients with glaucoma or ocular hypertension treated with prostaglandin analogues, were using tear substitutes and that in one of two patients, the tear substitute contained a preservative. This is not rational given that the preservative has probably played a major role in the development of the OSD [85].

A combination approach to manage the OSD in patients with severe OSD and inadequately controlled primary open angle glaucoma was described recently in 4 case-reports. Measure to control OSD included twice-daily lid hygiene measures, a 3 months course of 50 mg daily oral cycline, topical artificial tears 4 to 6 times daily, and preservative-free equivalents of topical antiglaucoma medications. Patients were reviewed for a maximum of 24 months after intervention. In all patients treatment resulted in a marked symptomatic and clinical improvement in the ocular surface with a reduction in hyperaemia, meibomian gland dysfunction and superficial keratopathy. A reduction in the IOP also occurred in all patients, obviating the need for glaucoma drainage surgery during the study period [71].

THE ADDITION STRATEGY IS NOT CONVENIENT FOR THE PATIENT AND IS NOT THE BEST SOLUTION SINCE IT DOES NOT TARGET THE ORIGIN OF THE OCULAR SURFACE DISEASE.

5.2 The subtraction strategy

For the reasons mentioned above, the preventive approach should be actually preferred over the addition strategy. This help in reducing or eliminating the origin of the ocular surface pathology. Different therapeutic approaches are possible when available [3] (Table 4):

- The use of ocular medication with a lower BAK concentration since the BAK-toxicity is clearly dose dependent.
- The reduction of the number of daily eye drops instillations using fixed combination rather than free association of several ocular medications or using once daily formulation rather than twice daily.
- The use of alternative, less toxic, BAK-free ocular medications.
- The use of preservative-free ocular medications either as single dose units (SDU) or mechanically-preserved multiple dose (MD) containers including COMOD® and ABAK® systems.

TABLE 4

Strategy to manage ocular surface disease [3]

Choose medications with lower BAK concentration
Choose medications requiring less instillations during the day
Use less toxic preserved medications
Use preservative-free medications

5.2.1 Alternative preserved eye drops

In recent years, new ocular medications containing a preservative with a lower toxicity than BAK have been developed by the pharmaceutical industry. Not all products are currently available on the European market. This includes polyquaternium-1 (Polyquad®), stabilised oxychloro complex (Purite®) and an ionic buffer solution (SofZia®) in the treatment of glaucoma. These products demonstrated clinical efficacy in clinical trials and higher tolerability. In toxicological studies, their antimicrobial efficacy is variable [72] and their long-term clinical safety is not yet known.

As shown by Meloni et al. [73] using a new in-vitro technic to determine the irritant and subclinical eye irritant potential of topical ocular medications, all preserved formulations have their own toxicity. This technic was based on the quantitation of occludin gene expression as a biological marker for the determination of eye irritation potential of ocular medications. The use of human corneal epithelial (HCE) model allowed the modelling of cumulative effects that may approach conditions obtained after long-term application of tear substitutes.

A modified multiple endpoint analysis (MEA), based on the assessment of the cellular viability of the basal epithelial layer, and histological analysis for the detection of both superficial and deeper morphological alterations has been proposed as a valuable and promising tool for in vitro assessment of eye irritation with the power to discriminate between mild irritants and subclinical eye irritant potential. In this study, it was shown that cellular viability was moderately reduced by Perborate and Polyquad®-preserved tear substitutes and dramatically reduced by BAK and by Thiomersal® and Oxyd® preserved tear substitutes. Thiomersal® also increased IL-8 release. Occludin expression profiles were modified by the four chemically-preserved tear substitutes and by the mechanically-preserved Comod®, but not by the mechanically-preserved ABAK® [73].

ALL PRESERVED FORMULATIONS HAVE THEIR OWN TOXICITY AND THEIR LONG-TERM SAFETY IS NOT KNOWN.

5.2.2 Unpreserved eye drops

One alternative to BAK in current uses includes single-use medications, and mechanically-preserved formulations with either a valve mechanism (COMOD®) or a antimicrobial filter (ABAK®) to prevent microbial contamination [3].

TABLE **5**

Benefits of preservative-free eye drops [3]

Less irritant for the ocular surface
Better treatment adherence
Improved quality of life
Success of filtration surgery
Improved disease control

There are now several preservative-free medications available for the treatment of glaucoma, including beta-blockers, carbonic anhydrase inhibitor (CAI), and prostaglandins analogues [74].

The benefits of preservative-free topical medication are clear:

- Better tolerability due to reductions in adverse events,
- Better adherence to treatment,
- Better clinical outcome for the patients,
- Lower costs due to reduced frequency of consultation.

Patients who may benefit from preservative-free treatment include:

- Patients with OSD that is independent of glaucoma, such as those with moderate to severe dry eye symptoms (e.g. keratoconjunctivitis sicca),
- Patients with moderate to severe blepharitis,
- Patients with allergic conjunctivitis or rosacea.

Patients with OSD caused by preserved glaucoma treatment, especially those who have had two or more medications, will also benefit. This group includes patients who are expected to receive long-term topical treatment for glaucoma and patients who may need glaucoma surgery (e.g. taking three to four drugs but IOP still not controlled) [75].

PATIENTS WITH GLAUCOMA SHOULD BENEFIT OF PRESERVATIVE-FREE THERAPY ESPECIALLY WHEN TWO OR MORE MEDICATIONS ARE USED AND WHEN PATIENTS NEED GLAUCOMA SURGERY.

Benefit of unpreserved eye drops in terms of toxicity

Experimental studies showed that unpreserved eye drops had very low or no pro-apoptotic, pro-necrotic, or pro-oxidative effects in-vitro compared to preservative-containing formulations [76].

Clinical studies have shown that patients treated with unpreserved eye drops had significantly less ocular symptoms and ocular signs compared to patients treated with preserved ocular medications [25, 26]. In a large multicentre cross-sectional survey which enrolled 9658 patients using preservative or preservative-free beta-blocking eye drops, the prevalence of ocular signs and symptoms was significantly higher in patients treated with preserved eye drop [26].

PATIENTS TREATED WITH UNPRESERVED EYE DROPS HAD SIGNIFICANTLY LESS OCULAR SYMPTOMS AND OCULAR SIGNS COMPARED TO PATIENTS TREATED WITH PRESERVED OCULAR MEDICATIONS.

A meta-analysis of randomised controlled trials [77] was recently performed to assess the safety of prostaglandin analogues in the treatment of OAG or OHT. The risk of hyperaemia was statistically significantly lower with the preservative-free prostaglandins than with prostaglandins preserved with polyquaternium, Sofzia® and BAK.

THE RISK OF HYPERAEMIA IS SIGNIFICANTLY REDUCED WITH PRESERVATIVE-FREE PROSTAGLANDINS.

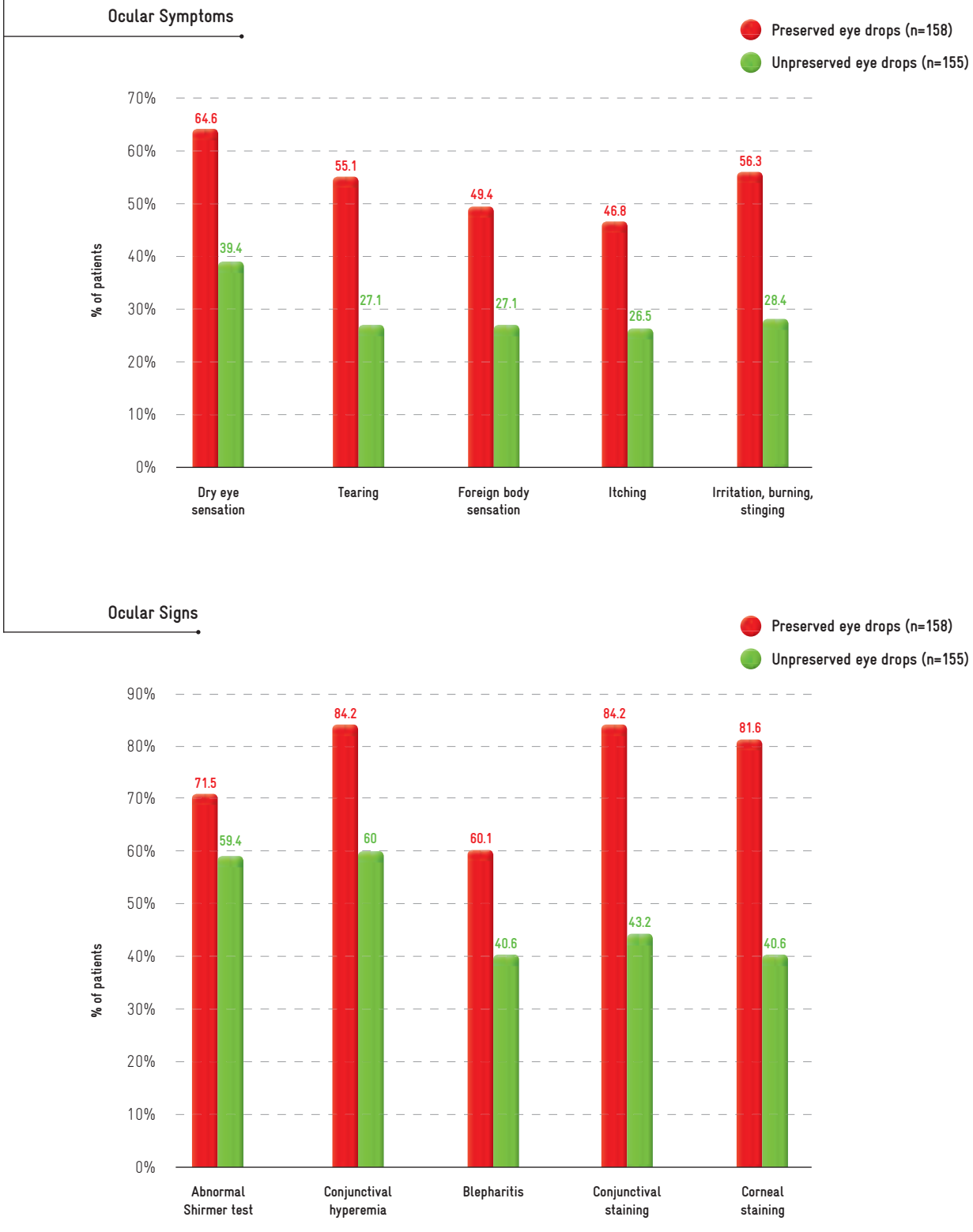
Clinical studies have confirmed that removal of BAK substantially benefit the patients' ocular surface. Improved ocular surface was shown in glaucoma patients who switched from preserved to unpreserved eye drops (Figure 15). After 3 months, the switch to unpreserved prostaglandin reduced the rate of patients with irritation/burning/stinging (from 56.3 to 28.4%), itching (from 46.8% to 26.5%), foreign body sensation (from 49.4% to 27.1%), tearing (from 55.1% to 27.1%) and dry eye sensation (from 64.6% to 39.4%). The rate of abnormal fluorescein corneal staining was reduced from 81.6% to 40.6%, conjunctiva from 84.2% to 43.2%, blepharitis from 60.1% to 40.6%, conjunctival hyperaemia from 84.2% to 60% and abnormal Shirmer tests from 71.5% to 59.4%. The TBUT was improved from 4.5±2.5 sec to 7.8±4.9 sec [78].

OCULAR SIGNS AND SYMPTOMS ARE CLEARLY IMPROVED WHEN PRESERVED THERAPY IS SWITCHED TO UNPRESERVED EYE DROPS.

FIG.**14**

Reduction of ocular symptoms and signs in patients with glaucoma or ocular hypertension after switching from preserved to unpreserved eye drops

Adapted from Uusitalo et al. [78]



Patients previously treated with preserved prostaglandin were switched with unpreserved prostaglandin for 3 months. Results showed a clear reduction of ocular signs and symptoms.

Recently, in prospective, longitudinal, open-study in 132 patients with POAG treated with preserved beta-blockers, the switch to a preservative-free beta-blockers 0,1% gel led to a statistically significant reduction in the corneal and conjunctival fluorescein staining, as well as eyelid erythema, conjunctival hyperaemia, and follicular hyperplasia (Table 6). A statistical difference was shown for the TBUT (from 9.4±4.7 sec to 10.6±4.7 sec after 3 months) and Schirmer test (from 12.9±5.6 mm to 14.2±5.8 mm after 3 months). Dryness and foreign body sensation were also improved [79].

TABLE **6**

Improvement of ocular signs when preserved beta-blockers are switched to unpreserved beta-blockers 0,1% gel in patients with POAG

	Baseline Mean (SD)	1 month Mean (SD)	3 months Mean (SD)	Baseline vs. 1 month P-value	1 month vs. 3 months P-value	Baseline vs. 3 months P-value
Eyelid erythema	0.46 (0.82)	0.23 (0.55)	0.13 (0.37)	<0.001	<0.001	<0.001
Conjunctival hyperemia	0.97 (0.94)	0.58 (0.64)	0.33 (0.52)	<0.001	<0.001	<0.001
Follicular hyperplasia	0.36 (0.62)	0.16 (0.40)	0.08 (0.31)	<0.001	<0.001	<0.001
Break-up time(s)	9.82 (0.31)	10.9 (3.24)	11.5 (3.38)	<0.001	<0.001	<0.001
Schirmer's test (min)	13.46 (6.28)	14.72 (6.44)	15.41 (6.32)	<0.001	<0.001	<0.001

Ocular surface epithelial staining was evaluated according to the NEI grading system (0–15 for fluorescein corneal staining).
Results showed a statistically significant reduction of ocular surface signs.
Bonferroni post hoc test was used to compare the 3 groups.
SD, standard deviation.

Adapted from Iester et al. [79]

In some studies, patients treated for less than 3 months showed no significant difference in ocular tolerability between BAK and preservative-free medications. But, the benefit was shown at long term as suggested in a recent prospective, open-label, multicentre study in patients with OAG/OHT. A total of 114 patients participated in this study. Transition from preserved prostaglandin to another prostaglandin BAK-free showed no significant effect on hyperemia at 1 month, but showed significant decreases at 3 and 12 months compared with baseline ($p<0.05$). The prevalence of superficial punctate keratitis (SPK), especially its severity score, at all points were significantly reduced compared with baseline ($p<0.05$). The IOP at baseline and at 12 months after transition was 14.9±3.4 and 14.3±3.3 mmHg, indicating a significant reduction after the change in regimen compared with baseline ($p<0.05$). Thus, treatment for 12 months with BAK-free prostaglandin after BAK-preserved prostaglandin resulted in fewer ocular surface complications, as indicated by the reduced prevalence of SPK and decreased hyperaemia, and no clinically relevant changes in IOP. BAK-free prostaglandin may have beneficial effects on the ocular surface while showing IOP-lowering efficacy comparable with BAK-preserved eye drops [80].

Efficacy of unpreserved eye drops

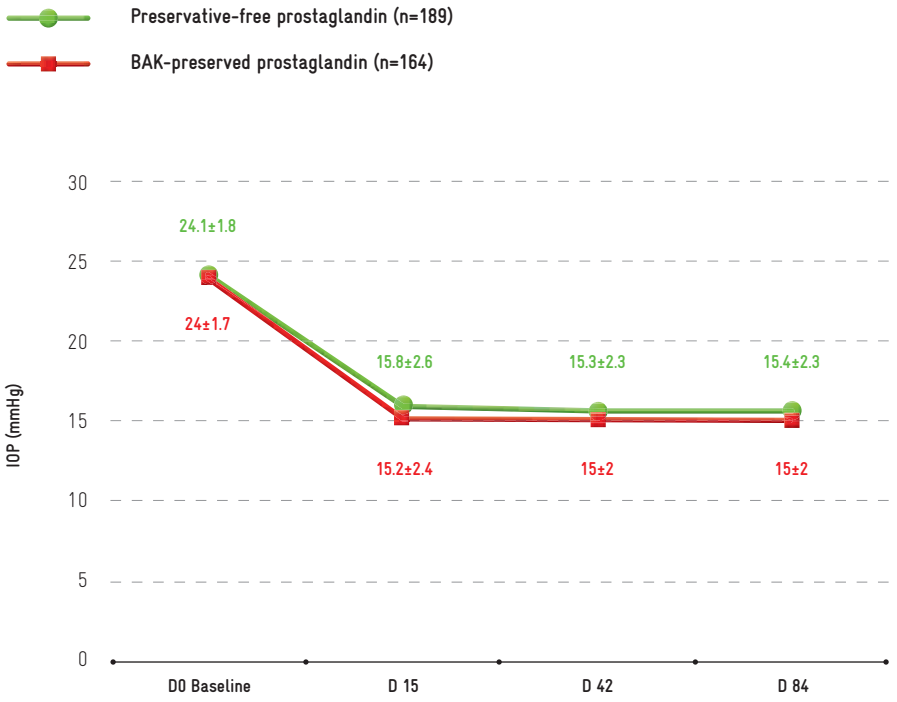
It has been suggested that through its detergent activity BAK could aid drug penetration into the eye and thus aid efficacy. This raised the hypothesis that BAK-free ocular medication may be less effective than BAK-preserved medications. In fact, this was not clearly demonstrated, and several randomised double-masked controlled studies have shown equivalence or non inferiority in terms of efficacy of unpreserved medication compared to formulation containing BAK [4, 81].

In a 3-month study comparing preserved and unpreserved prostaglandins in formulations in multidose containers, the non inferiority in IOP reduction of the unpreserved eye drops to the preserved formulation was demonstrated. The mean IOP reduction (±SD) after 3 months was -8.6±2.6 mmHg in patients treated with the unpreserved eye drops and -9.0±2.4 mmHg in patients treated with preserved eye drops. Non-inferiority of the unpreserved to preserved eye drops was demonstrated after 3 months of treatment (primary endpoints), but also after 15 days of treatments (Figure 16) [4].

FIG.**15**

IOP measurements at baseline and during 3-month treatment with preservative-free prostaglandin compared to BAK-preserved prostaglandin

Adapted from Rouland et al. [4]



In an open-labelled randomised two parallel groups clinical study, the efficacy and safety of unpreserved beta-blocker 0,1% gel was compared to preserved prostaglandin in patients with signs of ocular intolerance. At inclusion, all patients had ocular signs of intolerance to preserved-prostaglandin as defined by the association of at least two ocular symptoms and the presence of at least one mild or moderate ocular signs. The primary criteria was the responder rates defined as the reduction of at least 20% of the sum of ocular signs and symptoms score and IOP-lowering effect considered by the investigator as satisfactory or acceptable. After 3 months of treatment, the responder rate was 91.5% in the unpreserved beta-blocker gel group versus 48.6% in the preserved prostaglandin eye drop group ($p<0.001$). Thus, unpreserved beta-blocker 0.1% gel maintained the efficacy of preserved prostaglandin and reduced signs and symptoms of intolerance in almost all glaucomatous/OHT patients on preserved prostaglandin [81].

In another open-labelled randomised parallel-group controlled study, the IOP-lowering effect of beta-blocker 0.1% gel in single dose unit (SDU) was compared with beta-blocker 0.1% gel (in multidose (MD) containers) in patients with OHT or OAG. Treatments were administered once daily for 12 weeks. The mean IOP reduction (±SD) after 12 weeks was -5.6±2.8 mmHg in patients treated with the unpreserved SDU beta-blocker and -5.6±2.9 mmHg in patients treated with preserved MD beta-blocker gel. The study showed the non-inferiority of the unpreserved SDU gel to the preserved MD gel over the 12 weeks period of treatment [81 bis].

CLINICAL STUDIES DEMONSTRATED THAT EFFICACY OF UNPRESERVED GLAUCOMA MEDICATIONS WERE EQUIVALENT OR NON-INFERIOR COMPARED WITH PRESERVED EYE DROPS IN TERMS OF IOP REDUCTION.

6

Barriers to the development of unpreserved eye drops

Nowadays, most ocular medications on the market still contain toxic preservatives. Beside the research and development cost to develop new ocular medications without preservative or medications with minimally toxic preservative, this required important resources to adapt the manufacturing industrial processes.

For the pharmaceutical industry, it is more cost effective to get regulatory approval and manufacture a single formulation for global use and it is more cost effective to make multiple-dose vials than unit dose packaging.

Preservative-free medications are poorly reimbursed by the Health Authorities, and for the ophthalmologists, although it is essential to treat the ocular surface disease, it is more difficult to propose a not refunded treatment in some patients.

PRESERVATIVE OR PRESERVATIVE-FREE?

THAT SHOULD BE THE QUESTION TO ASK

7

Conclusion

In conclusion, it should be kept in mind that preservatives in ocular medications are toxic for the ocular surface. These effects are dose- and time-dependent and the risk to develop ocular surface disease is increased particularly in those patients who received long-term multi therapy with several eye drops daily like glaucoma or dry eye disease. Beyond the ocular discomfort, and the subjective problem of quality of life, chronic inflammation of the ocular surface may produce severe sight-threatening adverse effects and is an important risk factor of the filtering surgery failure.

Although, the priority is to treat the primary ocular disease, defects of the ocular surface may compromise the efficacy of the ocular treatment in terms of adherence to treatment. For these reasons, ophthalmologists should evaluate the risks and benefits of ophthalmic medications before initiating therapy, identify the minimum dosages necessary to achieve a therapeutic benefit, and monitor patients for ocular surface disease. When the patients present a severe ocular surface disease, the diminution of preserved ocular medications may improve both the ocular surface and the intra-ocular pressure measurements.

OPHTHALMOLOGISTS SHOULD EVALUATE THE RISKS AND BENEFITS OF OPHTHALMIC MEDICATIONS BEFORE INITIATING THERAPY, IDENTIFY THE MINIMUM DOSAGES NECESSARY TO ACHIEVE A THERAPEUTIC BENEFIT, AND MONITOR PATIENTS FOR OCULAR SURFACE DISEASE.

Since two decades, cumulative evidences based on laboratory, experimental and clinical studies support the interest to use preservative-free eye drops in the treatment of ocular disease. A preservative-free medication should be considered as soon as the therapy initiation [82]. It is accepted by the scientific and medical community that preservative-free treatment in glaucoma is a sensible and realistic aim [55]. According to the last recommendations of the European Glaucoma Society in June 2014 [89], "ocular surface should be evaluated and considered in clinical management of glaucoma patients. In case of ocular surface disease, preservative-free formulations should be considered".

A PRESERVATIVE-FREE MEDICATION SHOULD BE CONSIDERED AS SOON AS THE THERAPY INITIATION.

Health Authorities seem also more and more concerned by toxicological issues and should favour the development by the industrials of new preservatives or preservative-free alternatives. In 2009, the European Medicines Agency (EMA) addressed the interest of avoiding preservatives in "patients who do not tolerate eye drops with preservatives" and those with long term treatment, of using "concentration at the minimum level consistent with satisfactory antimicrobial function in each preparation", of promoting "new ophthalmic preparations without any mercury-containing preservatives" although a general recommendation not to use preservatives in eye drops was not given (EMEA statement, 2009).

The future for the patients is to treat efficiently their ocular diseases in terms of efficacy and ocular surface safety.

HEALTH AUTHORITIES RECOGNISE THE INTEREST TO AVOID PRESERVATIVES IN PATIENTS WITH LONG TERM THERAPY.

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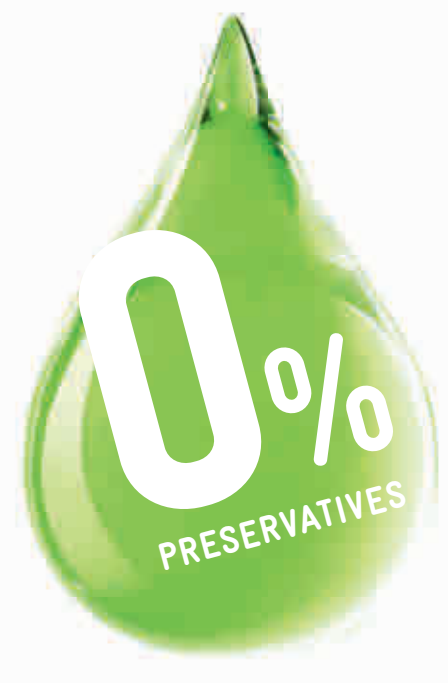

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