Comparison of the goblet cells’ density and the quality of tears in treatment with sodium hyaluronate 0.1% benzalkonium chloride preservative-free eye drops for patients with glaucoma and dry-eye syndrome: A double-blind randomized clinical trial

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Abstract. This study aims to compare the efficacy of benzalkonium chloride (BAK) on different goblet cells densities and to enhance the quality of tears in patients with glaucoma and dry-eye syndrome treated with preservative-free and preserved sodium hyaluronate (SH) 0.1% eye drops. In this double-blind, randomized clinical trial, we randomly assigned patients with glaucoma and dry-eye syndrome into two groups: group I, treated with SH 0.1% eye drops comprising BAK preservative (a positive comparator group); group II, treated with preservative-free SH 0.1% eye drops. We followed patients for 3 months after enrollment and assessed the quality of tears by examining symptoms and signs, including the Schirmer’s test, tear film break-up time (TFBUT), Ocular Protection Index (OPI), and conjunctival impression cytology pre- and post-treatment. Any ocular discomfort was assessed by using the Ora Calibra Ocular. We enrolled 40 eyes from 20 patients with glaucoma and dry-eye syndrome. Each group comprised 20 eyes of 10 patients. We observed increased values of the mean TFBUT, OPI, and Schirmer’s test pre- and post-treatment, with no significant difference between both groups. While the conjunctival impression cytology revealed similar results of goblet cells in both groups, we observed lower scale of the Ora Calibra Ocular. Both groups elicit a comparable improvement in the quality of tears as demonstrated by augmented results of the Schirmer’s test, TFBUT, OPI, and Ora Calibra Ocular Score of patients with glaucoma and dry-eye syndrome.

1. Introduction

Most patients with glaucoma require prolonged treatment with anti-glaucoma eye drops to maintain their target intraocular pressure (IOP) and prevent permanent blindness. Nearly all anti-glaucoma eye drops contain a preservative ingredient, benzalkonium chloride (BAK), which is necessary for the mainstay of glaucoma treatment [1,2]. However, BAK might cause side effects, including exacerbation of dry eyes and mild inflammation in conjunctival and corneal epithelial cells [3-5]. Furthermore, it is
likely to cause tear film instability, reduction in tear secretion, lower the Schirmer’s test, increase tear evaporation, shorten the tear film break-up time (TFBUT), apoptosis of conjunctiva cells, damage of corneal epithelial cells, and the loss of conjunctival goblet cells [4-9]. However, the needs for using BAK as a preservative agent in eye drops should not be ignored, as it exerts effective bactericidal and fungicidal effect that can minimize the growth of harmful microorganisms.

Several studies have estimated the prevalence of patients with glaucoma and dry-eye syndrome to be 16.5%–52.6% [10-12]. Most patients seek treatment with artificial tear eye drops, and one of them contains sodium hyaluronate (SH), which can improve the tear film stability and stimulate the proliferation of corneal and conjunctival cells [13,14]. Artificial SH-containing tear eye drops are of two types, with and without a preservative. In eye drops, BAK has been extensively used as a preservative agent. To date, no data are available about the effect of using BAK-preserved and preservative-free SH 0.1% eye drop for patients with glaucoma and dry-eye syndrome. Thus, this study aims to compare the effect of BAK on different goblet cells densities and the quality of tears in patients with glaucoma and dry-eye syndrome treated with preservative-free and preserved SH 0.1% eye drops.

Prevention efforts and treatment of halitosis are brushing the teeth and the tongue, using mouthwash, and improving one’s diet. Treatments such as brushing the teeth and tongue or using an antiseptic mouthwash have been proved to reduce hydrogen sulfide and methyl mercaptan, which are the components of VSC [5].

The use of mouthwash is a simple effort to overcome halitosis. Particularly in Indonesia, the market offers a wide variety of brands with different active ingredients of mouthwashes. Chlorhexidine is the most commonly used antibacterial agent. However, although chlorhexidine is one of the most effective oral antiseptic agents, research shows that the long-term use of chlorhexidine has some side effects, such as staining on the teeth and tongue and reduced sensitivity of taste buds [6,7].

Developments in dentistry have produced several discoveries of new products that can be used as supporting periodontal treatment, one of which is chlorine dioxide (ClO₂). ClO₂ is a strong oxidizing agent that can kill bacteria through a protein synthesis mechanism [8]. It contains oxygen, which can be used as an antiseptic on wounds and accelerates healing, and is effective for halitosis, gingivitis, periodontitis, and bleeding gums [10-12]. ClO₂ and chlorite anion (ClO₂⁻) together can oxidize VSC to become a non-malodor product and destroy amino acids, such as cysteine and methionine, which are VSC precursors, in the process of oxidation [12]. Mouthwashes containing ClO₂ have been widely used in developed countries, such as Japan and the United States, and ClO₂ mouthwash has been reported to be effective in reducing bad breath in the morning (morning breath malodor) up to 4 h after application in healthy subjects [13]. In Indonesia, mouthwashes containing ClO₂ are not too popular among the public. Moreover, studies on the efficacy of the use of mouthwash containing ClO₂ against halitosis in Indonesia have not yet been conducted. Therefore, this research is conducted to analyze the efficacy of using mouthwash containing ClO₂ as the active ingredient to address halitosis. The results are expected to improve the knowledge of dentists in dealing with halitosis and to help people to choose the right mouthwash to overcome halitosis.

2. Materials and Methods
2.1 Trial design
We conducted a double-blind, randomized clinical trial at the Departement of Ophthalmology, Cipto Mangunkusumo Hospital (Jakarta, Indonesia). The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of the institution. Furthermore, we obtained written informed consent from all patients before enrolment.

2.2 Participant
We enrolled patients with primary glaucoma aged >50 years and using <2 anti-glaucoma eye drops in the last 6 months with the TFBUT score of <10 s and the Schirmer’s test result of <15 mm. The exclusion criteria of this study were as follows: patients who had a history of ocular surgery other than
trabeculectomy and/or cataract extraction; using any psychotropic agent, antiandrogenic, antiarrhythmic agents; diagnosis with Parkinson’s disease; anti-histamine; a history of local ocular disease; and using the soft lens.

2.3 Randomized and Blinding
We randomly categorized patients with glaucoma into two groups that were later given preserved or preservative-free artificial tear eye drop based on the number they obtained from a sealed envelope. The packaging of the eye drops was concealed with a white paper label. In addition, patients in both groups and the evaluating investigator were blinded to the treatment given.

2.4 Intervention
Patients in the first group were treated with an artificial tear eye drop containing an active ingredient of 0.1% SH and a preservative of BAK 0.003% (Hialid®, Santen Pharmaceutical). While the first group served as a positive comparator, the second group was treated with a preservative-free artificial tear eye drop containing 0.1% SH (Hyalub®, Cendo, Indonesia). The eye drops were given at a dose of one drop four times daily, and the patients were followed for 3 months after the enrollment.

All patients were instructed to use their daily anti-glaucoma eye drops to maintain their IOP. We performed several eye examinations for each patient to establish the diagnosis of primary glaucoma, which were then followed by some tests for assessing the quality of tears, including the Schirmer’s test, TFBUT, Ocular Protection Index (OPI), and conjunctival goblet cell impression cytology both pre- and post-treatment. Any subjective ocular discomfort was measured by using the Ora Calibra Ocular Discomfort & 4 symptoms Questionnaire, which was presented on a discomfort scale recorded weekly in a score diary. In addition, the patients were asked to grade the ocular discomfort that they experienced, including burning sensation, drying, grittiness, and stinging, on a scale of 0–4. The TFBUT was defined when the conjunctiva was stained with a fluorescein dye, the time elapsing of the tear film can be observed (in seconds) from a complete blink to the disruption of the tear film layer; we performed this test three times and recorded the average set times (seconds). The Schirmer's test was performed with a strip of thin filter paper hooked over the lower eyelid and the length of paper wetted settled for 5 min was measured (mm). Meanwhile, we calculated the OPI of the TFBUT divided by the interblink interval (IBI); if OPI < 1, the eye was considered at the risk of dry eye. The conjunctival impression cytology was attained when the cellulose-acetate filter was pressed on the bulbar conjunctiva; then, its epithelial cells was fixed and stained by periodic acid Schiff. Finally, we assessed the cell goblet population density using an ultra-light microscope.

2.5 Study endpoints
In this study, the efficacy endpoints were symptoms and signs, including the Schirmer’s test, TFBUT, OPI, and impression cytology pre- and post-treatment [15].

2.6 Statistical analysis
Statistical analyses were performed using a computer software program, i.e., SPSS version 22.0. Different results of the Schirmer’s test and TFBUT were analyzed further using a paired t-test for the within-group analysis and unpaired t-test for the between-group analysis. Meanwhile, we analyzed the conjunctival impression cytology using the Mann–Whitney U-test. In addition, varied OPI results were analyzed and presented as a proportion. We considered $P < 0.05$ as statistically significant with a power of 20%

3. Results and Discussion
3.1 Results
In this study, we enrolled 40 eyes from 20 patients, with 20 eyes in each group. While 11 patients were with primary open-angle glaucoma (POAG), 9 patients were with primary angle-closure glaucoma (PACG). While 16 eyes had no history of ocular surgery, 9 eyes had a history of combined cataract
and trabeculectomy surgery. All patients continued to use timolol 0.5%, and brinzolamide eye drops for their glaucoma medications. Table 1 summarizes the patients’ characteristics.

Table 1. Patients’ characteristics

|                | Group I | Group II |
|----------------|---------|----------|
| **Sex**        |         |          |
| Male           | 5       | 5        |
| Female         | 5       | 5        |
| **Age (in years)** |       |          |
| Median (min–max) | 63 (55–83) | 65 (58–77) |
| **Diagnosis**  |         |          |
| POAG           | 6       | 5        |
| PACG           | 4       | 5        |
| **History of surgery** |   |          |
| None           | 10      | 6        |
| Phaco          | 8       | 6        |
| Phaco+trabec   | 2       | 8        |

POAG, primary open-angle glaucoma; PACG, primary angle-closure glaucoma

Before the treatment, the median values of the conjunctival impression cytology, Schirmer’s test, TFBUT, and OPI results were comparable between both groups. The increased Schirmer’s test, TFBUT, and OPI results were significantly different between pre- and post-treatment using artificial eye drops within the groups (P = 0.022, 0.001, and 0.02, respectively); however, no significant difference was observed in the goblet cell density (P = 0.93; Table 2).

Table 2. The ocular surface test Pre- and Post-Treatment within Groups after 3-Month Treatment

| Ocular surface test          | Group I (with BAK) | Group II (without BAK) | p   |
|------------------------------|--------------------|------------------------|-----|
| Impression cytology (median, min–max) | 88.2 (25–185)       | 67.8 (25–200)          | 0.48* |
| Schirmer’s test mm(mean ± SD, mm) | 16.33 ± 6.79        | 15.72 ± 10.26          | 0.10* |
| TFBUT seconds (mean ± SD)     | 14.45 ± 7.8         | 13.91 ± 7.46           | 0.37**|
| OPI number                   |                    |                        | 0.28***|
| >1                           | 13                 | 16                     |     |
| <1                           | 7                  | 4                      |     |

*McNemar’s test.  
**Paired t-test.  
***Chi-square test.  
BAK, benzalkonium chloride; TFBUT, tear film break-up time; OPI, Ocular Protection Index

The pre- and post-treatment analysis between groups revealed that no statistical significant difference in the results of the TFBUT (P = 0.37; Fig. 1), median values of the goblet cell density (P = 0.48; Fig. 2), results of the Schirmer’s test (P = 0.17; Fig. 3), and the value of OPI (P = 0.28; Fig. 4). Table 3 shows improved clinical outcomes as more ocular comfort scale were found post-treatment in both groups.
Figure 1. The mean TFBUT before and after 3-month treatment. BAK, benzalkonium chloride; TFBUT, tear film break-up time.

Figure 2. The median number of the conjunctival impression cytology before and after 3-month treatment. BAK, benzalkonium chloride.
**Figure 3.** The mean Schirmer’s test before and after 3-month treatment. BAK, benzalkonium chloride

**Figure 4.** The number of OPI result before and after 3-month treatment. OPI, Ocular Protection Index
Table 3. The Ora Calibra Ocular Discomfort Scale pre- and post-treatment between both groups

|          | Group I Before | After | P    | Group II Before | After | P    |
|----------|----------------|-------|------|-----------------|-------|------|
| Discomfort | 4              | 0.4   |      | 4               | 0.3   |      |
| Burning  | 2.1            | 1.1   |      | 2.1             | 0.1   |      |
| Grittiness | 2.1           | 1.1   |      | 2.1             | 1.1   |      |
| Stinging | 3.2            | 0.2   | 0.03 | 3.6             | 0.5   | 0.02 |

Figure 5. An increased density of goblet cells after treatment in the second group, which was compared with the condition before treatment

3.2 Discussion

Dry eye syndrome are more prevalent among patients with glaucoma, as most anti-glaucoma eye drops contain preservatives that might cause the ocular surface toxicity. Some studies reported a marked increase in inflammatory biomarker levels, including TNF, IL-1, IL-12, IL-10, and C-reactive protein, when the eyes were exposed to the prolonged use of BAK [3,16].

This study demonstrates that both clinical ocular comfort and statistical improvement were observed in both groups treated with SH eye drops, as shown by different values of the Schirmer’s test, TFBUT, and OPI within groups pre- and post-treatment, but not between groups. The increased value of diagnostic dry eye test might account for the characteristics of SH eye drops that can enhance the tear film stability by maintaining a good surface of the tear film, mucoadhesive properties, and its capacity to decrease the evaporation of aqueous component and protect the ocular surface damage [17,18]. In addition, this study established an increased goblet density post-treatment in both groups regardless of the presence of BAK as a preservative agent; however, the increase was not statistically significant.

Our findings can be described by comprehending the role of SH that can stimulate the cell proliferation in conjunctival epithelial cells by binding to CD4 receptors [19]; its viscoelastic properties might provide protection against mitotic cessation, inflammatory cytokinesis, and could create a protective layer against the cell membrane by binding to the receptors in conjunctival epithelial cells. In addition, SH is also rich in hydroxyl that might absorb the reactive oxygen species, in which an excess of this product can cause oxidation and reduction imbalance inside the cells, possibly leading to DNA damage [20,21]. All these mechanisms might contribute to the increased density of goblet cells after patients received artificial tear eye drops, either with or without preservative agents [22]. When a low-value goblet cell density was observed, it was attributed to the inability to produce a sufficient mucus of the tear film, as it is mostly produced by goblet cells. In this
study, we obtained a wide range of the goblet cells density, which indicated that the tear film layer stability was not merely dependent on the goblet cells’ mucus production, and other cells might contribute to the tear film stability.

In this study, we used BAK at a concentration level of 0.003% per De Saint Jean et al. [21] who revealed that the treatment with BAK at 0.1% and 0.05% concentration levels might cause direct lysis of conjunctival cells. Thus, we concluded that the BAK concentration of <0.005% in the eye drop treatment would be safe and provide beneficial effects. To date, the role of SH in neutralizing the toxic effect of BAK remains debatable; thus, our study glaucoma medication eye drops continued administering the same BAK concentration. However, >50% of patients had OPI results of >1 after they were treated with SH eye drops, suggesting that the tear film in both groups offered better protection. Furthermore, good protection, which was exerted by the tear film, suggested a stable tear film layers as expressed by clinically ocular comfort scale results in both groups [18].

This study has some limitations, including some biases in compliance and measuring techniques. Nevertheless, it also has some strengths, as it used an objective measuring technique such as the computed conjunctival goblet cell impression cytology. Overall, this study clearly validated an achievement that a local product displayed a comparable improvement in the ocular surface test and clinical symptoms.

4. Conclusion
This study suggests that both groups elicit comparable improvement in the values of Schirmer’s test, TFBUT, OPI, and ocular discomfort within 3 months in patients with glaucoma and dry eyes syndrome, with no significant difference in both groups.

Abbreviations

| Abbreviation | Definition |
|--------------|------------|
| BAK          | benzalkonium chloride |
| IBI          | interblink interval |
| IOP          | intraocular pressure |
| OPI          | Ocular Protection Index |
| POAG         | primary open-angle glaucoma |
| PACG         | primary angle-closure glaucoma |
| TFBUT        | tear film break-up time |

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