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Topical glaucoma medications – Clinical implications for the ocular surface

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ABSTRACT

Glaucoma is a leading cause of irreversible blindness. The use of topical eye drops to reduce intraocular pressure remains the mainstay treatment. These eye drops frequently contain preservatives designed to ensure sterility of the compound. A growing number of clinical and experimental studies report the detrimental effects of not only these preservatives but also the active pharmaceutical compounds on the ocular surface, with resultant tear film instability and dry eye disease. Herein, we critically appraise the published literature exploring the effects of preservatives and pharmaceutical compounds on the ocular surface.

1. Introduction

The tear film is classically divided into three individual layers: inner mucin, intermediate aqueous and outer lipid. However, the Tear Film and Ocular Surface Society Dry Eye Workshop II (TFOS DEWS II) tear film subcommittee recently advocated a two-layer model with an inner mucoaqueous layer and a superficial lipid layer [1]. The superficial lipid layer consists of meibum, the secretory product of the meibomian glands (MGs), which are modified sebaceous glands dispersed along the tarsal plates of the upper and lower eyelids [2]. Meibum helps stabilize the tear film, prevents evaporation and protects against external harmful agents [3]. Dry eye disease (DED) is the end result of a multifactorial pathway leading to disruption of tear film homeostasis [4]. The main underlying aetiologies include aqueous-deficient and evaporative dry eye, entities that often coexist and overlap along a spectrum, where the evaporative form is the most frequent. Meibomian gland dysfunction (MGD) is the most common cause of evaporative dry eye.

Glaucoma is a chronic, progressive optic neuropathy frequently associated with increased intraocular pressure (IOP) and is the leading cause of irreversible blindness globally [5]. The aqueous humour in the

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eye is produced in the ciliary body and mainly drains through the trabecular meshwork. The resulting pressure gradient determines the IOP, which has an average physiological range of 10–20 mmHg [6]. The use of topical IOP-lowering eye drops remains the primary treatment for glaucoma [7,8]. The objective of this review is to critically assess the clinical effects of topical medications on the ocular surface.

2. Methods

A PubMed and EMBASE search was conducted on April 1st of 2022. The search included any combination of the three keyword groups: (i) 'dry eye disease', 'meibomian gland dysfunction', or 'meibography', and (ii) 'topical medications' or 'eye drops' and (iii) 'glaucoma'. The PubMed search yielded 5027 results, while 4015 articles were identified in EMBASE. The inclusion criteria included relevance, full-text access and English language, and the exclusion criteria included lack of relevance, non-English language and abstract only. Letters to the editor, conference abstracts, review articles and case reports were excluded for clinical evaluation, although review articles have been used for background information. The remaining articles were evaluated for relevance, first based on the title and second on the abstract. Finally, the full text was appraised for inclusion based on relevance: studies including topical glaucoma medications and specification regarding the use of preservatives and quantitative results and outcome measures pertaining to the ocular surface (including symptoms, Schirmer test, tear film break up time, ocular surface staining, tear osmolarity and inflammatory markers). Both animal and *in vitro* studies describing the effect of topical glaucoma medications or preservatives on the ocular surface, tear film or meibomian glands were generally not included, although some are used for supplementary information. A critical appraisal of the included articles' reference sections was performed as well. In total, 253 research articles were included in the present review.

3. Effect of topical glaucoma medications on the ocular surface

3.1. Effect of preserved topical glaucoma medications on the ocular surface

Most available topical IOP-lowering medications contain preservatives, among which benzalkonium chloride (BAC) is one of the most common [8–11]. BAC is a quaternary ammonium exhibiting detergent-like qualities, affecting the cell membrane and metabolic processes [12]. BAC induces apoptosis and necrosis and reduces the amount of occludin in the corneal epithelium [13,14]. Furthermore, BAC has been shown to result in upregulation *in vitro* of the proinflammatory cytokines interleukin-1 (IL), IL-10, IL-12, C-reactive protein and tumor necrosis factor [12]. A recent murine and *in vitro* study examined the effects of BAC on the cornea and cultivated primary mouse corneo-limbal epithelial cells [15]. The mice received 5 μl BAC topically either once or twice daily for seven days and were randomly assigned BAC concentrations of 0% (phosphate buffered saline controls), 0.05%, 0.1% or 0.2%. Analysis of the murine corneas revealed a dose-dependent BAC toxicity ranging from punctate fluorescein staining to severe epithelial defects. Furthermore, cell contraction, vacuolation and necrosis were noted in cultured corneo-limbal epithelial cells upon exposure to BAC. Upon analysis of 27 common ocular hypotensives, the pH was 4.0–7.4 (mean = 6.25), and the free radical concentration was 0–4.54 mmol/L (mean = 0.66 mmol/L) [16].

Several studies have revealed a higher prevalence of dry eye symptoms among patients using IOP-lowering eye drops when compared to healthy, age matched controls [17–19]. Significant alterations in the Schirmer test [17,20–23], tear film break-up time (TBUT) [17,20–22,24], conjunctival staining [17,20–22], corneal and conjunctival sensitivity [25,26], corneal clarity [27], ocular redness [28,29], lipid layer thickness [30], and tear osmolarity [23] have been found in patients under glaucomatous treatment. Moreover, the use of topical antiglaucoma medications is the most common cause of drug induced cicatrizing conjunctivitis [31]. A recent case series including 23 patients with this diagnosis reported BAC as the most common instigating agent [31]. One case, however, was caused by stabilized oxychloro complex preserved antiglaucoma medication, while another subject reacted to various PF topical medications, including apraclonidine, bimatoprost, dorzolamide with timolol and timolol in isolation. Compared to healthy controls, proteomic analysis revealed upregulation of S100-A8, S100-A9, mammaglobin B and 14-3-3 ζ/δ in patients treated with preserved topical medications [32]. Alterations of clinical parameters among patients treated with preserved glaucoma medications are listed in Table 1. A short term randomized study reported significantly decreased tear film stability 3 h and three days following instillation of preserved carteolol, with no such findings among patients receiving the unpreserved equivalent [33]. Positive correlations between the number of topical IOP-lowering medications administered and the severity of signs and symptoms have been reported [34–37]. Further, a positive correlation between the number of BAC-containing eye drops, Ocular Surface Disease Index (OSDI), TBUT and osmolarity was described [38]. Fukuchi et al. reported superficial punctate keratitis in 51% of 749 eyes treated with IOP-lowering eye drops [39], whereas Rossi et al. found superficial punctate keratitis in 31.7% and ocular surface disease in 41.6% of 233 patients, these data correlated to the daily number of instillations, age, lower IOP values, total BAC exposure, and the Glaucoma Symptom Scale questionnaire [40,41]. No difference in corneal staining was observed in patients treated for five days with either latanoprost/0.02% BAC, travoprost/0.015% BAC or bimatoprost/0.005% BAC [42]. The use of preserved IOP-lowering medications was associated with an increased incidence of ocular signs of dry eye such as conjunctival redness, superficial punctate keratitis and blepharitis [35]. Additionally, both signs and symptoms of dry eye decreased upon changing from preserved eye drops to a preservative-free (PF) alternative [35,43].

Furthermore, an increase of TUNEL + cells was reported in human conjunctival cells among patients treated with BAC-preserved ocular hypotensives compared to healthy, age- and gender-matched controls [44].

A decreased number of sub-basal nerves and impaired corneal sensitivity through esthesiometry were found in patients treated with IOP-lowering eye drops preserved with BAC [45]. However, Kaminski et al. found no changes in mean corneal thickness, corneal endothelial cell count or corneal sensibility following 90 days of treatment with 2% dorzolamide in 20 patients [46]. In addition, latanoprost did not appear to disrupt the corneal epithelial barrier function as measured by fluorophotometric fluorescin uptake in 10 patients treated for 1 month nor in 14 patients treated for 6 months [47]. On the contrary, both BAC preserved and PF timolol caused a significant increase in fluorophotometric corneal fluorescin uptake in 20 healthy volunteers 30 minutes after instillation, indicating corneal epithelial barrier function disruption [48]. This effect was more pronounced in preservative-containing (PC) timolol which also decreased non-invasive break up time (NiBUT), contrary to the PF formulation. Similar findings were reported in patients treated with BAC preserved timolol when compared to untreated glaucomatous patients and healthy controls [49], as well as in patients treated with BAC preserved timolol compared to healthy controls and patients with cataract treated with pyrenoxine [50].

When glaucomatous subjects treated with various PC PGAs were compared to healthy controls, the glaucoma group demonstrated lower keratocyte density and decreased central corneal thickness (515.2 vs 549.6 μm) measured with IVCM [51]. However, a prospective randomized study including 369 glaucoma patients treated with either latanoprost, timolol or latanoprost/timolol fixed combination (FC) for 12 months found no changes in corneal endothelial cell density or central corneal thickness [52]. A prospective study including 66 patients treated with latanoprost and 42 patients treated with latanoprost and timolol (preservative status not described) reported a significant increase in central corneal thickness and corneal hysteresis but no change

Table 1
Alterations of clinical parameters among patients treated with preserved glaucoma medications.

Author/Year/ Reference	Subjects	Controls	Topical formulations	Schirmer test (mm)	TBUT (s)	OSS (Oxford scheme if not otherwise specified)	Symptoms (OSDI if not otherwise specified)	Study design	Additional information
Ramli et al., 2015 [17]	105	102 on no topical medications	Latanoprost (Xalatan), timolol (Timo-Comod), brinzolamide (Azopt), and brimonidine (Alphagan-p)	Abnormal in 39% vs 25%*	Abnormal value increased with number of medications. Abnormal in 90% on BAC drops and 94% on PF.	Present in 63% vs 36%*	Moderate OSDI symptoms in 17% vs 7%*	Cross-sectional	BAC gave OR of 2.94 for abnormal OSDI compared to purite preserved of PF eye drops
Rossi et al., 2009 [19]	61. Grouped 1–3 (G1-G3) according to number of topical medications	20, healthy, age and sex matched (G0)	G1: PGA; G2: betablocker; G3: PGA and FC timolol/ dorzolamide		Abnormal findings: G0:40%; G1: 29.4%; G2: 54.5%; G3: 60%	Abnormal findings: G0: 15%; G1: 33.3%; G2: 43.5%; G3: 50%	Normal OSDI: G0:65%; G1:66.7%; G2:47.8%; G3:50%. GSS avg: G0: 75.9; G1: 87.5; G2: 75; G3: 63.5	Cross-sectional	GSS total mean: G0: 76.9; G1: 85.0; G2: 77.5; G3: 61.8
Leung et al., 2008 [20]	101		0: 8%; 1: 45%; 2: 36%; 3: 10%	Pathological in 61%	Pathological in 78%	Present in 22%	Pathological OSDI in 59%	Cross-sectional	Each additional formulation containing BAC was associated with 2 times higher odds ratio for abnormal staining
Agnifili et al., 2013 [21]	80. Grouped according to number of formulations: G1 PC/PF 15/15; G2 23; G3 27	20 healthy, age and sex matched	G1: PF timolol: 7, PC timolol: 8, PC latanoprost: 8, PF tafluprost: 7; G2: latanoprost/timolol FC: 7, bimatoprost/timolol UF: 7, brimonidine/timolol UF: 4, dorzolamide/timolol FC: 5; G3: bimatoprost/ brimonidine/timolol: 14, latanoprost/timolol/ dorzolamide: 7, bimatoprost/brimonidine/ timolol/dorzolamide: 6	Controls: 18.0* G1PC: 9.4* G1PF: 9.1* G2: 7.2 G3: 6.8	Controls: 12.0* G1PC: 6.1* G1PF: 7.7* G2: 4.1 G3: 3.9	Controls: 0.3* G1PC: 1.9 G1PF: 1.9 G2: 2.3 G3: 2.2	OSDI:Controls: 9.5* G1PC: 18.7* G1PF: 9.4* G2: 30.8 G3: 32.6	Case-control	Contact meibography revealed increased MG dropout among glaucomatous patients. IVCM parameters were significantly worse when comparing groups 2 and 3 to the controls. PC formulations resulted in more morphological changes than PF formulations.
Agnifili et al., 2018 [22]	60 treated with prostaglandin/ timolol FC and 15 treated with latanoprost/timolol UF	15 healthy, age and sex matched	Latanoprost/timolol FC (LTFC): 15; travoprost/ timolol FC (TTFC): 15; bimatoprost/timolol FC (BTFC): 15; PF-BTFC: 15; latanoprost/timolol UF (LTUF): 15	Controls: 18.4*; LTFC: 8.6; TTFC: 9.4; BTFC: 8.1; PF-BTFC: 9.8*; LTUF: 5.2*	Controls: 12.5*; LTFC: 5.8; TTFC: 4.9; BTFC: 5.8; PF-BTFC: 6.2*; LTUF: 3.8*	Controls: 0.3*; LTFC: 1.4; TTFC: 1.5; BTFC: 1.4; PF-BTFC: 0.9*; LTUF: 2.3*	OSDI: Controls: 9.5*; LTFC: 28.5; TTFC: 27.4; BTFC: 28.7; PF-BTFC: 23.7*; LTUF: 35.8*	Cross-sectional	IVCM parameters were worse in LTUF compared to FCs and PF-BTFC. PF formulations showed better IVCM parameters than PC products
Wong et al., 2018 [23]	33 patients with unilateral treatment, fellow eye serving as control		Bimatoprost (24%), latanoprost (24%), travoprost (18%), timolol (3%), betaxolol/latanoprost (3%), bimatoprost/timolol (3%), bimatoprost/ brimonidine/timolol (3%), bimatoprost/dorzolamide/ timolol (9%), brimonidine/ timolol (6%), dorzolamide/	Treated eye 14*, fellow eye 16	NiBUT: treated eye 7.5*, fellow eye 10.1	Treated eye 0, fellow eye 0	OSDI: treated eye 5.0, fellow eye 6.3	Cross-sectional	Bulbar hyperemia score treated eye 1.5*, fellow eye 1.3. No difference in number of expressible glands or meibum grade. No difference in lid wiper epitheliopathy grade. No difference in meibography grade.

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Table 1 (*continued*)

Author/Year/ Reference	Subjects	Controls	Topical formulations	Schirmer test (mm)	TBUT (s)	OSS (Oxford scheme if not otherwise specified)	Symptoms (OSDI if not otherwise specified)	Study design	Additional information
			timolol (3%), timolol/ travoprost (3%)						TFLL grade: treated eye 3, fellow eye 3. Osmolarity treated eye 313*, fellow eye 305
Lee et al., 2019 [30]	30 patients with unilateral treatment, fellow eye serving as control.							Cross-sectional	LLT avg. Glaucoma eye: 64.83*; LLT avg. normal eye 77.26. Correlation between LLT and total glaucoma medications: −0.554*; number of daily drops: −0.543*; treatment duration: −0.689*
Baudouin et al., 1998 [33]	30 healthy volunteers. Examination performed 30, 60 and 180 minutes after instillation and after 3 days of preservative treatment		2% Carteolol with and without BAC	PF baseline/3 days: 25.7/13.3; PC baseline/3 days: 20.6/17.03	PF baseline/3 days: 9.1/8.4; PC baseline/3 days: 10.4/7.7*	PF baseline/3 days: 0.0/0.1; PC baseline/3 days: 0.0/0.1	100 mm visual analogue scale PF/ PC: 2.83 mm/3.66 mm	Randomized, double blind crossover study	
Fechtner et al., 2010 [34]	630 patients from 10 sites						Mean OSDI on 1 formulation: 12.9*; 2 formulations: 16.7; 3 formulations: 19.4	Prospective observational study	
Pisella et al., 2002 [35]	4107 patients at baseline, 1181 made second visit. Multicentre from 249 ophthalmologists		84% used PC, 13% used PF and 3% used both			Baseline presence of SPK: PC: 19%; PF 9%*. Presence of SPK visit 1/2 in PC/ PF crossover: 25.4%/5.3%*	Baseline discomfort upon instillation PC/ PF: 43%/17%*, symptoms between instillations PC/PF: 61%/36%*; PC to PF crossover visit 1/2 discomfort on instillation 57.6%/ 11.7%*, symptoms between instillation 82.7%/35.8%*	Prospective epidemiological study	Baseline presence of conjunctival signs PC/ PF: 49%/26%*; baseline presence of palpebral signs PC/PF: 22%/9%*; PC to PF crossover visit 1/2 palpebral sign 35.7%/14.5%*, conjunctival sign 68.9%/21.9%*
Sahlu et al., 2021 [36]	160 glaucoma patients	160 age and sex matched controls	Timolol (53%), timolol/ dorzolamide (41%), pilocarpine (36%), latanoprost (12%), brimonidine (25%), betaxolol (2%), dorzolamide (1%)	Glaucoma/ control (%): normal 43/48, mild to moderate 16/14, severe 41/38	Glaucoma/ control (%): normal 51*/55, mild to moderate 18/21, severe 31/24	Glaucoma/ control (%): normal 33/50*, mild 56/41, moderate 9/9, severe 1/0	Abnormal OSDI glaucoma/control (%): 30.8/36.1	Cross-sectional	
Labbè et al., 2012 [38]	40 patients, 28 with glaucoma and 12 with OHT. 20 women, 20 men. Mean age 63.92 years		Mean number of molecules 2.15, mean number of instillations 2.50, number of preserved eyedrops 1.87	Mean 14.19 mm. Normal (>10) 50%, mild to moderate (6–10) 35%, severe (<6) 15%	Mean 8.45 s. Normal (>9) 32.5%, mild to moderate (5–9) 57.5%, severe (<5) 10%	Oxford schema: mean 0.45. Normal (0) 60%, mild to moderate (1–2) 40%, severe (>2) 0%	OSDI. Mean 21.03. Normal (<13) 40%, mild to moderate (13–32) 30%, severe (>32) 30%	Cross-sectional (?)	Osmolarity: mOsm/L. Mean 308.8. Normal (<309) 47.5%, mild to moderate (309–328) 35%, severe (>328) 17.5%. Correlations with osmolarity: OSDI:

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Table 1 (*continued*)

Author/Year/ Reference	Subjects	Controls	Topical formulations	Schirmer test (mm)	TBUT (s)	OSS (Oxford scheme if not otherwise specified)	Symptoms (OSDI if not otherwise specified)	Study design	Additional information
									0.486*; TBUT: −0.495*; ST: −0.231; OSS: 0.251
Martone et al., 2009 [45]	84 patients. G2: 14; G3: 9; G4: 20; G5: 9; G6: 12	20 healthy, age-matched controls (G1)	G2: PC betablockers; G3: PF betablockers; G4: PC latanoprost; G5: PC timolol/ latanoprost FC; G6: PC timolol/latanoprost UF	Mean: G1: 16.9*; G2: 12.4; G3: 14.3*; G4: 10.5; G5: 10.3; G6: 9.8.	Mean: G1: 11.8*; G2: 8.2; G3: 11.6*; G4: 8.3; G5: 8.9; G6: 7.7.			Retrospective, single masked clinical study	Esthesiometry: G1: 58.2*; G2: 45.1; G3: 55.8*; G4: 41.2; G5: 49.4; G6: 39.1. IVCM: superficial epithelium, basal epithelium, activated keratocytes, number of nerves, numbers of beadings, reflectivity and tortuosity grades all better in controls
Mastropasqua et al., 2013 [56]	30 treatment naïve glaucoma patients. G1: 15; G2: 15; G3: 15; G4: 15	30 healthy, age and sex matched control	G1: PF tafluprost; G2: PC latanoprost; G3: vehicle of latanoprost including 0.02% BAC; G4: PBS	Mean: BL/1st/ 6th month. G1: 16.68/16.75/ 15.87; G2: 17.06/17.56/ 14.0*; G3: 17.96/ 12.12*/9.06*; G4: 17.06/ 17.56/17.5	Mean: BL/1st/ 6th month. G1: 11.5/12.06/ 12.12; G2: 12.06/12.62/ 10.18*; G3: 12.62/8.43*/ 6.43*; G4: 12.06/ 12.87/13.5		OSDI mean: BL/1st/ 6th month. G1: 4.55/ 4.68/5.85*; G2: 5.46/8.58/12.75*; G3: 4.42/13.51*/ 36.32*; G4: 4.55/ 6.99/5.59	Prospective, randomized, observer masked study	GCD BL/1st/6th month. IVCM: G1: 240.69/ 284.16*/282.8*; G2: 232.65/297.86*/ 227.55*; G3: 237.71/ 205.88*/166.32*; G4: 240.98/238.68/235. IC: G1: 162.10/230.62*/ 237.96*; G2: 164.71/ 221.78*/156.06*; G3: 155.44/139.54*/ 120.76*; G4: 155.31/ 159.06/157.06
Ciancaglini et al., 2008 [57]	27 newly diagnosed, treatment naïve patients. OHT: 12. POAG: 15. Male: 14. Female: 13. G1: 14. G2: 13		G1: PC levobunolol; G2: PF levobunolol					Randomized single masked study	BL/6 months. IVCM GCD: G1: 88.1/25.2*; G2: 90.0/75.4*. IC grading score: G1: 7/ 39*; G2: 9/6*. Index of epithelial regularity: G1: 3/34*; G2: 4/8*
Herreras et al., 1992 [63]	41 patients. G2: 21 under ongoing treatment. G3: 20 treatment naive	40 healthy controls. G1A: aged 41–60; G1B: 61-80	G2: ongoing PC timolol; G3: initiating PC timolol	Percent normal: G1: 100; G2: 33.8*; G3 BL/1/ 2/3 months: 100/60.5*/6.2*/ 6.0*	Percent normal: G1: 100; G2: 4.8*; G3 BL/1/2/ 3 months: 100/ 20.1*/4.3*/4.0	Percent normal: G1: 100; G2: 0.0*; G3 BL/1/ 2/3 months: 100/0.0*/0.0*/ 0.0*		Prospective clinical study	IC. Epithelial cells (% normal): G1: 100; G2: 100; G3 BL/1/2/3 months: 100/100/100/ 100. GCD: G1: 119.9; G2: 44.2*; G3 BL/1/2/3 months: 104.2/86.9*/ 61.2*/48.1
Frezzotti et al., 2014 [64]	40 glaucoma/OHT patients. G1: 20; G2: 20	G3: 20 healthy, age and sex matched control	G1: PF 0.1% timolol maleate gel; G2: 0.5% timolol maleate drops preserved with 0.01% BAC	BL: G1: 17.0; G2: 16.9; G3: 17.6. 12 months: G1: 16.8; G2: 11.3*; G3: 17.8	BL: G1: 11.3; G2: 11.1; G3: 11.5. 12 months: G1: 11.27; G2: 8.12*; G3: 12.1			Randomized clinical study	IVCM GCD: BL: G1: 84.0; G2: 85.38; G3: 86.1. 12 months: G1: 86.83; G2: 48.25*; G3: 88.9. IVCM ELI: BL: G1: 0.3; G2: 0.37; G3: 0.40. 12 months: no numerical data printed. Claim significant increase in G1 and G2, not G3.

(*continued on next page*)

Table 1 (*continued*)

Author/Year/ Reference	Subjects	Controls	Topical formulations	Schirmer test (mm)	TBUT (s)	OSS (Oxford scheme if not otherwise specified)	Symptoms (OSDI if not otherwise specified)	Study design	Additional information
									Claim significant difference between G2 vs. G1 and G3
Zaleska-Zmijewska et al., 2019 [65]	G1: 60 patients with POAG	G2: 30 suspected of having POAG	G1A: 30 treated with PF tafluprost; G1B: 30 treated with PC latanoprost; G2: no POAG treatment	G1A: 12.07*; G1B: 8.27; G2: 11.63*	Percentage <5s: G1A: 10; G1B: 50*; G2: 10	No conjunctival staining %: G1A: 83.3; G1B: 60.0; G2: 76.7. No corneal staining %: G1A: 96.7; G1B: 80.0; G2: 93.3	OSDI: G1A: 17.15; G1B: 23.0; G2: 21.62. Percentage reporting grade 0 on McMonnies scale: G1A: 43.3; G1B: 26.7; G2: 70	Prospective (although single visit), unblinded, single centre study	MMP-9% positive: G1A: 16.7*; G1B: 46.7; G2: 16.7*
Kim et al., 2020 [70]	G1: 67 POAG patients	G2: 47 healthy, age and sex-matched control	Tafluprost + BAC: 11, latanoprost + BAC: 8, bimatoprost + BAC: 2, travoprost + PQ: 2, timolol/ dorzolamide + BAC: 1	Percentage <11 mm: G1: 73.1; G2: 53.2*	Percentage <10s: G1: 82.1; 65.9*	Percentage >0: G1: 34.3; G2: 2.13*	DEQ-5, percentage >5: G1: 62.7; G2: 40.4*	Prospective case-control study	MMP-9, percentage positive: G1: 71.6; G2: 31.9*
Denoyer et al., 2012 [75]	G1: 20 glaucoma patients treated with BAC preserved formulations; G2: 20 glaucoma patients treated with PF formulations				G1: 7.5s; G2: 9.2s	van Blijsterveld index: G1: 1.7*; G2: 0.7		Cross-sectional	Conjunctival hyperemia (0–5): G1: 1.75; G2: 0.45
Zhivov et al., 2010 [82]	20 healthy volunteers		1 eye treated with 0.01% BAC solution (G1) and fellow eye with placebo solution (G2)	BL/Wk1/Wk6/ Wk12/Wk16: G1: 14.8/13.1/14.4/ 13.6/13.7; G2: 15.1/13.4/15.4/ 14.7/13.1	BL/Wk1/Wk6/ Wk12/Wk16: G1: 12.4/11.4/11.7/ 12.7/12.6; G2: 12.8/11.0/11.2/ 12.5/12.7			Randomized, double-blind clinical trial	LC density central cornea. BL/Wk1/Wk6/ Wk12/Wk16: G1: 37.1*/ 74.0/57.9*/94.9/59.7; G2: 59.6/91.3/64.6/ 98.6/80.1. LC density peripheral cornea. G1: 92.3/116/100/144*/ 81.3; G2: 102/118/118/ 120/94.9
Abelson et al., 2003 [96]	39 patients with OAG or OHT		Bimatoprost 0.03% preserved with BAC added to or replaced current treatment			No significant changes		Prospective clinical trial	Hyperemia scores peaked 1 day after the first instillation with consequent consistent decrease. At day 28 conjunctival hyperemia was still elevated while ciliary and episcleral hyperemia had returned to baseline
Alagöz et al., 2008 [97]	73 newly diagnosed POAG patients		G1: bimatoprost, 33; G2: travoprost, 40	BL/day 30/90/ 180: G1: 14.7/ 13.7/13.6/13.2; G2: 14.5/13.5/ 13.6/13.6	BL/day 30/90/ 180: G1: 12.6/ 12.1/11.9/12.7; G2: 12.5/11.8/ 12.2/12.1		Total discomfort score BL/day 30/90/ 180: G1: 0/5/3/2; G2: 0/6/3/1	Randomized, single masked study Case-control	BL/day 30/90/180: total conjunctival hyperemia score: G1: 2/29*/18*/ 14*; G2: 5/31*/22*/ 13*; impression cytology total grading: G1: 4/ 11*/30*/32*; G2: 4/ 10*/13*/24*

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Table 1 (*continued*)

Author/Year/ Reference	Subjects	Controls	Topical formulations	Schirmer test (mm)	TBUT (s)	OSS (Oxford scheme if not otherwise specified)	Symptoms (OSDI if not otherwise specified)	Study design	Additional information
Alves et al., 2014 [98]	G1: 20 glaucoma patients	G2: 24 age and gender matched controls	G1: BAC preserved topical eye drops	G1: 15.58; G2: 24.42	G1: 5.38*; G2: 10.50	G1: 0.60; G2: 0.08	OSDI: G1: 33.60; G2: 12.83		Osmolarity: G1: 301.2; G2: 295.3
Arici et al., 2000 [99]	77 POAG patients	30 healthy, age matched controls	G1: controls, 30; G2: betaxolol, 24; G3: timolol, 27; G4: betaxolol/ dipivefrin, 26; all preserved with BAC	G1: 18.6; G2: 9.6*; G3: 7.4*; G4: 5.68*	G1: 12.5; G2: 8.2*; G3: 6.9*; G4: 8.1*			Case-control	Impression cytology scores were significantly worse in G2, 3 and 4 compared to G1; there were no differences between G2, 3 and 4
Awe et al., 2020 [100]	103 POAG patients treated with BAC preserved medications for at least 6 months	103 healthy, age and sex matched controls	Timolol 24.3%; latanoprost 5.8%; timolol/latanoprost 54.4%; timolol/latanoprost/ pilocarpine 8.7%; timolol/ latanoprost/dorzolamide 2.9%; timolol/latanoprost/ brimonidine 2.9%; timolol/ pilocarpine 1%	Glaucoma*/ control: normal: 69.9%/82.5%; mild to moderate: 14.6%/3.9%; severe: 15.5%/ 13.6%	Glaucoma*/ control: normal: 16.5%/40.8%; mild to moderate: 34.0%/23.3%; severe: 49.5%/ 35.9%	Glaucoma*/ control: normal: 37.9%/68.9%; mild to moderate: 46.6%/27.2%; severe: 15.5%/ 3.9%		Case-control	
Baffa et al., 2008 [101]	21 patients using ocular hypotensives for at least 8 months	20 healthy, age and sex matched controls	Timolol 38.1%; timolol/ brimonidine 14.29%; timolol/pilocarpine 9.52%; pilocarpine 9.52%; latanoprost/brimonidine 4.76%; brimonidine 4.76%; timolol/brimonidine/ bimatoprost 4.76%; timolol/ brimonidine/dorzolamide/ latanoprost 4.76%; timolol/ dorzolamide/pilocarpine/ bimatoprost 4.76%; brinzolamide 4.76%	Glaucoma/ controls: 14.5mm/14.8 mm	Glaucoma/ controls: 6.4s*/ 14.1s	Glaucoma/ controls: 2.9*/ 0.6	Ocular discomfort questionnaire with no difference between groups	Case-control	Glaucoma/controls: lissamine green staining: 1.7*/0.4; no statistical difference concerning impression cytology scores between groups
Bartolome et al., 2018 [102]	211 patients with OAG or OHT	51 healthy age and sex matched controls			NiBUT: no significant correlation with redness score		OSDI weakly correlated with limbal nasal redness score	Authors describe as an observational, prospective, cross-sectional study	Ocular redness score was associated with age, use of prostaglandins and three or more daily instillations
Cui et al., 2016 [103]	30 patients with OAG or OHT, all treated with PGAs		Subjects randomized to receive either vitamin A palmitate eye gel or carbomer eye gel in addition to previous glaucoma treatment. G1: PGA + vitamin A (10); G2: PGA + carbomer (10); G3: PGA alone (10)	BL/1/3/6 months: G1: 13.4/11.3/12.8/ 13.1; G2: 8.9/ 8.6/10.5/9.0; G3: 7.7/9.1/7.1/ 7.3	BL/1/3/6 months: G1: 4.3/ 4.2/4.5/5.0; G2: 5.36/3.8/4.2/ 6.0; G3: 6.6/4.0/ 4.3/3.6*		BL/1/3/6 months: G1: 18.6/12.4/9.49/ 7.2*; G2: 18.7/12.5/ 8.67/3.3*; G3: 12.5/ 12.8/11.51/14.1	Prospective randomized study	No changes in impression cytology or density of epithelial non-goblet cells. Changes in goblet cell density measured with IVCM BL/1/3/6 months: G1: 40.4/33.4/47.6/69.1*; G2: 44.4/44.3/49.2/ 49.8; G3: 59.9/49.4/ 35.4/32.9*
Cvenkel et al., 2015 [104]	G1: 79 glaucoma patients treated with preserved topical medications	G2: 30 sex but not age matched controls with untreated OHT		G1: 10 mm; G2: 10 mm	G1: 6.0s*; G2: 9.5s	G1: 1.0*; G2: 0.0	G1: 11.1; G2: 9.2	Cross-sectional case-comparison	Impression cytology score: G1: 1.0*; G2: 0.6
Di Zazzo et al., 2017 [105]	G1: 15 glaucoma patients	G2: 15 glaucoma patients	G1: treated with topical palmitoylethanolamide in				BL G1/G2: 42.4/ 22.8*	Single masked prospective study	

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Table 1 (*continued*)

Author/Year/ Reference	Subjects	Controls	Topical formulations	Schirmer test (mm)	TBUT (s)	OSS (Oxford scheme if not otherwise specified)	Symptoms (OSDI if not otherwise specified)	Study design	Additional information
			addition to ongoing treatment; G2: treated with topical PF hyaluronic acid in addition to ongoing treatment	BL/1 month: G1: 5/6.4*; G2: 4.6/ 4.8	BL/1 month: G1: 4.3/5.6*; G2: 4.9/4.8				Hyperemia score BL/1 month: G1: 2.2/1.4*; G2: 1.8/1.8
Dogan et al., 2020 [106]	G1: 153 glaucoma patients using preserved eye drops	G2: 110 healthy, age and sex matched controls		G1: 10*mm; G2: 18 mm	G1: 12s*; G2: 16s			Case-control	Central corneal thickness: G1: 536*; G2: 552; central corneal epithelial thickness: G1: 56*; G2: 60
Figus et al., 2014 [107]	60 glaucoma patients treated with 0.03% bimatoprost monotherapy		G1: bimatoprost 0.01% with 0.02% BAC; G2: bimatoprost 0.03% with 0.005% BAC	BL G1: 10.1 mm; G2: 9.8 mm. Authors report significant improvement in G1 from BL at 6 and 12 months and compared to G2, no values reported	BL G1: 9.8s; G2: 9.3s. Authors report significant improvement in G1 from BL at 6 and 12 months and compared to G2, no values reported		Global clinical score improved in G1 from BL to 6 and 12 months with significant difference to G2.	Randomized prospective clinical study	GCD G1 BL/6 months/ 12 months: 351.8/ 425.6*/428.5*
Ghosh et al., 2012 [108]	G1: 300 glaucoma patients	G2: 100 age and sex matched controls	Brimonidine, brimonidine-P, timolol, betaxolol, pilocarpine, brinzolamide, dorzolamide, latanoprost, bimatoprost, travoprost, brimonidine/timolol, dorzolamide/timolol. BAC used as preservative in 93% of prescriptions	Prevalence <5 mm: G1: 13.0%; G2: 9.0%	Prevalence <5s: G1: 57.3%*; G2: 35.0%	Prevalence of corneal staining grade 2–3: G1: 51.3%*: G2: 17.0%	Glaucoma patients had higher prevalence of sticky sensation and crusting on eyelashes while controls had a higher prevalence of burning feeling	Cross-sectional case-comparison	Pathological Schirmer test correlated with duration of therapy and number of meds; TBUT correlated with gender, duration of therapy and number of meds; corneal staining correlated with duration of therapy and number of meds; conjunctival staining correlated with duration of therapy and number of meds; MGD correlated with duration of therapy
Güclü et al., 2021 [109]	G1: 47 glaucoma patients	G2: 48 healthy, age and sex matched controls	BAC preserved latanoprost: 9; dorzolamide/timolol/ BAC: 20; dorzolamide/ timolol/brimonidine/BAC/ purite: 13; latanoprost/ dorzolamide/timolol/ brimonidine/BAC/purite: 5	G1: 8.5; G2: 11.7*	G1: 6.5; G2: 8.7*		G1: 25.3; G2: 16.3*	Case-control	Central corneal epithelial thickness: G1: 48.5; G2: 54.5*; limbal epithelial thickness: G1: 64.1; G2: 76.0*
Horsley et al., 2009 [110]	20 patients treated with BAC preserved latanoprost changed to SofZia preserved travoprost				BL/8 weeks after switch: 2.02/ 6.34*	BL/8 weeks after switch: 2.40/1.38*	BL/8 weeks after switch: 26.31/ 16.56*	Prospective, open label	
Ilechie et al., 2016 [111]	60 healthy patients without glaucoma, OHT or DED		G1: PBS; G2: PF timolol; G3: timolol/0.01% BAC; G4: 0.01% BAC		NiBUT BL/30/ 60/90 minutes: G1: no change; G2: no change; G3: NiBUT			Randomized double blind	

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Table 1 (*continued*)

Author/Year/ Reference	Subjects	Controls	Topical formulations	Schirmer test (mm)	TBUT (s)	OSS (Oxford scheme if not otherwise specified)	Symptoms (OSDI if not otherwise specified)	Study design	Additional information
					decrease with 3.52s* at 90 minutes; G4: BL not given/-2.18s*/-4.46*/-6.28*. No other numerical values given				
Inoue et al., 2003 [112]	110 glaucoma patients		G1: no medications; G2: 1 medication; G3: >2 medications	G1: 16.9; G2: 12.9*; G3: 12.4*	G1: 11.1; G2: 9.4*; G3: 8.4*	G1: 0.39; G2: 0.57; G3: 0.86		Cross-sectional	SPK more common in patients using timolol compared to carteolol. Severity of SPK and TBUT worse in patients using timolol than carteolol. No differences between latanoprost and unoprostone
Inoue et al., 2003 [113]	G1: 19 glaucoma patients with diabetes mellitus	G2: 28 glaucoma patients without diabetes mellitus. No difference between groups regarding age, sex, kind of or number of medications		G1: 11.6; G2: 13.2	G1: 9.1; G2: 9.0	Incidence of SPK G1: 36.1%; G2: 27.7%		Cross-sectional	Mean grade of lipid layer status G1: 2.1; G2: 1.8
Inoue et al., 2018 [114]	43 glaucoma patients		Initially all used latanoprost in the evening and carteolol in the morning. All switched to latanoprost/carteolol FC		BL/1 month/3 months: 7.6/ 8.1*/8.6*	BL/1 month/3 months: 1.2/ 0.6*/0.5*	80.5% of patients preferred the FC	Prospective, single arm, open label study	IOP BL/1 month/3 months: 15.0/15.1/15.0. No changes in blood pressure or pulse rate
Inoue et al., 2020 [115]	30 patients with POAG, NTG or OHT		All patients initially used latanoprost/timolol FC preserved with BAC, changed without washout to latanoprost/carteolol preserved with edetate disodium and boric acid		BL/1 month/3 months: 7.9/ 8.8*/8.9*	SPK BL/1 month/3 months: 2.9/ 1.0*/0.8*		Prospective	No significant change in IOP. Systolic and diastolic blood pressure decreased. No change in pulse rate
Jin et al., 2016 [116]	138 glaucoma patients with DED		Type of medications not described, all treated for 6 months or longer. At inclusion all patients started treatment with diquafosol 6 times daily in both eyes	BL/1 week/1 month/3 months/9 months/1 year: 4.52/6.10*/ 4.86/5.39*/ 5.44*/5.64*	BL/1 week/1 month/3 months/9 months/1 year: 3.79/4.30*/ 4.56*/4.72*/ 4.53*/4.70*	BL/1 week/1 month/3 months/9 months/1 year: 2.84/2.30*/ 2.12*/1.93*/ 1.90*/1.89*	BL/1 week/1 month/3 months/9 months/1 year: 52.17/51.32/ 47.34*/45.61*/ 49.71/48.77*	Prospective	Goblet cell density through impression cytology BL/1 week/1 month/3 months/9 months/1 year: 445.1/ 511.0*/520.5*/504.8*/ 512.4*
Kocabeyoglu et al., 2014 [117]	100 glaucoma patients. G1: 37 patients without conjunctivochalasis	G2: 63 glaucoma patients with conjunctivochalasis	Type of medications not described, all preserved. All used 1–2 medications	G1: 13.3; G2: 7.7*	G1: 10.1; G2: 7.2*	Lissamine green staining G1: 0.3; G2: 1.6*	G1: 6.7; G2: 19.4*	Prospective	Increased severity of conjunctivochalasis associated with more pronounced signs and symptoms of DED
Konstas et al., 2014 [118]	42 OAG patients		G1: travoprost/timolol FC preserved with polyquaternium-1; G2: latanoprost/timolol FC preserved with BAC. Both	G1: 9.95; G2: 9.23*	G1: 5.15; G2: 4.65*	Corneal staining by van Blijsterveld score: G1: 1.53; G2: 1.78*		Prospective, observer masked, active controlled, cross-over, comparison study	24-h mean IOP: G1: 18.9*; G2: 19.3

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Table 1 (*continued*)

Author/Year/ Reference	Subjects	Controls	Topical formulations	Schirmer test (mm)	TBUT (s)	OSS (Oxford scheme if not otherwise specified)	Symptoms (OSDI if not otherwise specified)	Study design	Additional information
			groups treated for three months prior to cross-over						
Kumagami et al., 2014 [119]	27 glaucoma patients treated with PGA switched to tafluprost with 0.001% BAC		G1: latanoprost with 0.02% BAC (10); G2: travoprost with SofZia (17); both groups switched to tafluprost with 0.001% BAC (27) and re-examined at 1 and 2 months		BL/1 month/2 moths: G1: 5.20/ 5.20/5.33; G2: 5.53/5.94/5.88	BL/1 month/2 moths: G1: 3.90/2.80/ 2.56*; G2: 3.53/2.06*/ 1.53*		Prospective	Conjunctival hyperemia: BL/1 month/2 moths: G1: 0.30/0.40/0.33; G2: 0.65/0.47/0.35. IOP BL/ 1 month/2 moths: G1: 12.80/12.80/11.44*; G2: 12.71/12.06/12.59
Lajmi et al., 2021 [120]	155 glaucoma patients		52.3% used ≥2 medications; 42.6% used FC; 80.6% used PGAs; 58.6% used beta-blockers; 80% preserved with BAC; 20% used polyquternium-1 alone	<10 mm in 52.9%	<10 s in 84.5%	SPK present in 69.7%; lissamine green staining in 49.7%; filamentous keratitis present in 2.6%; corneal ulcers present in 1.3%	OSDI defined as normal <13; mild 13–22; moderate 23–32; severe 33–100. Normal: 38.7%; mild: 22.6%; moderate: 16.1%; severe: 22.6%. Score associated with age, treatment duration, number of medications and BAC	Cross-sectional	Prevalence of signs: blepharitis 21.9%; MGD 66.5%; palpebral telangiectasia 43.2%; palpebral eczema 6.5%; keratinization 3.9%; symblepharon 1.3%; conjunctival hyperemia 72.3%; LIPCOF 82.6%; corneal neovascularization 8.4%; corneal hypoesthesia 7.7%; altered tear film 65.8%
Lee et al., 2013 [121]	G1: 50 medically treated glaucoma patients; G2: 31 glaucoma patients post trabeculectomy	G3: 49 controls	G1: ≥1 PC glaucoma medication for ≥6 months; G2: no topical medications, previously treated with topical hypotensives; G3: no topical medications	G1: 9.7; G2: 4.5; G3: 10.7	G1: 3.6; G2: 4.4; G3: 4.9		Symptom score: G1: 23.4; G2: 23.3; G3: 6.5*	Authors describe as prospective case-control study	Osmolarity: G1: 307.0; G2: 307.4; G3: 301.4*
Lee et al., 2016 [122]	G1: 34 patients with OAG and DED	G2: 51 patients with DED without glaucoma	G1: all on PC medications for at least 3 months; G2: no topical medications		G1: 6.2; G2: 6.4	G1: 0.95; G2: 0.77	G1: 21.7; G2: 18.5	Retrospective case-control	LLT: G1: 69.2; G2: 81.1*
Liu et al., 2015 [123]	G1: 30 glaucoma patients	G2: 28 glaucoma patients	G1: sodium hyaluronate; G2: PBS; both groups continued treatment of BAC preserved topical medications	BL/3 months: G1: 3.27/7.33*; G2: 3.76/4.18	BL/3 months: G1: 5.18/10.78*: G2: 4.69/4.87	Fluorescein staining score BL/3 months: G1: 5/1*; G2: 5/5; rose Bengal staining score BL/3 months: G1: 3/ 1*; G2: 3/3	Significantly improved OSDI in G1 with a change from 0 in normal group at BL to 16 in normal group at 3 moths	Prospective	GCD BL/3 months: G1: 29.30/63.06*; G2: 32.66/38.95
Marsovszky et al., 2014 [124]	G1: 19 glaucoma patients; G2: 19 glaucoma patients	G3: 19 healthy age matched controls	G1: BAC preserved travoprost; G2: PQ preserved travoprost	G1: 6.3*; G2: 9.4*; G3: 12.4	G1: 9.3*; G2: 11.3; G3: 12.9		G1: 21.6*; G2: 18.9; G3: 11.7	Case-control	LIPCOF: G1: 2.05*; G2: 1.6; G3: 1.3. central LC: G1: 64.3*; G2: 48.4*; G3: 25.9. peripheral LC: G1: 127.0*; G2: 116.1*; G3: 66.7
Muz et al., 2021 [125]	44 patients newly diagnosed with POAG or OHT		G1: 22 patients treated with BAC preserved latanoprost; G2: 22 patients treated with PQ preserved travoprost	BL/1/3/6/12 months: G1: 13.41/9.45/ 9.91/9.689.77; G2: 9.14*/7.82/	BL/1/3/6/12 months: G1: 6.09/4.36/4.41/ 4.27/3.86; G2: 4.91/3.73/	BL/1/3/6/12 months: G1: 1.73/2.23/ 2.50/2.55/ 2.59; G2: 1.68/	BL/1/3/6/12 months: G1: 45.12/ 55.75/56.66/57.37/ 57.20; G2: 44.15/ 54.42/55.41/56.02/	Prospective randomized study	No inter-group difference in IOP lowering effect

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Table 1 (*continued*)

Author/Year/ Reference	Subjects	Controls	Topical formulations	Schirmer test (mm)	TBUT (s)	OSS (Oxford scheme if not otherwise specified)	Symptoms (OSDI if not otherwise specified)	Study design	Additional information
				7.55/7.09/6.73*. Both groups deteriorated from BL	3.32*/3.55/3.55. Both groups deteriorated from BL	2.32/2.36/ 2.55/2.45. Both groups deteriorated from BL, no inter-group difference	56.58. Both groups deteriorated from BL, no inter-group difference		
Naito et al., 2016 [126]	30 treatment naïve NTG patients		All treated with SofZia preserved travoprost			SPK BL/4/8/12 weeks: 0.7/0.7/ 0.4/0.6		Prospective	Incidence of conjunctival hyperemia SPK BL/4/8/12 weeks: 0.3/0.6*/0.5/0.6*
Nakakura et al., 2012 [127]	36 glaucoma patients previously treated with PGA, betablocker and carbonic anhydrase inhibitor		G1: latanoprost/timolol/ brinzolamide; G2: latanoprost/timolol/ dorzolamide; all BAC preserved			SPK BL/4/12 weeks: G1: 0.80/0.55/ 0.45; G2: 0.38/ 0.13/0.13	No significant differences in questionnaire regarding symptomatology	Randomized, open prospective multicentre study	Hyperemia score BL/4/ 12 weeks: G1: 0.16/ 0.16/0.11; G2: 0.17/ 0.17/0.11
Nebbioso et al., 2012 [128]	62 POAG patients		Initially treated with dorzolamide/timolol FC, all switched to brinzolamide/ timolol FC	Before/after switch: 7.5/9.2*	Before/after switch: 3.4/3.5	Before/after switch: 3.0/ 2.4*	Before/after switch: 19.8/14.6*	Prospective, single masked study	Ocular protection index before/after switch: 0.4/ 0.5*
Nebbioso et al., 2013 [129]	38 POAG patients with DED treated with BAC preserved medications		G1: placebo; G2: oral forskolin/rutin supplementation	BL/1 month: G1: 4.7/4.5; G2: 4.4/ 9.7*	BL/1 month: G1: 4.7/4.6; G2: 4.5/ 7.9*		BL/1 month: G1: 35.6/36.0; G2: 38.7/ 22.5*	Randomized, double blind study	Ocular protection index BL/1 month: G1: 1.7/ 1.6; G2: 1.6/2.3*
Rahmatnejad et al., 2018 [130]	28 treatment naïve glaucoma patients and 27 glaucoma patients treated with BAC preserved latanoprost monotherapy		G1: 28 treatment naïve; G2: 27 previously treated. Both groups treated with BAC preserved latanoprost in the right eye and SofZia preserved travoprost in the left eye	BL right/left, 1 month right/left, 2 months right/ left: G1: 21.8/ 23.0, 20.8/21.5, 21.8/24.2; G2: 16.1*/18.2*, 15.5*/19.3*, 16.0*/18.9*. Significance between groups, not treatments	BL right/left, 1 month right/left, 2 months right/ left: G1: 8.1/8.7, 7.1/8.0, 6.2/6.7; G2: 6.3*/5.9*, 5.6*/5.5*, 6.7/ 6.0. Significance between groups, not treatments	Corneal staining score BL right/left, 1 month right/ left, 2 months right/left: G1: 0.7/0.8, 0.7/ 0.8, 1.0/1.3; G2: 1.0/1.0, 1.3/1.2, 1.0/1.0	BL right/left, 1 month right/left, 2 months right/left: G1: 10.8/11.7, 10.8/ 11.7, 11.1/11.8; G2: 9.8/14.2, 7.0/8.1, 6.6/11.2	Authors describe as prospective, open-label, nonrandomized cohort study	
Roberti et al., 2018 [131]	39 glaucoma patients treated with various preserved topical medications		G1: PF 0.4% hyaluronic acid and 0.5% taurine (19); G2: 0.2% hyaluronic acid (20). Both groups continued hypotensive treatment	BL/1month/ 3months: G1: 12.05/11.21/ 12.78; G2: 11.25/11.10/ 11.40	BL/1month/ 3months: G1: 7.63/7.15/8.84*; G2: 7.25/7.35/ 7.65	BL/1month/ 3months: G1: 1.31/0.84*/ 0.89*; G2: 1.15/1.05/1.00	BL/1month/ 3months: G1: 43.76/ 37.79/34.47*; G2: 40.82/38.55/39.78	Prospective, randomized, single masked, parallel study	GCD BL/1month/ 3months: G1: 50.89/ 53.47/67.47*; G2: 49.65/52.15/53.60
Rossi et al., 2009 [132]	309 POAG and OHT patients		Initially all used concomitant latanoprost and timolol, switched to travoprost/timolol FC		BL/6months: 8.4/9.2*			Prospective, multicentre, observational cohort study	IOP BL/6months: 18.3 mmHg/16.3 mmHg*
Ruangvaravate et al., 2018 [133]	109 glaucoma patients		Mean number of topical medications was 3.2, all preserved	Percentage abnormal: 73.4%	Percentage abnormal: 99.1%	Percentage abnormal: 32.1%	Percentage abnormal: 38.5%	Single centre cross-sectional	Number of topical instillations and OSDI correlated with fluorescin staining

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Table 1 (*continued*)

Author/Year/ Reference	Subjects	Controls	Topical formulations	Schirmer test (mm)	TBUT (s)	OSS (Oxford scheme if not otherwise specified)	Symptoms (OSDI if not otherwise specified)	Study design	Additional information
Russ et al., 2013 [134]	33 treatment naïve patients with OAG or OHT		G1: travoprost/timolol FC (11); G2: bimatoprost/ timolol FC (11); G3: latanoprost/timolol FC (11); all preserved	BL/3 months: G1: 8.95/7.18*; G2: 12.45/9.95*; G3: 13.77/9.09*	BL/3 months: G1: 11.95/9.54*; G2: 11.90/11.18; G3: 13.86/11.68*	Lissamine green staining BL/3 months: G1: 2.36/6.0*; G2: 2.0/3.27*; G3: 0.86/1.41	BL/3 months: G1: 33.74/39.94*; G2: 29.76/30.30; G3: 8.06/11.16	Prospective, randomized, single-blind, multicentre, parallel group interventional study	HLA-DR BL/3 months: G1: 39.50/88.77*; G2: 126.86/95.79; G3: 176.79/172.95. IL-6 BL/ 3 months: G1: 54.48/ 150.52*; G2: 93.97/ 120.59; G3: 171.75/ 198.16. IOP BL/3 months: G1: 24.72/ 14.0*; G2: 22.32/ 12.10*; G3: 20.32/ 11.59*
Saade et al., 2015 [135]	31 POAG patients	30 healthy, sex, but not age matched controls	G1: POAG patients treated with at least 1 topical medication; G2: controls		Percentage abnormal: G1: 68%; G2: 17%*	Percentage abnormal lissamine green staining: G1: 67%; G2: 3%*	G1: 18.97; G2: 6.25*	Prospective, controlled cohort study	Intensity index (drops per week x duration of therapy) correlated with OSDI
Saini et al., 2015 [72]	16 glaucoma patients	15 healthy, age and sex matched controls	G1: BAC preserved timolol/ brimonidine FC with or without latanoprost; G2: controls. Cyclosporine A added to G1	BL/6 months G1: 7.28/10.78*; G2: 12.86	BL/6 months G1: 8.67/12.24*; G2: 11.80	BL/6 months: conjunctival staining score G1: 3.38/1.50*; G2: 0.84. Corneal staining score G1: 5.19/1.81*; G2: 1.10	BL/6 months G1: 30.63/14.76*; G2: 6.02	Prospective comparative study	Corneal sensation BL/6 months G1: 4.64/4.94*; G2: 5.07. IVCM parameters: nerve number: G1: 3.58; G2: 5.40*; nerve length: G1: 1101.44; G2: 1963.70*; nerve density: G1: 8811.35; G2: 12273.15*. Nerve density in G1 increased to 10335.13* after treatment
Saini et al., 2017 [136]	25 glaucoma patients	25 healthy, sex, but not age matched controls	G1: 10 eyes on timolol/ brimonidine, 8 eyes on latanoprost/brimonidine, 32 eyes on timolol/latanoprost; G2: controls	G1: 7.63; G2: 12.86*	G1: 9.44; G2: 11.80*	Conjunctival staining: G1: 5.06; G2: 0.84*; corneal staining: G1: 5.7; G2: 1.1*	G1: 35.89; G2: 6.02*	Prospective comparative study	Corneal sensitivity: G1: 4.68: G2: 5.07. IVCM parameters: nerve number: G1: 3.58; G2: 5.40*; nerve length: G1: 1101.44; G2: 1963.70*: nerve density: G1: 6883.94; G2: 12273.15*
Shimazaki et al., 2000 [137]	40 glaucoma patients included, 9 lost to follow-up		G1: timolol/0.005% BAC; G2: unoprostone/0.01% BAC	BL/12 weeks/24 weeks: G1: 12.5/ 9.5*/7.82*; G2: 16.4/17.1/16.1	BL/12 weeks/24 weeks: G1: 7.33/ 6.09/4.5; G2: 7.77/7.56/6.78. Values excluded for patients with diabetes or DED	No change, values not given		Prospective, randomized, open-label study	
Su et al., 2021 [138]	44 newly diagnosed, treatment naïve glaucoma patients		Latanoprost preserved with 0.02% BAC	BL/1 month/4 months: 9.42/ 7.11*/6.84*	NiBUT first BL/1 month/4 months: 8.2/8.13/8.31. Percent of area <10s: 6.21/6.44/ 9.11*	BL/1 month/4 months: 0.07/ 0.20*/0.26*	BL/1 month/4 months: 6.85/4.23*/ 5.28	Prospective	Conjunctival hyperemia increased significantly the first month, but stabilized after 4 months
Suzuki et al., 2018 [139]			Initially all treated with latanoprost/timolol FC for		BL/Wk6/Wk12: G1: 4.63/4.32/	SPK: BL/Wk6/ Wk12: G1: 0.8/	Significant decrease in eye irritation	Prospective, randomized,	

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Author/Year/ Reference	Subjects	Controls	Topical formulations	Schirmer test (mm)	TBUT (s)	OSS (Oxford scheme if not otherwise specified)	Symptoms (OSDI if not otherwise specified)	Study design	Additional information
	115 glaucoma patients, 109 completed the study		four weeks, then randomized to: G1: latanoprost/timolol/0.02% BAC FC (51); G2: tafluprost/ timolol/0.001% BAC (58)		4.34; G2: 4.13/ 4.27/4.32	0.8/0.7; G2: 0.7/0.5/0.4*	score and eye pain score for G2 at 6 weeks, no other significant differences in symptomatology	multicentre, controlled study	Hyperemia score BL/ Wk6/Wk12: G1: 0.3/ 0.3/0.3; G2: 0.3/0.3/0.4
Thygesen et al., 2000 [140]	35 glaucoma patients		G1: latanoprost (16); G2: timolol (19). Preservative status not described	BL/day 1/day 28: G1: 13.0/12.5/ 13.9; G2: 15.0/ 10.6*/12.8*	BL/day 1/day 28: G1: 17.5/19.1/ 16.4; G2: 17.8/ 15.8/13.8*	Increase of Rose-Bengal staining with latanoprost, not timolol		Prospective, randomized, double-masked, parallel group study	No change in impression cytology
Tomic et al., 2013 [141]	40 newly diagnosed POAG patients		BAC preserved travoprost		BL/3 months: 11.70/8.30*		BL/3 months: 31.63/ 44.41*	Prospective	IOP BL/3 months: 23.80/16.78*
Vagge et al., 2015 [142]	20 glaucoma patients		All treated with PGA preserved with BAC in monotherapy. All started with PF artificial tears	BL/1 month/3 months: 6.3/ 7.88/7.82*	BL/1 month/3 months: 5.9/ 6.45*/9.45*			Prospective, single-arm study	Central corneal sensitivity BL/1 month/ 3 months: 4.26/4.79*/ 5.58*. IVCM: number of fibres: 6.15/7.28*/ 8.75*; tortuosity: 1.79/ 2.14*/2.03; reflectivity: 2.18/2.34/2.42*
Valente et al., 2011 [143]	50 patients with OAG or OHT screened with OSDI, 26 included due to pathological values		All treated with at least one topical medication, all BAC preserved	12.6	6.1	Fluorescin staining: 2.3; lissamine green staining: 5.5	Normal: 48%; mild-moderate: 18%; severe: 34%	Prospective, cross-sectional	Lissamine green conjunctival staining correlated with number of instillations. Correlation between OSDI and staining in patients on betablocker monotherapy
Whitson et al., 2010 [144]	106 glaucoma patients		Initially all on latanoprost monotherapy, randomized to: G1: bimatoprost/0.005% BAC; G2: latanoprost/0.02% BAC; travoprost/SofZia		BL/1Wk/ 1month/ 3months: G1: 9.1/9.0/10.7/ 9.7; G2: 8.6/8.8/ 9.5/9.2; G3: 7.9/ 7.7/8.1/9.7	BL/1Wk/ 1month/ 3months: G1: 0.59/0.61/ 0.63/0.71; G2: 0.70/0.74/ 0.45/0.47; G3: 0.48/0.47/ 0.52/0.36		Prospective, randomized, multicentre, investigator masked, parallel study	Hyperemia BL/1Wk/ 1month/3months: G1: 0.74/0.95/0.83/0.80; G2: 0.74/0.78/0.78/ 0.74; G3: 0.86/0.94/ 0.98/0.98
Yalvac et al., 1995 [145]	40 POAG patients	20 healthy, age and sex matched controls	G1: controls (20); G2: timolol/BAC (20); G3: timolol/dipivefrin/BAC	G1: 12.7; G2: 10.4*; G3: 8.2*	G1: 14.4; G2: 8.0*; G3: 6.9*			Case-control	GCD: G1: 43; G2: 17*; G3: 15*. Impression cytology gradings G1<G2<G3
Yoon et al., 2019 [146]	10 glaucoma patients with toxic corneal epitheliopathy refractory to PF artificial tears		All treated with BAC preserved topical medications and initiated on autologous serum eyedrops	10.5 at baseline, authors describe no change, no value given	BL/1 month: 3.1/ 5.4*	BL/1 month: 7.7/1.8*	BL/1 month: 25.5/ 10.5*	Prospective	Corneal sensitivity BL/1 month: 4.6/5.8*; decrease in MMP-9 levels
Zhu et al., 2015 [147]	80 POAG patients	20 healthy, age and sex matched controls	G1: controls; G2: betablocker/0.005% BAC; G3: alpha adrenergic agonists/0.005% BAC; G4:	G1: 11.9*; G2: 7.7; G3: 9.6; G4: 6.2; G5: 6.2	G1: 11.0*; G2: 4.7; G3: 5.0; G4: 3.8; G5: 3.3		G1: 8.1*; G2: 12.0; G3: 18.6; G4: 17.5; G5: 31.4	Cross-sectional observational study	IVCM parameters: epithelial cell density: G1: 4299; G2: 4457; G3: 4518; G4: 4547; G5: 4432; GCD: G1: 408*;

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Table 1 (*continued*)

Author/Year/ Reference	Subjects	Controls	Topical formulations	Schirmer test (mm)	TBUT (s)	OSS (Oxford scheme if not otherwise specified)	Symptoms (OSDI if not otherwise specified)	Study design	Additional information
			PGAs/0.015% BAC; G5: combination therapy						G2: 267; G3: 264; G4: 336; G5: 252; dendritic cell density: G1: 15*; G2: 20; G3: 20; G4: 15; G5: 20; subepithelial fibre diameter: G1: 15*; G2: 20; G3: 20; G4: 15; G5: 20
Kobia-Acquah et al., 2021 [148]	100 glaucoma patients		Betablockers: 34; PGAs: 4; betablocker and PGA: 35; betablocker and alpha agonist: 8; betablocker, alpha agonist and PGA: 19; BAC only: 70; BAC and sodium chlorite: 30	Normal/mild/ moderate/ severe: 45/20/ 22/13	Normal/mild/ moderate/severe: 13/57/30/0		Normal/mild/ moderate/severe: 19/23/34/24	Cross-sectional study	Association between age, OSDI, Schirmer test and TBUT; association between number of medications, OSDI and Schirmer test
Aihara et al., 2015 [149]	103 glaucoma patients		Initially all treated with PGA + timolol, all switched to PGA + brimonidine			SPK BL/Wk4/ Wk12: 1.1/1.1/ 0.9*		Prospective, open-label multicentre study	BL/Wk4/Wk12: IOP: 15.7/14.3*/14.0*; no change in conjunctival hyperemia or conjunctival follicle formation
Kamath et al., 2007 [150]	88 treatment naïve glaucoma patients		G1: timolol (21); G2: pilocarpine (22); G3: brimonidine (22); G4: latanoprost (23); all BAC-preserved	Number <10 mm BL/6months/ 12months: G1: 0/ 0/8; G2: 0/0/3; G3: 0/0/2; G4: 0/ 0/2	Number <10s BUT BL/ 6months/ 12months: G1: 0/ 3/9; G2: 0/2/6; G3: 0/1/4; G4: 0/ 1/4			Prospective	Number with <50 goblet cells/HPF BL/6months/ 12months: G1: 0/6/12; G2: 1/4/7; G3: 1/2/5; G4: 0/2/4
Kuwayama et al., 2014 [151]	3901 glaucoma patients		G1: treatment naïve monotherapy (2213); G2: switch from prior treatment (1438); G3: add-on therapy (250); all started with PC tafluprost			Corneal fluorescin staining BL/ 1month/ 2months: G1: 0.06/0.07/ 0.06; G2: 0.12/ 0.09*/0.09*; G3: 0.08/0.09/ 0.08		Prospective, post-marketing observational study	Conjunctival hyperemia score BL/1month/ 2months: G1: 0.07/ 0.18*/0.15*; G2: 0.15/ 0.13*/0.13*; G3: 0.06/ 0.16*/0.10
Onoe et al., 2021 [152]	22 glaucoma patients		Initially all treated with brinzolamide and brimonidine, all switched to brinzolamide/brimonidine FC			SPK BL/ 1month/ 3months: 1.18/ 0.83/0.55*		Prospective, multicentred clinical trial	Hyperemia score BL/ 1month/3months: 0.43/ 0.61/0.48. No change in IOP
Uusitalo et al., 1994 [153]	71 glaucoma patients		G1: 35; G2: 36. Randomized to two different formulations of 0.5% timolol/2% pilocarpine FC	BL/10Wks: G1: 13.7/12.7*; G2: 13.9/9.3*		No ocular surface staining in any group at any point		Randomized, double blind study	IOP BL/10Wks: G1: 24.6/17.6*; G2: 23.4/ 17.2*. Mean pulse rate significantly decreased in both groups, no change in blood pressure in either group
Wong et al., 2018 [154]	51 glaucoma patients		All initially treated with latanoprost/0.02% BAC and				Subjective symptoms score BL/1month/		BL/1month/3months: bulbar hyperemia: 1.7/

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Table 1 (*continued*)

Author/Year/ Reference	Subjects	Controls	Topical formulations	Schirmer test (mm)	TBUT (s)	OSS (Oxford scheme if not otherwise specified)	Symptoms (OSDI if not otherwise specified)	Study design	Additional information
			switched to tafluprost/ 0.001% BAC		BL/1month/ 3months: 3.0/ 4.1*/3.7*	BL/1month/ 3months: 6.9/ 3.2*/3.3*	3months: 2.2/1.2*/ 0.7*	Prospective, single centre study	1.6*/1.5*; palpebral hyperemia: 1.8/1.8/ 1.5*; IOP: 14.4/14.2/ 14.3

BAC: benzalkonium chloride; BL: baseline; BUT: break up time; DED: dry eye disease; DEQ-5: Dry Eye Questionnaire-5; ELI: epithelial layer irregularity; FC: fixed combination; G: group; GCD: goblet cell density; GSS: Glaucoma Symptom Scale; HLA-DR: human leukocyte antigen-DR; HPF: high power field; IC: impression cytology; IL: interleukin; IOP: intraocular pressure; IVCM: in vivo confocal microscopy; LC: Langerhans cell; LIPCOF: lid-parallel conjunctival folds; LLT: lipid layer thickness; MGD: meibomian gland dysfunction; mm: millimetre; MMP-9: matrix metalloproteinase-9; NiBUT: non-invasive break up time; NTG: normal tension glaucoma; OHT: ocular hypertension; OR: odds ratio; OSDI: Ocular Surface Disease Index; OSS: ocular surface staining; PBS: phosphate buffered saline; PC: preservative containing; PF: preservative free; PGA: prostaglandin analogue; POAG: primary open angle glaucoma; PQ: polyquaternium-1; s: seconds; SPK: superficial punctate keratitis; TBUT: tear film break-up time; TFLL: tear film lipid layer; UF: unfixed combination; Wk: week; *: indicates statistical significance of at least $p < 0.05$.

in corneal resistance factor [53]. In a two-year follow-up study latanoprost was demonstrated to decrease the conjunctival thickness from 201.45 μm to 167.81 μm [54]. No significant difference was found in patients treated with carteolol with measures of 198.76 μm at baseline and 201.23 μm after two years. However, the preservative status in the included medications is not described. The conjunctival thickness in 49 glaucomatous eyes treated with various topical medications mostly preserved with BAC was 182.76 μm, significantly less than the 235.02 μm measured in the 51 healthy control eyes [55]. In a study employing IVCM and impression cytology, the authors reported a transient increase of goblet cell density in patients treated with BAC-preserved latanoprost after one month, returning to baseline at the 6-month follow-up [56]. However, in patients treated with PF-PGA, the effect of increased goblet cell density remained, also at the 6-month measurements. Conversely, levobunolol has been found to cause a significant decrease in goblet cell density, which is exacerbated by the addition of BAC [57]. Cennamo et al. compared changes to the conjunctiva of 20 human specimens treated with BAC preserved ocular hypotensives and 20 healthy, age- and sex-matched controls through a ferning test and analysis of impression cytology with a light microscope as well as a scanning electron microscope [58]. The mean grades of the ferning test, impression cytology from light microscopy and scanning electron microscopy were 2.52, 2.52 and 2.55, respectively, for the treatment group and were significantly lower in the control group (1.22, 1.25 and 1.15, respectively). The treatment duration was correlated with a reduced number of microvilli identified with scanning electron microscopy. Impression cytology from patients treated with timolol, latanoprost, dorzolamide, timolol/latanoprost FC and timolol/dorzolamide FC revealed higher cytology scores with a significant degree of metaplasia when compared to healthy controls [59]. These changes were more profound in patients treated with fixed combinations when compared to monotherapy. Conversely, no correlation between cytological grading, age, sex, type of medication, number of formulations or duration of treatment was found in conjunctival impression cytology samples from patients treated with betaxolol (24 eyes), levobunolol (20 eyes), timolol (32 eyes), pilocarpine (22 eyes), betablocker and pilocarpine (52 eyes), betablocker and dipivefrin (34 eyes) or maximum therapy (32 eyes) when compared to 51 healthy control eyes [60]. However, the degree of conjunctival metaplasia was significantly increased in eyes exposed to topical therapy. A later study compared the corneal epithelial thickness and number of microvilli in 16 patients treated with PC topical hypotensives and 6 healthy, age matched controls [61]. Following an initial decrease in corneal epithelial thickness, the authors report a gradual increase in this thickness as the number of microvilli decreased. Impression cytology was taken from 20 eyes of 12 patients prior to and after 1, 3 and 6 months of treatment with BAC preserved latanoprost [62]. There was no change in non-goblet epithelial cell density, but a significant reduction in cell size was observed. Goblet cell density increased during the first month and gradually returned to baseline with a longer treatment duration. Conjunctival impression cytology and mucus staining was performed on human specimens in a prospective study involving healthy controls, patients under ongoing treatment with BAC-preserved timolol and patients initiated with the same medication [63]. Patients under ongoing treatment demonstrated a significantly lower goblet cell density compared to healthy controls as well as a deteriorated mucus layer. In the cohort initiated on topical medications impression cytology analysis disclosed a gradual decrease of goblet cells and progressive degradation of the mucus layer. Moreover, both groups receiving BAC preserved timolol had worse Schirmer test, tear film stability and ocular surface staining. A later, randomized, prospective study comparing PF-timolol gel to BAC-preserved timolol drops and healthy controls reported a significant decrease in goblet cell density among patients receiving PC formulations [64]. However, no significant differences were found in patients in the PF cohort when compared to both baseline and controls.

Patients receiving BAC-preserved latanoprost presented with increased levels of the inflammatory marker matrix metallopeptidase 9

(MMP-9) as well as signs and symptoms of DED when compared to patients treated with PF tafluprost [65]. When comparing biomarkers in eyes treated with BAC preserved latanoprost or bimatoprost both medications caused an upregulation of MMP-2, MMP-9 and tissue inhibitors of metalloproteinase (TIMP)-1 when compared to non-glaucomatous controls with cataract [66]. The levels of MMP-1 were 1404, 1304, and 1079 pg/ml in bimatoprost, controls and latanoprost, respectively. Moreover, the eyes treated with latanoprost had an increased expression of cytokines related to tissue remodelling whereas the eyes treated with bimatoprost had an increased expression of cytokines associated with allergic reactions. Similarly, upregulation of MMP-2, MMP-3, MMP-9, TIMP-1, TIMP-2 and TIMP-3 were noted upon comparing 25 glaucoma patients treated with various BAC-preserved topical medications to 7 healthy controls [67]. Moreover, expression of MMP-1 and MMP-3 correlated with duration of treatment with pilocarpine. However, Pradhan et al. found no difference in the expression of MMP-2 or MMP-9 when comparing concentrations in the aqueous humour of glaucomatous patients treated with PGAs and non-glaucomatous controls [68]. A study examining the effects of BAC preserved latanoprost in patients with glaucoma compared to healthy controls including a murine study arm reported increased levels of MMP-9 and decreased levels of TIMP-1 in both humans and mice exposed to treatment [69]. These findings were corroborated in a later study, where the authors also reported a higher expression of MMP-9 with an increasing number of medications containing BAC [70]. An extracellular matrix metalloproteinase inducer and human leukocyte antigen (HLA)-DR were reportedly increased in the conjunctiva of 18 glaucoma patients treated with BAC preserved ocular hypotensives for at least six months when compared to eight healthy controls through impression cytology and flow cytometry [71]. These results were more pronounced in patients receiving higher doses of BAC. The same study had an *in vitro* arm which indicated that cyclosporin A could inhibit the inflammatory effects of BAC; these findings were corroborated clinically [72]. Furthermore, rebamipide demonstrated a protective effect against ocular surface damage induced by BAC preserved latanoprost and timolol [73]. In an *ex* vivo and *in* vitro study Pisella et al. compared the effects of BAC-preserved latanoprost and timolol to those of unpreserved timolol and healthy controls through impression cytology [74]. They reported an increased expression of HLA-DR and intercellular adhesion molecule-1 as well as a decreased MUC-5AC in the BAC receiving cohorts, more so in the PC-timolol group. No difference was described between healthy controls and patients treated with unpreserved timolol. In the *in vitro* arm of the study, a human conjunctiva-derived cell line was exposed to either latanoprost or timolol preserved with 0.02% BAC or 0.02% BAC alone. All three preparations caused apoptosis, although interestingly more so in the isolated BAC group. The authors hypothesize that the active pharmaceuticals may have a protective effect against the deleterious effect of BAC on conjunctival cells. Another study evaluating the inflammatory effects of BAC reported upregulation of the chemokine CX3CL1 and HLA-DR among patients receiving BAC preserved glaucoma medications when compared to patients on PF formulations, possibly through the TNF-α pathway [75]. The two chemokine receptors CCR5 and CCR4 were used as markers of the T helper 1 and T helper 2 cells, respectively [76]. The authors reported upregulation of both in patient groups treated with topical antiglaucomatous agents compared to healthy controls. However, overexpression of the HLA-DR class II antigen only reached statistical significance among patients on a multidrug regimen compared to controls. Comparison of cytokine levels in tears of 21 patients treated with PC ocular hypotensives and untreated healthy controls revealed increased concentrations of IL-1β, IL-6, TNF-α, T helper 1 associated interferon-γ and IL-2 as well as T helper 2 associated IL-4, IL-5 and IL-10 in the tears of treated eyes [77]. Upregulation of HLA-DR was described in patients on monotherapy with BAC-preserved latanoprost, betaxolol or timolol when compared to healthy controls [78], in patients treated with BAC-preserved latanoprost compared to controls instilling BAC-preserved artificial tears [79], and in patients treated with PC latanoprost, bimatoprost and travoprost [80]. Moreover, latanoprost preserved with BAC might increase the number of eosinophils, decrease the number of lymphocytes and cause alterations in both the ferning test and impression cytology in glaucomatous patients with allergic conjunctivitis [81]. In a randomized, double-blind clinical trial 20 healthy volunteers were treated with 0.01% BAC in one eye and placebo in the fellow eye three times daily for 12 weeks [82]. Langerhans cell density in the central and peripheral corneal epithelium was measured through confocal laser-scanning microscopy at baseline and after 1, 6, 12 and 16 weeks. In the BAC group, an increased Langerhans cell density was seen at 6 and 12 weeks in the central and peripheral cornea, respectively. The Langerhans cell density also increased in the peripheral cornea of the placebo group at week 12, although not to the same extent as in the BAC group. All values returned to normal after four weeks wash-out. A reversal of upregulated fibroblasts and inflammatory cells in human conjunctiva following topical antiglaucoma therapy was also reported following preoperative cessation of medical therapy and topical steroid treatment for 30 days [83]. Conversely, the number of collagen fibres in human eyes treated with latanoprost was significantly decreased in those treated with timolol but not when compared to controls [84]. Moreover, the amount of amorphous material was increased in both treatment groups compared to controls (but less in latanoprost when compared to timolol). MMP-1, MMP-3, TIMP-2, and TIMP-3 were upregulated in the epithelial and stromal cells of latanoprost-treated eyes, whereas timolol-treated eyes demonstrated infiltration of inflammatory cells and macrophages. A reduction of pro-inflammatory lipid mediators by as much as 50% was reported in patients treated with topical IOP lowering medications following trabeculectomy [85]. Recently, a Polish research team examined the effect of PGAs and BAC on parameters of oxidative stress in the tear film [86]. Compared to healthy controls, evidence of increased oxidative stress was found among patients receiving PGAs, a finding that was more profound in patients receiving PGAs containing BAC. The same research team also examined the effect of PF timolol as well as BAC-preserved brimonidine and timolol on the biomarkers of oxidative stress in the tear film [87]. In this study, the authors reported significant differences between patients treated with preserved medications vs those treated with PF timolol and healthy controls. In a later study the authors reported increased activity of superoxide dismutase and catalase as well as decreased content of sulfhydryl groups in patients treated with BAC preserved dorzolamide and brinzolamide when compared to healthy controls [88]. Additionally, the levels of oxidative stress were lower in patients treated with PF dorzolamide and controls compared to those treated with preserved formulations.

Among patients requiring maximum topical glaucoma therapy to lower IOP, dual maximum therapy (two fixed-drug combinations) has been shown to be non-inferior to triple maximum therapy (one fixed-drug combination and two single preparations) concerning IOP control; however, signs and symptoms of DED are significantly better with dual therapy [89,90].

Being a lipophilic molecule, BAC has the potential to penetrate and accumulate in deeper ocular structures. Mass spectrometric studies have revealed the presence of BAC in the trabecula and lens in both animal and human specimens after prolonged exposure [91,92]. In a combined animal and human experiment Baudouin et al. examined the histological and inflammatory changes in the conjunctiva and trabeculae of patients and rats treated with various ocular hypotensives [93]. The authors reported a dose-dependent infiltration of cells expressing inflammatory and/or fibroblastic markers in both the conjunctiva and trabeculae of human specimens. In rats, isolated BAC and BAC preserved timolol demonstrated similar changes, although, not in the PF or control cohort. A histological examination of conjunctival biopsies demonstrated a time- and dose-dependent effect on epithelial hyperplasia, keratinization and infiltration of lymphocytes, macrophages, fibroblasts, and mast cells in patients treated with preserved antiglaucomatous medications when compared to controls [94]. These findings do not agree with those

of another group comparing the histological changes in the conjunctiva of glaucoma patients treated with maximal medical therapy and healthy controls, in which no significant differences in any cell line were found [95].

3.2. Effect of preservative-free topical glaucoma medications on the ocular surface

Although the cumulative scientific evidence indicates worsening signs and symptoms with the use of BAC, it appears that several PF medications may also have deleterious effects on the ocular surface. A fluorophotometric study found a significant but transient decrease in tear production due to treatment with PF timolol in 24 patients [155]. Increased expression of HLA-DR, IL-6, IL-8 and IL-10 have been described as a result of unpreserved timolol, although not to the same extent as in PC formulations [156]. A recent study compared the expression of inflammatory cytokines in the tears of glaucoma patients treated with PC and PF ocular hypotensives to patients with DED and healthy controls [157]. The glaucoma cohort had significantly higher IL-6, TNF-α and vascular endothelial growth factor (VEGF) as well as lower IL-4 when compared to the DED group. When comparing glaucoma patients to controls, only IL-6 reached statistical significance. Between DED and controls, IL-1β, IL-6 and IL-10 were increased while VEGF were decreased in the DED group. Subgroup analysis found significantly increased levels of the pro-inflammatory and pro-apoptotic IL-1β in patients applying preserved medications compared to the PF cohort.

Several studies have revealed detrimental changes in the clinical parameters of glaucoma patients treated with PF compounds when compared to controls [17,21,22,158,159], whereas others have not [45]. Clinical alterations of the ocular surface among patients treated with unpreserved glaucoma medications are listed in Table 2. A prospective, randomized cross-over study demonstrated a superior IOP-lowering effect of PF bimatoprost when compared to PF latanoprost, although PF latanoprost was associated with slightly lower hyperaemia scores [160].

Multiple clinical studies have reported significant worsening of signs and/or symptoms among patients receiving BAC-containing eye drops compared to patients using PF medications [17,35,45,159,161–166]. However, Aptel et al. found no differences in efficacy or subjective tolerability when comparing BAC-preserved latanoprost to PF latanoprost in a 12 week follow up study with cross over between medications at 6 weeks [167]. Similarly, when comparing BAC-preserved bimatoprost/timolol to the PF equivalent in a randomized 12-week follow-up study, Goldberg et al. reported a comparable self-reported safety profile between the two and noninferiority of the PF formulation regarding IOP-lowering capability [168]. Four recent studies demonstrated an improvement of subjective and objective findings by the change from PC-PGA to PF-PGA [169–172]. A reduction in anterior chamber flare from 6.75 photon counts per millisecond (ph/ms) at baseline to 5.78, 5.41 and 5.50 ph/ms at 1, 2 and 3 months respectively (all $p < 0.05$) was reported in 22 patients changed from BAC preserved to PF latanoprost [173]. Similar findings with improved signs and symptoms of DED were found in a prospective study performed by Iester et al. comparing the change from PC to PF betablockers [174]. The reported changes in clinical signs at baseline vs. three-months were: eyelid erythema (0.46–0.13), conjunctival hyperemia (0.97–0.33), follicular hyperplasia (0.36–0.08), TBUT (9.82–11.5) and Schirmer test (13.46–15.41). Upon evaluating the effects of changing from BAC preserved latanoprost to PF tafluprost 158 patients experiencing ocular signs and/or symptoms of DED were included in a multicentre study that comprised 12 centres based in Finland, Sweden, and Germany [175]. No changes in IOP were recorded. Compared to baseline, the number of patients reporting various symptoms at 12 weeks were: irritation/burning/stinging (56.3%–28.4%), foreign body sensation (49.4%–27.1%), tearing (55.1%–27.1%), itching (46.8%–26.5%) and dry eye sensation (64.6%–39.4%). Percentage of patients with abnormal clinical parameters: TBUT (94.9%–71.6%), corneal fluorescin staining (81.6%–40.6%), conjunctival fluorescin staining (84.2%–43.2%), blepharitis (60.1%–40.6%), conjunctival hyperemia (84.2%–60.0%) and Schirmer test (71.5%–59.4%). All changes in subjective and clinical parameters reached statistical significance. Moreover, a reduction in the number of patients with increased values of HLA-DR positive epithelial cells were reported at six weeks; this reduction was not significant at the final control. The proportion of patients expressing an abnormal level of MUC-5AC-positive goblet cells improved at both 6- and 12-week follow-ups. In a multicentre study by Pisella et al., 4107 patients were included, among whom 956 underwent treatment modification and were seen at a second visit [35]. Seven hundred and eighty patients were initially on PC medications and switched to a PF formulation. Patient reported symptoms such as discomfort upon instillation and at least one symptom in-between instillations went from 57.6% and 82.7% to 11.7% and 35.8%, respectively. Clinical signs were as follows: palpebral sign (35.7%–14.5%), conjunctival sign (68.9%–21.9%) and superficial punctate keratitis (25.4%–5.3%). A second multicentre study included 9658 glaucomatous patients, 6083 of whom were seen at a second visit after modification of the treatment regime [176]. Among patients changing from PC to PF formulations a reduction in pain or discomfort from 52.4% to 7.8% was reported. Clinical signs of DED were changed as follows: anterior blepharitis (26.38%–8.71%), posterior blepharitis (9.13%–2.80%), eczema (11.00%–2.93%), hyperemia (64.36%–15.99%), follicles (21.13%–5.28%), fluorescin staining (13.65%–2.48%) and superficial punctate keratitis (28.99%–5.66%). Thirdly, 721 patients were included in a prospective multicentre study, 64.8% of whom the antiglaucomatous topical treatment was changed to PF latanoprost [177]. Subsequent to this alteration, a reduction in signs and symptoms of dry eye as well as the use of artificial tears was described. A prospective, multicentre clinical audit evaluating the effect of changing from PC to PF formulations reported findings contrary to the aforementioned trials [178]. The authors noted a significant reduction in symptom score and the number of patients with decreased TBUT when comparing baseline values to follow-up measurements. However, this reduction was observed in both patients switched to PF formulations and in those kept on PC medications with no significant between group differences. Nevertheless, patients who converted to unpreserved eyedrops experienced a decreased use of artificial tears. Finally, another recent multicentre study involving 577 patients reported significant improvement of IOP as well as signs and symptoms of DED among patients demonstrating insufficient response to betablocker or PGA monotherapy following transition to PF tafluprost/timolol fixed combination [179]. The number of participants treated with PC medications prior to transition was not described.

3.3. Impact of dry eye disease on the intraocular pressure

Multiple studies have shown reduced IOP upon treatment of DED in glaucoma patients, occasionally to an extent that allows for a decrease in the number of medications required for IOP control [202–205]. IOP reduction was seen following addressing DED through punctal plugs [202,206], change to PF equivalents [203,205], PF lubricants, eyelid hygiene and antibiotics [203–205], and non-steroidal anti-inflammatories or cyclosporine A [203]. Some authors have hypothesized that the association between an inflamed ocular surface and uncontrolled IOP may be due to penetration of inflammatory mediators from the surface to the deeper ocular structures or possibly due to direct detrimental effects of drugs or preservatives on the trabecular meshwork, sclera and episclera [203,205,207]. Such hypotheses are supported by a study by Baudouin et al. which described loss of trabecular cells and signs of inflammation in surgically excised trabecular meshwork of patients treated with PC IOP-lowering eye drops and induction of apoptosis and inflammation of trabecular cells exposed to BAC *in vitro* [207]. Furthermore, in that study the authors demonstrated that

Table 2
Alterations of clinical parameters among patients treated with unpreserved glaucoma medications.

Author/Year/ Reference	Subjects	Topical formulations	Schirmer test (mm)	TBUT (s)	OSS (Oxford scheme if not otherwise specified)	Symptoms (OSDI if not otherwise specified)	Study design	Additional information
Benitez-del-Castillo et al., 2019 [157]	G1: 41 POAG patients; G2: 30 DED patients; G3: 36 healthy, age and sex matched controls	Latanoprost + BAC; bimatoprost + BAC; travoprost + PQ; PF tafluprost	G1: 7.82*; G2: 4.26*; G3: 13.25	G1: 6.14*; G2: 4.35*; G3: 14.24			Prospective observational cohort study	Cytokines G1/G2/G3: IL1β 11.3/11.9*/9.6; IL4: 20.2*/21.8/19.7; IL6: 27.5*/18.0*/ 12.1; IL10: 4.0/5.3*/ 3.9; TNFα: 17.9*/ 14.3/16.3; VEGF: 609.3*/367.8*/581.6. NS: IL2, IL5, IL8, IL12, GMCSF, IFNγ. PC/PF: IL1β: 13.7*/4.9. Rest NS
El Hajj Moussa et al., 2018 [158]	32 POAG patients	G1: Bimatoprost + BAC: 8; G2: latanoprost + BAC: 7; G3: travoprost + PQ: 8; G4: PF-tafluprost: 9			Percentage with superficial keratitis: G1: 50; G2: 57.1; G3: 37.5; G4: 33.3	OSDI. G1: 21.76; G2: 32.13; G3: 10.68*; G4: 25.6	Prospective, open label, single centre study	Adverse effects in percentage: conjunctival hyperemia: G1: 75; G2: 71.4; G3: 50; G4: 100. Follicular conjunctivitis: G1: 25; G2: 57.1; G3: 37.5; G4: 55.5. iris hyperpigmentation*: G1: 0; G2: 28.6; G3: 0; G4: 0. Eyelash growth*: G1: 12.5; G2: 57.1; G3: 25; G4: 0
Kuppens et al., 1995 [159]	20 patients with POAG/ OHT	Timolol + BAC, crossover to PF-timolol		Number <10s: PC: 4; PF: 5			Crossover clinical study	Mean tear turnover measured with fluorophotometry: PC: 10.7; PF: 13.2*
Stalmans et al., 2016 [160]	67 glaucoma/ OHT patients	Before study: PC bimatoprost: 28; PC latanoprost: 28. Randomized to PF equivalents after washout with crossover after 3 months					Prospective, randomized, investigator masked, crossover clinical trial	No IOP efficacy difference from screening. PF bimatoprost gave 1.6 mmHg* lower IOP than PF latanoprost. PF bimatoprost gave higher hyperemia scores 0.85* vs. 0.71.
Katz et al., 2010 [161]	678 POAG/ OHT patients	All treated with latanoprost + BAC before study. At study start randomized to: G1: continue latanoprost + BAC (n = 335); G2: travoprost + SofZia (n = 343)			Absence of staining at 12 weeks: G1: 40.0%; G2: 37.1%	OSDI classes: <13: normal; 13–22: mild; 23–32: moderate: >32 severe. No between group difference at 12 weeks. Mild group at 12 weeks: G2: 11.6*; G1: 14.4.	Prospective, double-masked, randomized control trial at 66 clinics	Patients treated with latanoprost + BAC for >24 months prior to study more likely to improve to normal OSDI if in G2 (47.9%*) than in G1 (33.9%)
Rouland et al., 2013 [162]	402 POAG/ OHT patients	All treated with latanoprost + BAC prior to study. Randomized to G1: latanoprost + BAC (n = 189) or G2: PF latanoprost (n = 213)				Subjective ocular symptom score at day 84: G1: 0.46; G2: 0.18*	Prospective, randomized, investigator masked parallel group trial	Hyperemia score at day 84: G1: 2: 21.5%, 3–4: 7.6%; G2*: 2: 16.5%, 3–4: 4.9%. No difference between groups regarding OSS, anterior chamber flare, folliculopapillary conjunctivitis or palpebral abnormality
Aptel et al., 2019 [163]	242 glaucoma/ OHT patients	All treated with BAC preserved latanoprost/ timolol FC prior to study. Randomized to G1: PF latanoprost/ timolol (n = 127);			No difference between groups at day 84	Percentage of patients reporting improvement of total symptom score upon instillation at	Randomized, parallel group, investigator masked trial	No difference between groups regarding conjunctival hyperemia at day 84. No difference between groups in IOP lowering effect

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Table 2 (*continued*)

Author/Year/ Reference	Subjects	Topical formulations	Schirmer test (mm)	TBUT (s)	OSS (Oxford scheme if not otherwise specified)	Symptoms (OSDI if not otherwise specified)	Study design	Additional information
		G2: remain on BAC preserved latanoprost/ timolol (n = 115)				day 84: G1: 45.9*; G2: 33.6. Improvement during the day: G1: 41.0; G2: 38.2.		
Aptel et al., 2016 [167]	30 glaucoma/ OHT patients	Randomized to receive either PC or PF latanoprost with crossover after 6 weeks				No difference in reported symptoms	Randomized, investigator masked, crossover clinical trial	No difference in objective ocular findings through slit lamp examination. No difference in IOP lowering capability
Goldberg et al., 2014 [168]	561 glaucoma/ OHT patients	Randomized to PF (n = 278) or PC (n = 283) bimatoprost/ timolol				No difference in reported symptoms.	Randomized, parallel group study	Ocular adverse events reported in 33.1% of PF group and 33.7% of PC group. Only significant clinical difference was skin hyperpigmentation: PF group: 4.0%*; PC group: 1.1%. No reported differences in any other clinical parameter. No difference in IOP lowering effect
Lopes et al., 2019 [169]	11 POAG patients	Initially: PC bimatoprost (6), PC travoprost (2), PC latanoprost (3). All changed to PF tafluprost and re-evaluated after 6 weeks	BL/6 weeks: 5.09/4.36	BL/6 weeks: 6.68/5.5	BL/6 weeks: 6.27/3.04*	OSDI (only 10 questions) BL/ 6 weeks: 35.27/17.95*	Prospective observational study	
El Ameen et al., 2019 [170]	82 glaucoma/ OHT patients	Initially: 36.6% PF latanoprost, 30.5% PC latanoprost, 19.5% PC travoprost, 13.4% PC bimatoprost. 30/52 (57.7%) of patients on PC PGA switched to PF latanoprost due to intolerance		NiBUT: no difference between PF and PC at BL or 6 months after switch		BL symptoms on instillation: PF latanoprost 11.5%, PC latanoprost 38.1%*, PC travoprost 42.9%*, PC bimatoprost 33.3%. At least one symptom 6 months after switch PF vs. PC: 12% vs. 44%*	Observational cross sectional study	No difference in TMH between PF and PC at BL or after 6 months. Hyperemia at BL: PF latanoprost: 2.08; PC latanoprost: 2.50*; PC travoprost: 2.67*; PC bimatoprost: 2.68*. Hyperemia after switch, BL/6 months: PC latanoprost 2.36/ 2.15*; PC travoprost 2.56/2.20*; PC bimatoprost 2.79/ 2.31*. No significant change in IOP after switch
Iester et al., 2014 [174]	132 POAG patients	Initially all treated with PC betablocker, all changed to PF timolol	BL/1 month/ 3 months: 13.46/ 14.72*/ 15.41*	BL/1 month/3 months: 9.82/ 10.9*/11.5*		OSDI BL/1 month/3 months: 30.69/21.53*/ 15.75*	Prospective, longitudinal, open labelled study	BL/1 month/3 months: Eyelid erythema: 0.46/ 0.23*/0.13*; conjunctival hyperemia: 0.97/ 0.58*/0.33*; follicular hyperplasia: 0.36/ 0.16*/0.08*. No difference in IOP after switch
Uusitalo et al., 2010 [175]	158 glaucoma/ OHT patients	Initially all treated with PC latanoprost, switched to PF tafluprost and re-evaluated after 6 and 12 weeks	Percentage pathological at BL/6 weeks/12 weeks: 71.5/ 61.5*/59.4*	Percentage pathological at BL/6 weeks/ 12 weeks: 94.9/76.9*/ 71.6*	Percentage pathological corneal staining at BL/6 weeks/ 12 weeks: 81.6/52.6*/ 40.6*	Percentage pathological at BL/6 weeks/12 weeks: irritation/ burning/ stinging: 56.3/ 30.8*/28.4*; foreign body sensation:	Open label multicentre clinical trial	HLA-DR BL/12 weeks: 61.2/52.0*; MUC-5AC BL/12 weeks: 3.8/6.7*

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Table 2 (*continued*)

Author/Year/ Reference	Subjects	Topical formulations	Schirmer test (mm)	TBUT (s)	OSS (Oxford scheme if not otherwise specified)	Symptoms (OSDI if not otherwise specified)	Study design	Additional information
						49.4/28.8*/ 27.1*; tearing: 55.1/25.6*/ 27.1*; itching: 46.8/25.6*/ 26.5*; dry eye sensation: 64.6/35.3*/ 39.4*		
Jaenen et al., 2007 [176]	9658 glaucoma patients	Initially: PC: 74%; PF: 12%; PC/PF combination: 10%; unknown: 4%. 6083 patients had a change in treatment regime and seen at a second visit			Staining present in percentage of patients changing from PC to PF visit 1/visit 2: 13.65/2.48*	Percentage of patients presenting symptoms after changing from PC to PF visit 1/visit 2: pain or discomfort during instillation: 52.42/7.82*; foreign body sensation: 49.44/8.37*; stinging or burning: 53.77/8.97*; dry eye sensation: 43.16/11.18*; tearing: 31.14/ 10.13*; eyelid itching: 26.95/ 5.41*	Multicentre cross sectional epidemiological study	Percentage of patients presenting signs after changing from PC to PF visit 1/visit 2: anterior blepharitis: 26.38/8.71*; posterior blepharitis: 9.13/ 2.80*; eczema: 11.0/ 2.93*; hyperemia: 64.36/15.99*; follicles: 21.13/5.28*; superficial punctate keratitis: 28.99/5.66*
Goldberg et al., 2015 [178]	375 glaucoma patients enrolled, completion rate 64%	280 patients were switched to BAC free medication (although not PF)		Described significant improvement of TBUT in both BAC free and BAC groups from BL with no difference between groups. Values not presented		Significant decrease of McMonnies dry eye questionnaire in both groups from BL with no difference between groups	Prospective clinical audit	Significant association between change of treatment and reduction of ocular lubricants
Oddone et al., 2020 [179]	577 glaucoma/ OHT patients	PGA or betablocker monotherapy initially, switched to PF tafluprost/ timolol FC	BL: 10.0. Described no significant change, values not presented	BL: 6.0. Authors report an average increase by 1s per each later visit*. This difference was also reported for patients on BL PGA, but not for those on BL betablockers	BL/4 weeks/ 12 weeks/6 months: 0.76/0.55*/ 0.54*/0.47*	30.8% reported improved symptoms, 59.3% reported no change, 9.8% reported increased severity	Prospective multicentre study	IOP BL/4 weeks/12 weeks/6 months: 21.5/16.2*/15.7*/ 15.8*
Aihara et al., 2012 [180]	114 patients included initially, 67 completed the study	Changed from BAC preserved latanoprost to SofZia preserved travoprost			SPK present in number of eyes at BL/1 month/3 months/12 months: 36/ 9*/9*/19*		Prospective, open-label multicentre study	Hyperemia decreased gradually at 3 and 12 months. IOP BL/12 months: 14.9 mmHg/ 14.3 mmHg*
Bourne et al., 2019 [43]	123 patients with OAG or OHT, 114 patients completed the study	G1: BAC preserved bimatoprost/ timolol: 71; G2: PF bimatoprost/ timolol: 43. All changed to PF tafluprost/timolol	Abnormal findings at BL G1/G2: 61.8%/64.4%	Abnormal findings at BL G1/G2: 68.4%/71.1%	Abnormal findings at BL G1/G2: 82.9%/93.3%		Open label phase IV clinical study	Authors describe significant improvement of signs and symptoms of DED in both groups after switching medications

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Table 2 (*continued*)

Author/Year/ Reference	Subjects	Topical formulations	Schirmer test (mm)	TBUT (s)	OSS (Oxford scheme if not otherwise specified)	Symptoms (OSDI if not otherwise specified)	Study design	Additional information
Campagna et al., 1997 [181]	20 OAG patients	Changed from BAC preserved timolol to PF timolol		BL/1/2/3 months: 7.9s/ 8.3s/9.1s*/ 9.3s*	Rose Bengal staining at BL/1/2/3 months: 1.49/1.37/ 0.83*/0.94*	The authors report improved symptoms after medication change	Prospective, single blind study	Impression cytology scores at BL/1/2/3 months: 1.52/1.31*/ 0.94*/0.89*
Delval et al., 2013 [182]	G1: 63; G2: 67	All initially treated with preserved latanoprost and showing signs of intolerance. Randomly divided into G1 (PF timolol gel) and G2 (BAC preserved latanoprost)		Presence of pathological finding day 0/ day 84: G1: 53.9%/38.0% *; G2: 48.0%/ 40.0%	Presence of SPK day 0/ day 84: G1: 48.1/19.1*; G2: 54.8/32.8	The authors report significant improvement of global symptom score after change to PF timolol	Prospective, phase IV randomized study	Conjunctival hyperemia day 0/day 84: G1: 64.9/2.7*; G2: 56.2/25.7; blepharitis day 0/day 84: G1: 50.7/15.1*; G2: 43.8/ 32.9
Duru et al., 2020 [183]	21 treatment naïve POAG or OHT patients	G1: PF brimonidine (21 eyes); G2: brimonidine preserved with purite (21 eyes)	BL/1 month: G1: 12.23/ 11.33; G2: 11.8/10.71	BL/1 month: G1: 9.95/ 6.38*; G2: 9.38/5.76*		Burning sensation upon instillation significantly higher in PF formulation	Prospective randomized study	No difference in IOP lowering efficacy.
Fogagnolo et al., 2015 [184]	40 newly diagnosed, treatment naïve glaucoma patients	G1: PF tafluprost (20); G2: BAC preserved latanoprost (20)	BL/3/6/9/12 months: G1: 16.8/15.7/ 14.3/17.8/ 16.9; G2: 16.6/15.9/ 18.1/17.8/ 18.0	BL/3/6/9/12 months: G1: 7.7/7.6/7.8/ 7.1/7.3; G2: 7.7/7.1/7.5/ 7.1/7.0	SPK n = yes BL/3/6/9/12 months: G1: 0/2/83/3; G2: 2/5/0/5/ 1		Randomized prospective study	Both groups reduced IOP by 3.6–4.2 mmHg, no difference between groups. According to IVCM, patients treated with latanoprost developed branching pattern of sub-basal nerves and beading. Both groups developed increased activation of anterior stromal keratocytes, reaching significance at 3 months for G2 and 6 months for G1
Hagras et al., 2021 [185]	30 newly diagnosed POAG patients treated with monotherapy	G1: PF tafluprost to BAC preserved latanoprost (15); G2: BAC preserved latanoprost to PF tafluprost. Cross-over after 2 months, 1 month wash-out period		TBUT: 0= >10s; 1 = 5–10s; 2= <5s; 3 = immediate. G1 PF/PC: 0.67/ 1.5*; G2 PC/ PF: 1.7/0.93*	Corneal erosion 0–3: G1 PF/PC: 0.14/0.57*; G2 PC/PF: 0.53/0.2*	Gritty sensation, redness and watering at all times worse in patients receiving PC latanoprost. No difference in blurring of vision	Prospective, randomized cross-over study	Conjunctival hyperemia: G1 PF/PC: 0.85/1.35*; G2 PC/ PF: 1.06/0.46*
Hommer et al., 2018 [186]	30 POAG or OHT patients treated with preserved PGA for at least 6 months	All patients switched to PF tafluprost		BL/4 weeks/ 12 weeks: 5.1/ 7.2*/10.1*	BL/4 weeks/ 12 weeks: 1.8/1.4*/0.7*	BL/4 weeks/12 weeks: OSDI: 9.3/6.1/6.7; DEQS: 11.4/ 5.7*/4.7*	Prospective, observer masked	Tear film thickness BL/4 weeks/12 weeks: 4.7/5.0*/4.8
Kim et al., 2021 [187]	51 patients with OAG or OHT	G1: BAC preserved latanoprost (26); G2: PF latanoprost (25)		BL/4 weeks/ 12 weeks: G1: 6.0/6.03/5.57; G2: 6.57/ 5.22/5.16	Corneal staining BL/4 weeks/12 weeks: G1: 0.88/1.00/ 0.96; G2: 0.84/0.80/ 0.72	BL/12 weeks: G1: 17.07/ 13.22; G2: 17.33/8.25	Randomized, multicentre, parallel-grouped, investigator-blind, phase 4 clinical trial	Hyperemia BL/4 weeks/12 weeks: G1: 2.12/2.38/2.46; G2: 1.92/2.40/1.01*. G2 demonstrated better adherence and less stinging/burning sensation
Kurna et al., 2014 [188]	43 patients with glaucoma	G1: PF timolol (8); G2: timolol/ benzododecinium bromide (7); G3: latanoprost/BAC (7); G4: bimatoprost/BAC (7); G5: travoprost/ BAC (7); G6:	BL/1 week/1 month/3 months/6 months/12 months: G1: 11.53/13.73/ 10.2/11.67/ 11.33/11.27; G2: 12.86/ 13.07/12.93/	BL/1 week/1 month/3 months/6 months/12 months: G1: 14.57/11.67/ 10.27/11.0/ 11.47/12.07; G2: 14.5/ 12.21/12.36/	Numbers not given. Authors describe increased corneal staining in G1 and G2 at 3, 6 and 12 months. Nasal		Prospective	G1 and G2 had squamous metaplasia in superior and inferior conjunctiva at all time points. G5 had squamous metaplasia at 3 months, G6 at 3 and 6 months. All groups except G4 had decreased goblet cells

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Table 2 (*continued*)

Author/Year/ Reference	Subjects	Topical formulations	Schirmer test (mm)	TBUT (s)	OSS (Oxford scheme if not otherwise specified)	Symptoms (OSDI if not otherwise specified)	Study design	Additional information
		brimonidine/ purite (7)	11.86/11.29/ 12.0; G3: 16.14/13.71/ 13.71/13.14/ 15.5/14.0; G4: 10.93/ 10.93/9.79/ 11.14/11.29/ 10.79; G5: 10.93/9.43/ 10.5/8.79/ 9.43/9.14; G6: 11.86/ 11.21/11.21/ 13.43/12.5/ 11.43	10.21/10.71/ 10.21; G3: 18.0/18.86/ 18.0/18.5/ 18.64/19.71; G4: 16.5/ 14.14*/ 14.36*/15.36/ 16.5/18.29; G5: 19.29/ 16.57*/ 15.64*/ 16.43*/ 15.64*/ 16.21*; G6: 16.0/16.71/ 17.36/16.57/ 17.64/18.21	staining was increased in G1 at 1 month, G1 and G2 at 3 months, G2 at 6 months, G1 and G4 at 12 months			at one or more time points
Konstas et al., 2017 [189]	43 OAG patients	G1: BAC preserved latanoprost; G2: PF tafluprost; G3: PF tafluprost and PF dorzolamide/ timolol FC	G1: 8.2; G2: 9.1*; G3: 8.2	G1: 6.0; G2: 6.7*; G3: 6.1	Corneal staining van Blijsterveld score: G1: 2.2; G2: 1.3*; G3: 1.7*		Randomized, observer masked, cross-over, comparison study	Mean 24-h IOP: G1: 22.2; G2: 21.9*; G3: 17.3*
Lee et al., 2017 [190]	20 OAG and NTG patients	G1: PF tafluprost switched to tafluprost/0.001% BAC; G2: tafluprost/0.001% BAC switched to PF tafluprost. Switch after 6 months	BL/1/3/6/7/ 9/12 months: G1: 5.80/ 2.62/4.22/ 4.60/4.83/ 5.37/4.60; G2: 3.33/ 5.66/5.28/ 5.14/4.40/ 6.85/5.00	BL/1/3/6/7/ 9/12 months: G1: 5.80/ 3.25*/4.33/ 5.00/4.83/ 5.00/3.60; G2: 4.55/5.44/ 5.00/4.42/ 3.60/4.14/ 4.75	Cornea erosions BL/ 1/3/6/7/9/ 12 months: G1: 0.30/ 0.25/0.22/ 0.40/0.50/ 0.50/0.60; G2: 0.55/ 0.33/0.28/ 0.14/0.80/ 0.42/0.25	Subjective discomfort: BL/1/3/6/7/ 9/12 months: G1: 0.70/ 1.87*/1.11/ 0.80/0.50/ 0.25/0.60; G2: 1.33/1.55/ 1.85/1.14/ 1.60/1.28/ 0.87*	Randomized, prospective cross-over study	
Manni et al., 2005 [191]	20 glaucoma patients	G1: PF timolol switched to BAC preserved timolol; G2: BAC preserved timolol switched to PF timolol. Patients treated for 60 days with each drug, 3-week washout between		BL/1month/ 2months/BL/ 1month/ 2months: G1: 8.8/8.5/9.0/ 9.0/7.2*/7.2*; G2: 8.9/7.5*/ 7.6*/9.2/9.0/ 8.9	Authors report no significant changes in any group, numbers not given		Prospective, randomized, cross-over, single masked study	IL-1β levels BL/ 1month/2months/BL/ 1month/2months: G1: 49.9/46.9/57.1/51.6/ 59.8*/95.5*; G2: 32.4/53.2*/88.5*/ 36.3/43.4/46.1
Martinez-de-la-Casa et al., 2017 [192]	40 glaucoma patients and 39 healthy age and sex matched controls	G1: BAC preserved latanoprost (20); PF latanoprost (20); G3: control (19)		NiBUT: G1: 11.37; G2: 8.84		G1: 6.44; G2: 11.22	Authors describe as prospective observational study	IL-2, IL-5, IL-10, IL-12, IL-13, IL-15, IL-17, fibroblast growth factor basic, PDGF-BB and TNF-α were upregulated in G1, no such changes were seen in G2
Murugesan et al., 2019 [193]	77 glaucoma patients and 30 healthy controls	G1: BAC preserved ocular hypotensives; G2: BAC free ocular hypotensives; G3: controls	G1: 11.97; G2: 13.0; G3: 32.33*	G1: 6.65; G2: 6.88; G3: 13.51*	G1: 3.30; G2: 3.32; G3: 0*		Prospective case-control study	SP: G1: 9.06; G2: 10.15; G3: 3.70*; NGF: G1: 256.07; G2: 216.04; G3: 154.26*; CGRP: G1: 2.95; G2: 2.87; G3: 3.88*; VIP: G1: 1.18; G2: 1.12; G3: 2.66*; NPY: G1: 1.79; G2: 1.70; G3: 3.39*
Perez-Bartolome et al., 2017 [194]	211 glaucoma patients and 51 healthy, age and sex matched controls	G1: PF medications; G2: BAC preserved medications; G3: PQ and BAC preserved medications; G4: controls		NiBUT: G1: 10.41; G2: 9.48; G3: 9.98; G4: 10.37	Percentage with staining present: G1: 36.36; G2: 68.1*; G3: 79.4*; G4: 33.33	G1: 5; G2: 9.1; G3: 12.5; G4: 2.5	Authors describe as prospective, observational, cross-sectional study	LTMH: G1: 290.16; G2: 277.71; G3: 285.42; G4: 291.69
Rolle et al., 2012 [195]	75 POAG or OHT patients	Initially all treated with timolol				BL/3months after change:	Prospective	No difference in IOP lowering capability

(*continued on next page*)

Table 2 (*continued*)

Author/Year/ Reference	Subjects	Topical formulations	Schirmer test (mm)	TBUT (s)	OSS (Oxford scheme if not otherwise specified)	Symptoms (OSDI if not otherwise specified)	Study design	Additional information
		preserved with 0.01% BAC, all changed to PF timolol gel	BL/3months after change: 9.42/14.31*	BL/3months after change: 8.25/12.0*		burning/ stinging/ itching: 23%/ 14%*; eye dryness: 35%/ 12%*; foreign body sensation: 42.6%/15.4 *; blurred vision: 13.8%/ 18.5%; mean percentage: 28.6%/15.5%*		
Rolle et al., 2017 [196]	51 patients with POAG or OHT and 20 healthy, age and sex matched controls	G1: PF tafluprost (27); G2: PF timolol (24); G3: controls (20)	G1: 16.48; G2: 14.13; G3: 15.63	G1: 10.09; G2: 10.31*; G3: 12.12		OSDI: G1: 10.0; G2: 12.01; G3: 0* GSS: G1: 85.88; G2: 84.79; G3: 99.75*	Single-masked, observational, cross-sectional study	IVCM parameters: basal epithelial cell density: G1: 5700; G2: 5745; G3: 5535*. Stromal reflectivity: G1: 2.11; G2: 2.17; G3: 1.65*. Number of sub-basal nerves: G1: 4.33; G2: 4.23; G3: 4.78*. Sub-basal nerve tortuosity: G1: 2.17; G2: 2.0; G3: 1.3*. Sub-basal nerve reflectivity: G1: 2.07; G2: 2.04; G3: 1.8. Endothelial cell density: G1: 2476.17; G2: 23.94.13; G3: 2360.80
Ruangvaravate et al., 2020 [197]	30 glaucoma patients	All initially treated with PGAs except tafluprost. Randomized to: G1: tafluprost/ 0.001% BAC in one eye (30); G2: PF tafluprost in one eye (30)	BL/24 weeks: G1: 5.45/ 6.18; G2: 7.63/7.13	BL/24 weeks: G1: 5.21/ 7.45*; G2: 5.38/8.10*	BL/24 weeks: G1: 0.97/ 0.87; G2: 0.93/0.87		Prospective, randomized, investigator-masked, single-blinded, open-label study	Conjunctival hyperemia BL/24 weeks: G1: 1.27/1.40; G2: 1.27/1.37. No changes in IOP
Saito et al., 2021 [198]	22 POAG and OHT patients	All treated with PGA initially, randomized to: G1: BAC free carteolol/ latanoprost FC (11); G2: BAC preserved latanoprost/ timolol FC (11)			Corneal staining BL/ 1month/ 2months: G1: 1.4/0.3/0.5; G2: 1.4/0.8/ 0.6	Subjective symptoms score without change for both groups	Prospective, randomized, single-centre, open-label, cross-over	G2: lower heart rate at all time-points
Villani et al., 2016 [199]	G1: 100 POAG patients; G2: 50 controls	BAC preserved: 72; PQ preserved: 8; PF: 20; betablocker: 36; PGA: 14; combination therapy: 50	G1: 10.77; G2: 12.08. BAC group: 8.85; PQ group: 13.62*; PF group: 11.30*	G1: 6.02; G2: 6.56. BAC group: 5.62; PQ group: 5.62; PF group: 7.55*	G1: 0.56; G2: 0.49	G1: 11.91; G2: 12.76	Case-control	Significant IVCM parameters: corneal subbasal dendritic cell density: G1: 86.84; G2: 41.01*; corneal subbasal nerve length: G1: 2814.74; G2: 2077.68*; corneal subbasal nerve tortuosity: G1: 2.0; G2: 1.2*
Wu et al., 2021 [200]	150 glaucomatous eyes	G1: PF bimatoprost (76); G2: latanoprost/0.02% BAC	BL/1month/ 4months: G1: 8.38/9.15/ 9.28; G2: 9.42/7.11*/ 6.84	NiBUT first BL/1month/ 4months: G1: 7.67/7.61/ 7.85; G2: 8.63/ 8.19/8.28	BL/1month/ 4months: G1: 0.07/0.15/ 0.21; G2: 0.07/0.20/ 0.26	BL/1month/ 4months: G1: 7.02/5.26/ 3.83; G2: 6.85/ 4.23/5.28	Prospective, randomized, controlled study	IOP BL/1month/ 4months: G1: 17.58/ 14.45/15.42; G2: 17.26/14.23/13.95*
Seong et al., 2021 [201]	27 POAG patients	All used BAC-preserved PGAs initially, all switched to PF latanoprost		BL/45 days/90 days: 7.4/7.4/ 6.9	Corneal staining BL/ 45 days/90 days: 1.6/ 1.0/0.8*;	OSDI BL/45 days/90 days: 26.4/19.8/ 15.7*	Prospective, open-label, observational study	BL/45 days/90 days: IOP: 14.0/13.9/13.7; bulbar injection: 1.3/ 1.2/1.0*; limbal injection: 1.2/1.1/1.0

(*continued on next page*)

Table 2 (*continued*)

Author/Year/ Reference	Subjects	Topical formulations	Schirmer test (mm)	TBUT (s)	OSS (Oxford scheme if not otherwise specified)	Symptoms (OSDI if not otherwise specified)	Study design	Additional information
					conjunctival staining: 5.9/ 5.6/4.6*			

BAC: benzalkonium chloride; BL: baseline; CGRP: calcitonin gene-related peptide; DEQS: dry eye quality of life score; DEQ-5: Dry Eye Questionnaire-5; ELI: epithelial layer irregularity; FC: fixed combination; G: group; GCD: goblet cell density; GMCSF: granulocyte-macrophage colony-stimulating factor; GSS: Glaucoma Symptom Scale; HLA-DR: human leukocyte antigen-DR; IC: impression cytology; IFNγ: interferon-γ; IL: interleukin; IOP: intraocular pressure: IVCM: in vivo confocal microscopy; LC: Langerhans cell; LLT: lipid layer thickness; LTMH: lower tear meniscus height; mm: millimetre; MMP-9: matrix metalloproteinase-9; MUC-5AC: mucin 5AC; NGF: nerve growth factor: NiBUT: non-invasive break up time; NPY: neuropeptide Y: NS: not significant; NTG; normal tension glaucoma; OAG: open angle glaucoma; OHT: ocular hypertension; OSDI: Ocular Surface Disease Index; OSS: ocular surface staining; PC: preservative containing; PF: preservative free; PGA: prostaglandin analogue; PDGF-BB: platelet-derived growth factor-BB; POAG: primary open angle glaucoma; PQ: polyquaternium-1; SP: substance P; s: seconds; SPK: superficial punctate keratitis; TBUT: tear film break-up time; TFLL: tear film lipid layer; TMH: tear meniscus height; TNFα: tumor necrosis factor-α; UF: unfixed combination; VEGF: vascular endothelial growth factor; VIP: vasoactive intestinal peptide; *: indicates statistical significance of at least $p < 0.05$.

topically administered BAC can reach the trabecular meshwork in rabbits using mass spectrometry imaging; additionally, increased IOP and apoptosis of trabecular cells was noted in rats given subconjunctival injections of BAC [207]. Moreover, increased corneal permeability following BAC exposure was demonstrated in DED patients treated with PC and PF artificial tears, in which case the PF cohort demonstrated decreased permeability whereas patients receiving BAC had increased corneal permeability [208]. Finally, the treatment of ocular surface disease among glaucoma patients appears to improve the measurement quality of optical coherence tomography and measured thickness of the retinal nerve fibre layer [209], decrease visual field testing times and enhance results [210], as well as bringing the number of conjunctival goblet cells detected through IVCM closer to normal [211].

4. Critical appraisal of preservatives

Alternative preservatives exist, such as purite, polyquaternium-1 and SofZia. Purite is reportedly well tolerated, with mild adverse effects [212,213]. Animal and *in vitro* studies indicate a lower cytotoxicity of polyquaternium-1 when compared to BAC [214–220]. An impression cytology study found polyquaternium-preserved travoprost to be better tolerated and safer than BAC-preserved travoprost [221]. A later randomized study found upregulation of IL-1β, IL-6 and IL-8 in patients treated with BAC preserved medications when compared to patients treated with PF of polyquaternium-1 preserved medications [222]. Some clinical studies have noted increased tolerability and decreased adverse effects with polyquaternium-1 when changed from BAC [223–225], while others have found a comparable safety profile between the two preservatives [125,226–229]. It is worth noting that among the studies reporting a favourable adverse effect profile after changing preservative, the active pharmaceutical had been changed as well. Thus, it is unclear whether the positive effect stems from the change of preservative, antihypertensive or both. This uncertainty was further exemplified by a study comparing bimatoprost/BAC, latanoprost/BAC, travoprost/polyquaternium-1 and PF tafluprost, with tolerability measured using the OSDI symptom score [158]. Travoprost/polyquaternium-1-treated patients had significantly better scores, whereas latanoprost/BAC -treated patients had the worst scores. It is noteworthy that even though tafluprost was unpreserved, it did not display a superior side-effect profile. In a study by Kim et al., polyquaternium-1 did not show a significantly favourable profile concerning objective signs of DED and MMP-9 expression compared to BAC [70]. A recent retrospective study analysed the effects of latanoprost/BAC, travoprost/polyquaternium-1, bimatoprost/BAC, brimonidine/purite and brimonidine/BAC on corneal hysteresis and corneal resistance factor as compared to healthy, age-matched controls [230]. Corneal hysteresis and resistance factor in controls were 10.26 and 10.60, respectively. Significant differences were reported among patients using bimatoprost/BAC (8.50 and 8.77) and brimonidine/BAC (8.77 and 8.93) with insignificant findings in the other groups.

In vivo and *in vitro* studies have indicated a better safety profile for SofZia as compared to BAC [215,231–234]. Some clinical studies have reported improved symptoms and/or clinical parameters with SofZia when compared to BAC [110,235–238], while others have not [144,239,240]. Among the clinical studies reporting improvement after changing from BAC to SofZia, the active pharmaceutical component was changed from latanoprost to travoprost. Hence, determining the precise clinical effect produced by changing preservative is complicated due to the manipulation of two factors concomitantly.

5. Design of included studies

Table 5 summarizes the included studies' different study designs. The majority of the included clinical studies fall within Levels 1 and 2 of evidence as delineated by the modified American Academy of Ophthalmology Preferred Practices guidelines [241] (Table 4). Furthermore, several *in vitro* and animal studies have been performed, elucidating aspects not possible or considered not ethical to evaluate clinically.

6. Discussion

Upon reviewing the literature, examples from both the active pharmaceuticals and the preservatives in topical glaucoma medications appear to exert deleterious effects on the ocular surface. Interventional studies have shown a decrease in symptoms and improvement in clinical signs upon conversion from PC to PF PGA and betablockers [159,161,242]. However, the underlying pathophysiological mechanisms responsible for the detrimental effects of BAC and ocular hypotensives on the ocular surface are generally still in need of further elucidation. Inflammatory pathways such as induction of apoptosis, cytokines and oxidative stress have been shown to be involved [12–14,86,87,157,207,260]. Moreover, through its detergent properties, BAC may compromise the lipid layer, thus predisposing treated eyes for excessive evaporation,

Table 4
Study design grading scheme [241].

Clinical studies	
Level 1	Evidence obtained from at least one properly conducted, well-designed, randomized controlled trial or evidence from well-designed studies applying rigorous statistical approaches
Level 2	Evidence obtained from one of the following: a well-designed controlled trial without randomization, a well-designed cohort or case-control analytic study, preferably from one or more centres, or a well-designed study accessible to more rigorous statistical analysis
Level 3	Evidence obtained from one of the following categories: descriptive studies, case reports, reports of expert committees, and expert opinion

Table 5
Study design of included studies.

Prospective studies	
Prospective, randomized trials, n = 48	[33,42,52,56,57,64,73,80,82,97,103,107,111,125, 127,129,131,134,137,139], [140,144,153,160,162, 163,167,168,182–185,187,189–191,197,198,200, 206,208,222,227,229], [235,236,239,240]
Other prospective studies, n = 87	[27,29,34,35,43,46,47,53–55,62,63,65,68,70,72,77, 79,81,83,85,96], [102,110,114–119,121,123,126, 128,130,132,135,136,138,141–143,146,149–152, 154,155,157–159,161,164–166], [169,171–175,177, 179–181,186,188,192–195,201,204,209–213,221, 223–226,237,238,242]
Retrospective studies	
Case control, n = 37	[21,25,32,44,45,49,50,59–61,66,67,71,78,84,87, 93–95,98–101,104,106,108,109,122,124,145,156, 199,202,228,230,243,244]
Cross-sectional, n = 35	[17–20,22–24,26,28,30,36–41,48,51,69,75,76,86,88, 112,113,120,133,147,148,170,176,196,207,245,246]
Case report/series, n = 5	[31,89,90,203,205]
Experimental studies	
In vitro studies, n = 25	[12,14,15,71,74,207,215,217,219,231,232,234, 247–259]
Animal studies, n = 20	[15,69,91–93,207,214,216,218,220,233,250,252, 260–266]

causing dehydration and subsequent inflammation, initiating the vicious cycle of DED through several mechanisms, as illustrated in Fig. 1. The resulting hyperosmolarity and tear film instability may further instigate damage to the ocular epithelium with succeeding inflammation, exacerbating tear film instability, evaporation and hyperosmolarity [23, 267–269].

Purite, polyquaternium-1 and SofZia appear to have a less deleterious effect on the lipid layer of the tear film [247]. It has been noted that topical medications may induce subclinical inflammation of the conjunctiva [243,246], increased inflammatory cells and mast cells [245,246], as well as increased immunoglobulin E (IgE) [244]. Whether these alterations result from active pharmaceutical compounds, preservatives, or both, remains unknown.

More than 110 million people worldwide are expected to have glaucoma by 2040 [270]. With current treatment regimens, the prevalence of ocular surface disease among patients receiving topical glaucoma medications ranges from 38.5% to 75% [9]. This illustrates the current need for prospective longitudinal studies comparing the effects of different pharmaceuticals and preservatives on the ocular surface and MGs. Several studies have shown that a change from preserved to unpreserved formulations may decrease the signs and symptoms of DED in glaucoma patients. Moreover, treating DED in afflicted glaucoma patients could improve IOP and reduce the number of formulations needed to reach treatment goals. Therefore, it is vital that clinicians recognize DED in this population and provide appropriate treatment. A decrease in the DED prevalence among glaucoma patients may increase compliance and efficacy of glaucoma medications, thereby improving the prognosis and help to preserve sight.

Conflicts of interest

Fredrik Fineide: Nothing to declare.
Neil Lagali: Nothing to declare.
Yasin Adil Muhammed: Nothing to declare.
Reiko Arita: Reiko Arita is a founder and the chair-person of Lid and meibomian gland working group (LIME)in Japan, and also a founder and the chair-person of international LIME working group. She delivers

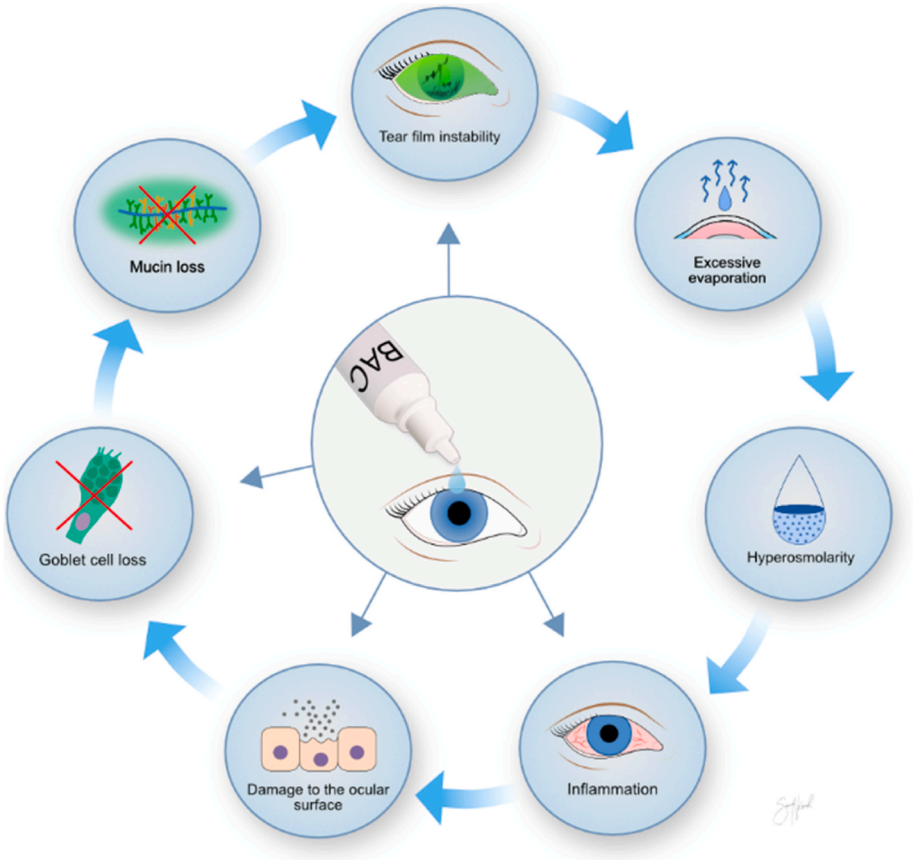

Fig. 1. The vicious circle of dry eye disease can be propagated by multiple interconnected mechanisms that collectively promote dry eye disease and may be hard to break. Benzalkonium chloride could potentially initiate the vicious circle by causing tear film instability, inflammation, damage to the ocular surface and goblet cell loss. Illustration by Sara Nøland.

for her talk for the following: Alcon, Senju, Santen, Inami, Johnson & Johnson and Lumenis. She has served on the global scientific advisory board for Novartis and Alcon.

Miriam Kolko: Miriam Kolko serves on the advisory boards of Thea Laboratories, Santen and Abbvie. She delivers talks for the same industries. Finally, she is a consultant for Thea Laboratories.

Jelle Vehof: Dr Vehof is a consultant for Alcon, Santen, Thea Pharma, Horus Pharma and Tramedico.

Tor Paaske Utheim is co-founder and co-owner of The Norwegian dry eye clinic and the Clinic of eye health, Oslo, Norway, which delivers talks for and/or receives financial support from the following: ABIGO, Alcon, Allergan, AMWO, Bausc'h&Lomb, Bayer, European school for advanced studies in ophthalmology, InnZ Medical, Medilens Nordic, Medistim, Novartis, Santen, Specsavers, Shire Pharmaceuticals and Thea Laboratories. He has served on the global scientific advisory board for Novartis and Alcon as well as the European advisory board for Shire Pharmaceuticals. Utheim is the Norwegian Global Ambassador for Tear Film and Ocular Surface Society (TFOS), a Board Member of the International Ocular Surface Society, an International Member of the Japanese Lid and Meibomian gland working group (LIME), a Consultant at the Norwegian Association for the Blind and Partially Sighted, the President of the Oslo Society of ophthalmology, and the Editor-in-Chief of Oftalmolog, an eye journal distributed to all eye doctors in the Nordic region since 1980. Besides publishing articles of presumed interest to our readers, Oftalmolog publishes advertisements from pharmaceutical companies, companies selling ophthalmological equipment, and associations organizing conferences and events in ophthalmology. For more information, visit: oftalmolog.com.

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