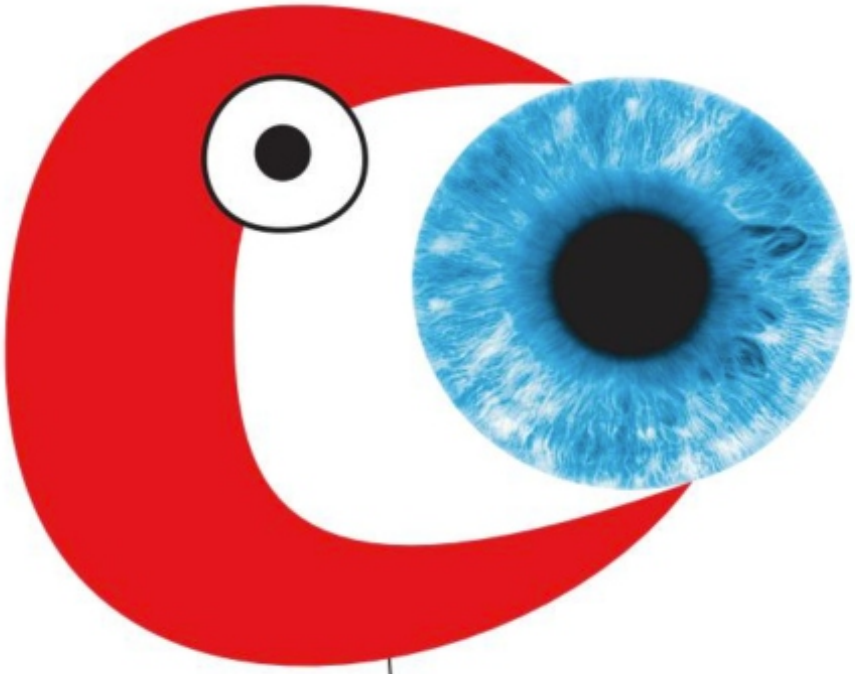
10 ans de Révolution sans Conservateur



volume 3

Volume 3

Preuves Cliniques

Pr. Christophe Baudouin, Centre Hospitalier National d'Ophtalmologie des XV-XX et Institut de la Vision, Paris.

LABORATOIRES Théa

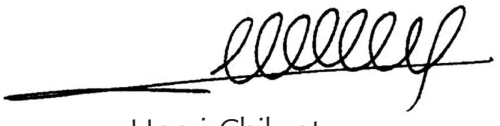
Professor Christophe Baudouin, renowned worldwide for his research on the ocular surface, brings you a summary of the experimental, epidemiological and clinical studies of the effects of preservatives as published in international literature.

Following **a first volume on the elements of assumption** introducing us to the harmful effects of eye drop preservatives on the cornea, **a second volume provided the experimental evidence**.

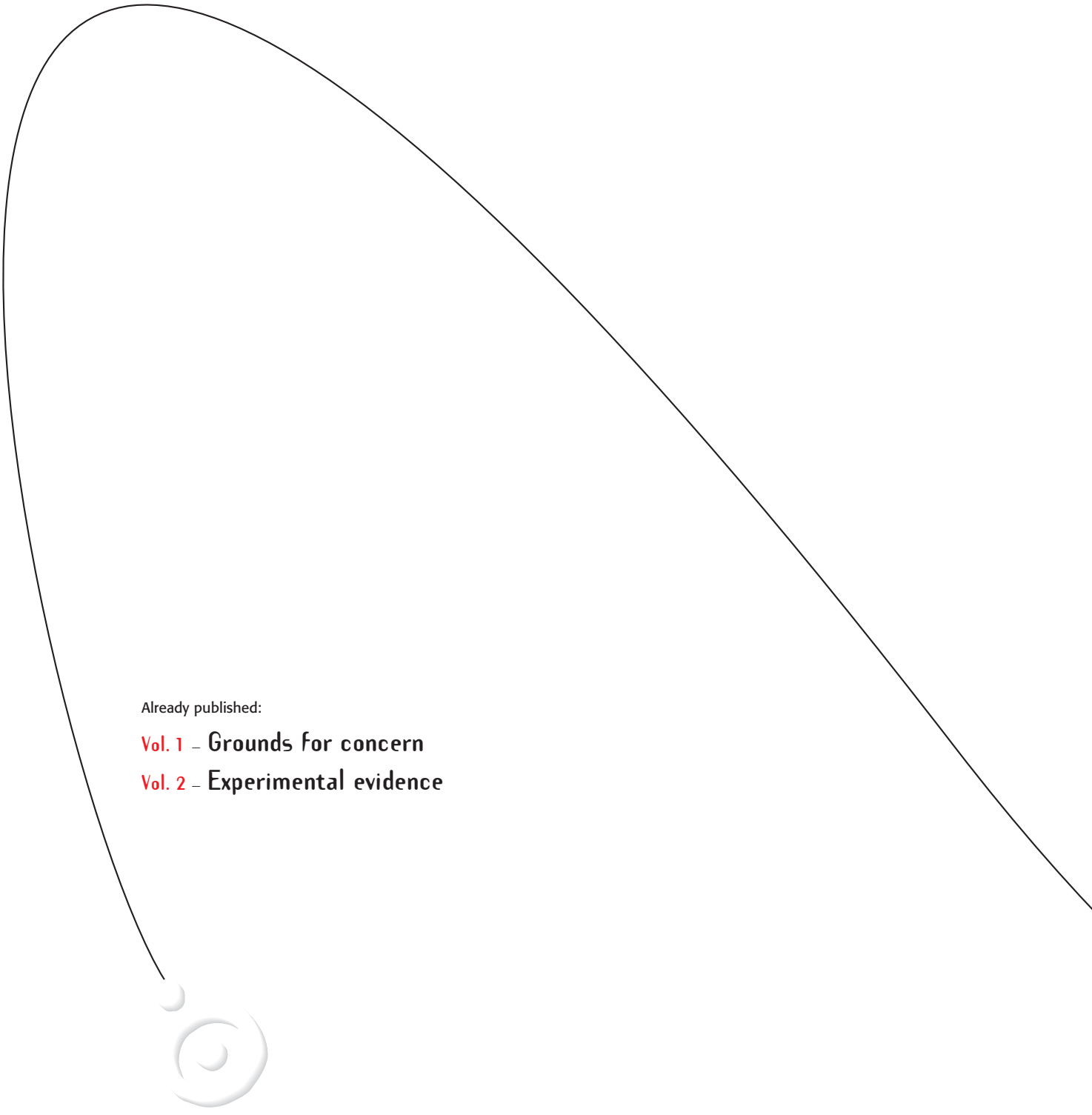
I am pleased to present **this third volume which is certainly the most long-awaited as it discusses the clinical proof on this subject**. All the advantages of preservative-free eyedrops in the daily treatment of various ophthalmic pathologies (dry eye syndrome, allergies, inflammation, glaucoma etc.) are illustrated here.

I wish you a pleasant read,

Best regards.



Henri Chibret



Already published:

Vol. 1 – Grounds for concern

Vol. 2 – Experimental evidence

10 years of preservative-free eyedrops

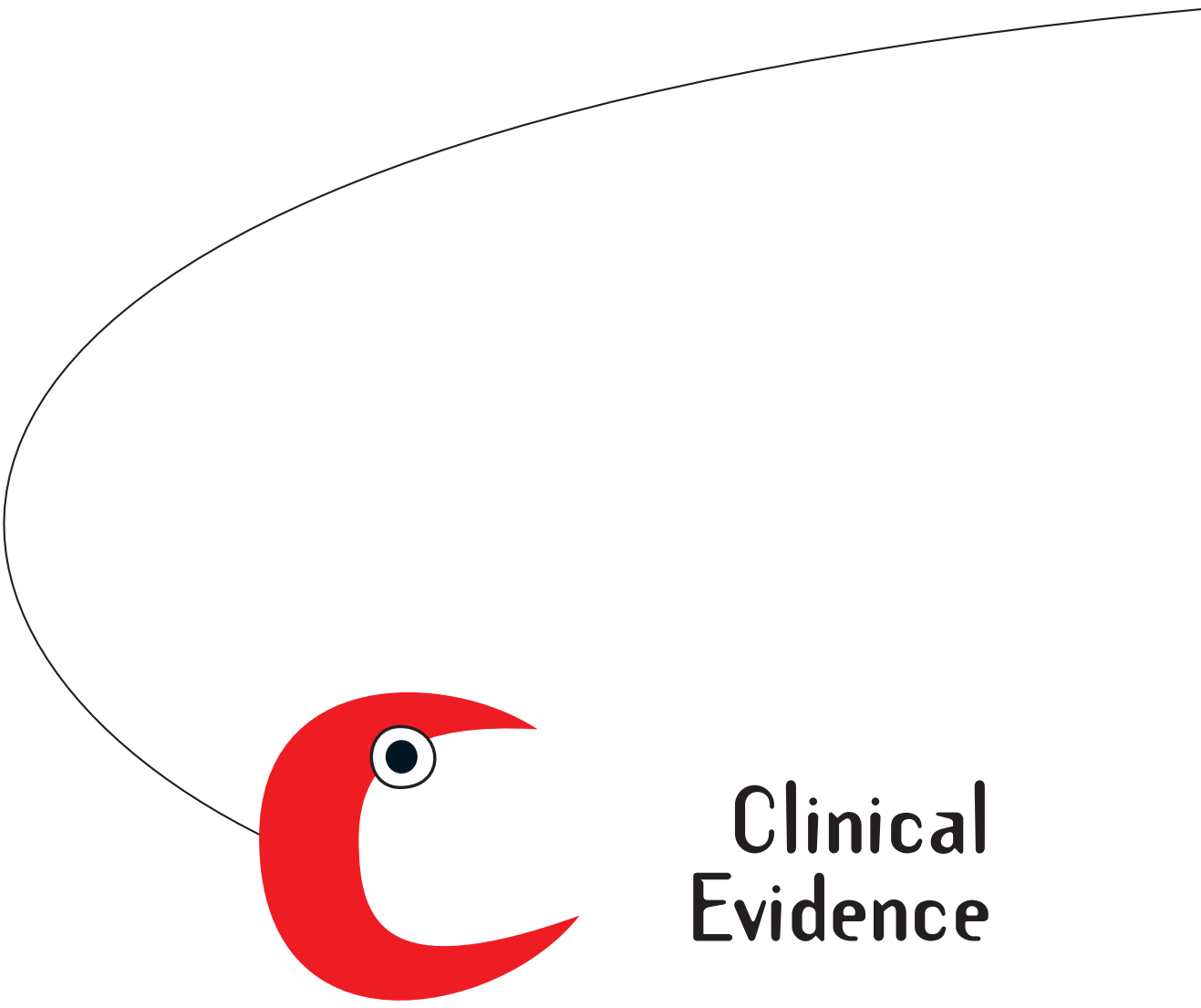
Vol. 3 – Clinical evidence

Prof. Christophe Baudouin, CHNO des XV-XX (National Eye Hospital) and Vision Institute, Paris

Introduction

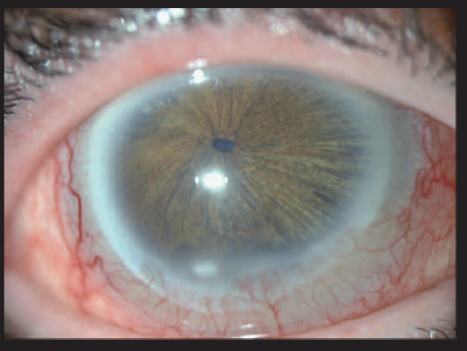
Although highly suspected on the basis of in vitro studies or studies in animals and by a number of clinical observations, the role of eye drop preservatives in corneoconjunctival toxicity had not been formally identified in humans until recently. The lack of preservative-free commercial preparations, single dose eyedrops aside, did not enable a comparative evaluation of the local tolerance of the two formulations to be made. The development and subsequent commercialisation of systems administering preservative-free multidose eyedrops devoid of all contamination (protected by a membrane filter with very low porosity), have made it possible to determine the specific tolerance to unpreserved eyedrops over the last few years.

This brochure presents a summary of the clinical studies aiming to determine the ocular tolerance of preservative-free eyedrops compared to preserved eyedrops. We will notably observe that in patients intolerant to preserved drops, changing to preservative-free drops considerably improves all functional signs and symptoms along with lachrymal function. Double-blind, randomized, controlled clinical trials confirm the results of the retrospective studies showing better corneoconjunctival tolerance of preservative-free eyedrops.



Clinical Evidence

1. Retrospective studies



Glaucoma: a disease of the ocular surface

A number of cross-sectional epidemiological surveys have determined the prevalence of functional signs and symptoms in large populations of patients treated by preserved or preservative-free eyedrops [5, 23, and 32]. The surveys show a strong prevalence of ocular surface changes in these patients: alteration of the ocular surface being less frequent in patients using preservative-free eyedrops.

1.1- Subjective symptoms and clinical signs

These studies were conducted by private ophthalmologists and produced similar results [23, 32]. The patients involved in the study presented ocular hypertension and/or chronic open-angle glaucoma treated by one or more eye drop solutions containing a preservative or not. Out of 4,107 patients enrolled in the second study [32], 84% were receiving one or more preserved eye drop solutions, 13% were receiving preservative-free treatment in monotherapy and 3% a combination of preserved and preservative-free eyedrops. Median treatment duration was 3.9 years. Discomfort on instillation was reported more frequently in patients receiving preserved eyedrops (43% vs. 17%) and in particular stinging or burning sensations (40% vs. 22%) and a grittiness sen-

sation in the eye (31% vs. 14%) (Figure 1). Discomfort between instillations was also reported twice as frequently in the group of patients treated by one or more eye drop solutions containing a preservative (61 % vs. 36 %, $p < 0.001$). The same applied concerning clinical signs of conjunctival alteration (49% vs. 26%, $p < 0.001$), superficial punctate keratitis (19% vs. 9%, $p < 0.001$) and eyelid pruritis (22% vs. 9%, $p < 0.001$) (Figure 2). It was also observed that the frequency of the signs and symptoms increased with the number of preserved products instilled by the patients (Figure 3).

Another epidemiological study conducted in general medical practice confirmed these results recently. This study aimed to determine the side effects experienced by patients treated over a 7-day period for allergic conjunctivitis. Retrospectively 2,712 patients had been treated by preservative free eyedrops and 121 by preserved eyedrops. Adverse ocular events were considerably more frequent in patients treated by preserved eyedrops (89.3%) than in patients receiving preservative-free eyedrops (23.8%). The adverse ocular events reported the most frequently in the preservative group were burning or stinging (47.1%), foreign body sensation (31.4%) and tearing (26.4%). In comparison, these effects were reported by only 10.4%, 3.7% and 9.3% of patients treated by preservative-free eyedrops. Analysis of the compliance data showed that the patients treated by preserved eyedrops were less consistent in taking their treatment (significant reduction in the average number of instillations per day and significant increase in forgetting to take the treatment), probably due to the increased feeling of discomfort on instillation or between instillations.

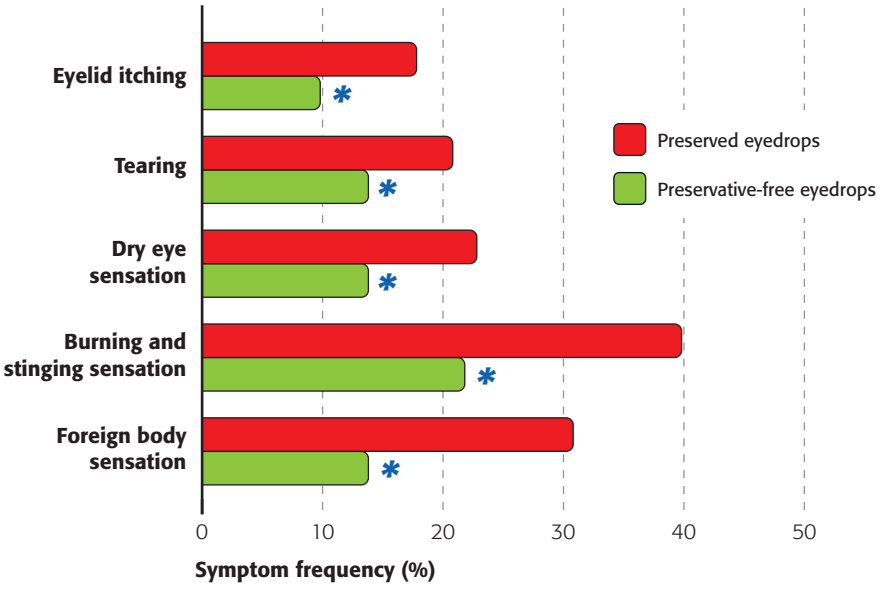
Points to remember:

Wide scale retrospective studies have showed that preserved eyedrops may lead to alteration of the ocular surface (feeling of discomfort on instillation and between instillations, conjunctival, corneal or palpebral signs). More important, even the slightest discomfort may lead patients to neglect taking their treatment correctly.

These side effects are minimised in patients treated by preservative-free eyedrops, suggesting significant involvement of the preservative in the occurrence of these functional signs and symptoms.

Figure 1

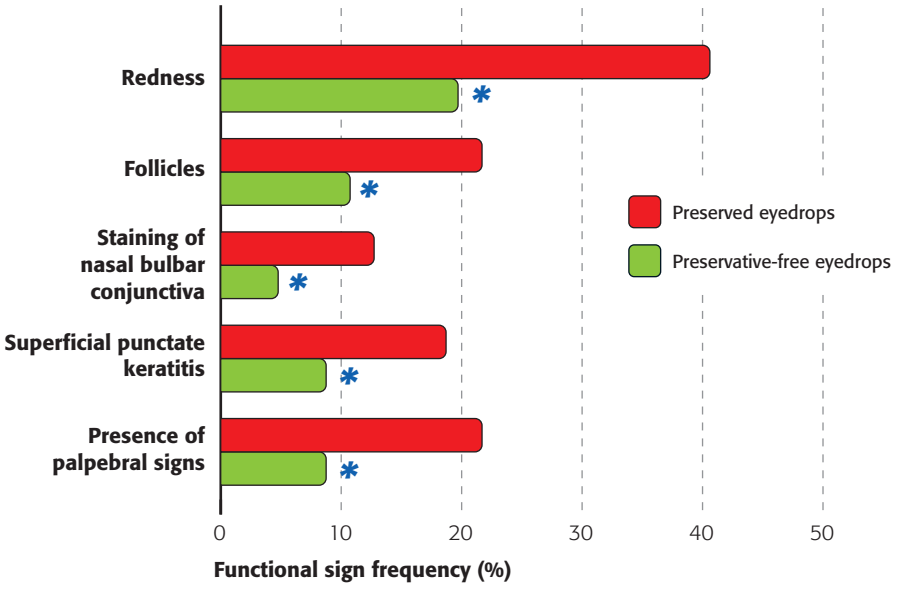
Prevalence of symptoms reported by glaucoma patients treated by preserved and preservative-free eyedrops



* $p < 0,001$

Figure 2

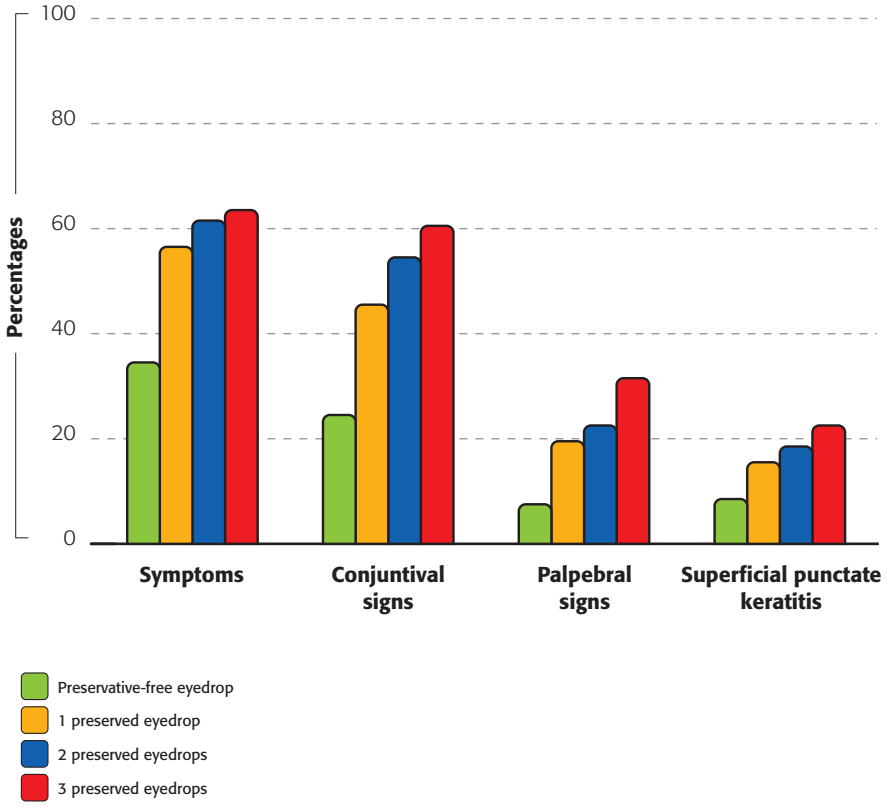
Prevalence of functional signs in glaucoma patients treated by preserved and preservative-free eyedrops



* $p < 0,001$

Figure 3

Prevalence of signs and symptoms according to the number of preserved eye drop solutions received by the patients



1.2- Cytology and conjunctival inflammation

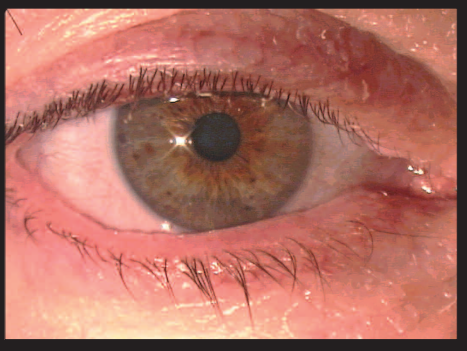
Pisella and coll. [31] showed that preservative-free eye-drops were much better tolerated by the conjunctiva in cytologic terms. In patients treated for chronic open angle glaucoma for at least one year, the expression of inflammatory markers (HLA-DR and ICAM-1 membrane antigens) was significantly higher in patients treated by preserved timolol than in those receiving preservative-free timolol (Table 1).
In parallel, mucus cell markers (MUC5AC) were less well expressed in patients receiving preserved eye-drops. This suggests a significant loss of mucus cells in this group.

Table 1

	Preserved Timolol (n = 15)	Preservative-free Timolol (n = 17)
HLA-DR		
% of positive cells	35,7 ± 24,6	7,4 ± 6.4*
ABC (average ± standard deviation)	62,541 ± 75,720	6,788 ± 12,542*
ICAM-1		
% of positive cells	23,9 ± 18,8	5,8 ± 4,2*
ABC (average ± standard deviation)	33,524 ± 37,676	5,559 ± 8,236*
MUC5AC		
% of positive cells	2,8 ± 1,4	11,9 ± 12*
ABC (average ± standard deviation)	76,620 ± 41049	289,908 ± 183,759*

*ABC : antibody binding capacity (fluorescence intensity unit); * p<0,02.*
According to Pisella and coll. [31]

Expression of inflammatory cell and mucus cell markers on the conjunctiva of glaucomatous patients treated by preserved or preservative-free timolol (flow cytometry analysis)



Allergic blepharitis

In sicca syndrome, Albietz and coll. [1] demonstrated a significant increase in the nucleocytoplasmic ($p = 0.011$) and HLA-DR and CD23 membrane antigen expression ratio (respectively, $p = 0.0001$ and $p = 0.0001$), with a significant decrease ($p = 0.0001$) in mucus cells in 57 patients untreated for three months, compared to healthy control subjects ($n = 21$). In 30 other patients receiving preservative-free treatment, HLA-DR and CD23 antigen expression was significantly less marked (respectively, $p = 0.0026$ and $p = 0.0001$) and mucus cell density was shown to have increased ($p = 0.0003$) compared to patients receiving preserved treatment ($N = 47$).
The extent of inflammation of the conjunctival epithelium is therefore less in patients treated by preservative-free eyedrops than in patients receiving preserved eyedrops. The integrity of the ocular surface and in particular the mucus cells, is also more effectively protected.

Points to remember:

In histopathological terms, the preserved eyedrops may generate subclinical inflammation of the conjunctiva or lead this to persist. This is characterised by infiltration of inflammatory cells, epithelial hyperplasia and a loss in mucus cells.

Preservative-free eyedrops protect the integrity of the ocular surface.

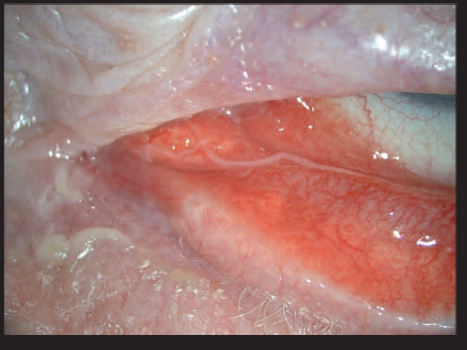
1.3- Allergic reactions

The symptoms of conjunctival allergy (congestion, tearing, photophobia, burning and stinging sensations) induced by the instillation of preserved eyedrops are various. Simple conjunctival congestion or papillary conjunctivitis may be observed with or without eczema, the worst reaction being patent giant papillary conjunctivitis. In certain cases, a severe allergic reaction of type IV may develop. Switching to preservative-free eyedrops is often curative, thus suggesting involvement of preservatives [7].

Among preservatives, mercurial derivatives are the most allergenic and may frequently lead to delayed hypersensitivity of type IV, thus limiting their use. Mondino and coll. [28] describe three cases of hypersensitivity to mercurial derivatives, responsible for conjunctival congestion and corneal infiltrates in contact lens wearers using a solution containing thiomersal as a disinfectant. The presence of a cell infiltrate containing neutrophilic leukocytes, some mononuclear cells and rare eosinophilic leukocytes was detected by corneal scraping. Cutaneous tests clearly revealed delayed hypersensitivity reactions to thiomersal. The symptoms were resolved by removal of the contact lenses for a few days and by storing the lenses in a saline solution and then subjecting them to heat-disinfection.

Three cases of hypersensitivity to benzalkonium chloride confirmed by allergy tests were also reported. Among the cases described, that of a women presenting with a family history of allergy to quaternary ammonium compounds and having suffered immediate hypersensitivity confronting her with vital risk involving chemosis and angioneurotic oedema [11]. Chronic allergy may also develop in contact lens wearers with conjunctivitis treated by eyedrops containing benzalkonium chloride [20]. The development of pronounced superficial keratitis, leads to loss of visual acuity. In certain conditions, neovascularisation of the cornea accompanies the development of giant papillary conjunctivitis.

Non-specific chronic conjunctivitis



Removal of contact lenses induces disappearance of the symptoms.

Roth [35] reported the results of a retrospective study on 320 patients with giant papillary conjunctivitis after wearing hard or soft contact lenses. Many of the patients had a history of allergy or immunological disorder and dry eye syndrome. The subjective disorders begin in both eyes with a feeling of burning, foreign body sensation, itching and a feeling of dryness. Visual acuity declines over the day and photophobia appears. On waking the conjunctiva are congested and the eyelids stuck together by the secretions. The symptoms subside completely following removal of the contact lenses and instillation of preservative-free cromoglycate eyedrops.

In any case, it is recommended using preservative-free eyedrops to avoid treatment-induced complications, to treat associated pathologies (notably eye dryness) and any irritant factors by [17].

Points to remember:

Preservatives may be allergenic and are sometimes the cause of severe conjunctivitis notably in contact lens wearers.

They may also induce inflammatory reactions and eye dryness through lachrymal instability in the treatment of allergic conjunctivitis or keratoconjunctivitis.

Preservative-free eyedrops should be preferred for treating these pathologies.

2. Switching studies

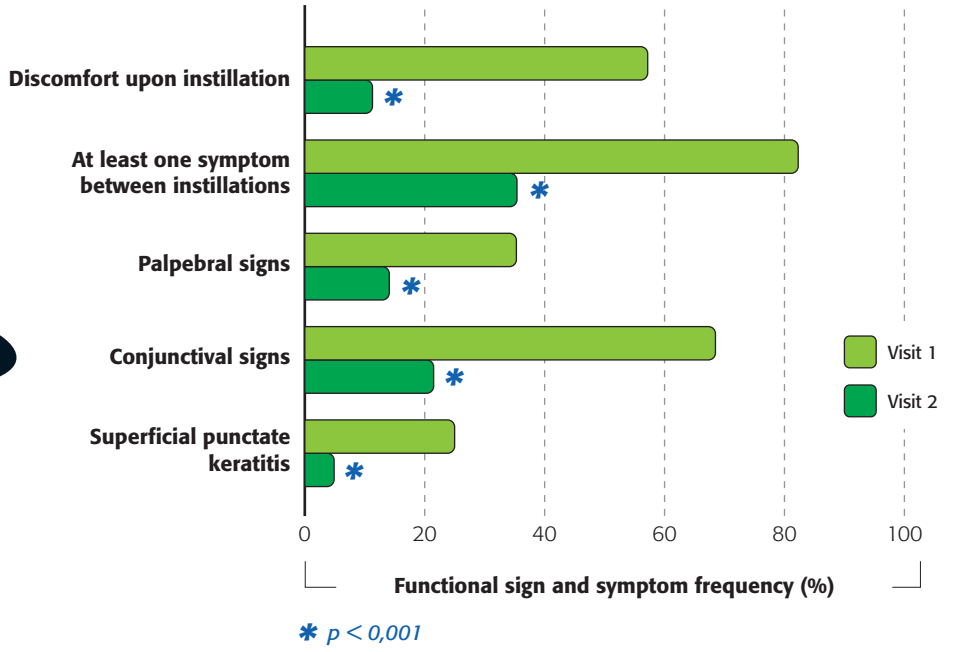
Cross-sectional study observations have been confirmed by switching studies. In patients treated by poorly-tolerated preserved eyedrops, presenting with impairment of the ocular surface (functional symptoms, conjunctival, corneal or palpebral signs) or dry eye, changing to preservative-free eyedrops leads to a rapid improvement in the ocular symptoms [8, 9, 13, 23, 32] and/or tear film [6, 9, 22, 24]. Such improvement does not come at the expense of efficacy [8].

2.1- Improvement of clinical signs and symptoms

Changing to a preservative-free solution reduces the signs and symptoms by a factor of 3 to 4 [23, 32].

In the Pisella and coll. [32] study, conducted in private practices on patients treated for chronic open-angle glaucoma, ophthalmologists prescribe preservative-free eyedrops to 349 patients presenting with the signs and symptoms of ocular impairment and previously treated by preserved eyedrops. After four months' monitoring, a sizeable and significant decrease ($p < 0.001$) of all functional signs and symptoms was observed (Figure 4). A less marked improvement was also obtained in approximately fifty patients that had reduced the number of preserved eye drop solutions used with respect to the first visit (Figure 5). However, the patients having continued their previous treatment, be it preserved (374 patients) or preservative-free (176 patients), did not show any improvement or worsening of the functional signs and symptoms.

Figure 4



Prevalence of functional signs and symptoms upon enrolment (visit 1) and 3 months after switching to preservative-free eyedrops (visit 2) in glaucomatous patients [32].

Bron and coll. [8] report similar results in another multicentre, prospective, open-label study conducted on 435 patients with open angle glaucoma or ocular hypertension, by replacing a preserved solution by a preservative-free solution of the same type (timolol).

After three months, changing to preservative-free eyedrops leads to a notable improvement in local tolerance, while maintaining good pressure balance. On instillation, irritation, dry eye or foreign body sensations and blurred vision or stuck eyelids are diminished. Between instillations, the dry eye or foreign body sensations are reduced by half (15.4% vs. 8.0%). The percentage of conjunctival congestion dropped from 24.4% to 14.6% (p = 0.0002). Folliculopapillary and superficial punctate keratitis rates were also reduced by half.

The improvements described above are related to a significant decrease in the cytologic and inflammatory processes of the conjunctiva and the cornea. In a single blind study, Campagna and coll. [9] analysed the cytologic impression of 20 glaucoma patients before and after three months' use of preservative-free timolol to replace preserved timolol. **Switching to a preservative-free solution led to a significant increase in mucus cells and to a significant improvement in conjunctival epithelial cell impairment** (rose Bengal staining). The subjective symptoms (stinging, foreign body sensation) present on enrolment had

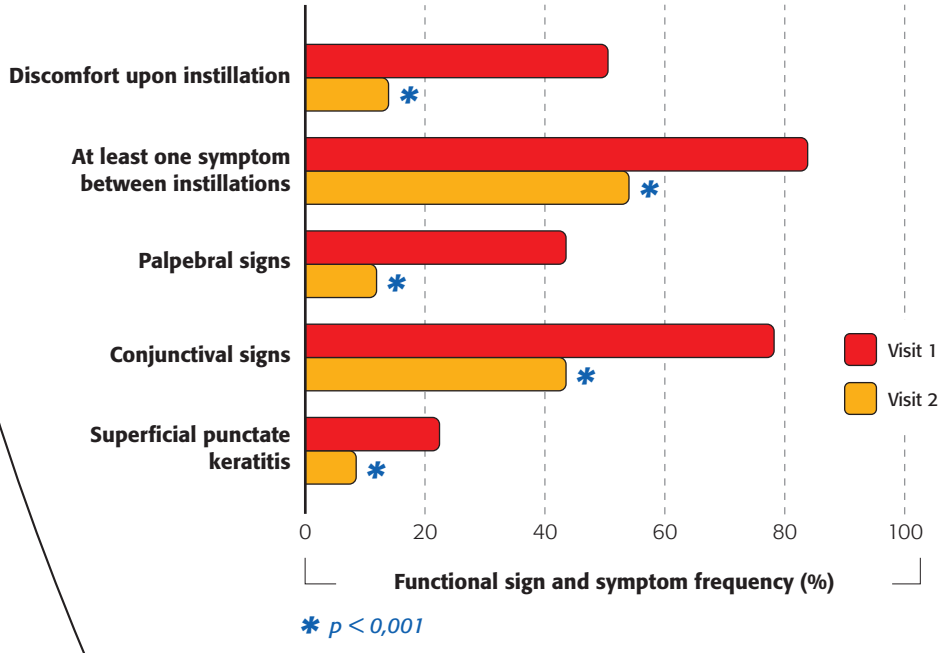
also diminished. These improvements were significant as of the second month of treatment. Intraocular pressure control was also maintained during the change of treatment.

In another study of 21 patients treated by preserved timolol (benzalkonium chloride), two weeks' treatment by preservative-free timolol resulted in partial normalisation of corneal permeability as measured by fluorophotometry (increase of +27%, $p = 0.025$) [13]. In parallel, improvement or disappearance of symptoms was obtained in eight out of ten patients complaining of a burning or dry eye sensation.

A recent international (France, Portugal, Italy, Belgium), epidemiological, multi-centre cross-over study [19] carried out by private practice ophthalmologists included 9,658 patients with open angle glaucoma or ocular hypertension treated by preserved or preservative-free beta blockers between December 2003 and June 2007.
Among the patients enrolled, 74% received preserved eyedrops, 12% preservative-free eyedrops and 10% a combination of preserved eyedrops and preservative-free eyedrops Four percent did not know which treatment they were taking.

Figure 5

Prevalence of functional signs and symptoms upon enrolment (visit 1) and 3 months after reduction of number of preserved eye drop solutions (visit 2) in glaucomatous patients [32].

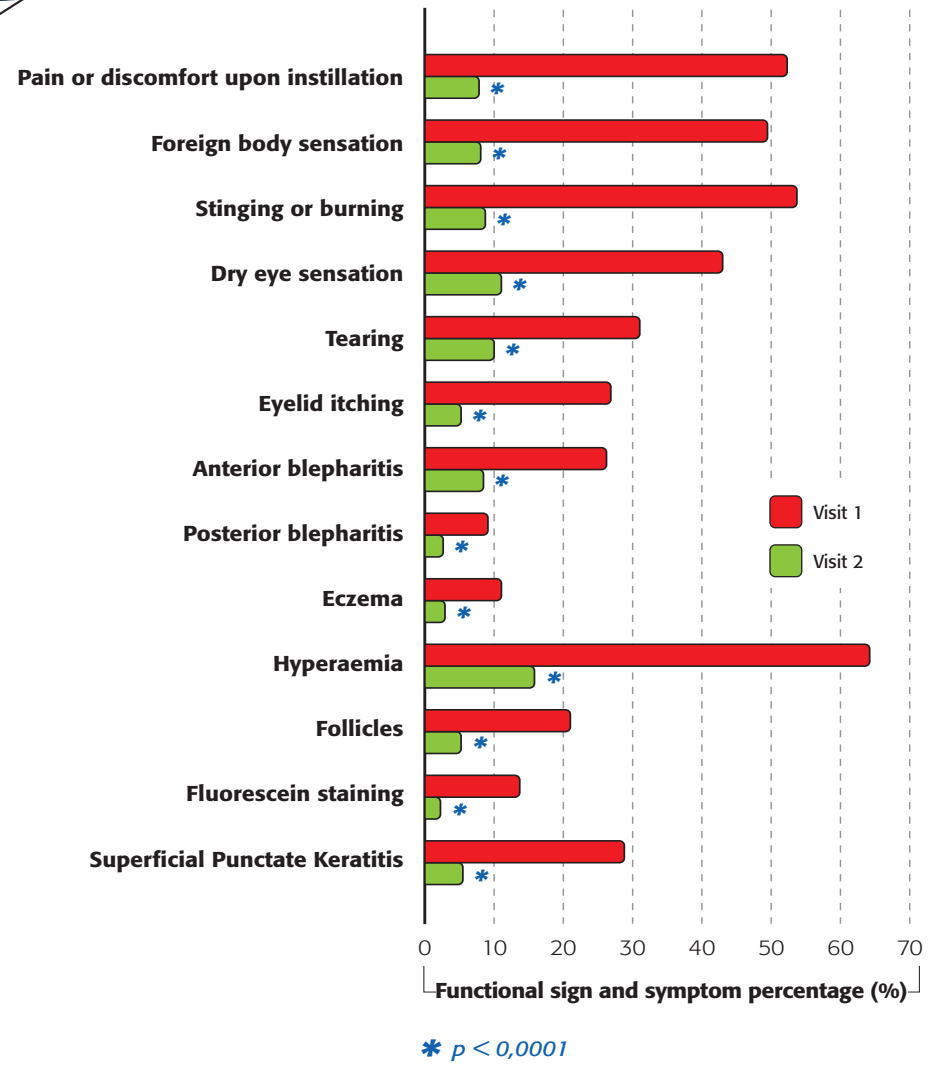


Analysis of the symptoms and signs shows that the latter are significantly more frequent in the patients receiving preserved eyedrops ($p < 0.0001$) than in those receiving preservative-free eyedrops. The results respectively show: pain or discomfort on instillation (42 vs. 19%), foreign body sensation (48 vs. 15 %), stinging or burning sensation (48 vs. 20%) and dry eye sensation (35 vs. 16%).

In a second visit scheduled for 6,088 patients for whom the number of preserved solutions was reduced or replaced by preservative-free eyedrops, the authors observed a significant decrease in all signs and symptoms (Figures 4, 5 and 6).

Figure 6

Prevalence of functional signs and symptoms at visit 1 and visit 2 following reduction of the number of preserved solutions or replacement by preservative-free solutions.



* $p < 0,0001$

Points to remember:

Replacement of a poorly-tolerated preserved solution by a preservative-free solution leads to a rapid improvement of all ocular signs and symptoms.

This is observed in cytologic terms by an increase in mucus cells and normalisation of the changes in conjunctival epithelial cells.

2.2- Improved lachrymal function

Switching preserved eyedrops by preservative-free eyedrops leads to a notable improvement in lachrymal function. This was verified in patients treated by preserved eyedrops for open angle glaucoma or ocular hypertension.

Changing to a preservative-free solution for two weeks led to a significant improvement in lachrymal secretion, highlighted by an increase in turnover as evaluated by fluorophotometry (average increase of +28%, $p = 0.04$) [21]. Campagna and coll. [9] report a significant improvement ($p < 0.01$) in tear film break-up-time (BUT), rising from 7.9 seconds before substitution to 9.1 seconds and 9.3 seconds respectively after two months and three months.

In non-controlled dry eye syndrome or where preserved artificial tears are poorly tolerated, changing to preservative-free artificial tears over a period of several weeks 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 mucus and better acceptance by patients [22, 24]. Brewitt [6] observes a significant improvement in the BUT as of the 2nd week (9.5 seconds), then in the 4th week (12.3 seconds) and in the 12th week (13.3 seconds) in patients with a significantly reduced BUT prior to substitution.

Points to remember:

Replacement of preserved eyedrops by preservative-free eyedrops leads to a notable improvement in lachrymal function: increase in the number of mucus cells and tear film break-up-time recovery.

3. Controlled studies

A number of controlled studies on healthy volunteers and patients proved that preservative-free eyedrops were better tolerated by the corneoconjunctival surface than preserved eyedrops. **Preservative-free solutions are less cytotoxic with respect to the corneal epithelium, less harmful to the tear film and significantly reduce the symptoms reported by patients, bringing increased comfort of use.**

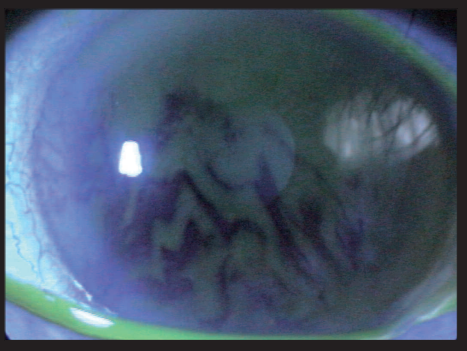
3.1- Healthy volunteers

Tolerance to preservative-free eyedrops was made the subject of a short-term evaluation (single or repeated instillations over a few days) and a long-term study (continued instillation over several weeks) of volunteers. These studies confirm that preservative-free eyedrops are better tolerated by the ocular surface [29, 33] and more effectively protect the tear film [2, 3, and 39] compared to preserved eyedrops.

Short-term single or repeated instillations

A double-blind, intra-individual, randomised controlled study evaluated the tolerance of two ocular lubricants used in a gonioscopic examination: preserved hydroxymethylcellulose (HMC) (0.01% benzalkonium chloride) and preservative-free carboxymethylcellulose (CMC) [30]. Both products were instilled in the eyes of 55 healthy volunteers (one product per randomised eye). The corneal epithelium was less affected by the preservative-free lubricant ($p<0.00005$). In total, 36.4% of eyes having received the preservative-free lubricant presented an epithelial score of ≥ 1 (at least one individual case of punctuate stippling on one area of the epithelium) versus 92.7% of eyes having received the preserved lubricant.

The preservative-free lubricant provided enhanced comfort, the subjects complaining less often of stinging or burning sensations, compared to the preserved lubricant.



Diminution of Break-up-time (BUT)

Similar results were produced in 12 healthy subjects without any history of eye disorders. Ramselaar and coll. [33] compared the effects of instillation of two local anaesthetics on the cornea (oxybuprocaine, tetracaine hydrochloride) with or without a preservative (chlorhexidine, benzalkonium chloride).

Following instillation of the anaesthetics (one drop five times at 2 minutes' interval in the conjunctival cul-de-sac of one of the two eyes, the contralateral eye receiving a control solution), corneal permeability was measured by fluorophotometry for one hour. The eyes having received the preserved anaesthetics showed increased corneal permeability ($p<0.05$), the most toxic effect being obtained with the benzalkonium chloride-preserved anaesthetics ($p<0.005$). The preservative-free anaesthetics however had a less serious effect on corneal permeability.

In another study conducted on 240 healthy volunteers (480 eyes) without ocular surface impairment, Avisar and coll. [2] analysed the effects of instillation of eight tear substitutes on the stability of the precorneal tear film (seven preserved substitutes and one preservative-free substitute). The results shown in Figure 7 show that the preservative-free artificial tear solution produces the lowest reduction in BUT, with respect to the base value measured prior to instillation.

Average value (± standard deviation) of the tear film break-up-time (BUT) 20 minutes after instillation of the preserved (+) or preservative-free (-) lubricant compared to the base value in healthy volunteers

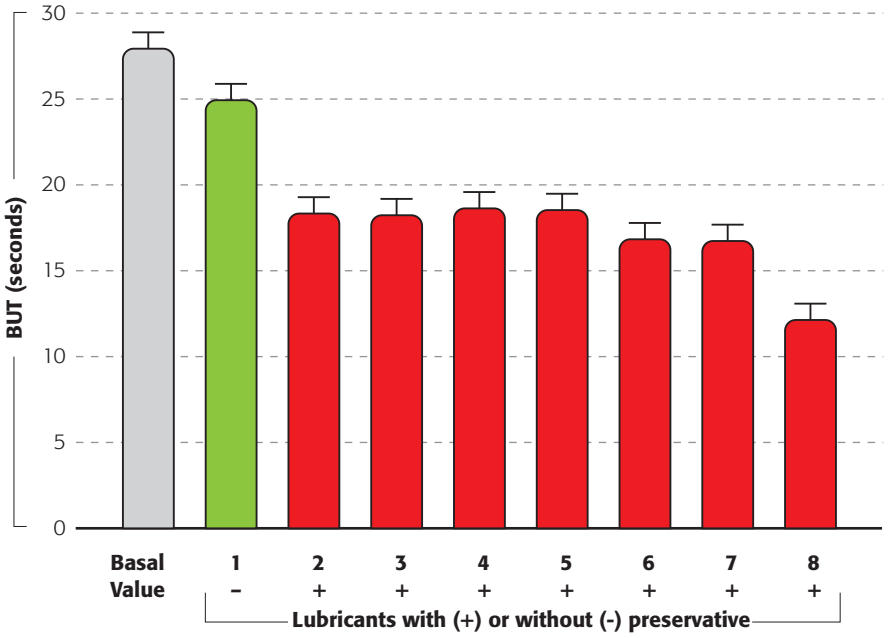


Figure 7

In a randomised, double-blind, phase 1 crossover clinical trial including 30 healthy volunteers without ocular impairment and not receiving any eye treatment, Baudouin and coll. [3, 4] compared the short-term tolerance of instillation of two 2 % carteolol formulas with or without a preservative (two drops per day for three days). Instillation of the first drop was very well tolerated by the two treatment groups. Moderate conjunctival congestion was observed however and was significantly more marked in the benzalkonium group than in the preservative-free group ($p<0.001$). The subjects in the benzalkonium group also showed a decrease in BUT after three hours of - 4.6 seconds, a statistically significant difference compared to the preservative-free group (-1.15 seconds, $p = 0.04$) (Figure 8).

After repeated instillation over a three-day period, a tendency ($p = 0.068$) to a more marked decrease in BUT was observed in the preserved carteolol group (-2.69 seconds) compared to the preservative-free carteolol group (-0.73 seconds).

Tear film break-up-time before and after a single instillation of preserved carteolol (benzalkonium chloride) or preservative-free carteolol in 30 healthy volunteers

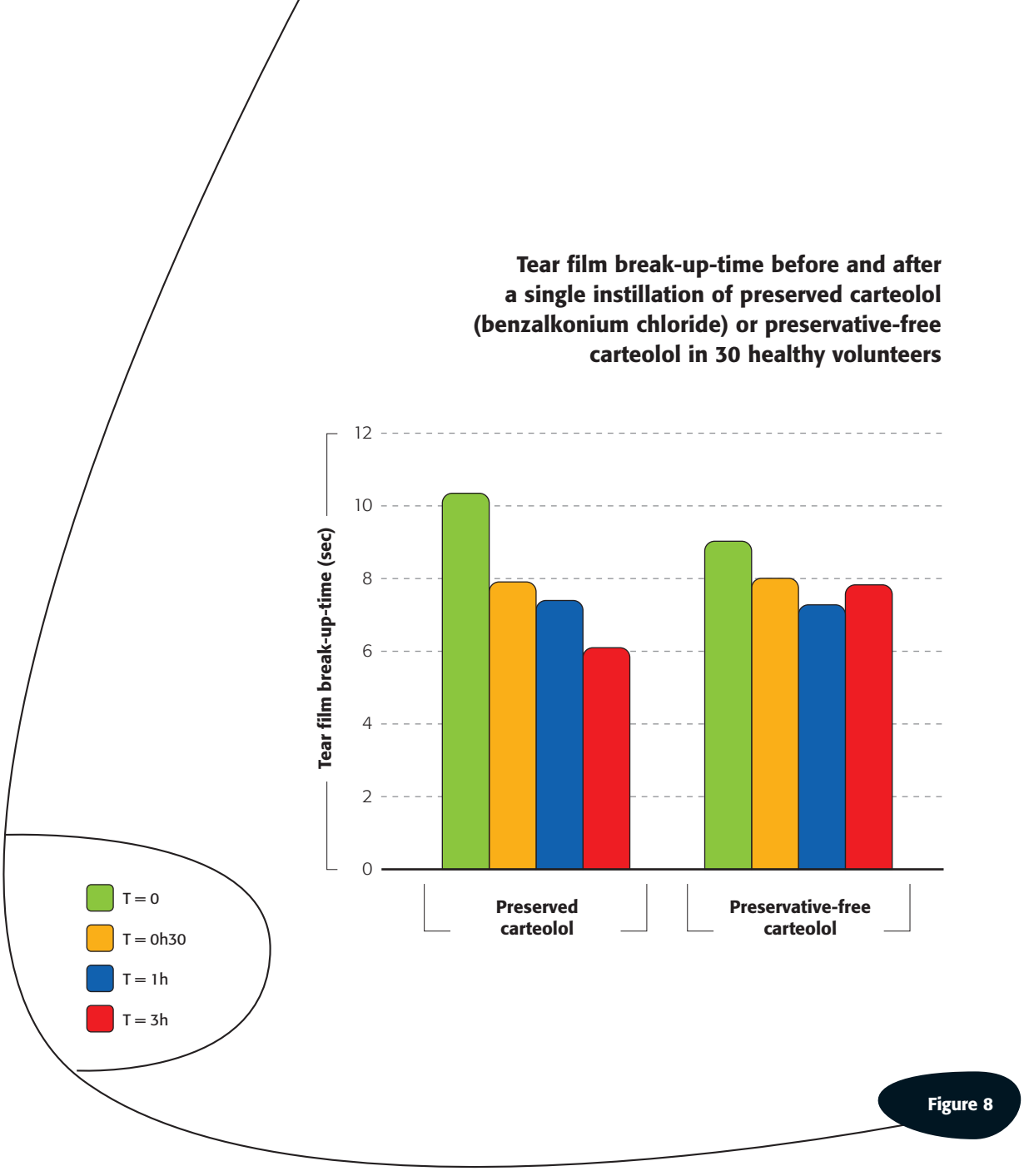
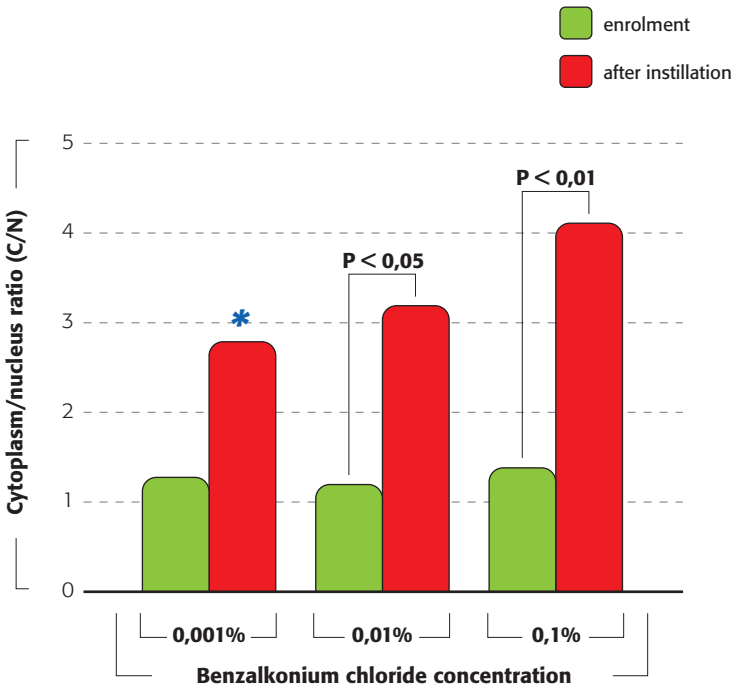


Figure 8

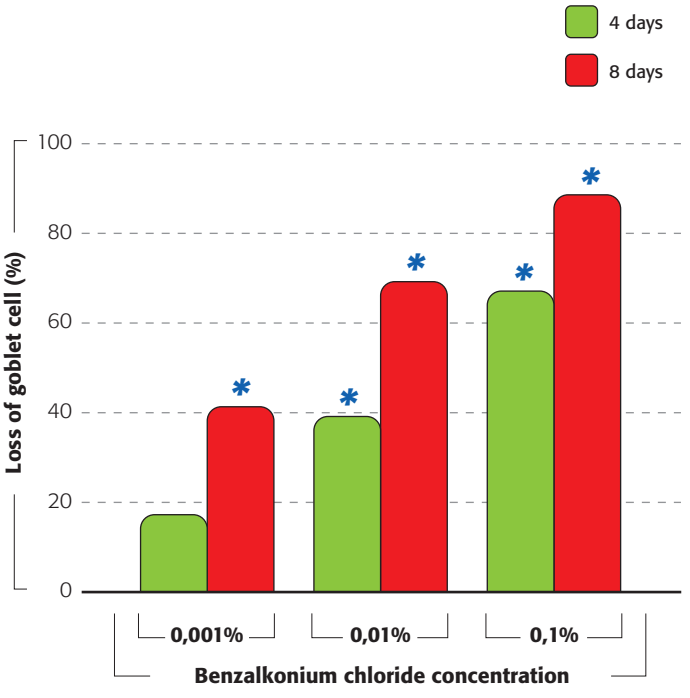
In a double-blind prospective study of three parallel groups [34], administration (eight times per day for seven days) of a lachrymal gel containing various concentrations of benzalkonium chloride (0.1 %, 0.01 % and 0.001 %) produced a significant increase in the degree of cell metaplasia (Figure 9) and a significant reduction in mucus cell density (Figure 10), in particular for the highest concentration (0.1 %), compared to administration of a preservative-free lachrymal gel in the fellow eye. This controlled study in healthy volunteers confirms the toxicity of benzalkonium chloride on the cytologic aspect.

Figure 9



* *Not statistically significant.*

Epithelial metaplasia of the temporal conjunctiva on enrolment and following instillation of various concentrations of benzalkonium chloride over 8 days in 21 healthy volunteers



* *p < 0,05 compared to control eyes treated with preservative-free tear-gel*

Reduction in mucus cell density in the temporal conjunctiva induced by instillation of various concentrations of benzalkonium chloride over 4 days and 8 days in 21 healthy volunteers

Figure 10

In order to evaluate the effects of 0.5% timolol with or without preservatives on tear film stability, a randomised, comparative clinical study was conducted on 20 healthy volunteers [18]. The preservative-free timolol was instilled in the right eye and the preserved timolol in the left eye. The tear film stability index was evaluated before instillation then 30 minutes after instillation. The results revealed a significant decrease in BUT in the eye treated by the preserved timolol. The permeability of the corneal epithelial barrier as measured by fluorophotometry significantly increased following instillation of the two products but was more marked in the group treated by the preserved timolol (Figure 11).

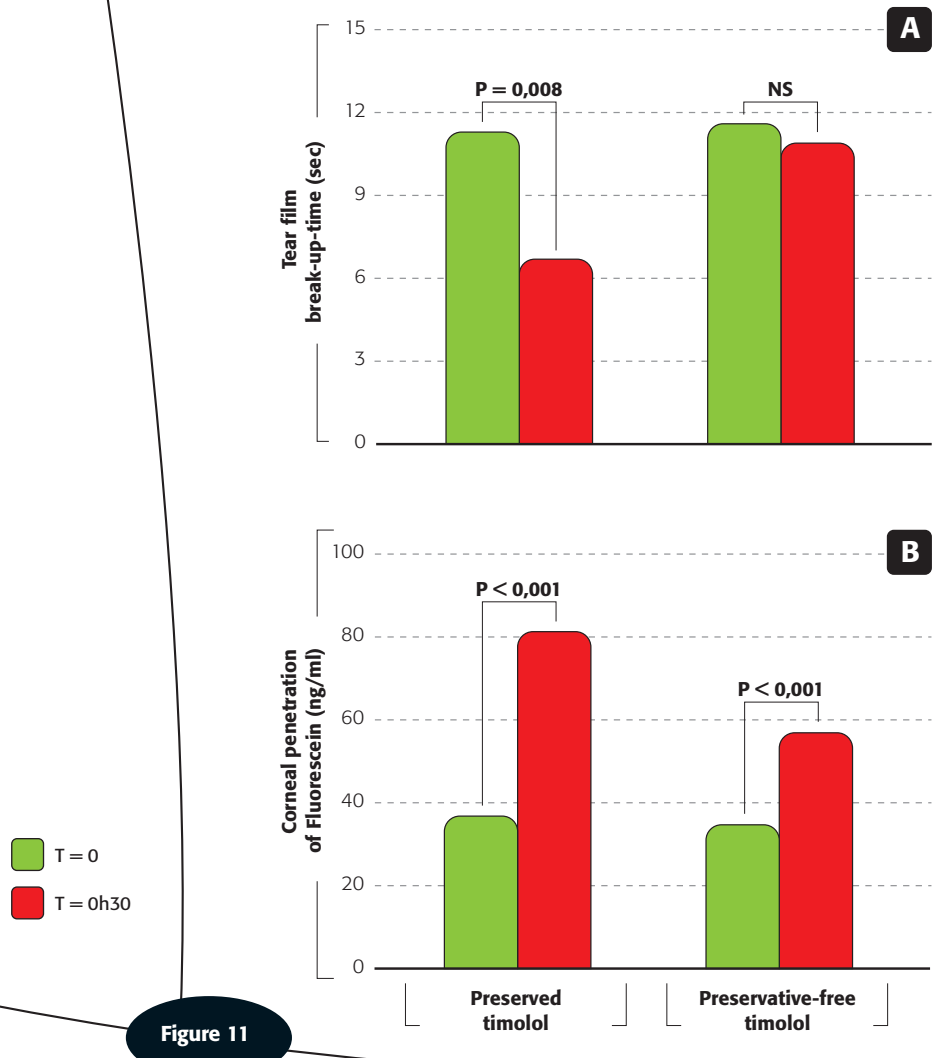


Figure 11

Tear film break-up-time (A) and corneal permeability (B) before (T0) and 30 minutes (T30) after instillation of the preserved or preservative-free timolol in 20 healthy volunteers

Points to remember:

Controlled clinical tolerance studies conducted in healthy volunteers without ocular impairment proved that preservative-free eyedrops were better tolerated than preserved eyedrops.

Preservative-free eyedrops more effectively preserve the tear film and corneal permeability in particular.

Repeated instillations over several weeks

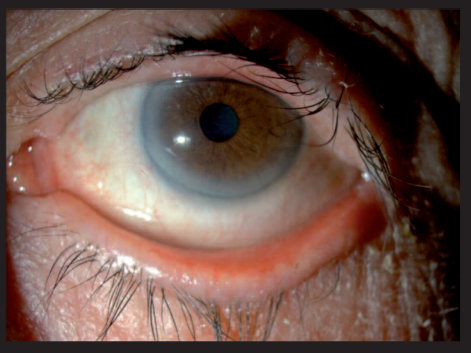
The good tolerance of preservative-free solutions was also verified in prospective studies conducted over several weeks. A phase 1 [37], randomised, double-blind, intra-individual study of healthy volunteers compared the ocular tolerance of a vitamin B12, preservative-free solution to a preserved vitamin B12 solution (benzododecinium bromide). The tolerance of the preservative-free solution was slightly higher (Table 2): in particular, evolution of discomfort upon instillation between D0 and D28, the appearance of functional symptoms (irritation, burning and/or stinging) and the anomalies observed with the slit-lamp, were more favourable in the preservative-free group. A decrease in the total lissamine green test score was observed in the preservative-free vitamin B12 group and a slight increase in the preserved vitamin B12 group.

Table 2

Tolerance of a preservative-free vitamin B12 solution compared to the preserved vitamin B12 solution in 30 healthy volunteers

	Preserved Vitamin B12	Preservative-free Vitamin B12	P
Evolution of discomfort on instillation according to the visual scale (VAS) (mm)	+3,0 ± 13,1	-1,1 ± 5,9	0,047*
Number of eyes having presented at least one functional symptom between D0 and D28	9/60 (15 %)	3/60 (5 %)	0,054**
Number of eyes having presented at least one functional symptom between D0 and D14	9/60 (15 %)	2/60 (3 %)	0,020**
Number of subjects having presented at least one ocular anomaly between D0 and D28	18/30 (60 %)	10/30 (20 %)	0,048**
Evolution of lissamine green test score between D0 and D28	+0,1 ± 0,9	-0,4 ± 0,9	0,051***

** paired series student test, ** Cochran-Mantel-Haenzel test, *** Student test. According to [37]*



Drug allergy

A study [12] comparing the ocular tolerance of preservative-free diclofenac versus preserved diclofenac (thiomersal) was conducted in 40 healthy volunteers. The treatment was instilled in an identical manner over a 28-day period (5 drops per day during 7 days, then 3 drops per day during 20 days). The results revealed better tolerance to the preservative-free diclofenac. In particular, the overall symptom score per subject was statistically improved on day 7 (D7) in the preservative-free diclofenac group (Table 3). This difference was also clinically significant after 4 weeks' treatment. The biomicroscopical examination showed better tolerance of the ocular surface in the preservative-free diclofenac group. Cases of folliculopapillary conjunctivitis were more numerous and of more severe intensity in the preserved diclofenac group ($p = 0.031$ on D14). Tolerance of the ocular surface, highlighted by the lissamine green test, was better in the preservative-free diclofenac group. In the same way and concerning corneal symptoms, after the initial dose on D7 only one subject in the preservative-free diclofenac group presented moderate superficial keratitis punctata compared to 5 subjects in the preserved diclofenac group. The results suggest that the preservative is greatly responsible for the toxic keratoconjunctivitis cases observed.

Tolerance of a preservative-free diclofenac solution compared to a preserved diclofenac solution in 40 healthy volunteers

	Preserved diclofenac	Preservative-free diclofenac	P*
Overall symptom score on Day 7	0,85 ± 1,14	0,45 ± 0,71	0,022
Overall symptom score on Day 28	0,35 ± 0,07	0,20 ± 0,46	0,063
Evolution of the lissamine green test score between D0 and D28	+1,88	+1,00	0,001

** Wilcoxon test, according to [12]*

Table 3

Points to remember:

**In healthy volunteers
without ocular impairment,
repeated instillation over several weeks
of preservative-free solutions
is better accepted and tolerated
compared to preserved solutions
which cause conjunctival
and corneal impairment more frequently.**

3.2- Patients

The success of ocular pathology treatment or of surgery depends on effective corneo-conjunctival tolerance and on the integrity of the epithelium and its permeability. Controlled studies made it possible to verify that preservative-free solutions are less cytotoxic for the corneoconjunctival epithelium and that they more effectively preserve tear film qualityal.

Ocular Hypertension and/or glaucoma

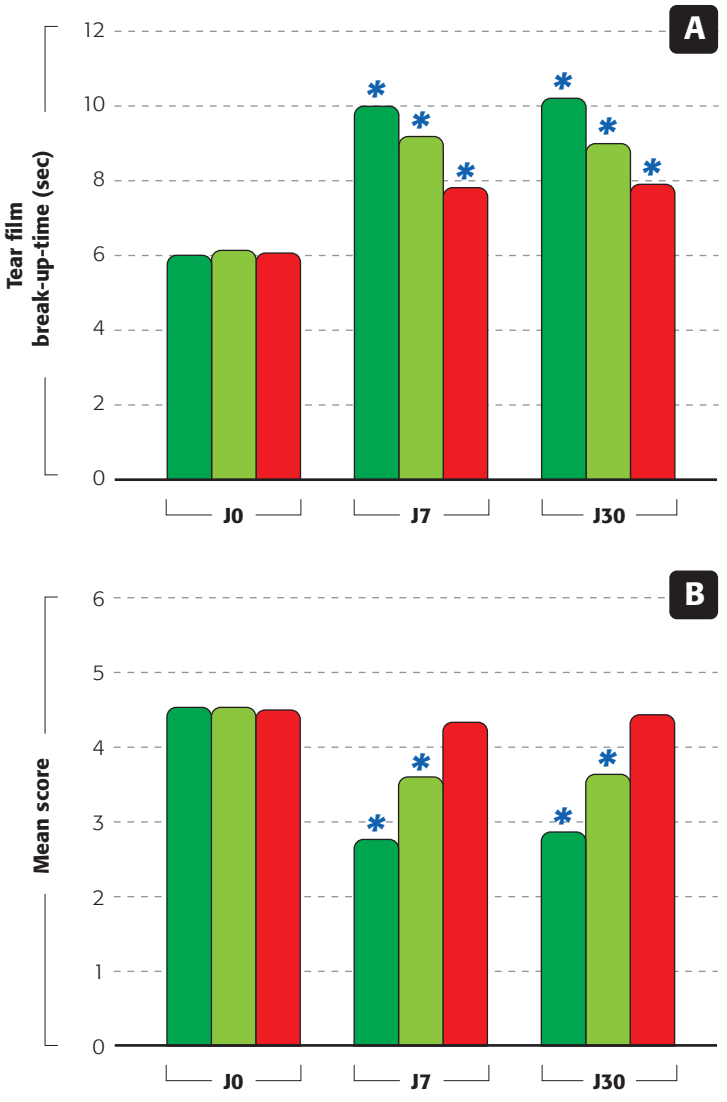
In patients with open-angle glaucoma [38], instillation of various preservative-free preparations of 0.5 % timolol over thirty days (timolol in solution in hyaluronic acid or aqueous solution) or preserved preparations (carbopol solution) resulted in a significant increase of BUT in all three treatment groups with a higher increase in the preservative-free groups (Figure 12A). A significant decrease in rose Bengal staining (Figure 12B) was seen in patients receiving the preservative-free eyedrops ($p < 0.001$), but not in patients receiving the preserved eye-drops. This suggests less epithelial distress in the preservative-free group.

Points to remember:

**In glaucomatous patients,
controlled clinical studies
showed that the preservative-free solutions
more effectively preserve the tear film
and corneal epithelium
compared to preserved solutions.**

Figure 12

Effect of the administration of various preserved or preservative free timolol solutions on tear film break-up-time (A) and the rose Bengal staining test (B) in glaucomatous patients. Randomised, single-blind, controlled study of three parallel groups (16 patients per group)



✱ *Intra group difference, p < 0,05 compared to D0*

Preservative-free timolol/hyaluronic acid
Preservative-free timolol
Preserved timolol/carbopol

Dry eye syndrome

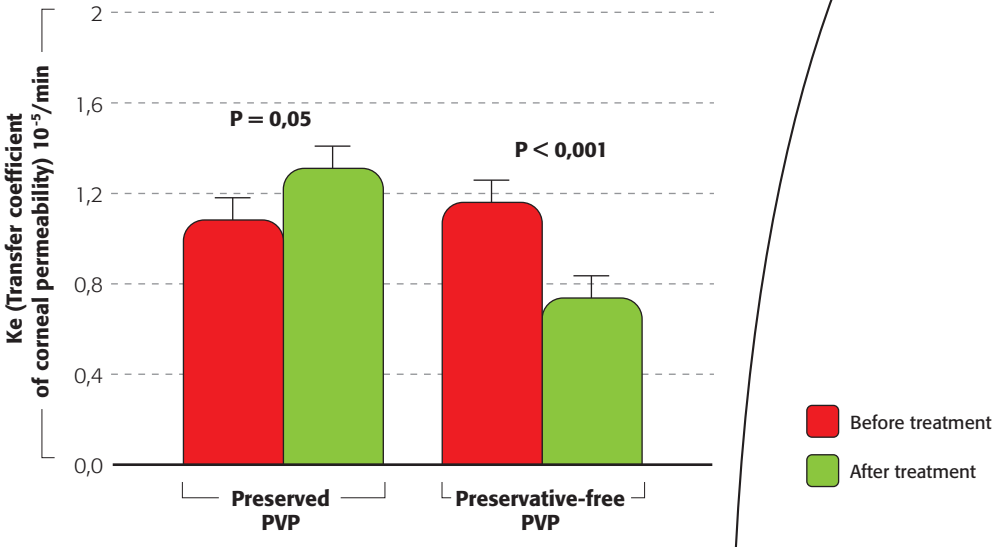
In dry eye syndrome, preservative-free eyedrops more effectively preserve the integrity of the corneal epithelium already in distress.

Forty patients complaining of at least two severe symptoms (burning, pruritis or foreign-body sensation) were enrolled on a controlled and randomised study [14]. On enrolment they presented with moderate metaplasia of the conjunctival epithelium and received, over a six-week period, either a 2% polyvinylpyrrolidone (PVP) solution with preservative (benzalkonium chloride 0.005%), or a preservative free PVP solution in six to nine instillations per day in both eyes.

Fluorophotometry measurements revealed a significant improvement in epithelial permeability post-treatment (reduction of 37%, $p < 0.001$). In comparison, the patients having received the preserved artificial tears showed deterioration of corneal permeability (increase of +21%, $p = 0.05$) (Figure 13). **These results indicate that the preservative-free artificial tears produce an objective improvement of the corneal surface of patients with dry eye syndrome.**

Figure 13

Average corneal permeability (± SD) before and after 6 weeks' treatment with artificial tears containing preserved (40 eyes) or preservative-free PVP (40 eyes)



These results confirm the first observations made by Göbbels and coll. [15] in another controlled study. Patients treated with preservative-free artificial tears showed a significant decrease (-43.4%) in corneal permeability, compared to patients treated by preserved artificial tears (chlorbutanol or 0.005% benzalkonium chloride).

In 56 patients presenting with keratoconjunctivitis sicca [16], instillation of preservative-free carboxymethylcellulose-based (CMC) artificial tears (eight instillations per day over eight weeks) led to a significant improvement in functional symptoms, of superficial punctate keratitis and squamous metaplasia compared to the patients treated by preserved artificial tears (Figure 14).

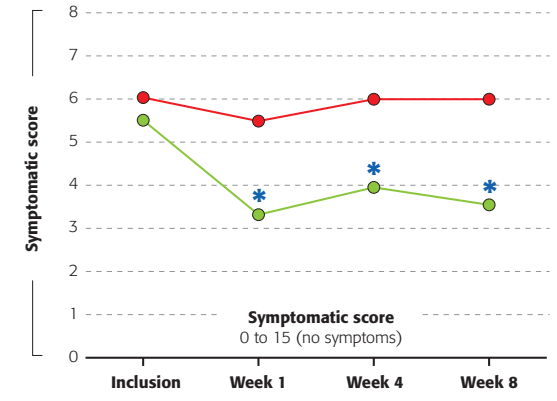
These results are also confirmed by Smith and coll. [36] in a randomised, open-label, controlled intra-individual study. Thirty patients, with dry eye syndrome, ineffectively managed using preserved artificial tears, were asked to instil preservative-free hydroxyethylcellulose drops in one of the two eyes, the fellow eye continuing to receive the eyedrops containing a preservative. After two weeks of treatment, 63% of patients declared that they preferred the preservative-free artificial tears. The eye treated by the preservative-free solution showed a significant decrease in grittiness sensations ($p = 0.007$), dry eye sensations ($p < 0.0001$) and rose Bengal staining ($p = 0.004$). No significant difference with respect to the enrolment visit was demonstrated for the eyes treated by the preserved tears.

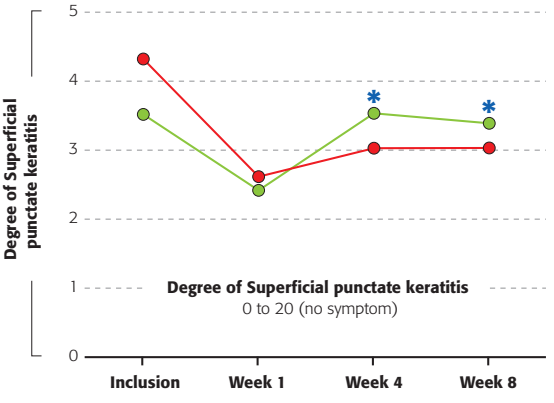
Points to remember:

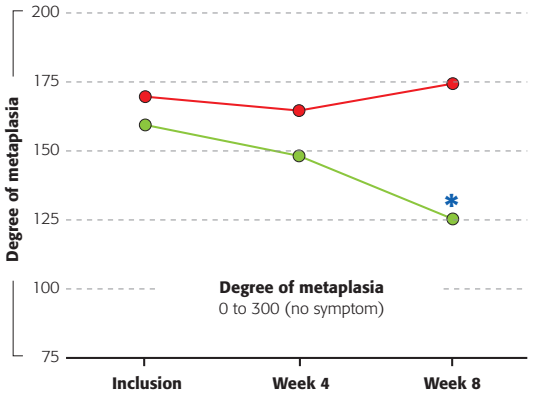
In patients treated for dry eye syndrome, controlled clinical studies showed that artificial tears containing a preservative were less well tolerated than preservative-free tears.
Preservative-free solutions significantly improve ocular symptoms, superficial keratitis punctata and epithelial metaplasia.

Figure 14

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







Preservative-free CMC
Preserved HMC

✱ *Statistically significant difference (CMC vs. HMC)*

Preservatives and cataract surgery

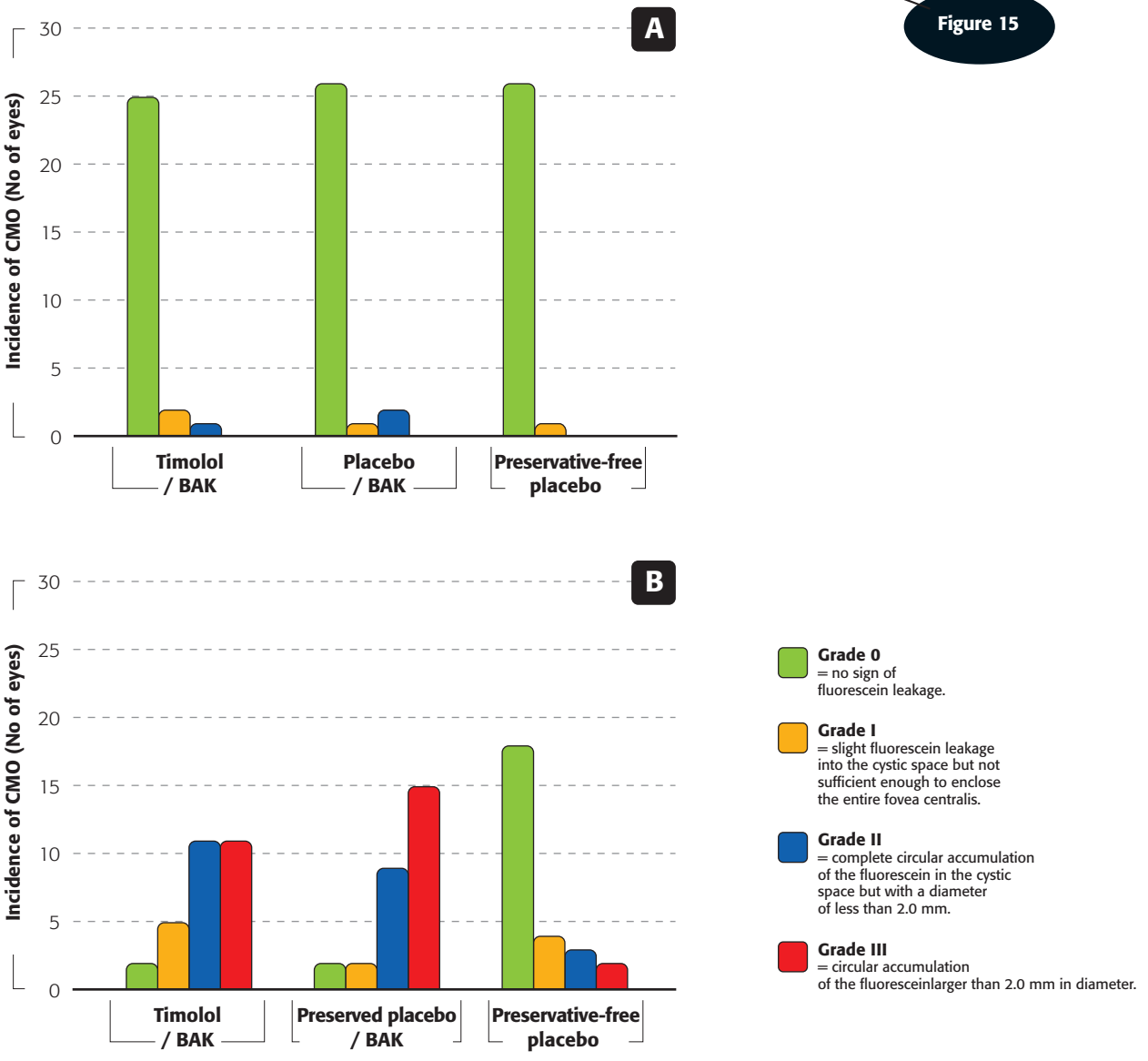
The development of a cystoid macular oedema further to phacoemulsification is always possible. An inflammatory reaction, eventually brought on by the presence of preservatives in post-surgical treatment, increases the risk of rupture of the blood-aqueous barrier induced by surgery [25].
Two placebo-controlled, double-blind randomised studies were conducted in patients presenting with ocular hypertension or glaucoma and having undergone cataract surgery. The aim of these studies was to determine the role of the solution (timolol or latanoprost) and of the preservative (benzalkonium, BAK) in the rupture of the blood-aqueous barrier as measured by laser flare meter and in the development of cystoid macular oedema (CMO) as measured by angiography. Any anti-glaucoma treatment was interrupted three weeks before the cataract surgery. Following the operation, the patients received either anti-glaucoma eyedrops or a placebo containing benzalkonium chloride or a preservative-free placebo. In addition, the patients also received anti-inflammatory treatment in the form of diclofenac drops (chlorobutanol-preserved), or fluorometholone drops (benzalkonium chloride-preserved). The treatment was administered over a five-week period.

In the first study [27], no difference was observed between the timolol/BAK, placebo/BAK, and preservative-free placebo groups in patients receiving diclofenac (Figure 15A). In the patients receiving the fluorometholone, a significant increase in the cystoid macular oedema angiographic score was observed between the timolol/BAK and unpreserved placebo groups and between the placebo/BAK and unpreserved placebo groups (Figure 15B). In the timolol/BAK and placebo/BAK groups, a significant increase in rupture of the blood-aqueous barrier on day 3, week 1 and week 2 is observed following the cataract operation compared to the preservative-free placebo-group (Figure 16).

These results indicate that complications relating to cataract surgery are more frequent when the anti-glaucoma treatment contains benzalkonium chloride. The presence of benzalkonium would appear to encourage rupture of the blood-aqueous barrier following surgery and would appear to increase the risk of cystoid macular oedema after five weeks. The use of a benzalkonium chloride-free non-steroid anti-inflammatory drug (diclofenac) could prevent such complications. Fluorometholone containing benzalkonium chloride would not appear to have this protective action.

Comparison of the prevalence of cystoid macular oedema (CMO) five weeks after cataract surgery in patients treated by timolol or a placebo with/without a preservative, in combination with diclofenac (A) or fluorometholone (B)

Figure 15



Significant difference ($p<0.01$) between the groups timolol/BAK and preservative-free placebo and between the groups placebo/BAK and preservative-free placebo

Evolution of aqueous flare in patients receiving timolol, or a placebo with or without a preservative in combination with fluorometholone

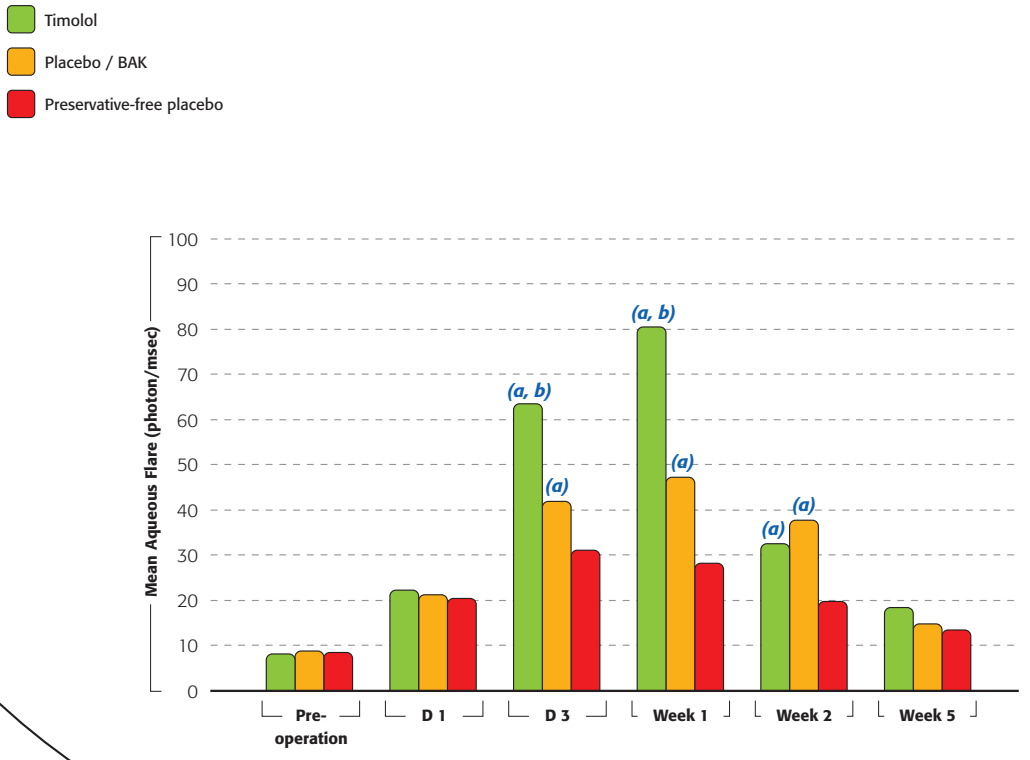
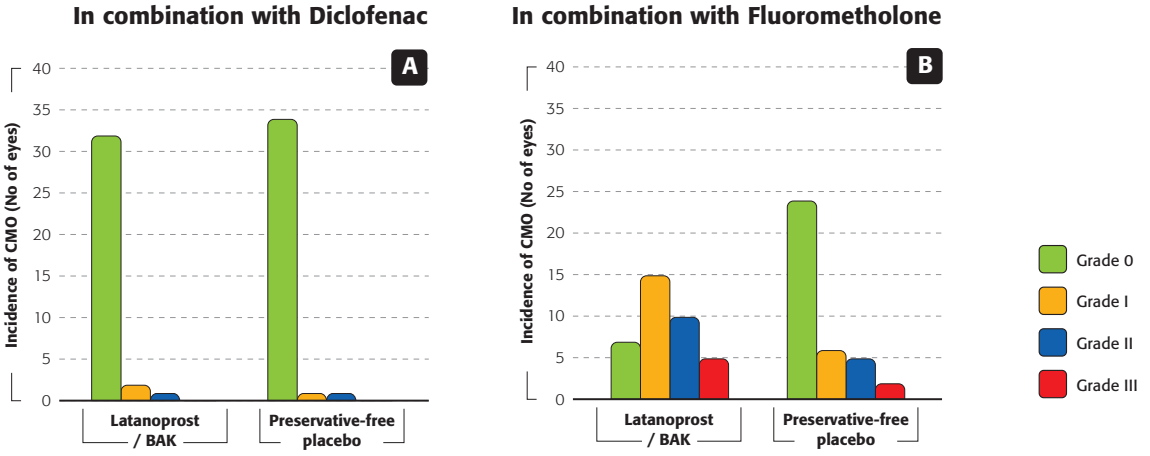


Figure 16

(a) p < 0,01 timolol versus preservative-free placebo
(b) p < 0,01 preserved placebo versus preservative-free placebo

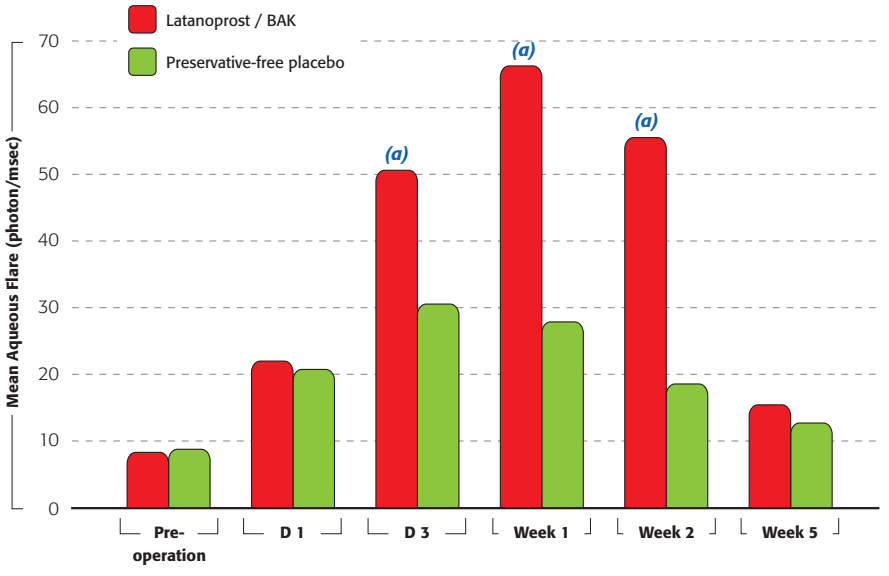
Similar results were obtained with latanoprost [25]. In patients receiving diclofenac drops, cystoid macular oedema angiographic scores were similar in the latanoprost group and in the preservative-free placebo group (Figure 17A). In patients receiving fluorometholone, a significant increase in cystoid macular oedema was observed in the latanoprost/BAK group compared to the preservative-free placebo group (Figure 17B). A significant increase in flare was demonstrated on day 3, in week 1 and in week 2 in the latanoprost/BAK group compared to the preservative-free placebo group (Figure 18).

Comparison of the prevalence of cystoid macular oedema (CMO) five weeks after cataract surgery in patients treated by latanoprost-based eyedrops with/without BAK, in combination with Diclofenac (A) or fluorometholone (B)



Significant difference ($p < 0.01$) between the two groups

Figure 17



(a) *$p < 0,01$ latanoprost versus preservative-free placebo*

Figure 18

Evolution of aqueous flare in patients receiving latanoprost, or a preservative-free placebo in combination with fluorometholone

Points to remember:

Preservatives, by releasing proinflammatory mediators, are the cause of the prevalence of cystoid macular oedema in pseudophakic subjects.

The effect is more significant in patient receiving preserved long-term anti-glaucoma treatment and can be avoided by prescribing preservative-free anti-glaucoma treatment

The combined results of the two studies support the significant contribution of benzalkonium chloride over that of the type of antiglaucoma agent (timolol or latanoprost) in the increased risk of blood-aqueous rupture and cystoid macular oedema.

In a special report published in 2003, Miyake and coll. analysed (among four clinical and laboratory studies) the prevalence of cystoid macular oedema onset immediately after extraction of the cataract [26]. This analysis shows that preservatives, and in particular benzalkonium chloride, would appear to lead to an increase in prostaglandin synthesis and synthesis of other substances and is said to intensify post-operative inflammation. Hence the name "pseudophakic preservative maculopathy".

Conclusion

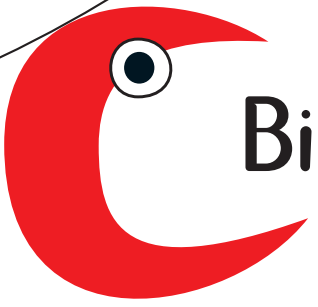
In the past, a certain number of observations suspected preservatives to be the cause of failed trabeculectomy and to be at the origin of certain serious ocular disorders (prolonged superficial keratitis, corneal ulcers, conjunctival scarring, pseudopemphigoid), thus corroborating the first preclinical in vitro studies and and studies conducted in animals.

The trials presented in this brochure, conducted on healthy volunteers, glaucomatous patients, allergic patients or patients with dry eye syndrome show that in the very short-term preservatives lead to significant changes in the structures of the ocular surface and even in deeper tissue, leading to reduced tolerance and a higher prevalence of side effects.

Preservatives, BAK in particular, are not an essential component of the formulation in terms of patient benefit. Preservative-free eyedrops were shown to be equally effective as preserved formulations.

New imaging techniques and future studies now aim to focus on the long-term evaluation of the effects of preservatives on the human eye.

The development of preservative-free solutions already represents real progress and these solutions should be preferred where available.



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