Ocular Surface Morbidities in Glaucoma

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Abstract

Introduction: Ocular surface disease (OSD) is a major cause of ophthalmological consultation. Within this group, glaucoma patients constitute an important percentage due to the nature of their chronic topical treatment. Advances in the understanding of ocular surface pathology on both a cellular and molecular level, and the interaction to the toxic nature of some topical medication solution compounds, are stimulating the development of better alternatives in topical treatment.

Materials and Methods: Extensive review of available literature and current research on topical ophthalmological treatments in both OSD and glaucoma was performed. Emphasis was made on inflammatory modulators of the ocular surface and pathological changes associated with the use of preservatives in solutions, in particular benzalkonium chloride (BAK). Independent research is been also performed by the authors, using a rubbing-induced reflex tears collecting method with capillary tubes. Cytokines in tear samples were subsequently analysed with the multi-Plex System (Luminex® R-200). The 12 cytokines analysed using this method are the interleukins (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, the tumour necrosis factor alpha (TNFα), the vascular endothelial growth factor (VEGF), the granulocyte-macrophage-colony stimulating factor (GM-CSF) and the interferon gamma (IFg).

Results: Our unpublished, preliminary results trend to show higher values in IL-5 and IL-6 in glaucoma patients than in normal controls. Also, higher values in IL-2, IL-5 and IL-12 in dry eye syndrome (DES) patients were found versus normal controls.

Conclusions: Inflammation is an important element in OSD and strong association seems to exist between this and the use of BAK preservative. New kinds of preservative molecules, as well as single-dose eye drops and sophisticated dispensing bottle mechanisms, are currently available on the market in order to avoid the secondary effects of this topical treatments, increasing patient compliance and adherence to chronic topical glaucoma treatment.

Keywords

Ocular surface, glaucoma, benzalkonium chloride, preservatives, conjunctiva, cornea, inflammation

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Ocular surface disease (OSD) represents one of the major causes for ophthalmological consultation worldwide.[1] It involves all sorts of pathological alterations of conjunctiva and cornea from the minor such as punctuate keratitis (see *Figure 1*), to the extreme such as symblepharon, or loss of limbal stem cells with corneal conjunctivalisation. Within this group, dry eye syndrome (DES) constitutes a well-defined, yet not completely explained entity, with multifactorial aetiology but clearly defined symptoms. Redness, itching, foreign-body sensation, tearing and pain are some of the frequent complaints ophthalmologists face on a daily basis from this group of patients. Ocular lubricants constitute the main treatment, but recently, advances in the understanding of the DES as an inflammatory condition have modified our view on the correct way to approach this problem.

Up to the present, no definitive single diagnostic test for DES has been available in clinical practice. It is essential to optimise face-to-face interviews and ophthalmological examination of DES patients for assessing diagnosis and progression of the disease. The clinical diagnosis of objective tests for DES includes the following: 1) the Schirmer test (ST), with or without anaesthesia, which determines tear production; 2) tear break-up time (TBUT) that reflects tear film stability; and 3) dye staining tests for evaluating the tissue integrity, by using the conjunctival lissamine green or the corneal fluorescein staining.[2–4]

Recent research has helped in understanding DES pathophysiology. A multitude of diagnostic techniques have been developed to assess tear film and ocular surface optimising DES diagnosis and therapy. Biochemical and molecular assays have arisen to better diagnose and treat these patients. A significantly different pattern of autoantibodies against ocular antigens in the tear fluid of patients suffering from DES has been demonstrated. With these non-invasive analyses in tear samples, potential clues for underlying autoimmune processes in these cases can be obtained.[5–8] In general, two sampling methods have been utilised to assess biochemical determinations of tear fluid composition, both in healthy and pathological eyes. These techniques are the yawn collection and the eye-flush collection procedures.[9] Schirmer strips have been extensively used for tear fluid evaluation and collection.

A less-invasive technique, the glass capillary micropipettes extracting method, has been proposed as that most adequate for obtaining reflex tear samples.[9,10] Although it is easier to collect tears using this method, it has also been demonstrated that even when basal tears are obtained using the standard protocol of Schirmer strips and an anaesthetised cornea, some degree of reflex tearing still occurs.

Glaucoma is a chronic disease of the optic nerve, with progressive loss of the retinal nerve fibre layer. The main factor associated with this damage

Figure 1: Punctuate Keratitis

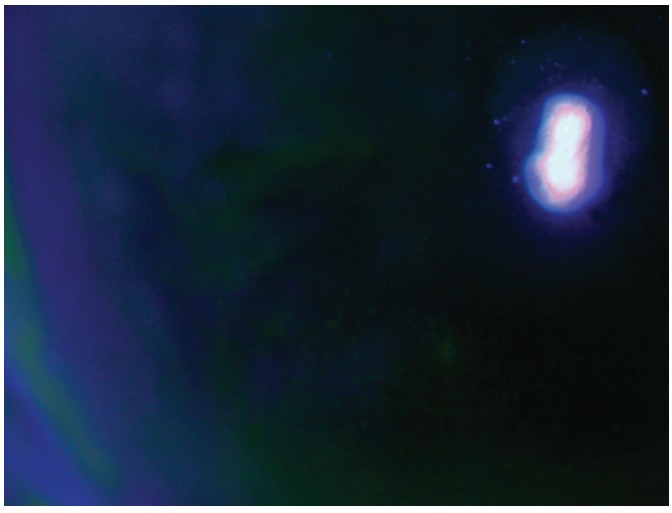
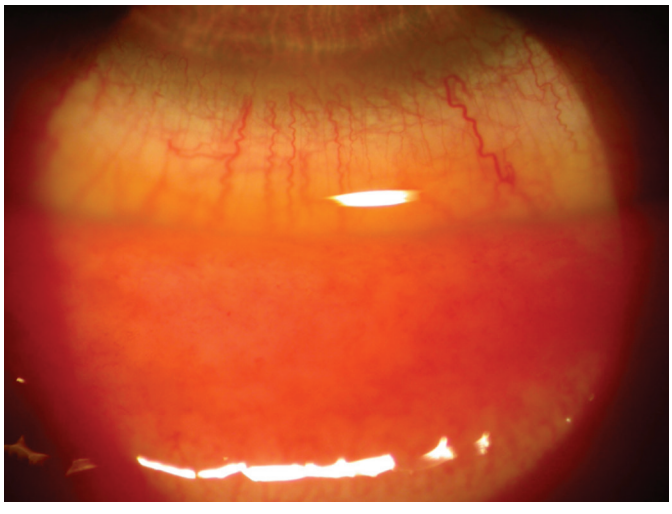


Figure 2: Acute Conjunctivitis-like Reaction



is an elevated intraocular pressure (IOP). Glaucoma constitutes the first cause of blindness in Europe and one of the main causes worldwide.[7] The first line of treatment typically constitutes topical medical treatment that either decrease aqueous humor production and/or facilitates its outflow. Just as with many eye drops, these medications generally contain preservatives to warranty their safety. Recent evidence has showed that some of the ingredients used as preservatives in many ophthalmic medications, (even some for the treatment of DES), are at least partially responsible for the problem.[8] In particular, glaucoma patients, who are exposed to a chronic use of preservatives in their treatment, represent a very susceptible population.

Preservatives and Effects

The most commonly used preservative in ophthalmic preparations is benzalkonium chloride (BAK).[8] It is a nitrogenous cationic surface-acting agent belonging to the quaternary ammonium group. Quaternary ammoniums are bipolar compounds which are highly hydrosoluble and have surfactant qualities. They behave as detergents, dissolving microbial cell walls, thus, they function as preservatives for ophthalmic solutions. The problem comes from this same detergent effect acting upon the ocular surface. There is a delicate balance in the homeostasis of the tear film and cellular function within both conjunctiva and cornea. It is well known that deficient tear production, low quality of the tear film and increased evaporation can lead to DES. In addition to this, damaging effects of preservatives such as BAK are accountable for much of the inflammation found in these eyes. The ocular surface of glaucoma patients on chronic medication has been reported to express inflammatory markers specific for the recruitment of T-cells that could be responsible for generating the early events of inflammation and wound healing. The inflammatory tear protein profile present in chronically treated glaucoma patients appear to be different than that found in primary dry eye.[9]

Animal and human studies have shown the adverse effect of prolonged exposure to topical conservatives on the conjunctiva, cornea and trabecular meshwork.[10] In particular, BAK is known to disrupt the tight junctions on the corneal epithelium.[11] Pauly et al. conducted an experiment involving exposure for 24 hours to increasing concentrations of BAK (0.001 %–0.5 %) on a human reconstituted corneal epithelium model. After 24 hours, dose dependant disappearance of junction protein occluding was found.[12] This is consistent with the kind of epithelial damage expected from chronic use of BAK associated topical formulations. However, the effect of the main active ingredient in these formulations cannot be ruled out. As an example, non-preserved formulations such as timolol can induce the presence of inflammatory markers and cytokines (human leukocyte antigen-DR region [HLA-DR], interleukin [IL]-6 and IL-8) in conjunctival epithelium of glaucoma patients.[13]

Recent studies have examined the prevalence of OSD in ocular hypertension and DES. Leung et al. conducted a study among 101 glaucoma patients to assess the prevalence of OSD.[14] A majority (59 %) of patients in the study reported symptoms of dry eye. Sixty-one per cent of patients had a decrease in tear production according to Schirmer tests. Positive lissamine green tests were found in 22 % of patients. Abnormal tear quality (TBUT) was present in 78 % of patients.

Another large-scale study was carried out on a population of 4,107 patients measuring the incidence of ocular toxicity from glaucoma medications.[15] Eighty-four per cent of the patients used preserved eyedrops. Ocular symptoms were much more evident in patients taking preservative solutions compared to those using preservative-free medications ($p<0.001$). The symptoms included, itching, foreign-body sensation, eyelid itching, tearing and burning. Patients on the preserved eyedrops had a more than twofold presence of these symptoms. Ocular signs and symptoms, as one can expect, increased directly proportional to the number of preserved solutions applied by the patient.

In Europe, a multicentre epidemiologic study was carried out to compare the presence of side effects in about 10,000 patients on a topical either preserved or non-preserved beta-blocker.[16] Ocular side effects, such as discomfort upon instillation, itching, foreign-body sensation or burning, were significantly more frequent in the preservative-medication group ($p<0.0001$). The most commonly used preservative at that time was BAK.

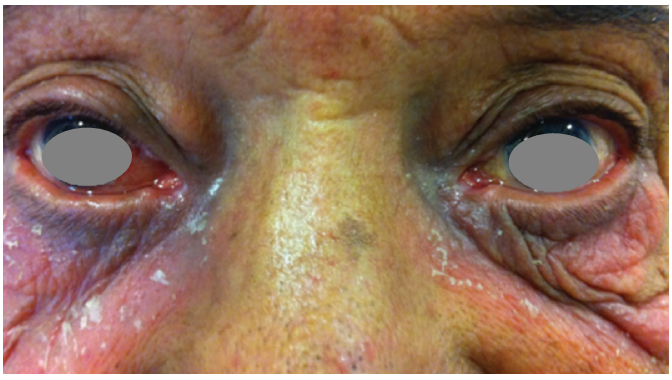
The relatively high incidence of OSD among glaucoma patients is clearly noticed when comparing it to the presence of dry eye in the normal population. A cross-sectional survey among 25,444 men in the US found that the prevalence of DES increased with age from about 3.9 % among men aged 50–54 years, to 7.7 % on those aged >80 years.[17] Another survey performed on 40,000 women in the US reported 5.7 % of women <50 years old suffered DES symptoms, compared to

9.8 % of those with age above 75.[18] These rates are much lower than those described on IOP-lowering medications.

Other adverse events observed with the use of antiglaucoma medications may result from either an allergic or a toxic reaction. Allergy is the most prominent reaction from a clinical point of view, but it occurs in fact in a small proportion of patients. Toxicity is probably the predominant cause, manifesting as chronic subclinical inflammatory changes induced throughout the ocular surface by the repeated use of eyedrops. As mentioned earlier, the side-effects may be caused by either the active compound of the antiglaucoma medication, or, as it is more currently accepted, the preservatives included within the formulation. Clinical manifestations of drug-induced ocular surface side-effects have been well described and classified by Badouin.[19,20] They can be resumed as follows:

1. Drug-related allergic reactions, in most clinical trials it remains as a relatively rare situation, happening mostly in the early course of treatment, affecting a few per cent of patients and mainly consisting of an acute conjunctivitis-like reaction (see *Figure 2*). From the different formulations, Latanoprost eyedrops showed ocular allergic reactions in only 1.5 % of patients.[21] Higher rates of ocular allergy (13.5 %) have been found on brimonidine eyedrop treatment (mean time of occurrence after initiation of around 15 days).[22] But, other clinical manifestations could be related to drug toxicity, e.g. corneal punctuate staining, without involving any allergic reaction.[23] So, it is important to discriminate early, acute allergic reactions from other more delayed toxic and non-specific inflammatory mechanisms.
2. Periocular dermatitis – as Baudouin refers,[20] in addition to allergic conjunctivitis, (which rarely corresponds to type I hypersensitivity) the most frequent drug-induced allergic reaction is a type IV delayed cell-mediated hypersensitivity. This could explain why many reactions occur at the eyelid level, (such as allergic blepharitis) which are often difficult to distinguish from other causes of eyelid inflammation or contact dermatitis (see *Figure 3*). There are even some reported cases which present with an atypical lichenoid eruption.[24]
3. Drug-induced ocular pseudopemphigoid – characterised by a very severe toxic scarring reaction of the ocular surface and clinically similar to that seen in cicatricial ocular pemphigoid or Stevens-Johnson syndrome. While the latter have a clear immunological origin, pathological examinations of these drug-induced scarrings usually fail to find any auto-antibodies in the conjunctiva.[25] This rare, severe scarring conditions can also involve development of important corneal opacities.[26, 27]
4. Failure of filtration surgery – although not having much immediate clinical relevance, occurrence of a certain degree of subepithelial conjunctival fibrosis, and an inflammatory infiltrate in patients with long term use of anti-glaucoma drugs, has also been reported. It has been widely suspected that the failure of filtration surgery could be related to theses drug-induced changes.[28–32] A study performed by Flach[33] attempted to answer this issue; however, because of heterogeneity of the design, (involving mostly retrospective uncontrolled case series) and disparity of the treatments, the author could not draw definitive conclusions from the literature analysed. So, it seems we still lack strong evidence to support this hypothesis; Anyway, all previous pathological studies cited in the paper and more recent molecular analysis oriented studies[34–36] are consistent with the fact that the conjunctiva of glaucoma patients is affected with this subclinical inflammation after a certain time of medical treatment, and that this could in turn influence the success of future filtration surgical treatments.

Figure 3: Periocular Dermatitis



Materials and Methods – Preliminary Research by the Authors

As mentioned earlier in this article, much work has been done in recent years to develop a practical system to analyse tear film molecules associated with inflammation and with DES. The Luminex Multi-analyte profiling assay system (LabMap, Austin, TX) is an outstanding new technology based on flow citometry.[37] A major characteristic of this system is that it permits a wide spectrum of measuring procedures, ranging from one single determination to simultaneously assaying various analytes. Another main advantage is that very small sample volumes can be utilised. It has recently been demonstrated that the Luminex system is useful in analysing cytokine levels in human tears collected with capillary tubes[38] or by Schirmer test strips.[39] Our main goal is to study and compare tear cytokine/chemokine concentrations in Glaucoma patients under medical treatment, DES patients and control individuals to determine changes in immune-response and inflammation mediators.

We used a gentle rubbing-induced reflex tears collecting method with capillary tubes for specimen collection (see *Figure 4*). Reflex tears were collected simultaneously from both eyes of each individual by capillary tubes as follows: a disposable capillary tube (Drummond Scientific Co., Broomall, PA) was used to collect yawn-induced reflect tears from the right and left inferior fornix. Approximately 50–60 µl of the reflex tears collected by capillary tube from each eye were transferred directly into a cryotube that was labelled and frozen until processing. Cytokines in tear samples were subsequently analysed with the multi-Plex System (Luminex® R-200). Here, polystyrene beads coupled covalently to specifically directed antibodies (Human Cytokine/Chemokine panel) are allowed to react with 40 µl of each tear sample containing either an unknown amount of cytokine, or a standard solution containing a known amount of cytokine, at room temperature for 1 hour. The 12 cytokines analysed using this method, as instructed by the manufacturers, are the interleukins (IL)-1 β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, the tumour necrosis factor alpha (TNFα), the vascular endothelial growth factor (VEGF), the granulocyte-macrophage-colony stimulating factor (GM-CSF) and the interferon gamma (IFg). The amount of tear obtained from the patients in our study usually allows detection in up to 90 % of the samples.

Results

So far, our unpublished, preliminary results trend to show higher values in IL-5 and IL-6 in glaucoma patients than in normal controls. Also, higher values in IL-2, IL-5 and IL-12 in DES patients were found versus normal controls. Other researchers have also shown this kind of differences in the tear cytokine profile of both medicated glaucoma patients[40,41] and DES patients[42–47] versus control groups, using different techniques.

Figure 4: Tear Collecting Method with Capillary Tubes

Such findings point towards the presence of an increased subclinical inflammation within the pathological groups involved. Subtle differences between the tear inflammatory cytokine profile of Glaucoma and DES patients could mean that although the clinical manifestations of OSD may be similar in both dry eye and in chronically treated glaucoma patients, their aetiologies may be different. More work and larger studies are required to establish the significance and role of these tear biomarkers in the management of glaucoma patients.

Conclusions – Practical Implications in Glaucoma Management

Medical topical treatment of glaucoma is generally the first line of management, applied either as a unique treatment or in combination with surgery and/or laser during the course of the glaucomatous disease. Because of this and of the chronic nature of the disease, medical hypotensive treatment should be considered as a long-term requirement. Besides, medical treatment is typically carried out on a combined medication scheme.[48,49] The ultimate purpose of efficient glaucoma management is preserving visual function, while sustaining good quality of life at a reasonable cost. This is empowered by avoidance of secondary effects and inconvenience of the topical medications.[50] It is known that patients with higher rates of secondary effects and worse quality of life, are those recognising to have poor adherence to treatment regime.[51] The impact of a lack of adherence to glaucoma treatment on its evolution has not yet been determined, but it seems reasonable to suggest that low adherence to treatment has important detrimental effects on the visual prognosis and general disease control. In order to increase adherence, simplification of therapeutic regime is essential, just as decreasing the secondary effects of medication; in fact, bad local tolerance has been described as the third major cause of non-adherence after forgetfulness and inadequate dose administration.[52,53]

Alternatives to Benzalkonium Chloride

The only way to totally eliminate BAK-related side effects, especially in the most sensitive patients, would obviously be to remove BAK from eye drops; however, this raises industrial and regulatory concerns.[8] Single-dose units are the most frequently used preservative-free preparations and depending on the country, some compounds are available such as tafluprost, a prostaglandin analogue, and most of the others consisting in different beta-blockers; this, mainly because they were the first active ingredients to became generics and could more easily be re-developed by the industry in a single-dose, preservative-free form.

However, some drawbacks have been pointed out regarding single-dose units, such as higher cost and difficult handling, especially in elderly patients.[54] Other study conclude that patients could manage single-dose units as least as well as the conventional eye drop bottles.[55] Anyway, multidose bottles have been developed, working by either allowing the preservative filtration and posterior absorption on a porous membrane, or by using a valve system that hinders penetration of bacteria into the bottle. The ABAK® (Laboratoires Théa, France) and COMOD® (Ursapharm, Germany) are examples of these systems, and have been patented and commercialised with various beta-blockers such as timolol, carteolol and non antiglaucomatous compounds.[56–58] Particularly, the ABAK® (Thea Laboratories, France) system contains a 0.2 micron nylon fibre membrane that filters the solution. The pressure exerted causes the solution to pass through the antibacterial filter in the ABAK® system, forming a drop that falls from the tip of the dispenser. When pressure is released, the solution is re-absorbed and filtered from bacteria and air, ensuring the protection of the solution throughout its use. Hence, the ABAK® system filter provides a double protection from microbial contamination without using preservatives.

Extensive research has been conducted to develop less toxic preservatives than BAK and quaternary ammoniums.[8] However, since preservatives must be potent antimicrobial agents, while not being cytotoxic, only very few have been proposed and are now commercially available. For example, research performed in rabbit eyes studied the effects of Purite® (Allergan, Inc, Irvine, California, US), a stabilised oxychloro complex available in US, to topical BAK-containing antiglaucoma medications.[59] After 30 days, BAK-containing dorzolamide, timolol and latanoprost produced significantly more changes in rabbit corneas and conjunctivas, than rabbit eyes treated with artificial tears containing Purite® and brimonidine Purite® ($p<0.001$).

Two other approaches have been developed for avoiding BAK as a preservative. In the US, prostaglandin-analogue solution travoprost, (which used to be preserved with BAK), is available with a new preservative system, called Sofzia® (Alcon, Fort Worth, TX), comprising boric acid, propylene glycol, sorbitol and zinc chloride. A toxicological study in conjunctival cells confirmed that Sofzia® preserved travoprost induced significantly less apoptosis and fewer alterations of cell viability and membrane integrity than did BAK-containing latanoprost or travoprost.[60] The third candidate as a preservative that could be a less toxic alternative to BAK is polyquaternium, a polycationic polymer. At least 37 different polymers exist under the polyquaternium designation, but polyquaternium-1, commercially known as Polyquad® (Alcon, Fort Worth, TX) is now commonly used as a multipurpose solution for contact lens care, showing good safety and tolerance compared with other multipurpose solutions.[61] This compound is commercialised in Europe preserving travoprost solution eyedrops as an alternative to BAK.[62]

As a conclusion, given that glaucoma is a non-curable sight-threatening disease and that its effective control relies mainly on chronic topical treatment with good patient adherence to the prescribed medical regimen, minimisation of ocular surface side effects is essential. Alternatives for eye-drops that avoid harming ocular surface and simultaneously act effectively in lowering intraocular pressure, are crucial milestones for ideal glaucoma management. ■

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