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# Effect of Eyewash Solution (Commercial Washing Solution) on the Corneal Epithelium: Adverse Effects of Benzalkonium Chloride on the Eye Surface

**Masanori Iwashita[1,2], Dogru Murato[3], Hiroko Yano[4], Yasuko Santo[4], Manabu Nozaki[4] and Hiroshi Fujishima[1,2]***

[1]*Department of Ophthalmology, School of Dental Medicine, Tsurumi University, Kanagawa, Japan*
[2]*Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan*
[3]*Department of Ophthalmology, Tokyo Dental College Ichikawa General Hospital, Chiba, Japan*
[4]*Kobayashi Pharmaceutical Co., Ltd, Osaka, Japan*

## Abstract

**Purpose:** In recent years, there has been an increasing interest in eye washing in healthy individuals due to pollinosis and an increase in the number of people wearing contact lenses. Previously, commercial eyewash solutions contained a preservative (benzalkonium chloride [BAK]) that was shown to be associated with epithelium disorders. However, currently, a reduction in corneal epithelium disorders is assumed to be due to the popularization of eyewash solutions lacking BAK (over-the counter drugs).

**Methods:** We performed a comparative study between current eyewash solutions and those utilized in the past, and examined the effects of BAK on the cornea in both rabbit and human eyes.

**Results:** Eyewash solutions containing BAK were associated with corneal epithelium disorders and collapse of the mucin layer in the cornea. However, eyewash solutions lacking BAK were not associated with corneal epithelium disorders and did not affect the mucin layer.

**Conclusion:** The study highlights the adverse effects of a preservative and the safety of eyewash solutions not containing a preservative.

**Keywords:** Benzalkonium chloride (BAK); Ocular surface; Eye wash solution; Mucin layer; Corneal epithelitis

## Introduction

There are increasing opportunities for itching and foreign-body sensations in and around the eyes in healthy individuals due to an increased numbers of patients with pollinosis and contact lens users, and environmental changes such as an incoming of particles with a particle mass of 2.5 μm (PM 2.5) and yellow sand [1-5]. It is extremely important, as a self-care practice, to evade or eliminate antigens to prevent the occurrence of itching [6] and, in Japan; practicing washing to eliminate antigens in and around the eyes is becoming general practice. Alongside a popularization of commercial eyewash solutions (over-the-counter [OTC] drugs), the interest in eye washing in healthy individuals and patients is increasing.

Looking back at the history of commercial eyewash solutions, a cup-type eyewash solution, Eyebon® (Kobayashi Pharmaceutical Co., Ltd.; Osaka, Japan), was launched in 1995 triggering wide recognition of eyewash solutions. Since previous eyewash solutions contained a preservative (benzalkonium chloride [BAK]), and with Eyebon®, with the largest share in the market, also containing BAK until 2002, corneal epithelium disorders were found to be associated with the use of eyewash solutions [7]. At present, however, eyewash solutions free of BAK have become popular, reducing the risk of corneal epithelium disorders.

Therefore, in this study, we observed the effects of eyewash solutions on the corneal epithelium experimentally using rabbits, to examine the presence or absence of BAK in the eyewash solutions currently on the market, and their effects on the corneal epithelium. We also conducted a clinical study on healthy individuals and comparatively examined objective findings in different eye wash solution groups.

## Methods

### Eyebon®

Eyebon® was the commercial eyewash solution used in the animal experiment and human clinical examinations, the details of which are as follows. The maximum frequency of daily use is six times, based on the manufacturing/marketing approval standards of OTC drugs, [8] and duration of washing is specified at not more than 30 seconds per administration based on a report on corneal epithelium disorders [7]. The solution does not contain BAK. The utilization method and photograph of the eyewash cup are shown in Figure 1.

### Attention

- Do not wash the opposite eye with the solution used already for washing.
- Do not wash the eye for longer than 30 seconds.
- The color of the solution is the color of vitamin B12. If the solution adheres on your clothes accidentally, wash with water as quickly as possible.

### Other attention

- Be sure to remove contact lenses, if worn, before use of the eyewash solution.
- Wipe off cosmetics and blots around the eyes before use.

***Corresponding author:** Hiroshi Fujishima, Department of Ophthalmology, Tsurumi University School of Dental Medicine, 2-1-3 Tsurumi, Tsurumi-ku, Yokohama, Kanagawa 230-8501, Japan, Tel: +81-45-581-1001; Fax: +81-45-573-9599; E-mail: fujishima117@gmail.com

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(1) Pour the solution up to the line (5 mL) inside the attached eyewash cup, press firmly to the eye.

(2) While pressing the cup to the eye, face up by laying your head back taking care not to spill the solution, and wash your eye with several blinks.

**Figure 1:** Instructions for administration.

- Wash the eye washing cup thoroughly before and after use.
- Wipe lightly the pink crystals that may adhere around the mouth of the container depending on the storage conditions with clean gauze.

**Active ingredients (in 100 mL):** Dipotassium glycyrrhizinate 25 mg, chlorpheniramine maleate 3 mg, taurine 100 mg, pyridoxine hydrochloride (vitamin B6) 10 mg, cyanocobalamin (vitamin B12) 1 mg, and chondroitin sulfate sodium 10 mg.

**Excipients (in 100 mL):** Boric acid, borax, polysorbate 80, sodium edetate, propylene glycol, l-menthol, dl-camphor, and pH regulator.

**Dosage and administration:** Wash the eyes three to six times daily using 5 mL of the eyewash solution per administration.

## Animal experiment

**Animals:** Female Japanese white rabbits (weighing 1.95 to 2.42 kg) were utilized. The experiment was performed in 21 rabbits randomized into three groups each comprising seven animals and the results were summarized after completion.

**Test solutions (Table 1):** Physiological saline solution and three types of eyewash solution were selected:

- Physiological saline solution
- Eyebon® (hereinafter referred to as solution A)
- Eyebon® containing BAK (referred to as solution B)
- Physiological saline solution containing BAK (referred to as solution C)

The active ingredient in the solution A was as mentioned previously, the ingredients in the solution B comprised the ingredients in the solution A mixed with BAK (10 mg/100 mL), and those in the solution C comprised physiological saline solution mixed with BAK (10 mg/100 mL).

## Evaluation methods

A disposable cup approximately 26.5 mm in diameter containing 5 mL of one of the test solutions was pressed around the right and left eyes of rabbits without anesthesia to expose the eye surface to the test solution for 30 seconds with the eyelids open, and allowed to stand. Eye washing was performed nine times in 1-hour intervals, assuming a severer condition than the upper limit of six washings per day in the dosage and administration for humans.

Corneal epithelium disorders were assessed before eye washing (7 days before the day of eye washing) and 1 hour after eye washing by dropping 2 µL of fluorescein sodium solution and observing the area of corneal abrasion (area stained with a yellow-green fluorescent color) using a slit lamp (SL-2, Kowa Company, Ltd.). The cornea was divided into nine blocks (Figure 2) and the areas stained were scored with nine points at the highest (Table 2). The staining was also performed with lissamine green stain solution and the areas stained were evaluated by the same criteria to observe any defect in the mucin layer.

| | Name | Description | Preservative |
|---|---|---|---|
| - | Physiological saline solution | A clear liquid | Not added |
| A | Eyebon® | A pink liquid | Not added |
| B | Eyebon® with benzalkonium chloride | A pink liquid | Benzalkonium chloride (10 mg/100 mL) |
| C | Physiological saline solution with benzalkonium chloride | A clear liquid | Benzalkonium chloride (10 mg/100 mL) |

**Table 1:** Test solutions.

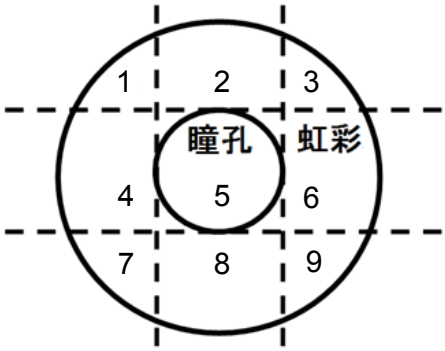



**Figure 2:** Cornea evaluation sections.

| | |
|---|---|
| **1.** | The cornea was divided into nine areas with the pupil as the indicator and the areas stained were scored (nine points at the highest). |
| **2.** | Fluorescein staining and lissamine green staining were evaluated. |

**Table 2:** Corneal staining evaluation.

Confocal microscopy was performed before eye washing (7 days before the day of eye washing) and 1 hour after administration using a confocal microscope (Heidelberg Retina Tomograph HRT2R, Rostock Cornea Module Model: HRT2R/RCM, HEIDERBERG ENGINEERING GmbH) and the corneal epithelial cells, keratocytes, and corneal endothelial cells in both eyes were observed and their densities determined. The measurement of the corneal epithelial cells 1 hour after the administration was performed at three sites.

For statistical analysis, the scores at each measurement time interval were compared between the groups using analysis of variance and paired t-test, and the cases with a risk rate of 5% or lower were statistically evaluated.

### Clinical examination

**Subjects:** The study was conducted in healthy individuals who visited the outpatient Department of Ophthalmology, Tsurumi University, between February and April 2014, after obtaining the approval from the Ethical Review Committee at Tsurumi University. This study was based on the study protocol and the ethical principles of the Declaration of Helsinki. Thirty subjects aged between 23 and 60 years with a mean age of 39.7 years participated in the study. Inclusion criteria comprised the following:

- Healthy individuals who had not used contact lenses for at least 1 day before the study.
- Healthy individuals aged 20 years and older at the time of informed consent.
- Healthy individuals that received a full explanation on the objectives and methods of the study, expected effects and adverse drug reactions, respect for the privacy, recompense and compensation, and who themselves or their legal representatives voluntarily provided consent for study participation.

**Test solutions (Table 1):** The test solutions were administrated blinded and the results were summarized after completion. The following three types of eyewash solutions were selected as the test solution:

- Eyebon® (hereinafter, referred to as solution A)
- Eyebon® containing BAK (referred to as solution B)
- Physiological saline solution containing BAK (referred to as solution C)

**Evaluation methods:** The subjects washed the right eye with the physiological saline solution as the control and the left eye with the respective test solutions without anesthesia. The eyes were washed for 30 seconds with 5 mL of the eyewash solution at a time using the eye cup for commercial eyewash solutions (eye cup attached to Eyebon®). Eye washing was performed six times in total in 1-hour intervals, based on the upper limit specified in the dosage and administration.

Corneal epithelium disorders were observed using fluorescein vital staining and fluorophotometry. In addition, lissamine green vital staining was performed to evaluate the corneal epithelial mucin layer. Fluorescein staining (2 μL) was performed and evaluated initially, by an ophthalmologist before eye washing. Eyes were washed with 20 mL of BSS-plus after 10 minutes to wash away the fluorescein stain solution and, 20 minutes later (30 minutes after fluorescein staining), fluorescein remaining on the cornea was measured using the fluorophotometry. Following this, evaluation by lissamine green staining (2 μL) was performed. After eye washing, fluorescein staining evaluation, fluorophotometry measurements, and lissamine green staining evaluation were performed in the same manner as before eye washing, and the results were compared before and after eye washing.

For statistical analysis, the inter-group comparison of scores and two-sample comparisons were performed using the Tukey multiple comparison test and non-paired t-test, and cases with a risk rate of 5% or lower were statistically evaluated.

## Results

### Animal experiments

In the corneal epithelium observations performed 1 hour after the last eye washing (session 9) (Figure 3), the fluorescein staining score, lissamine green staining score, and the corneal epithelial cell density by confocal microscopy in solution A group were all comparable to those in the physiological saline and fluorescein staining groups (with a significant difference). The lissamine green staining group had lower scores than solution B group. Solution B group showed higher fluorescein

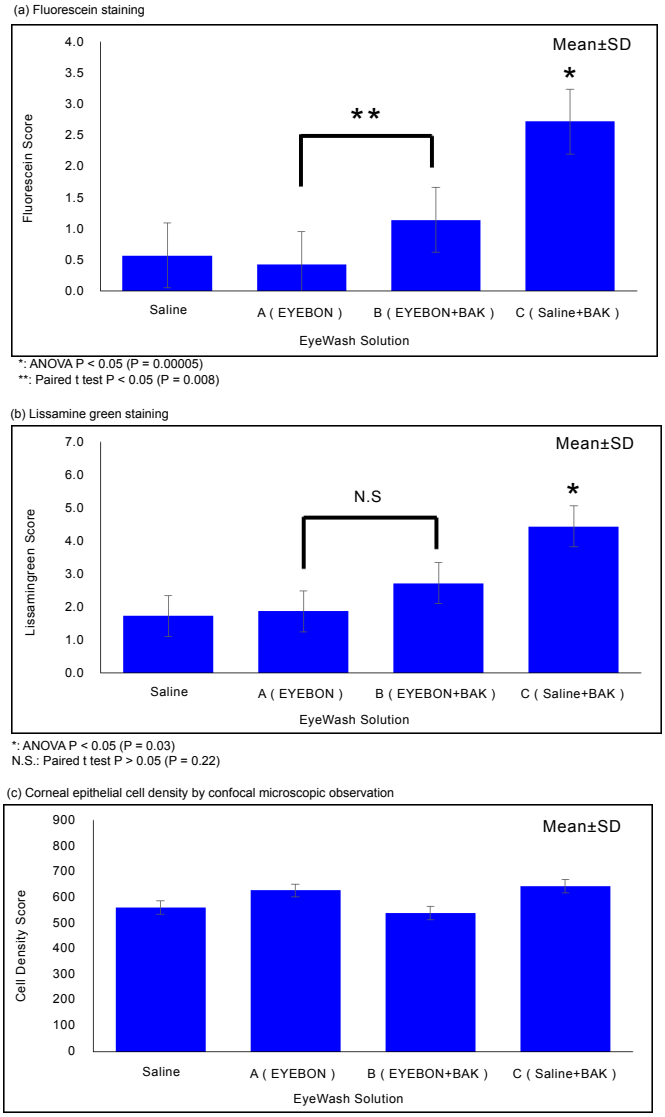



**Figure 3:** Animal experiment results.

and lissamine green staining scores compared in the physiological saline group; however, the differences were not significant. Solution C group showed significantly higher fluorescein and lissamine green staining scores than in other groups. Confocal microscopy showed no significant differences in the respective groups compared with the physiological saline group. For reference, photographs of fluorescein and lissamine green staining (Figure 4) and confocal microscopic observations (Figure 5) are presented.

### Clinical examination

**Before and after eye washing comparisons (Figure 6):** The cornea was observed before eye washing and immediately after the last eye washing (session 6). The scores generally decreased in solution A group. The scores were comparable or increased in solution B group; however, values were variable. In solution C group the scores increased.

**Inter-group comparison of scores after eye washing (Figure 7):** The cornea was observed immediately after the last eye washing (session 6). Solution A group showed a significantly lower score in the fluorescein and lissamine green staining compared with solution B group. Although no significant difference was noted in fluorophotometry, score were lower than that in other groups. Solution C group showed a significantly higher score in all evaluation items. For reference, photographs of fluorescein and lissamine green staining (Figure 8) are presented.

## Discussion

In the experiment using rabbits, the effects of eyewash solutions on the cornea were examined. Compared with eye washing using the physiological saline solution (control), eye washing with the physiological saline solution containing the preservative BAK showed a significant difference in fluorescein and lissamine green staining scores, suggesting corneal epithelium disorders and collapse of the mucin layer associated with BAK. With Eyebon®, no significant differences were recorded in any of the items compared with the physiological saline solution even though eye washing was performed nine times, exceeding the upper limit specified in the dosage and administration for humans. There were also no significant differences in the observations of the corneal epithelium, keratocytes, and corneal endothelium by confocal microscopy. This may indicate that the corneal disorders associated with BAK were limited to the uppermost layer of the cornea. In the comparison between Eyebon® and Eyebon® containing BAK, a significant difference was recorded in the fluorescein staining, indicating corneal disorders caused by BAK. In lissamine green staining, however, the lack of significant difference indicated absence of a disorder in the mucin layer. Eyebon® contains ingredients such as sodium chondroitin sulfate used for the treatment of dry eyes and this may have reduced the collapse in the mucin layer by BAK. These findings suggest that the eyewash solutions lacking BAK had less influence on the corneal epithelium and can be used safely. We also performed a clinical study in healthy humans, referring to the results of the animal experiment.


**Figure 4:** An example of fluorescein and lissamine green staining scoring in a rabbit after washing nine times with physiological saline containing BAK.

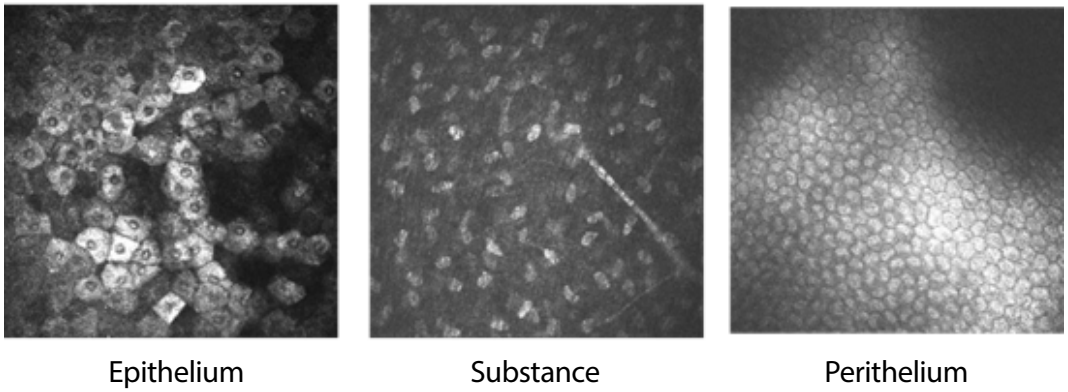
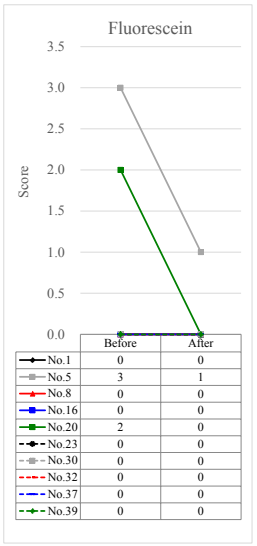
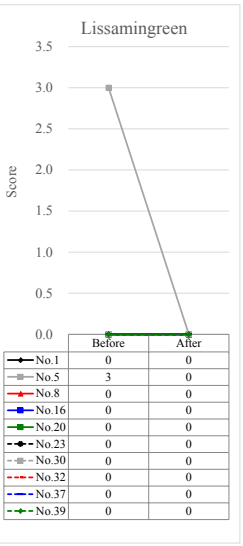



**Figure 5:** An example of confocal microscopic observations in a rabbit after washing nine times with physiological saline containing BAK.

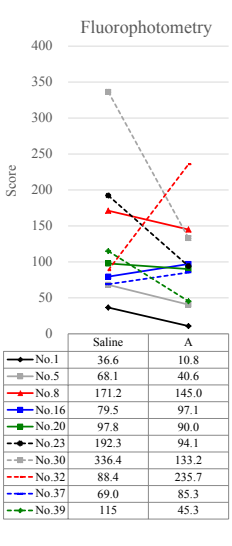
(Test solution A) Eyebon®




| | Before | After |
|---|---|---|
| No.1 | 0 | 0 |
| No.5 | 3 | 1 |
| No.8 | 0 | 0 |
| No.16 | 0 | 0 |
| No.20 | 2 | 0 |
| No.23 | 0 | 0 |
| No.30 | 0 | 0 |
| No.32 | 0 | 0 |
| No.37 | 0 | 0 |
| No.39 | 0 | 0 |

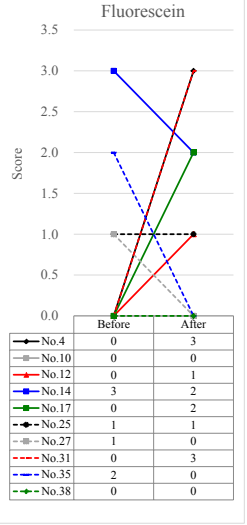



| | Before | After |
|---|---|---|
| No.1 | 0 | 0 |
| No.5 | 3 | 0 |
| No.8 | 0 | 0 |
| No.16 | 0 | 0 |
| No.20 | 0 | 0 |
| No.23 | 0 | 0 |
| No.30 | 0 | 0 |
| No.32 | 0 | 0 |
| No.37 | 0 | 0 |
| No.39 | 0 | 0 |

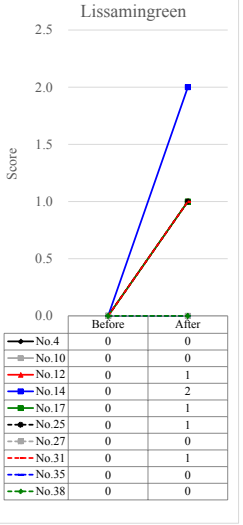



| | Saline | A |
|---|---|---|
| No.1 | 36.6 | 10.8 |
| No.5 | 68.1 | 40.6 |
| No.8 | 171.2 | 145.0 |
| No.16 | 79.5 | 97.1 |
| No.20 | 97.8 | 90.0 |
| No.23 | 192.3 | 94.1 |
| No.30 | 336.4 | 133.2 |
| No.32 | 88.4 | 235.7 |
| No.37 | 69.0 | 85.3 |
| No.39 | 115 | 45.3 |

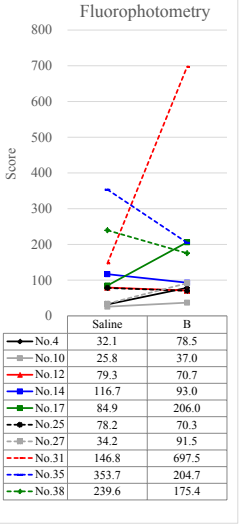
(Test solution B) Eyebon® containing benzalkonium chloride




| | Before | After |
|---|---|---|
| No.4 | 0 | 3 |
| No.10 | 0 | 0 |
| No.12 | 0 | 1 |
| No.14 | 3 | 2 |
| No.17 | 0 | 2 |
| No.25 | 1 | 1 |
| No.27 | 1 | 0 |
| No.31 | 0 | 3 |
| No.35 | 2 | 0 |
| No.38 | 0 | 0 |

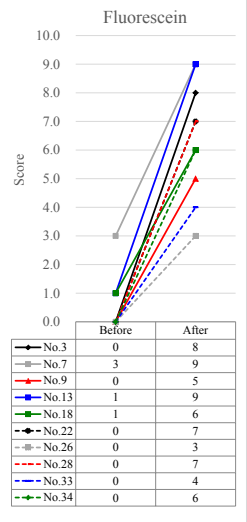



| | Before | After |
|---|---|---|
| No.4 | 0 | 0 |
| No.10 | 0 | 0 |
| No.12 | 0 | 1 |
| No.14 | 0 | 2 |
| No.17 | 0 | 1 |
| No.25 | 0 | 1 |
| No.27 | 0 | 0 |
| No.31 | 0 | 1 |
| No.35 | 0 | 0 |
| No.38 | 0 | 0 |

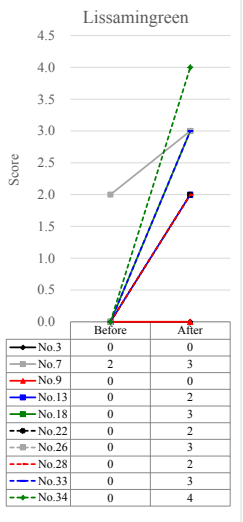



| | Saline | B |
|---|---|---|
| No.4 | 32.1 | 78.5 |
| No.10 | 25.8 | 37.0 |
| No.12 | 79.3 | 70.7 |
| No.14 | 116.7 | 93.0 |
| No.17 | 84.9 | 206.0 |
| No.25 | 78.2 | 70.3 |
| No.27 | 34.2 | 91.5 |
| No.31 | 146.8 | 697.5 |
| No.35 | 353.7 | 204.7 |
| No.38 | 239.6 | 175.4 |

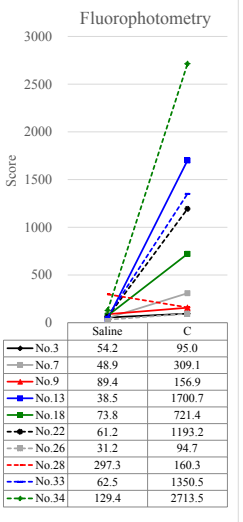
(Test solution C) Physiological saline solution containing benzalkonium chloride




| | Before | After |
|---|---|---|
| No.3 | 0 | 8 |
| No.7 | 3 | 9 |
| No.9 | 0 | 5 |
| No.13 | 1 | 9 |
| No.18 | 1 | 6 |
| No.22 | 0 | 7 |
| No.26 | 0 | 3 |
| No.28 | 0 | 7 |
| No.33 | 0 | 4 |
| No.34 | 0 | 6 |



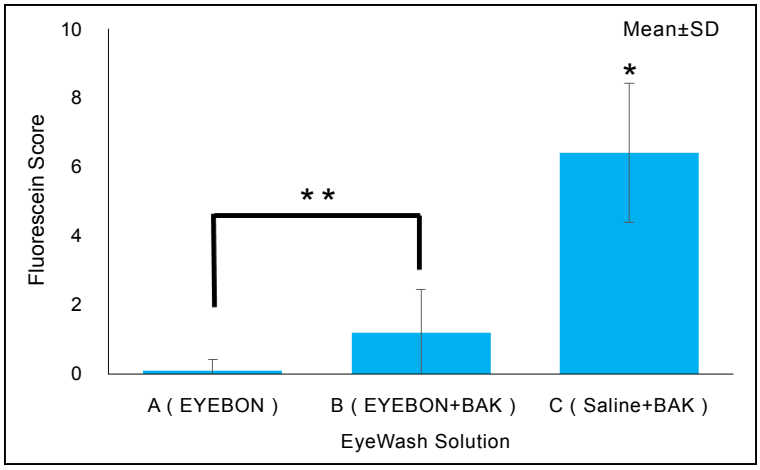

| | Before | After |
|---|---|---|
| No.3 | 0 | 0 |
| No.7 | 2 | 3 |
| No.9 | 0 | 0 |
| No.13 | 0 | 2 |
| No.18 | 0 | 3 |
| No.22 | 0 | 2 |
| No.26 | 0 | 3 |
| No.28 | 0 | 2 |
| No.33 | 0 | 3 |
| No.34 | 0 | 4 |




| | Saline | C |
|---|---|---|
| No.3 | 54.2 | 95.0 |
| No.7 | 48.9 | 309.1 |
| No.9 | 89.4 | 156.9 |
| No.13 | 38.5 | 1700.7 |
| No.18 | 73.8 | 721.4 |
| No.22 | 61.2 | 1193.2 |
| No.26 | 31.2 | 94.7 |
| No.28 | 297.3 | 160.3 |
| No.33 | 62.5 | 1350.5 |
| No.34 | 129.4 | 2713.5 |

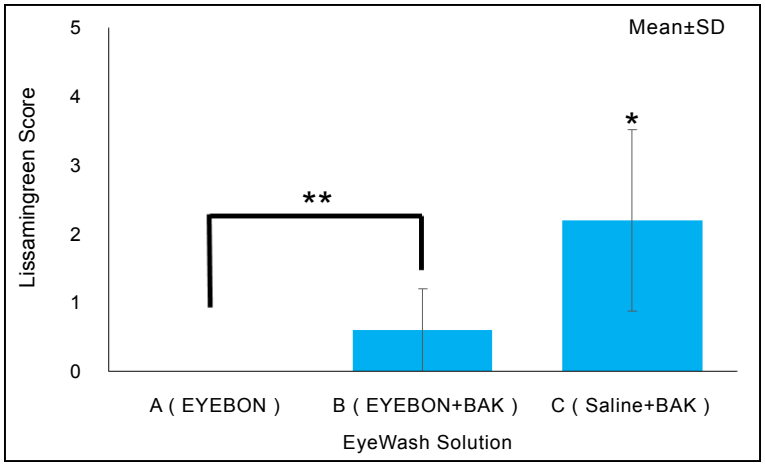
**Figure 6:** Clinical examination results (before and after eye washing comparisons).

(1) Fluorescein staining



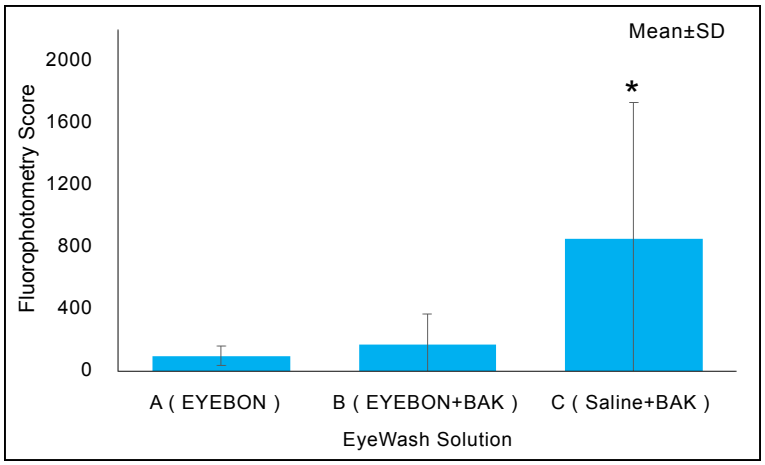

*: Tukey P < 0.05
**: non paired t test P < 0.05 (P = 0.013)

(2) Lissamine green staining




*: Tukey P < 0.05
**: non paired t test P < 0.05 (P = 0.014)

(3) Fluorophotometry




*: Tukey P < 0.05

**Figure 7:** Clinical examination results (inter-group comparison of scores after eye washing).

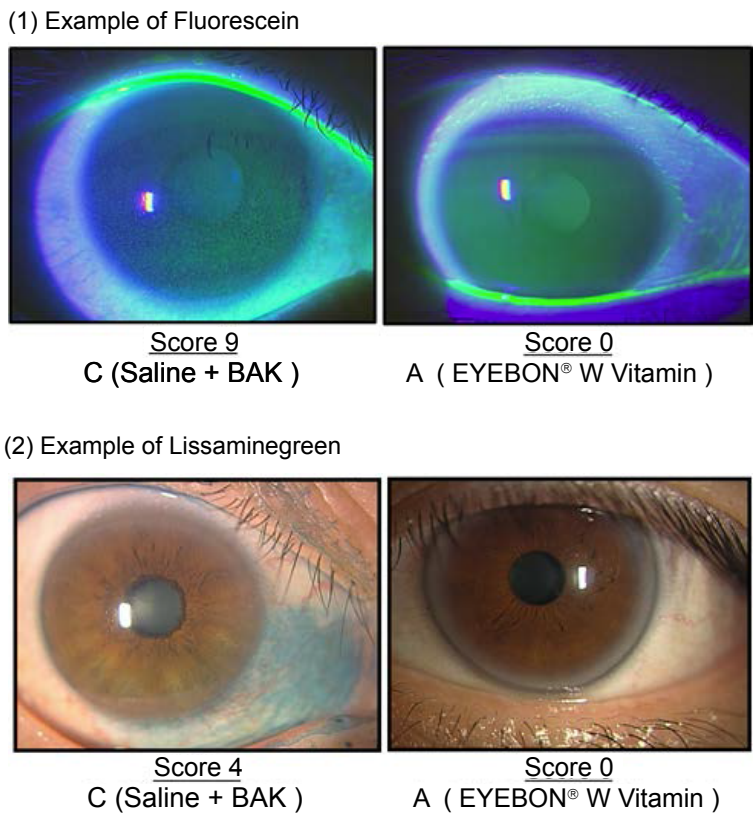



**Figure 8:** An example of fluorescein and lissamine green staining scoring in a human eye after washing nine times with physiological saline containing BAK or Eyebon®.

In the healthy individual study, eye washing was performed for 30 seconds durations, six times in total at 1-hour intervals, based on the upper limit specified in the dosage and administration for humans. The physiological saline solution containing BAK showed significant differences, not only in the fluorescein staining and lissamine green staining performed before and after the eye washing (similar to the animal experiment), but also in the fluorophotometry. Eyebon® showed significantly lower scores in the fluorescein and lissamine green staining compared with Eyebon® containing BAK. These results indicate corneal epithelium disorders and collapse of the mucin layer due to BAK, suggesting that BAK could aggravate eye diseases such as dry eyes and superficial punctate keratitis.

Akei et al. examined allergic reactions caused by the adhesion of pollens on the skin in percutaneously sensitized mice and reported systemic induction of Th2 immune response and susceptibility to induction of allergic rhinitis [9]. Other studies have reported aggravation of transbronchial allergic reactions in mice with atopic dermatitis induced by percutaneous sensitization [10], and complication of asthma and allergic rhinitis in 70% of patients with severe atopic dermatitis [11]. These results suggest that reduction of the percutaneous sensitization in the skin with pollen can also prevent the effects on other allergic disorders.

Furthermore, destruction of the tight junction in epithelial cells by diesel exhaust particles (DEP) has been reported [12,13]. DEP destroys the tight junction by inducing oxidation stress mediating production of reactive oxygen species instead of direct destruction of the tight junction by proteases. Since the destruction of the tight junction accelerates the contact between immune cells and allergens by increasing the penetration of allergens into the subepithelial tissue, protection of the epithelial barrier is important for prevention and treatment of allergic disorders [14,15]. Therefore, eyewash solutions are considered to be effective for the prevention and treatment of allergic disorders.

A new concept, eyelid skin pollinosis (pollen dermatitis or pollen blepharitis), has been proposed suggesting that adhesion of pollens to the thin and soft eyelid skin easily induces skin allergy [16-18]. In such patients, ocular instillation is not as effective because itching occurs in the eyelid skin rather than the eyeball. These patients are thought to require treatment of the eyelids [19,20] and eyewash solutions could be one of the methods for treating or preventing itching without any adverse drug reactions.

In recent years, a report on the optimization of BAK concentration in ophthalmic solutions for the treatment of glaucoma [21], and development of BAK-free ophthalmic solutions for the treatment of allergy and dry eyes have led to absence of precautions in the formulation of eyewash solutions in the package insert for contact lens users. While such modifications were taken due to the occurrence of corneal epithelium disorders with BAK in conventional ophthalmic solutions [22,23], eyewash solutions also contained BAK in the past and the occurrence of corneal epithelium disorders raised concern. While the examinations in rabbits and healthy volunteers in the present study revealed effects of BAK on the cornea in eyewash solutions, it was indicated that the occurrence of adverse drug reactions such as corneal epithelium disorders could be prevented by selecting BAK-free eyewash solutions similar to ophthalmic solutions.

There is an increasing interest in healthy individual eye washing due to pollinosis and an increase in the number contact lens users. Eyebon® has a big share in the commercial eyewash solution market and the results obtained in the present study are beneficial because they demonstrate the safety of the product, which does not contain BAK. In addition, eye washing could be one of the safest methods for washing the anterior ocular segment as a countermeasure against increasing trends of yellow sand and PM 2.5 in the future. We will continue to investigate the details of its safety, as well as its efficacy, in future studies.

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