

Therapeutic Effects of Sodium Hyaluronate on Ocular Surface Damage Induced by Benzalkonium Chloride Preserved Anti-glaucoma Medications

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Abstract

Background: Long-term use of benzalkonium chloride (BAC)-preserved drugs is often associated with ocular surface toxicity. Ocular surface symptoms had a substantial impact on the glaucoma patients' quality of life and compliance. This study aimed to investigate the effects of sodium hyaluronate (SH) on ocular surface toxicity induced by BAC-preserved anti-glaucoma medications treatment.

Methods: Fifty-eight patients (101 eyes), who received topical BAC-preserved anti-glaucoma medications treatment and met the severe dry eye criteria, were included in the analysis. All patients were maintained the original topical anti-glaucoma treatment. In the SH-treated group (56 eyes), unpreserved 0.3% SH eye drops were administered with 3 times daily for 90 days. In the control group (55 eyes), phosphate-buffered saline were administered with 3 times daily for 90 days. Ocular Surface Disease Index (OSDI) questionnaire, break-up time (BUT) test, corneal fluorescein staining, corneal and conjunctival rose Bengal staining, Schirmer test, and conjunctiva impression cytology were performed sequentially on days 0 and 91.

Results: Compared with the control group, SH-treated group showed decrease in OSDI scores (Kruskal-Wallis test: $H = 38.668$, $P < 0.001$), fluorescein and rose Bengal scores (Wilcoxon signed-ranks test: $z = -3.843$, $P < 0.001$, and $z = -3.508$, $P < 0.001$, respectively), increase in tear film BUT (t -test: $t = -10.994$, $P < 0.001$) and aqueous tear production (t -test: $t = -10.328$, $P < 0.001$) on day 91. The goblet cell density was increased (t -test: $t = -9.981$, $P < 0.001$), and the morphology of the conjunctival epithelium were also improved after SH treatment.

Conclusions: SH significantly improved both symptoms and signs of ocular surface damage in patients with BAC-preserved anti-glaucoma medications treatment. SH could be proposed as a new attempt to reduce ocular surface toxicity, and alleviate symptoms of ocular surface damage in BAC-preserved anti-glaucoma medications treatment.

Key words: Anti-glaucoma Medications; Benzalkonium Chloride; Ocular Surface Toxicity; Sodium Hyaluronate

INTRODUCTION

Topical anti-glaucoma medication is the most common treatment modality for glaucoma. Long-term use of these preserved eye drops is often associated with ocular surface toxicity, such as dry eye, allergic reactions, and subconjunctival fibrosis. Benzalkonium chloride (BAC), the most commonly used preservative in anti-glaucoma eye drops, is largely responsible for the toxicity.^[1-3] Prevalence of ocular surface symptoms was found to be very high in glaucoma patients using preserved drops. Pooled data from 9658 glaucoma patients in several European countries

showed that the incidence of ocular surface symptoms ranged between 30% and 50%.^[4] Likewise, in the United States, a cross-sectional study demonstrated that 59% of glaucoma

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patients reported dry eye symptoms, 61% of patients with decreased tear production, 78% of patients with decreased tear break-up time (BUT).^[5] Dry eye symptoms occurred more frequently in severe glaucoma patients when three or more anti-glaucoma medications were used and increased with the duration of glaucoma disease.^[6] Multiple studies concluded that ocular surface symptoms had a substantial impact on the glaucoma patients' quality of life and compliance and became a common reason noted by physicians for switching medications.^[7] Meanwhile, ocular surface damage induced by BAC-preserved anti-glaucoma medication was associated with a lower filtration surgery success rate.^[8] Unstable tear film and reduction in the production of mucin stimulate the inflammation cascade of the ocular surface epithelial cells.^[9] This vicious cycle lead to the occurrence of dry eye disease and the development of subconjunctival fibrosis.^[10] Hence, ocular surface symptom induced by BAC-preserved medications should not be ignored in the long-term treatment of glaucoma.

As a widely used artificial tear, sodium hyaluronate (SH) displays excellent effect in dry eye treatment.^[11] Furthermore, several *in vitro* studies showed that SH significantly reduces BAC-induced cytotoxic effects.^[12,13] We recently showed that topical application of SH significantly decreased the ocular surface toxicity, such as damages in the superficial structure and integrity, increasing inflammation and apoptosis rate, and reduction of aqueous tear production, induced by BAC-preserved latanoprost in rabbits.^[14] However, these results in animal models cannot reflect the whole ocular surface reactions in glaucoma patients, including subjective symptoms and tear film BUT. The purpose of this study was to investigate the therapeutic effects of SH on ocular surface damage induced by long-term BAC-preserved anti-glaucoma medications treatment in clinical setting.

METHODS

Study population

Fifty-eight patients diagnosed with primary open angle glaucoma (POAG), normal tension glaucoma (NTG), or ocular hypertension (OH) were enrolled between February 2013 and June 2014 from the Department of Glaucoma, Zhongshan Ophthalmic Center, Sun Yat-sen University, China. The research adhered to the tenets of the *Declaration of Helsinki*. The Institutional Review Board had approved the protocol prospectively. Informed consent had been obtained from all the participants.

All patients have received long-term topical BAC-preserved anti-glaucoma medications treatment and met the severe dry eye criteria according to the ODISSEY European Consensus Group algorithm.^[15] The criteria for diagnosis of severe dry eye disease were as follows: (a) Ocular Surface Disease Index (OSDI) ≥ 33 and corneal fluorescein staining (CFS) score ≥ 3 ; (b) OSDI < 33 and CFS ≥ 3 , additional criteria ≥ 1 or impaired corneal sensitivity; (c) OSDI ≥ 33 and CFS = 2, additional criteria ≥ 1 ; (d) OSDI ≥ 3 and CFS ≤ 1 , additional criteria ≥ 1 and BUT < 3 s.

Exclusion criteria were as follows: Any ocular surgery treatment, any ≥ 2 weeks topical eye drops treatment other than anti-glaucoma medications, any lacrimal apparatus and ocular surface diseases, use of contact lenses, any medical treatment that influence tear production, such as dopamine, amitriptyline, estazolam, and immunosuppressive agent, not completing the follow-up protocol.

Fifty-eight patients (101 eyes) were included in the analysis. All patients were maintained the original topical anti-glaucoma treatment. Commercially available unpreserved 0.3% SH (Hialid; Santen, Osaka, Japan) eye drops were administered with 3 times daily for 3 months in the SH-treated group (30 patients, 56 eyes). Control group (28 patients, 55 eyes) were administered by 50 μ l phosphate-buffered saline (PBS) 3 times daily for 3 months. PBS and 0.3% SH solutions were administered in the same container. Patients did not know the ingredients. The interval between SH/PBS and anti-glaucoma medications instillation were at least 1 h.

A follow-up observation of 90 days was performed. All patients underwent the ophthalmologic routine evaluation including best-corrected visual acuity, slit-lamp examination, intraocular pressure measurement and fundus examination on days 0 and 91. OSDI questionnaire, BUT test, CFS, corneal, and conjunctival rose Bengal staining, Schirmer test, and conjunctiva impression cytology were performed sequentially following the methods described below on days 0 and 91. The interval between these examinations and topical treatments were 1 h.

Ocular Surface Disease Index questionnaire

The OSDI is a validated, self-administered instrument for assessing the presence and severity of ocular surface disease symptoms.^[16] The OSDI questionnaire includes 12 questions about the respondent's past-week experience with ocular symptoms, vision related function, and environmental triggers.^[17] The total OSDI score ranged from 0 to 100. The scores classified ≤ 12 as normal, 13–22 as mild, 23–32 as moderate, ≥ 33 as severe.^[18]

Tear film break-up time

The BUT was measured by applying a slightly moistened fluorescein strip (Tianjin Jingming New Technology Development Co., Ltd., Tianjin, China) to the bulbar conjunctiva and asking the patient to blink. The tear film was then scanned under a slit-lamp with a cobalt filter while the patient refrains from blinking. The time that elapses before the first dry spot appears in the corneal fluorescein layer is the tear film BUT. Normally, the BUT is over 15 s.

Fluorescein and rose Bengal staining

Meanwhile, fluorescein will stain the eroded and denuded areas of the corneal epithelium. The scores were graded under a slit-lamp microscope with a cobalt blue filter. Three corneal areas were considered, the area of positive corneal staining was scored from 0 (absent) to 3 (diffuse loss of epithelium).^[19]

Thirty minutes later, the disappearance of fluorescein staining was observed under a slit-lamp microscope. Two microliter of 1% rose Bengal was instilled into the conjunctival sac. The dye will stain all eroded and denuded areas of the corneal and conjunctival epithelial cells. Fifteen seconds later, the scores were graded according to the van Bijsterveld grading system^[20] under a slit-lamp microscope.

Schirmer test

After topical application of proparacaine (ALCAINE®; Alcon, Fort Worth, TX, USA), tear production was measured by the Schirmer test using Whatman 41 filter paper strip (Tianjin Jingming New Technology Development Co., Ltd., Tianjin, China). The strip was placed into the mid and temporal thirds of the lower lid, and the wetted length of the strip was read after 5 min, less than 5 mm was abnormal.

Conjunctival impression cytology

After topical application of proparacaine, two 3.5 mm × 3.5 mm round nitrocellulose filter paper (Pall Corporation, New York, USA) were applied separately on the superior nasal and superior temporal bulbar conjunctiva, and pressed for 10 s with constant pressure. The specimens were fixed with 95% ethanol and stained with periodic acid-Schiff and hematoxylin. The number of goblet cells was counted under a light microscope (Olympus, Tokyo, Japan) in a masked fashion. Three nonadjacent high power fields (×400) of each specimen were observed randomly for counting; an average was calculated. The morphology of the conjunctival epithelium was graded according to the Nelson's method.^[21]

Statistical analysis

Statistical analyses were performed using the SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). Kruskal-Wallis test was applied to make comparisons of OSDI scores. Wilcoxon signed-ranks test was applied to make comparisons of fluorescein and rose Bengal staining scores. Student's *t*-test was applied to make comparisons of BUT, aqueous tear production, and conjunctival goblet cell density (GCD). A *P* < 0.05 was considered statistically significant.

RESULTS

General characteristics

A total of 58 patients (101 eyes), who were diagnosed with POAG, NTG, or OH, were included in this study. A general

characteristic of the patients, the average time of topical anti-glaucoma medications application, and the average number of category of anti-glaucoma drugs were presented in Table 1. The topical anti-glaucoma medications included latanoprost (0.02% BAC), travoprost (0.015% BAC), bimatoprost (0.005% BAC), carteolol (0.005% BAC), brimonidine (0.005% BAC), and brinzolamide (0.01% BAC). No significant difference was found between the two groups in all categories.

Ocular Surface Disease Index scores

Before administration of SH or PBS (day 0), no significant difference for symptoms was found between the two groups in the patients' OSDI scores. There were 36.7% of patients with burning or stinging sensation, 30% of patients with foreign body sensation, 23.3% of patients with dry eye, and 33.3% of patients with blurred vision on day 0. After administration of SH or PBS, the OSDI scores were improved significantly (Kruskal-Wallis test: *H* = 38.668, *P* < 0.001) in the SH-treated group, than the control group on day 91. The symptoms were relieved after SH treatment. There were only 20% of patients with burning or stinging sensation, 13.3% of patients with foreign body sensation, 3.3% of patients with dry eye, and 16.7% of patients with blurred vision in the SH-treated group [Table 2].

Break-up time

Before administration of SH or PBS (day 0), no significant difference was found between the two groups in the tear film BUT. After administration of SH/PBS, the tear film BUT were prolonged significantly (*t*-test: *t* = -10.994, *P* < 0.001) in the SH-treated group than the control group on day 91.

Fluorescein and rose Bengal staining

Before administration of SH or PBS (day 0), no significant difference was found between the two groups in the CFS scores and conjunctival rose Bengal staining scores. After administration of SH/PBS, the staining scores of fluorescein and rose Bengal were decreased significantly (Wilcoxon signed-ranks test: *z* = -3.843, *P* < 0.001, and *z* = -3.508, *P* < 0.001, respectively) in the SH-treated group than the control group on day 91.

Aqueous tear production

Before administration of SH or PBS (day 0), no significant difference was found between the two groups in the average aqueous tear production. After administration of SH/PBS, the

Table 1: General characteristic of the patients with anti-glaucoma drug treatment

Characteristics	SH-treated group (30 patients, 56 eyes)	Control group (28 patients, 55 eyes)	<i>P</i>
Age, years, mean ± SD (range)	46.76 ± 3.21 (18–76)	42.97 ± 4.06 (20–73)	0.662
Male/female, <i>n</i>	21/9	18/10	0.407
Diagnosis (POAG/NTG/OH), <i>n</i>	54/2/0	51/2/2	0.796
Time of anti-glaucoma treatment, months, mean ± SD (range)	39.13 ± 7.38 (6–144)	35.07 ± 6.67 (8–138)	0.336
Number of anti-glaucoma drugs, mean ± SD (range)	1.43 ± 0.08 (1–3)	1.87 ± 0.12 (1–4)	0.117

SD: Standard deviation; SH: Sodium hyaluronate; POAG: Primary open angle glaucoma; NTG: Normal tension glaucoma; OH: Ocular hypertension.

Table 2: Compared results of SH-treated group and Control group

Items	Day 0		P	Day 91		P
	SH-treated group (30 patients, 56 eyes)	Control group (28 patients, 55 eyes)		SH-treated group (30 patients, 56 eyes)	Control group (28 patients, 55 eyes)	
OSDI scores, n (%)			0.745			<0.001
Normal	0 (0)	0 (0)		16 (53.3)	0 (0)	
Mild	0 (0)	1 (3.6)		9 (30)	1 (3.6)	
Moderate	21 (70.0)	22 (78.6)		5 (16.7)	23 (82.1)	
Severe	9 (30.0)	5 (17.9)		0 (0)	4 (14.3)	
BUT (s), mean ± SD (range)	5.18 ± 0.38 (3–11)	4.69 ± 0.78 (2–10)	0.305	10.78 ± 0.51 (5–20)	4.87 ± 0.64 (2–11)	<0.001
Fluorescein staining scores, median (P ₂₅ –P ₇₅)	5 (3–6)	5 (3–7)	0.918	1 (0–2)	5 (3–7)	<0.001
Rose Bengal staining scores, median (P ₂₅ –P ₇₅)	3 (2–5)	3 (2–6)	0.877	1 (0–2)	3 (2–5)	<0.001
Aqueous tear production, mm, mean ± SD (range)	3.27 ± 0.32 (1–11)	3.76 ± 0.57 (2–10)	0.684	7.33 ± 0.35 (3–18)	4.18 ± 0.63 (3–11)	<0.001
GCD (HP), mean ± SD (range)	29.30 ± 3.15 (5–79)	32.66 ± 4.09 (11–68)	0.667	63.06 ± 2.37 (27–105)	38.95 ± 3.73 (9–81)	<0.001

SD: standard deviation; P₂₅–P₇₅: Lower quartile – upper quartile; SH: Sodium hyaluronate; OSDI: Ocular Surface Disease Index; BUT: Break-up time test; GCD: Goblet cell density.

aqueous tear production were increased significantly (*t*-test: *t* = -10.328, *P* < 0.001) in the SH-treated group than the control group on day 91.

Goblet cell density and conjunctival epithelium morphology

Before administration of SH or PBS (day 0), no significant difference was found between the two groups in the GCD. After administration of SH/PBS, the GCD was increased significantly (*t*-test: *t* = -9.981, *P* < 0.001) in the SH-treated group than the control group on day 91. The morphology of the conjunctival epithelium was also improved after SH treatment. Grading scores decreased from 3 to 1–2 after treatment with SH [Figure 1].

DISCUSSION

Numerous experimental and clinical studies^[1,2,22] showed that BAC, the most frequently used preservative of topical anti-glaucoma medications, may induce ocular discomfort, tear film instability, superficial structure and integrity impairment, conjunctival inflammation, subconjunctival fibrosis, and epithelial apoptosis. These ocular surface damages affect the patients' quality of life and treatment compliance and become the potential risk of failure for filtration surgery. Therefore, improvement of the ocular surface and reduction of inflammation prior to surgery should be considered.^[23,24] SH, a widely used tears substitute, showed a good effect in the treatment of dry eye even in Sjögren's syndrome patients.^[25] Our previous animal experiment has demonstrated that topical application of SH significantly decreased the ocular surface toxicity induced by BAC-preserved latanoprost.^[14] In the current study, we observed that the therapeutic effects of SH on ocular surface damage induced by long-term BAC-preserved anti-glaucoma medications treatment in clinical setting. All participants were received BAC-preserved anti-glaucoma medications treatment for at least 6 months and were diagnosed as severe dry eye according to the ODISSEY

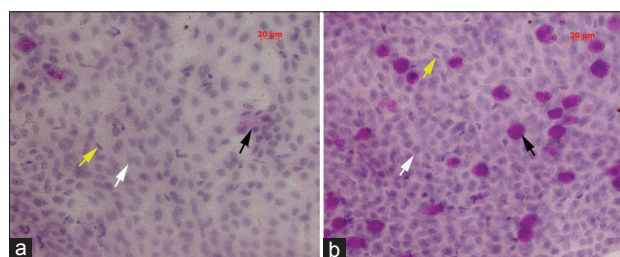


Figure 1: Representative images of conjunctival impression cytology (HP, ×400). (a) Before sodium hyaluronate treatment, epithelial cells (white arrow) appeared enlarged with a nucleocytoplasmic ratio of 1:4–1:5 and pyknotic nuclei (yellow arrow). The cell to cell junctions were loose. Goblet cells (black arrow) were smaller and fewer, less intensely periodic acid-Schiff positive with poorly defined cellular borders. The conjunctiva impression cytology grade was 3; (b) After sodium hyaluronate treatment, epithelial cells were small and round with a nucleocytoplasmic ratio of 1:2–1:3. Goblet cells were abundant, oval with intensely periodic acid-Schiff positive cytoplasm. The conjunctiva impression cytology grade was 1–2.

European Consensus Group algorithm.^[15] Several patients presented significant ocular damage signs, but symptom severity was relatively mild. We considered that impaired corneal sensitivity played a major role.

The OSDI is one of the most widely used questionnaires. It reliably assesses the severity, natural history, and effect of ocular surface diseases.^[26] The OSDI also has the necessary psychometric properties to be used as an end point in clinical trials of dry eye disease.^[16] Compared to the National Eye Institute Visual Functioning Questionnaire-25 and the McMonnies Dry Eye Questionnaire, the OSDI questionnaire obtains a sensitivity of 60% and specificity of 79%.^[16,27] Our result showed that all subjects receiving BAC-preserved anti-glaucoma drops treatment suffered from symptoms related to the ocular surface. However, SH treatment relieved markedly these symptoms such as burning or stinging sensation, foreign body sensation, dry eye, and blurred vision.

Alves *et al.*^[28] compared signs, symptoms and predictive tools used to diagnose dry eye disease in six different systemic well-defined and nonoverlapping diseases (i.e., Sjögren's syndrome, graft versus host disease, graves orbitopathy, facial palsy, diabetes mellitus, and glaucoma who received BAC-preserved topical drugs treatment), and found that the best combination of diagnostic tests for dry eye disease were OSDI, BUT and Schirmer test (sensitivity 100%, specificity 95% and accuracy 99.3%). The Schirmer test is a screening test for assessment of tear production. Schirmer test with topical anesthesia measure the function of the accessory lacrimal glands (the basic secretors). Measurement of the tear film BUT could sometimes be useful to estimate the mucin content of tear fluid. Deficiency in mucin may not affect the Schirmer test result, but may lead to instability of the tear film. BAC-preserved drugs induce damages in the accessory lacrimal gland cells and mucin-secretion cells, and lead to the deficiency of aqueous layer and mucus layer of tear film. Transmission electron microscopy of the anterior corneal surface demonstrated fixation of the mucus layer after exposure to 0.01% BAC for 5 min, whereas more prolonged exposure (60 min) to 0.01% BAC destroyed the mucus layer.^[29] In addition, as a tensioactive compound, BAC is also a detergent for the lipid layer of the tear film, causing evaporation of the aqueous tear film.^[1] SH is a linear polymer built from repeating disaccharide units containing N-acetylglucosamine and glucuronic acid.^[30] Disaccharides are hydrophilic molecules that confer to SH an excellent water holding capacity. Indeed, SH can incorporate a volume of water approximately more than 1000-fold its initial volume, constituting a viscous polymer.^[30,31] This property plays a major role in increasing tear film stability, reducing the evaporation rate of the tear, and long resident-time on the ocular surface.

Fluorescein staining means the disruption of the corneal epithelial cell to cell junctions and damage of corneal epithelial cells.^[32] Rose Bengal staining was apparent earlier than fluorescein staining. Rose Bengal stain live and dead epithelial cells that are not adequately protected by the tear film.^[33] Besides have damaging effects indirectly on the corneal and conjunctival epithelial cells by breakage of tear film, BAC can damage directly the corneal and conjunctival epithelial cells by biological interaction with proteins, lipids and G proteins in cell membranes.^[34] In this study, we observed that SH treatment significantly improved ocular surface staining of patients. We also demonstrated in the earlier animal research that SH could protect ultrastructures such as microvilli on the epithelial cells under the scanning and transmission electron microscopy.^[14] As a viscous biopolymer with negative charges, SH can neutralize the toxic cationic charge of the remaining BAC quaternary ammonium in conjunctival sac.^[13] Moreover, CD44, a transmembrane receptor linked to the actin cytoskeleton, express in human cornea.^[35] It binds to hyaluronate and is also capable of binding fibronectin, laminin, and collagen I. SH promoted human cornea epithelial cells migration and

wound healing by the adhesion between CD44, which coats the surface of the denuded areas.^[36]

GCD is a pivotal parameter that reflects the overall health of the ocular surface.^[37] We used conjunctival impression cytology, the most effective noninvasive technique for counting goblet cells, to evaluate the morphology of the ocular surface. Our study further supports existing data. BAC induced a direct toxicity on goblet cells and inhibited mucin production in goblet cells.^[14,38] Reductions of mucin production speed up the damage of tear film stability, thereby aggravating the ocular surface damage and stimulating the inflammation cascade of epithelial cells. The inflammatory immune environment caused apoptosis of goblet cells. Ultimately, this results in a vicious cycle of ocular surface damage.^[9,14] Our results showed that SH treatment significantly improved GCD and conjunctival squamous metaplasia of glaucoma patients. Brignole *et al.*^[39] found that SH treatment may decrease expression of apoptosis related inflammatory markers, including Fas, apoptosis 2.7, human leukocyte antigen-DR and CD40 through CD44 mediation in patients with moderate dry eye syndrome and superficial keratitis. SH possibly maintain GCD and stimulates mucin synthesis through its anti-oxidative and anti-inflammatory properties, and help the ocular epithelium damage break out of the vicious cycle.

As far as we known, this research demonstrated firstly the function of SH to decrease the toxicity induced by BAC-preserved anti-glaucoma drops in clinical setting. A limitation of this study was a lack of detecting the alteration of inflammatory cytokines expression and apoptosis rate. Further studies were limited due to the poor availability of living tissue. What's more, the impacts on the anti-glaucoma treatment (e.g., intraocular pressure reducing the value) need to be observed. On the other hand, even we have observed the use of SH had no effect on the single measurement of intraocular pressure, more parameters, such as curve of intraocular pressure, visual field, retinal nerve fiber layer thickness, should be compared.

In conclusion, our findings demonstrated that SH significantly improved both symptoms and signs of ocular surface damage in patients with BAC-preserved anti-glaucoma medications treatment. SH could become a new attempt to reduce ocular surface toxicity, alleviate symptoms of ocular surface damage, and promote the patients' quality of life in BAC-preserved anti-glaucoma medications treatment.

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Conflicts of interest

There are no conflicts of interest.

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