10 years of preservative-free eyedrops

Prof. Christophe Baudouin, Quinze-Vingts National Hospital Centre for Ophthalmology, Paris, France

volume 1

LABORATOIRES Théa

In the last 10 years the use of preservative-free eyedrops has spread throughout the world and Laboratoires Théa were the pioneers in this area.

On the tenth anniversary of Laboratoires Théa, we asked Professor Christophe Baudouin, whose research on the ocular surface is a reference, to make a review of the relevant experimental, clinical and epidemiological work published in the international scientific literature.

It gives me great pleasure to offer you this first volume, 'Grounds for concern', which gives the setting for two further volumes, 'Experimental evidence' and 'Clinical evidence'.

I trust you will find it of interest.

Best regards,

Henri CHIBRET

To be published:

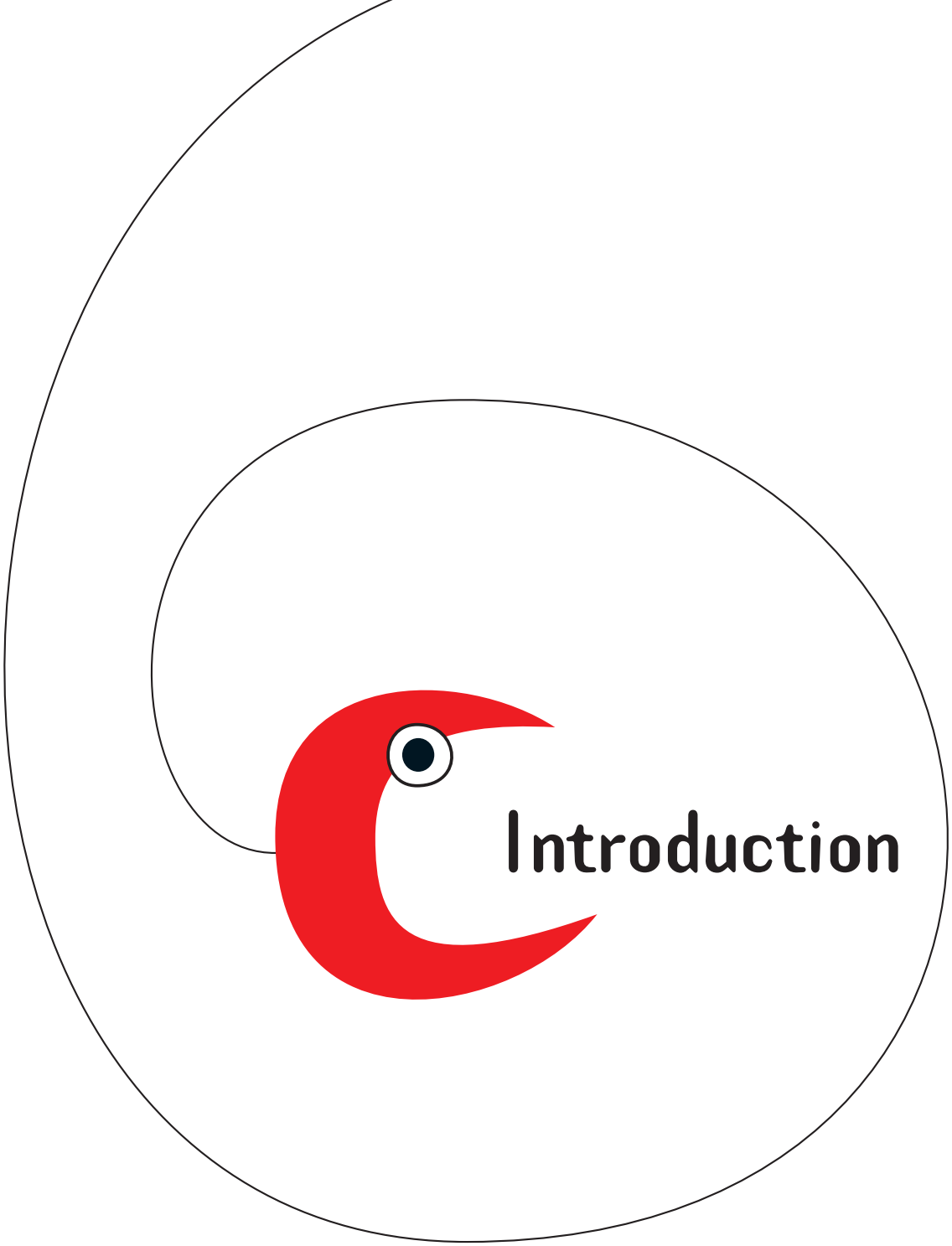
Vol. 2 – Experimental evidence

Vol. 3 – Clinical evidence

10 years of preservative-free eyedrops

Vol. 1 – Grounds for concern

Prof. Christophe Baudouin, Quinze-Vingts National Hospital Centre for Ophthalmology, Paris, France



Introduction

Pharmaceutical regulations require an ophthalmic solution to meet criteria of stability, tolerance, activity and sterility. To meet the sterility condition, multidose eyedrops contain a preservative, which is an antimicrobial agent compatible with the other components of the solution.

Although eyedrops are mostly well tolerated, especially when they are used in short-term treatments, in certain circumstances patients may frequently complain of **stinging, burning, discomfort, irritation or eye dryness**. In some cases, less frequently, conjunctivitis or corneal damage may occur, in particular in long-term treatment or during repeated concurrent use of several eyedrops.

These effects cannot be attributed in every case to the active ingredients present in the eyedrops. The toxicity of the preservatives, however, is well documented, especially by experiments *in vitro* and in animals. Mercury derivatives induce mainly delayed allergic reactions, which restrict their use. Quaternary ammonium salts, in particular benzalkonium chloride, are the most frequently used preservatives. They are also the most toxic for the ocular surface, even at low concentrations. They have detergent properties as quaternary ammonium molecules can be easily incorporated in the membranes of epithelial cells by their lipophilic chains, and create inroads for aqueous or ionic substances into the intracellular space. They can also break up the intercellular junctions, letting through aqueous or ionic substances [17].

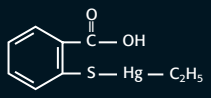
They are responsible for modification of the tear film causing eye dryness and patient discomfort.

In the last few years many observations and clinical studies have been published suggesting that preservatives (primarily benzalkonium chloride) are strongly implicated in the topical toxicity of eyedrops, especially when these are used in long-term treatment.

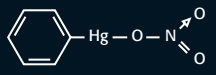
This brochure presents a review of the data on the toxicity of eyedrops containing preservatives and on the role of those preservatives in their toxicity.

Various particularly striking studies that suggest the implication of preservatives in adverse effects on eye structures (conjunctiva, cornea, lens) will first be examined. **The mechanisms involved in this toxicity (immuno-inflammatory response, underlying subepithelial fibrosis and modification of tear film) will also be described.**

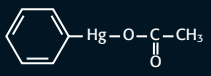
Mercury derivatives



Thiomersal

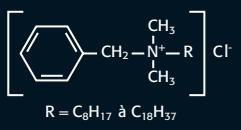


Phenylmercury nitrate



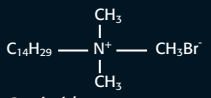
Phenylmercury acetate

Quaternary ammonium salts

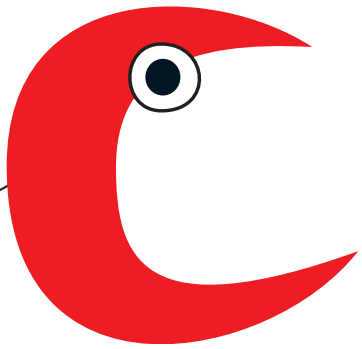


$R = C_8H_{17}$ à $C_{18}H_{37}$

Benzalkonium chloride

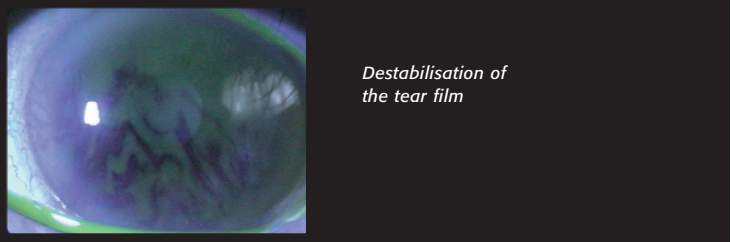


Cetrimide



Grounds for concern

The prolonged use of eyedrops containing preservatives can cause allergic or inflammatory reactions, either immediate or delayed, most often benign: **stinging** upon instillation, **pruritus, foreign body sensation in the eye, conjunctival hyperaemia, shortening of tear film break-up time, superficial punctate keratitis**, especially in the lower part of the cornea opposite the lachrymal lake. The use of preserved eyedrops has been linked to more serious conditions, in some cases leading to intraocular lesions: chronic conjunctival fibrosis (pseudopemphigoid), trabecular modifications, cataract, cystoid macular oedema, and failed glaucoma filtering surgery.



Destabilisation of the tear film

1. Preservatives and inflammation of the ocular surface

The toxicity of preserved eyedrops can cause changes to the tear film, damage to the corneo-conjunctival epithelium, and increased epithelial permeability [30]. At a deeper level, a chronic inflammatory response and subconjunctival fibrosis may be observed, sometimes extending to the trabeculum, in patients under long-term treatment with associations of topical antiglaucoma solutions [5].

Evidence suggests that the development of the infraclinical inflammatory response generates underlying fibrosis, epithelial cell lesions, and ocular dryness. It forms the common mechanism of the ocular complications induced by eyedrops [30].

The absence of specific effects of active ingredients, the link with the duration of treatment, and the close correlation with the number of concomitant eyedrops used, reported in certain studies, support the implication of the preservatives contained in most of these eye medications. Such implication is not unexpected as these preservatives are largely known to produce cytotoxic effects *in vitro* and in animals [17].

1.1- Inflammation and the tear film

The tear film has a protective and nutritional role that is essential for a healthy ocular surface. Disruption of its lipid component, lowered stability and solubilisation are the first adverse effects produced by preserved eyedrops, especially those containing a quaternary ammonium salt.

This detergent effect can cause an increased tear evaporation rate and eye dryness often reported by patients and can also worsen an existing sicca syndrome.

The tear film alteration is intimately linked to the toxic mechanisms exerted by preserved eyedrops, in particular a conjunctival inflammatory response and an epithelial metaplasia [30] affecting goblet cells and transmembrane mucins.

Table 1

Tear film change in glaucoma patients under monotherapy (timolol) and bitherapy (timolol + dipivefrin) compared to non-treated healthy subjects

	Means (+/- standard deviation)	
	Schirmer test (mm)*	Tear film break-up time* (s)
Control subjects ($n = 20$)	12.70 (2.21)	14.40 (2.67)
Monotherapy ($n = 20$)	10.40 (1.58)	8.00 (1.89)
Bitherapy ($n = 20$)	8.20 (1.55)	6.90 (1.97)

** Analysis of variance, $p < 0.001$. In Yalvaç et al. [42]*

A qualitative change in the tear film was suggested by Garcher *et al.* [21], who demonstrated a modification of the structure of mucin chains in glaucoma patients treated by a preservative-containing beta-blocking agent.

The loss of goblet cells and the change in tear film quality may be associated with the development of the subconjunctival fibrosis produced by preserved eyedrops [30]. A reduction of nearly 50% of the density of these cells has been observed in biopsies from glaucoma patients under long-term treatment [37]. Yalvaç *et al.* [42] report a reduction of more than 60% of the density of goblet cells in patients receiving long-term monotherapy (timolol) or bitherapy (timolol + dipivefrin), in contrast to healthy controls (conjunctival impression cytology).

Yalvaç *et al.* [42] also report a significant decrease in the Schirmer test value and a reduced tear film break-up time in patients with primary open angle glaucoma compared to healthy controls (Table 1).

Nuzzi *et al.* [35] confirm the alteration of the ocular surface by antiglaucoma eyedrops, and suggest that the duration of treatment influences the degree to which the tear secretion is reduced. This reduction, observed with timolol monotherapy, is greater when pilocarpine is associated. The changes affect both basal and reflex lachrymal secretion.

The implication of the preservative, in particular quaternary ammonium salts, is suggested by the detergent properties of these compounds [17], which can be readily integrated into the tear film and reduce its stability. The instillation of a single drop of 0.01% benzalkonium chloride in healthy volunteers halves tear film break-up time [41]. **Nuzzi *et al.* [35] have shown that benzalkonium chloride instilled for three months in control subjects with no ocular disease can produce tear and conjunctival modifications of the same intensity as those observed in patients with primary open-angle glaucoma receiving long-term monotherapy (timolol) or bitherapy (timolol and pilocarpine).**

In a randomised crossover study in glaucoma patients, Costagliola *et al.* [18] note that 0.005% latanoprost (1 drop per day for two weeks) containing a double concentration of benzalkonium chloride (0.02%) produced conjunctival modifications and reduced tear film quality (Ferning test) to a greater degree than 0.5% timolol instilled twice daily for two weeks containing half the concentration of benzalkonium chloride (0.01%).

Strempel [38] also found that the beta-blocking ophthalmic solution that was the least toxic in terms of reduced tear film break-up time measured 20 minutes after instillation in healthy volunteers was the one with the lowest concentration of benzalkonium chloride (0.004%).

Main points:

Preservatives reduce the stability of the tear film by their detergent effect on the lipid layer through the destruction of goblet cells.

The changes in the tear film induce an increased evaporation and ocular dryness.

The effects of preservatives on the tear film are closely linked to the development of inflammation and conjunctival metaplasia.

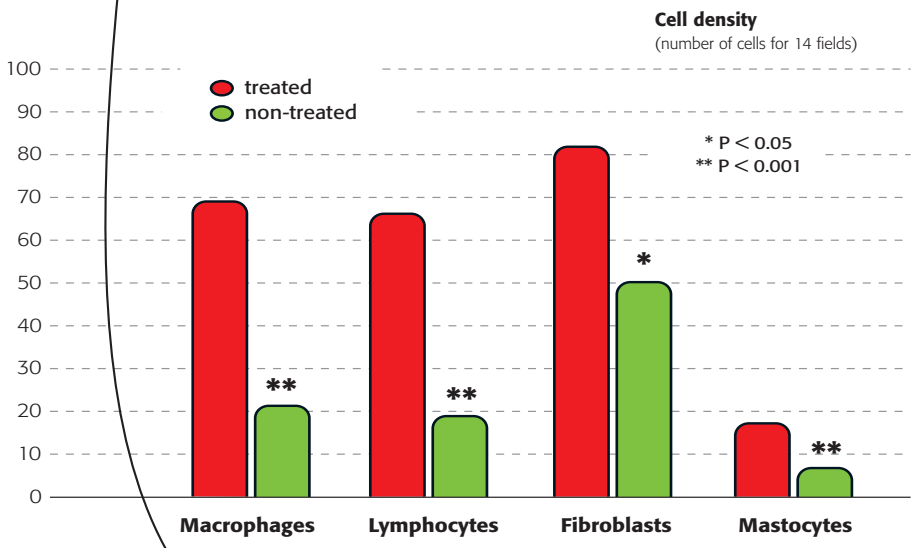


Figure 1

Comparison of the density of inflammatory cells in the substantia propria of the conjunctiva of glaucoma patients: non-treated (primary surgery) or treated with combined eyedrops containing a preservative

1.2- Markers of preservative-related inflammation

The infiltration of immunocompetent cells (Langerhans cells, macrophages, lymphocytes) in the outermost layers of the epithelium has been reported in patients under long-term treatment with preservative-containing antiglaucoma medications [7, 13].

Sherwood *et al.* [37] report a significant three to fourfold increase in the density of lymphocytes and macrophages in the conjunctiva (Figure 1) and the layers of Tenon's capsule in patients under long-term treatment with combination of different antiglaucoma eyedrops.

The intensity of the inflammatory reaction is related to the duration and the number of antiglaucoma medications used concomitantly [1]. A significant increase in the number of mastocytes is also observed. These cells can participate in inflammatory response, tissue repair and control of local blood flow [3]. **The immuno-inflammatory response has been especially well demonstrated by the expression of HLA-DR antigens, which are indispensable for cell immune reaction** [8].

More recently Baudouin *et al.* [5] studied the expression of immune markers of inflammation. Biopsies of conjunctiva from patients receiving long-term antiglaucoma monotherapy (30 patients) or multitherapy (6 patients) showed a low-level expression of auxiliary lymphocytes (CD4) and B lymphocytes (CD22) in conjunctival stroma, at a level that was, however, comparable to the expression found in 5 patients who had never been treated. But above all, immunocytological staining showed a significant increase in the expression of HLA-DR antigens, adhesion molecules, especially ICAM-3, and beta-2 integrins (CD11a and CD11b), and CD45RO (membrane phosphatase expressed by immune cells) in the substantia propria of patients receiving monotherapy or multitherapy, compared to patients who had never been treated.

Baudouin *et al.* [3, 4] also found an abnormal expression of antigens HLA-DR and CD23 (low affinity IgE receptor) at the surface of epithelial cells in conjunctival impression cytology specimens from patients treated with antiglaucoma medications for at least 6 months who displayed no corneal or conjunctival lesion. **This infraclinical reaction was found in nearly 50% of patients treated with one or more eyedrops containing benzalkonium chloride** (Table 2). In comparison, no markers were found on conjunctival impression cytology specimens in normal subjects.

These results were recently confirmed by another research team [19] who found an over-expression of HLA-DR at the surface of T lymphocytes in patients under long-term monotherapy with antiglaucoma eyedrops and presenting no signs of clinical inflammation.

Expression of membrane antigens HLA-DR and CD23 in conjunctival impression cytology specimens from glaucoma patients receiving long-term monotherapy or bitherapy (beta-blocker, pilocarpine and (or) dipivefrin containing preservative)

Table 2

	Number of positive impression cytology specimens		Mean % of reactive cells (+/- standard deviation)*	
	HLA-DR	CD23	HLA-DR	CD23
Patients treated	43/88 (49%)	26/68 (38%)	70 (28)	52 (28)
Patients non-treated	0/19	0/19		
Healthy volunteers	0/30	0/29		

** percentages calculated for positive cytology, standard deviation.*
In Baudouin et al. [3]

Another study [8] showed that the degree of overexpression of antigens HLA-DR at the surface of epithelial cells was higher in patients receiving a combination of preserved eyedrops. **By contrast, HLA-DR expression was not significantly increased in patients receiving a preservative-free beta-blocking agent compared to healthy subjects with no ocular disorders, suggesting a direct involvement of the preservative in the inflammatory reaction**. The presence of IL-6 and IL-8 in the conjunctival epithelial cells expressing antigens HLA-DR underlines the pro-inflammatory role of these cells.

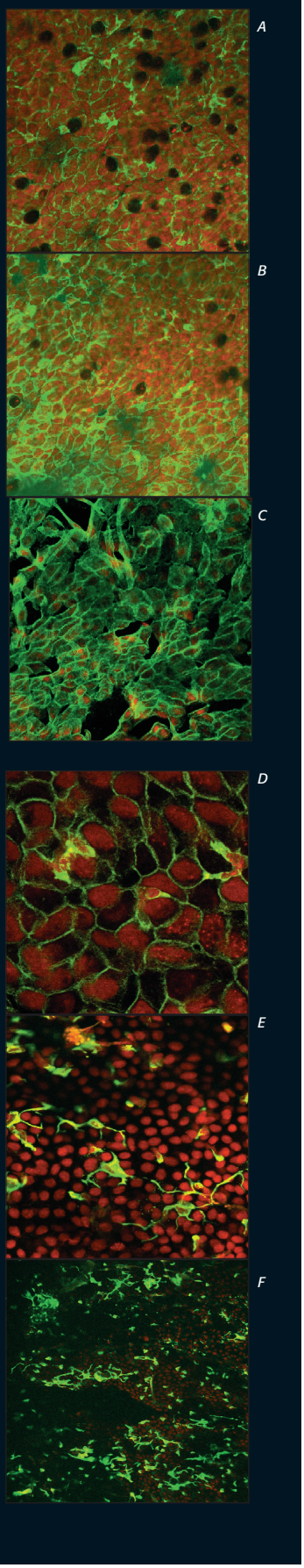
Main points:

Preservatives induce an infiltration of the conjunctiva by inflammatory cells (lymphocytes and macrophages).

The intensity of this inflammatory response is related to the duration or the number of preserved eyedrops used.

Patients receiving preservative-free eyedrops exhibit no inflammation.

Conjunctival impression cytology specimens viewed by confocal microscopy



The dark areas are goblet cells

***A**: Non-treated patient: numerous goblet cells*
***B**: Prolonged monotherapy: goblet cell rarefaction*
***C**: Multitherapy: metaplasia with disappearance of goblet cells*

Cells stained green express inflammation markers

***D**: Non-treated patient: rare immune cells*
***E**: Prolonged monotherapy: moderate inflammatory infiltration*
***F**: Multitherapy: very numerous immune cells*

1.3 - Consequences of the inflammatory response

Changes in the conjunctival epithelium

The conjunctival impression cytology specimens of patients under long-term treatment with antiglaucoma eyedrops show well-defined features (Table 3) [30]. **Disorganised conjunctival layers, loss of tissue cohesion, modification of the morphology of epithelial cells, keratinisation and loss of goblet cells are characteristic of cell metaplasia.**

Histopathological changes produced by preserved antiglaucoma medications.

Table 3

Reduction of goblet cells
Epithelial keratinisation
Squamous metaplasia
Loss of microvilli
Increased number of desmosomes
Bullous dystrophy of the epithelium
Increased number of subepithelial fibroblasts
Subepithelial fibrosis
Reduction of intravascular spaces
Increased number in subepithelial lymphocytes and plasma cells
Thickening of basal membrane
Basement membrane staining for immunoglobulin

From Liesegang [30]

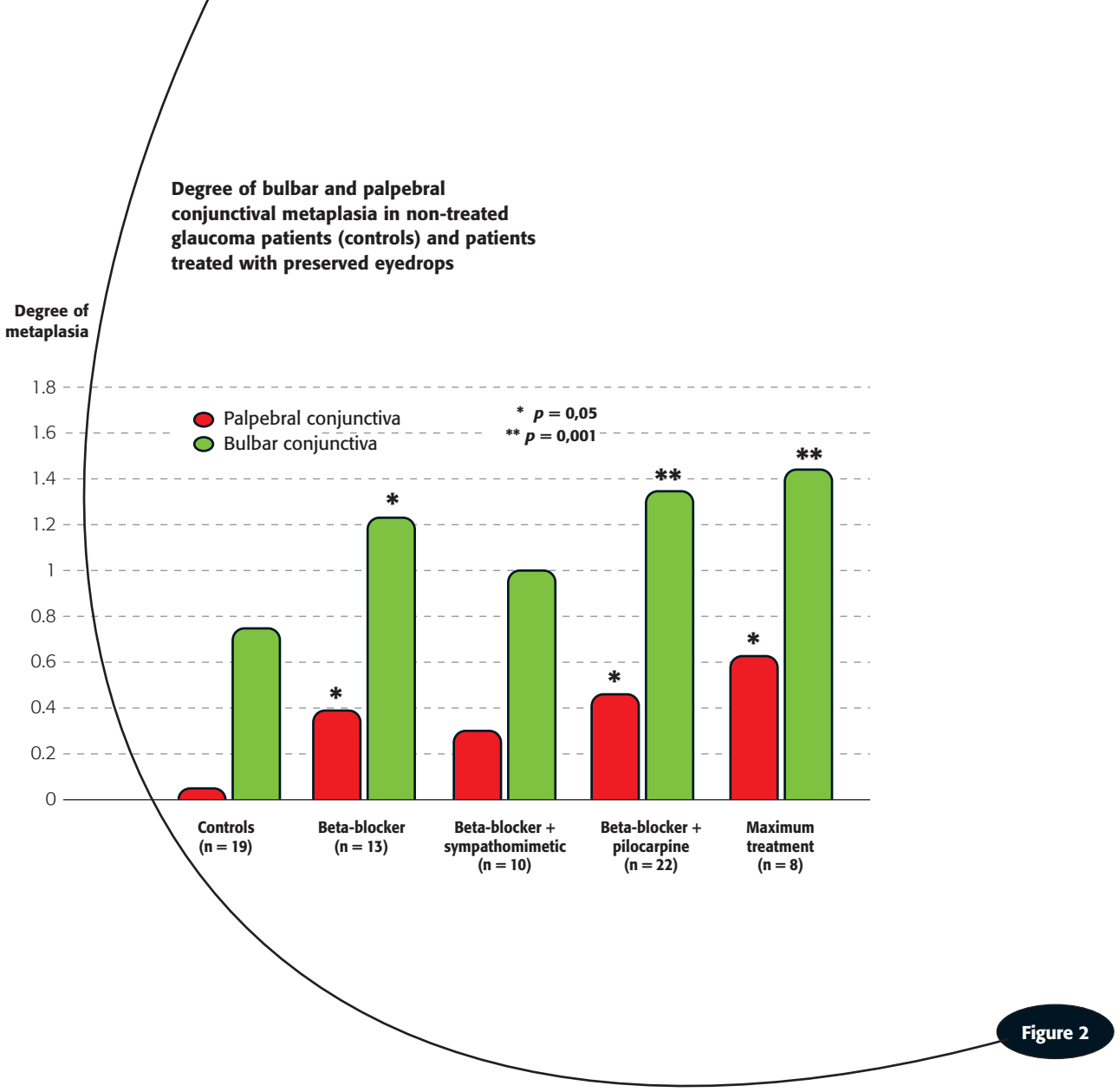
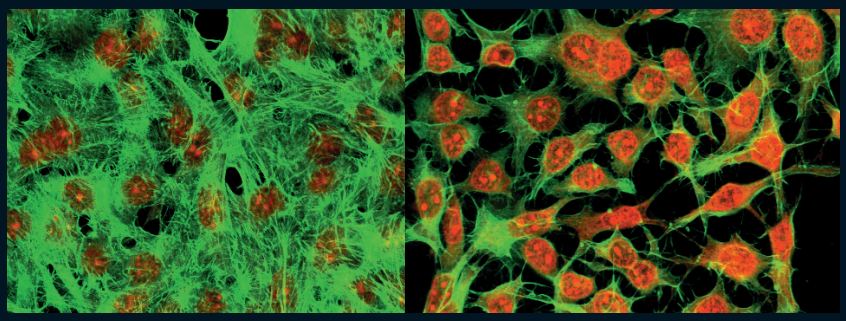


Figure 2

These patients present conjunctival epithelium metaplasia compared to non-treated glaucoma patients [10] (Figure 2) or control subjects with no ocular anomaly [42]. The epithelial metaplasia is observed on palpebral and bulbar conjunctiva [10].

The histomorphological changes of the epithelium can appear rapidly two weeks after the start of an antiglaucoma treatment [18].



Control *0.01% benzalkonium chloride*

Evidence of apoptosis induced by benzalkonium chloride (conjunctival cell culture)

Turaçli *et al.* found no relation between the degree of metaplasia and the duration of antiglaucoma treatment in patients treated for more than 3 months [40]. Conversely, several studies have clearly demonstrated an association between the number of antiglaucoma eyedrops used concomitantly and the degree of epithelial metaplasia [10, 35].

In a recent study Baudouin *et al.* [2] evidenced a relation between the degree of infraclinical inflammation measured by the expression of HLA-DR antigens and apoptosis of the conjunctival epithelial cells in glaucoma patients.
They showed that a cell marker specific to apoptosis (APO 2.7) was very weakly expressed by the conjunctival epithelial cells in normal subjects, moderately expressed (10% to 30% of the cells) in non-treated glaucoma patients, and very strongly expressed in practically all the cells in treated patients.

The loss of goblet cells is the first sign of squamous metaplasia, and is followed by an increase in cell stratification, and then keratinisation [30]. Patients under long-term treatment with antiglaucoma eyedrops often present a significant reduction of goblet cells compared to non-treated glaucoma patients [37] or healthy subjects with no ocular anomalies [42].

Preservatives, especially benzalkonium chloride, are strongly suspected of causing or maintaining morphological changes in the epithelium observed during the use of antiglaucoma eyedrops.

In healthy subjects with no ocular disorders, who had received benzalkonium chloride instillations twice daily for 3 months, the degree of metaplasia was comparable to that observed in patients under long-term treatment with antiglaucoma eyedrops, and higher than that found in controls [35].

In a randomised crossover study Costagliola [18] found that morphological changes in conjunctival impression cytology specimens produced by the instillation of timolol with preservative (twice daily) for 2 weeks were less marked than those produced by the instillation of latanoprost (once daily) preserved with benzalkonium chloride at twice the dose (0.02%).

Main points:

The preservative causes changes in the conjunctival epithelium. The loss of goblet cells is the first sign of squamous metaplasia.

These histological modifications appear rapidly, two weeks after the start of a treatment with a preserved ophthalmic solution.

Subepithelial fibrosis

The development of progressive subconjunctival fibrosis with no clinical sign of intolerance is also documented and is quite frequent in patients under long-term treatment with antiglaucoma eyedrops [36]. **The infiltration by inflammatory cells observed in these patients is generally associated with a significant increase in the fibroblast density in the subepithelial substantia propria** [5, 37]. Baudouin *et al.* [5] carried out an immunohistochemical analysis of conjunctival and trabecular biopsies from patients treated with preservative-containing and preservative-free antiglaucoma eyedrops. They found a significant increase in the expression of fibroblastic and inflammatory markers, which was more pronounced in patients under multiple treatment (Table 4).

No correlation was found with treatment duration. Fibroblast staining was also increased in the trabeculum biopsies (Table 5) in particular in patients receiving multiple treatment.

This finding suggests that the toxicity of eyedrops observed in the conjunctival epithelium may act on deeper ocular tissue.

Table 4

Histopathology of the conjunctiva of glaucoma patients: non-treated (primary surgery), treated with long-term monotherapy, and treated with multiple eyedrops (beta-blocker, pilocarpine and (or) sympathomimetic) containing a preservative

	Non-treated patients	Patients under monotherapy	Patient under multiple treatment
	$n = 5$	$n = 30$	$n = 26$
Normal	4/5 (80%)	11/30 (36.6%)	2/26 (7.7%)
Inflammatory	0/5 (0%)	16/30 (53.3%)	22/26 (84.6%)
Fibrosis	1/5 (20%)	3/30 (10%)	2/26 (7.7%)
Pathological	1/5 (20%)	19/30 (63.3%)	24/26 (92.3%)

Evaluation of inflammation and fibrosis based on immunological marking of antigens HLA-DR, CD45RO, vimentin and (or) integrins CD11a and CD11b.

The pathological picture combines inflammation and (or) fibrosis

In Baudouin et al. [5].

Histopathology of the trabeculum of glaucoma patients: untreated (primary surgery), treated with long-term monotherapy or treated with multiple eyedrops (beta-blocker, pilocarpine, and (or) sympathomimetics) containing a preservative

	Non-treated patients	Patients under monotherapy	Patient under multiple treatment
	n = 5	*n* = 22	*n* = 24
Normal	4/5 (80%)	13/22 (59%)	3/24 (12.5%)
Inflammatory	0/5 (0%)	3/22 (13.6%)	10/24 (41.6%)
Fibrosis	1/5 (20%)	6/22 (27.3%)	11/24 (45.8%)
Pathological	1/5 (20%)	9/22 (40.9%)	21/24 (87.7%)

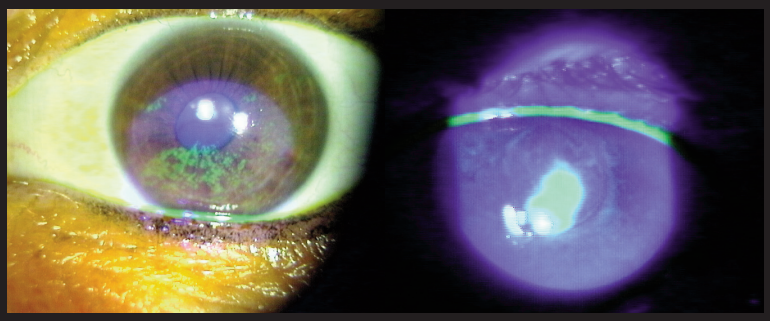
Evaluation of inflammation and fibrosis based on immunological marking of antigens HLA-DR, CD45RO, vimentin and (or) integrins CD11a and CD11b.
The pathological picture combines inflammation and (or) fibrosis.
In Baudouin et al. [5].

Table 5

Main points:

In parallel with the infiltration of the conjunctiva by inflammatory cells, the preservative induces an increase in the density of the subepithelial fibroblasts, resulting in a subconjunctival fibrosis.

The administration of preserved eyedrops also induces an increase in trabecular inflammatory and fibroblastic markers.



1- Inferior punctate keratitis
2- Corneal ulcer

1.4 - Severe corneal toxicity – clinical observations

Toxic keratopathies (prolonged superficial keratitis, corneal ulcers) attributed to preservatives, have been described in various situations, in particular in contact lens wearers [22] or patients with ocular dryness [26, 28, 32], or glaucoma, or after surgery [43].

Preserved eyedrops, whether of antiglaucoma agents, steroids or tear substitutes, are involved. The treatments are generally long-term, chronic or repeated at close intervals in the case of tear substitutes [28]. Superficial punctate keratitis is a known complication produced by the instillation of eyedrops.

Long-term exposure can be dangerous and cause a marked loss of epithelium, stromal oedema, infiltration and corneal opacity. Patients have hyperhaemic painful eye, and decreased visual acuity. Biomicroscopic examination reveals epithelial erosion and (or) vascularisation of the superficial layers of the cornea.

The symptoms improve or disappear when treatment or contact lens wear is stopped, or when preservative-free eyedrops are used instead, indicating that preservatives are implicated in these corneal disorders.

It is likely that during long-term use of preserved eyedrops the preservatives, by interfering with the cell metabolism, produce toxic effects leading to cell death, premature desquamation of the epithelial cells, rupture of stromal keratocytes, and

possibly degeneration of endothelial cells, and lead to marked ulcerative keratopathies [28].

Gasset [22] reports an ulcer of the cornea in a woman who had undergone extracapsular cataract extraction and who wore a protective soft contact lens cleansed with a solution containing benzalkonium chloride. The tests carried out *in vitro* showed the presence of benzalkonium chloride on the soft contact lens. It is likely that the prolonged contact with the benzalkonium chloride produced toxic effects on the patient's conjunctiva and cornea, resulting in a corneal ulcer.

Kilp *et al.* [26] report the case of a woman aged 46 instilling, for a dry eye syndrome, an artificial tear solution containing benzalkonium first every two hours and then every 30 minutes. She developed a superficial keratitis with a vortex-like arrangement of the hyperplastic epithelial areas. The symptoms worsened. A preservative-free treatment was substituted, whereupon the keratopathy regressed after one week. Skin tests showed no allergy to benzalkonium chloride.

Although in most toxic keratopathies the corneal endothelium is not damaged, Lemp *et al.* [28] report a case of severe toxic endotheliopathy clearly due to benzalkonium chloride in a man presenting a keratoconjunctivitis sicca treated for several years by instillation of artificial tears containing benzalkonium chloride. This patient showed an advanced degeneration of the corneal epithelium requiring keratoplasty.

The histopathology of the excised corneal button revealed stromal fibrosis and corneal oedema. After surgery, **the patient's symptoms persisted until the preservative-containing medication was totally replaced by a saline solution containing no preservative. A dramatic alleviation of the symptoms was observed after two weeks.**

During general anaesthesia the cornea is especially sensitive to reduced tear production, impaired tear film stability, and lagophthalmia. Ocular lubricants are often prescribed. Manecke *et al.* [32] report a severe corneal aggression (conjunctival hyperaemia, blurred vision, photophobia, reduction of visual acuity) accompanied by headaches in anaesthesia in a man aged 47 who had received an ocular lubricant containing 0.5% chlorobutanol, a preservative normally less toxic than benzalkonium chloride.

Ophtalmic examination showed a bilateral decrease in visual acuity together with conjunctival hyperaemia. Fluorescein staining showed de-epithelialised areas. Symptoms were alleviated after 3 days treatment with topical antibiotics and anti-inflammatory drugs, together with a preservative-free lubricant. Vision reverted to normal after 2 weeks with restoration of a normal corneal epithelium.

Schwab *et al.* [36] report the case of toxic ulcerative keratopathies in patients who had instilled or received an ophthalmic solution (anaesthetic, antibiotics, corticosteroids) containing preservatives. Most of the patients presented an ailment of the ocular surface (keratoconjunctivitis sicca, previous intraocular surgery, herpes or zona). The corneal defects were mostly inferior or inferonasal.

Severe keratopathies after cataract extraction have also been reported [43]. They evolve in well-defined phases: punctuate epithelial keratopathies (without ocular discomfort), followed by transient but recurrent pseudodendritis that can regress or progress rapidly towards a central epithelial ulcer and a central torpid ulcer of the stroma. Two patients presented an axial fibrosis and an epithelial defect causing a marked visual acuity loss. The cause of these disorders was attributed to the benzalkonium chloride contained in the eyedrops prescribed after surgery. These patients also had an history of chronic open-angle glaucoma or corneal dystrophy. It is possible that earlier preserved antiglaucoma treatments, defective tear film or corneal damages may have predisposed these patients to this complication.

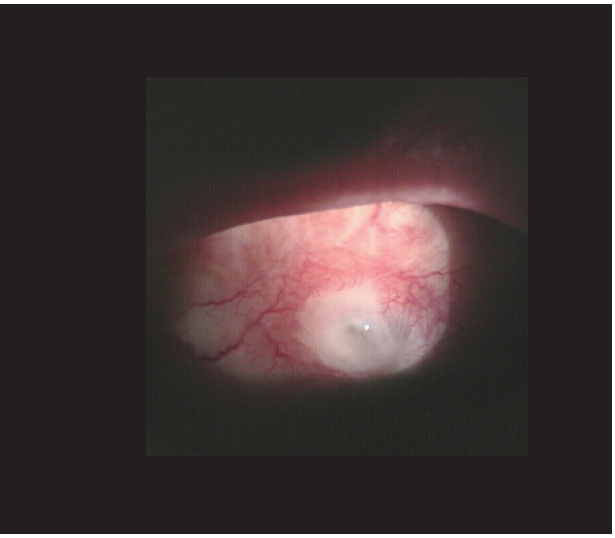
Liu *et al.* [31] report permanent corneal oedemas through endothelial damage in a series of patients who had undergone phacoemulsification. Research carried out after surgery pointed to the accidental administration of a solution containing 0.013% benzalkonium chloride in the anterior chamber of patients during surgery. Improvement of visual acuity occurred in only one patient after 6 months. The corneal changes observed included folds in Descemet's membrane and a thickened corneal stroma [20].

Main points:

The use of preserved eyedrops may in certain conditions (contact lens wear, ocular dryness, antiglaucoma treatment, during general anaesthesia or after surgery) be the cause of severe cell toxicity resulting in prolonged superficial keratitis and corneal ulcers.

Switching to an equivalent preservative-free solution can improve these conditions.

2 Preservatives and complications of chronic treatment



Fibrosis of a filtration bleb

2.1 - Preservatives and complications of glaucoma surgery

The treatment of glaucoma is based on the use of eyedrops given as monotherapy or in association. Filtration surgery is generally recommended when medication has failed. Hence most patients who have to undergo such surgery have often been treated for a long time with single or multiple antiglaucoma eyedrops.

Filtration surgery (trabeculectomy or deep non-perforating sclerectomy) lets the aqueous humour flow under the conjunctiva and Tenon's capsule, causing the formation of a filtration bleb. Despite the high performance of filtration surgery, the success rate of these procedures ranges widely [15]. In a series of 106 cases of filtration surgery, Broadway *et al.* [14] **report a success rate of 90% in patients who had undergone a primary trabeculectomy (non-treated patients), 93% in patients treated with single beta blocking medication, 72% in patients taking a beta-blocker plus a miotic agent, and 45% in patients receiving a miotic drug plus a sympathomimetic agent (Figure 3)**.

In addition, the success rate was significantly lower in the patients treated for more than 3 years (55%) than in the others (94%, $p < 0.001$), suggesting that **duration of antiglaucoma treatment and number of treatments are linked to the result of filtration surgery**.

In a retrospective study, Lavin *et al.* [27] report a particularly high success rate (97.9%) in patients who had received no long-term antiglaucoma treatment. In comparison, the patients treated for at least one year with an association of several antiglaucoma agents showed a statistically lower success rate (79.1% $p < 0.001$).

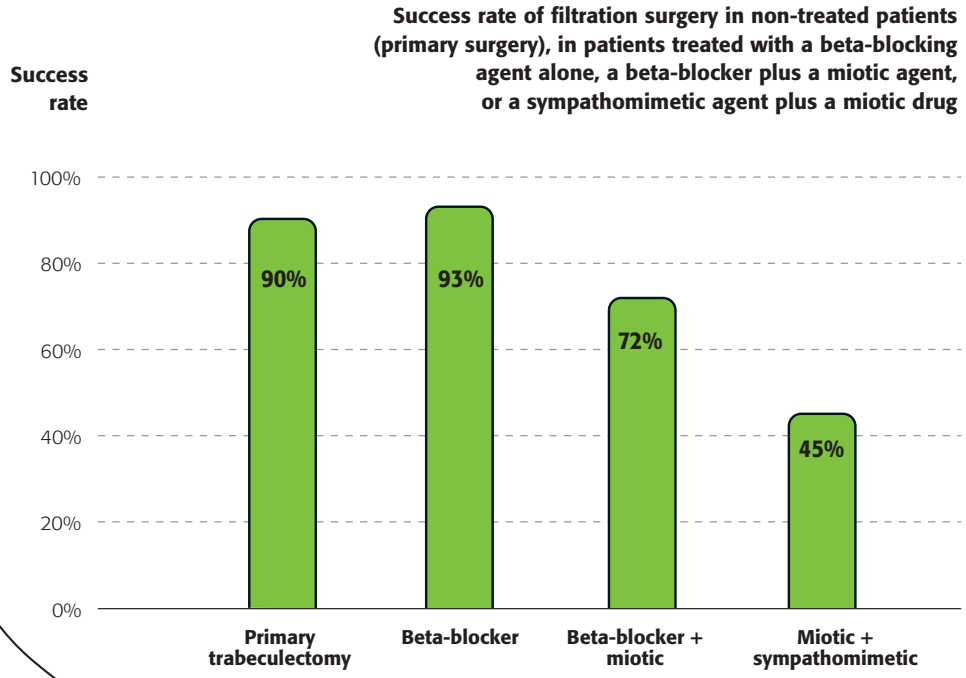


Figure 3

This study also shows that an association of several antiglaucoma treatments multiplies by five the failure risk of filtration surgery.

The long-term use of topical antiglaucoma medications can compromise trabeculectomy [6, 7, 15]. However, none of the active ingredients present in these eyedrops (beta-blockers, miotic agents, sympathomimetic agents, prostaglandin analogues) has been formally identified as being responsible for this adverse effect, at least not in monotherapy [14]. The toxic effects mostly appear when 2 or 3 ophthalmic medications are used concomitantly, even for short treatment durations [6, 7]. **Owing to the ocular toxicity of the preservatives, evidenced in preclinical trials, and their omnipresence in antiglaucoma eyedrops, these preservatives, in particular benzalkonium chloride, are strongly suspected of being a risk factor in trabeculectomy failure.**

It is accepted that the main cause of failed filtration surgery is the excessive development of a local fibrosis of the bleb, which hinders the flow of the aqueous humour [6, 12]. An intensive medical therapy could favour the infiltration by inflammatory cells and the proliferation of fibroblasts, thereby increasing the risk of fibrotic scarring and filtration surgery failure. Sherwood et al. [37] have shown a significantly increased infiltration by inflammatory cells, fibroblasts and hyalin bodies in the substantia propria of the conjunctiva and in the layers of Tenon's capsule in glaucoma patients undergoing long-term treatment (more than a year), compared to patients who had undergone a primary trabeculectomy.

Baudouin *et al.* [5] have also demonstrated an infiltration by inflammatory cells and fibroblasts in trabeculum biopsies from patients treated with antiglaucoma eyedrops.
In another study, Broadway *et al.* [14] found a direct relation between the duration of the topical antiglaucoma treatment, the treatment regimen, the cell profile of the conjunctiva (in particular the inflammatory cells) and the result of filtration surgery. Certain patients with no obvious signs of chronic underlying conjunctival inflammation [3] may thus be good candidate for trabeculectomy.

Main points:

The prolonged administration of antiglaucoma eyedrops increases the failure rate of filtration surgery.
No active ingredient has been incriminated in these failures in any of the different studies.

The preservative increases fibrotic scarring. This local fibrosis of the bleb hinders the flow of the aqueous humour, thus compromising the successful outcome of filtration surgery.

2.2 - Preservatives and cataract

Patients receiving long-term antiglaucoma treatment can develop cystoid macular oedema after cataract surgery. This effect was observed with different types of ophthalmic solution (epinephrin, dipivefrin, timolol, latanoprost). The causes of this susceptibility are not well established. A link to an inflammatory state has recently been suggested [33]. The mechanisms probably involved include the release of pro-inflammatory mediators (prostaglandins, cytokines). Miyake points to the role of preservatives and has demonstrated these mechanisms in several experimental studies, under the term pseudophakic preservative maculopathy [34].

An *in vitro* study [23] has recently shown that benzalkonium chloride exerts a dose-dependent toxic effect towards the cells of an epithelial line of human lens in culture, thereby inducing the expression of soluble inflammatory mediators (PGE2, IL-1a and IL-6). This cytotoxic effect was much stronger than that observed with timolol (5 mg/ml) or latanoprost (200 mg/ml). The implication of benzalkonium chloride is especially likely as it possesses a high power of impregnation of ocular structures [17].

In the light of these findings the implication of benzalkonium chloride in the aetiology of cataract itself has been suggested. In the analysis of 3 recent soundly-designed large-scale trials conducted on different populations, Brandt [11] notes that **the incidence of cataract is increased significantly in patients under long-term treatment with topical antiglaucoma agents compared to non-treated patients followed up for several years.**

In the Ocular Hypertension Treatment Study (OHTS) [25] the effect appears to be mild though significant: 6.4% of the patients treated developed a cataract *versus* 4.3% of non-treated patients ($p = 0.006$) during a 5-year follow-up. In the Early Manifest Glaucoma Trial (EMGT), nuclear opacities developed more rapidly and were more often associated with antiglaucoma treatments [24]. **In a study conducted in Barbados (BISED study) [29], it was shown that the treatments (mainly given in association) aiming at reducing intraocular pressure tripled the risk of developing a nuclear opacity in the following 4 years.**

Main points:

The preservative, by inducing the release of pro-inflammatory mediators, is responsible for an increase in the incidence of cystoid macular oedema in pseudophakic patients, especially those undergoing long-term treatment with preserved antiglaucoma eyedrops.

The implication of benzalkonium chloride in the aetiology of cataract is suggested by various studies. For example, a significant increase in the incidence of cataract is observed in patients undergoing long-term treatment with preserved antiglaucoma eyedrops.

2.3 - Conjunctival cicatrisation and pseudopemphigoid

The development of subconjunctival fibrosis during antiglaucoma treatment is moderately frequent [37, 30]. Schwab *et al.* [36] have shown a significant diminution in the depth of the lower conjunctival fornix in glaucoma patients treated with antiglaucoma eyedrops for at least 3 years compared to healthy subjects.

This effect has been observed with different types of preservative-containing ophthalmic solutions (beta-blockers, sympathomimetics or miotics administered in monotherapy or in association) and in the absence of any clinical sign of intolerance.

More rarely, exposure to antiglaucoma eyedrops is a risk factor for severe structural modifications such as ocular cicatricial pemphigoid (pseudopemphigoid or chronic progressive conjunctival cicatrisation).

These changes can leave serious irreversible sequelae: obstruction of lachrymal and Meibomian glands, modification of the tear film, trichiasis, keratopathies and possibly even blindness [9].

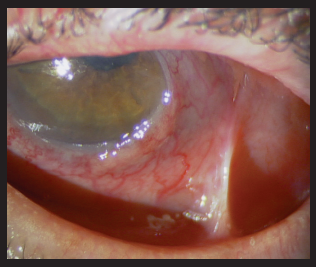
In addition, it is likely that exposure to eyedrops may accelerate abnormal cicatrisation in patients at risk, presenting a pemphigoid [16]. The mechanisms are largely unknown.

A genetic predisposition associated with a modification of the conjunctival epithelium, an immuno-inflammatory response and a chronic subconjunctival fibrosis, frequently observed after instillation of antiglaucoma eyedrops, may contribute to its development [30, 12].

Because of their chronic development, pseudopemphigoids appear after several years of exposure to antiglaucoma medications. The active drug in the antiglaucoma ophthalmic solution is irrelevant, pseudopemphigoids having been observed with solutions of beta-blockers, miotics and sympathomimetic agents [12].

Multiple medication is evidently one of the main risk factors. In a series of 145 patients presenting a pseudopemphigoid, Thorne *et al.* [39] recently showed that exposure to antiglaucoma eyedrops was the primary cause of pseudopemphigoid. A combination of antiglaucoma eyedrops was used in almost all the cases reported (97.4%).

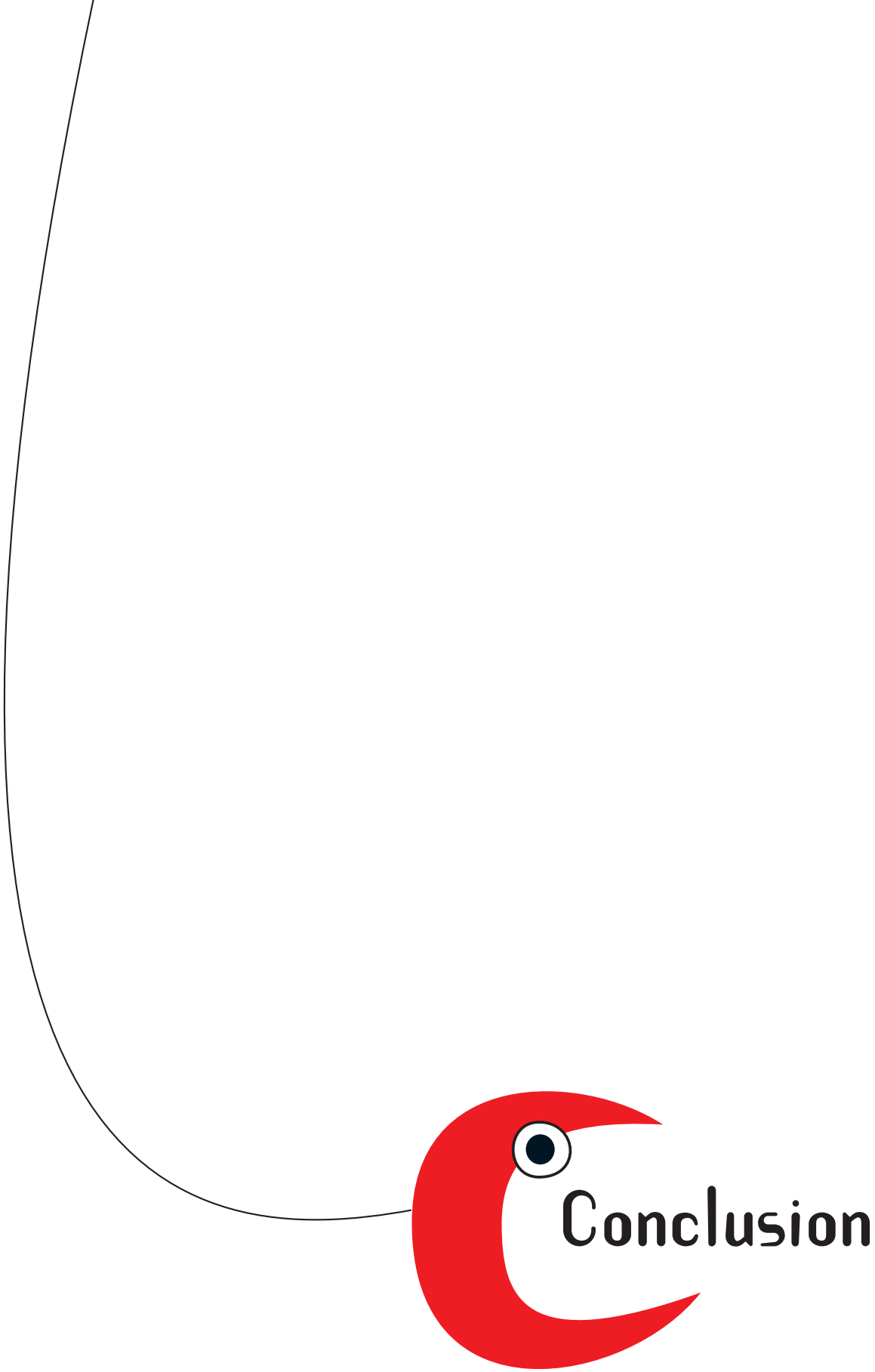
Pseudopemphigoid



Main points:

The prolonged administration of preserved eyedrops is the main cause of pseudopemphigoids.

The mechanisms are still unknown, but probably involve an inflammation responsible for abnormal cicatrisation.



Conclusion

Primum non nocere

The use of preserved eyedrops induces adverse effects of variable intensity and severity, which can diminish treatment compliance and even cause treatment failure (e.g. filtration surgery).

In humans, the prolonged administration of preserved eyedrops leads to alterations of the superficial ocular structures (conjunctiva, cornea) and deeper structures (trabeculum, crystalline lens).

The least severe ocular signs and symptoms are discomfort or pain on instillation: sensation of foreign body, stinging or burning, dry eye, itching of eyelids.

The most severe adverse effects include an inflammation of varying intensity, from a simple subclinical response to the progressive development of a fibrosis. This fibrosis is implicated in the failure of filtration surgery, and can, in certain patients, lead to severe ulcerative keratopathies or pseudopemphigoids.

Various studies have demonstrated the adverse effects of different types of preserved eyedrops. The preservatives, in particular benzalkonium chloride, are the common factor and thus are responsible for these adverse effects. Duration of treatment and the number of preserved eyedrops used are key variables, the common factor being the presence of a preservative. The major deleterious role of preservatives is confirmed by clinical trials comparing the use of preserved eyedrops and their preservative-free equivalents.

Hence considering the occurrence of adverse effects (stinging, burning and dryness sensations, itching, hyperaemia) of ranging severity, it is advisable to restrict the use of preserved eyedrops and to replace them with preservative-free alternatives devoid of preservative related adverse effects.

Primum non nocere.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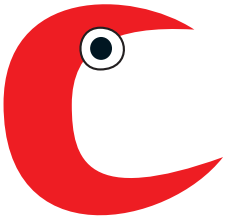

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