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Research article

Effects of Over-the-Counter Eyewash Products on Cedar Pollen Wall Rupture

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Abstract

Background: Cedar pollen-associated allergic inflammation is caused by antigenic proteins released from the pollen wall and the endosporium. The rupture of the pollen wall augments the release of antigenic proteins. Eye washing, particularly with eyewash agents that suppress pollen wall rupture, is one of the most useful preventive measures against pollinosis. In this study, we investigated the effects of the acid level and composition of over-the-counter eyewash solutions on the pollen rupture rate and antigenic protein elution. Methods: Japanese cedar pollen was suspended in various test solutions and the rupture rate was calculated using light microscopy. Cry j 1 and Cry j 2 levels were measured in the supernatant fluid. Test solutions included artificial tears, eyewash, leporine lacrimal fluid, as well as solutions with different pH levels and ingredients that were prepared to determine the factors contributing to pollen rupture. Results: The pollen rupture rate was lower when suspended in eyewash than in artificial tears or leporine lacrimal fluid. The addition of potassium chloride and sodium chloride was demonstrated to increase the pollen rupture rate. Conclusions: Salts such as sodium chloride and potassium chloride, as well as pH, were involved in pollen wall rupture. The eyewash used in this study had a low pH and did not contain any salts, thus presenting a decreased pollen rupture rate and release of antigenic proteins. Therefore, our results suggest that the use of eyewash suppresses pollen rupture and eliminates cedar pollen.

Keywords: cedar pollen, eyewash, ingredient, lacrimal fluid, pollen wall rupture.

Introduction

Antigenic proteins released from the pollen wall and endosporium infiltrate the conjunctival epithelium and bridge the 2 IgE antibody molecules on mastocytes underneath theconjunctival epithelium. This causes the degranulation of mastocytes and release of chemical mediators, including histamine, which is followed by cedar pollen-induced allergic inflammation. Cry j 1 (molecular weight: 45–50 kDa), which is localized in the pollen wall, and Cry j 2 (molecular weight: 37 kDa), which is localized in starch granules in the pollen, have been identified as the major cedar pollen antigens [1,2]. Their elution is affected by the fluid where the pollen granules exist. Antigen elution results from the rupture of the thinnest part of the pollen wall after water absorption. A previous study reported that the rupture rate may increase depending on the presence of proteins, mucins, and lysozymes in nasal discharge. Furthermore, the physicochemical properties such as alkaline conditions, temperatures close to that of the body, and the presence of in vivo secretion create an environment that promotes pollen rupture and elution of antigenic proteins[3].

Similar results were reported regarding the relationship between lacrimal fluid and pollen rupture. A study on the effects of four eyedrop products for the treatment of allergic conjunctivitis and associated eye dryness on pollen wall rupture showed that these eye-drops suppressed antigen elution in lacrimal fluid[4].

Among the body parts exposed to pollens, the eyes are covered with lacrimal fluid and frequently have direct contact with the external environment. Thus, the eyes are more prone to develop symptoms. Although washing out cedar pollen from the eyes to suppress itchiness is one ofthe useful preventive measures against hay fever [5], agents that not only eliminate the pollen but also suppress pollen wall rupture are preferable.

We aimed to investigate the effects of the acidity and composition of over-the-counter (OTC) eyewash products on pollen wall rupture and the elution of the major antigenic proteins Cry j 1 and Cry j 2. Furthermore, since as the lacrimal fluid promotes immediate pollen wall rupture and antigenic protein elution, we investigated the importance of washing out pollen residue from the eyes in an in vitro study by measuring Cry j 1 and Cry j 2 levels in the

pollen residue after the rupture.

Methods

Test solutions

The following test solutions were used (Table 1): OTC artificial tears (solution 1) at pH 7.1, and Eyebon®AL (solution 2) OTC eyewash at pH 6.0 (Kobayashi Pharmaceutical Co., Ltd., Osaka, Japan). In addition, an eyewash solution adjusted to pH 7.1 with NaOH (solution 3) was prepared to investigate the effect of pH.

Table 1. List of test solution contents

Test solution number	1†	2‡	3	4	5	6	7	8	9	10	11	12	13
Dipotassium glycyrrhizinate	-	✓	✓	✓	✓	-	-	✓	-	-	-	-	-
Sodium chondroitin sulfate	-	✓	✓	✓	✓	-	-	-	✓	-	-	-	-
Chlorpheniramine maleate	-	✓	✓	✓	✓	-	-	-	-	✓	-	-	-
Epsilon aminocaproic acid	-	✓	✓	✓	✓	-	-	-	-	-	✓	-	-
Potassium chloride	✓	-	-	✓	-	-	✓	-	-	-	-	-	-
Sodium chloride	✓	-	-	-	✓	-	✓	-	-	-	-	-	-
Boric acid	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Borax	-	✓	✓	✓	✓	-	-	-	-	-	-	-	-
Polysorbate 80	-	✓	✓	✓	✓	-	-	-	-	-	-	✓	✓
l-Menthol	-	✓	✓	✓	✓	-	-	-	-	-	-	-	✓
d-Borneol	-	✓	✓	✓	✓	-	-	-	-	-	-	-	✓
Edetate sodium	-	✓	✓	✓	✓	-	-	-	-	-	-	-	-
NaOH	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
pH	7.1	6.0	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1

✓ : Present
- : Not present
† : Over-the-counter (OTC) artificial tears
‡ : OTC eyewash (Eyebon®AL)

To investigate the effects of the ingredients contained in artificial tears and eyewash, potassium chloride and sodium chloride were added to eyewash solution (solutions 4 and 5, respectively). Potassium chloride/sodium chloride (solution 7), dipotassium glycyrrhizinate (solution 8), sodium chondroitin sulfate (solution 9), chlorpheniramine maleate (solution 10), epsilon aminocaproic acid (solution 11), polysorbate 80 (solution 12), and polysorbate 80/l-menthol/d-borneol (solution 13) were added to a basic boric acid solution (solution 6, Table 1). The pH of solutions that were numbered 4 to 13 was adjusted to 7.1.

Rabbit lacrimal fluid (pH 7.8) was obtained from the Drug Safety Testing Center Co., Ltd. (Saitama, Japan). They were collected from forced open eyes using a lid retractor under general anesthesia was used as a control.

Measurement of pollen rupture rate

Japanese cedar pollen (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) was added to each test solution at a concentration of 10 mg/mL and was then incubated at 37 °C. After 5, 30, and 60 min, 10 µL of each test solution was placed on a glass slide and covered with a cover slip. The number of ruptured pollen grains as well as the total number of pollen grains (≥200) were counted using a light microscope (ECLIPSE E600; Nikon, Tokyo, Japan). The pollen rupture rate was calculated using the following equation: pollen rupture rate (%) = number of ruptured pollen/total number of pollen grains × 100. The means of the three repeated procedures were used.

Microscopic observations of ruptured pollen

Microscopic images (100 × magnification) were captured after 5 and 60 min using a light microscope (ECLIPSE E600; Nikon).

Quantification of antigenic proteins

Japanese cedar pollen (FUJIFILM Wako Pure Chemical Corporation) was added to each test solution at a concentration of 10 mg/mL to measure Cry j 1, and 200 mg/mL to measure Cry j 2, before incubating at 37 °C. The solutions were immediately cooled using ice water after 5 min. The solutions were then centrifuged at 11,000 × g at 4 °C for 3 min before removing the supernatant, which was also centrifuged. The supernatant was analyzed using Cry j 1 and Cry j 2 enzyme-linked immunosorbent assay (ELISA) kits (Institute of Immunology Co., LTD, Tokyo, Japan) by a sandwich assay.

Extraction of antigenic proteins contained in pollen residue after rupture

Japanese cedar pollen (FUJIFILM Wako Pure Chemical Corporation) was added to an artificial tear solution at a concentration of 10 mg/mL to measure Cry j 1, and at 200 mg/mL to measure Cry j 2, before incubating at 37 °C for 3 h. The pollen rupture rate was measured to confirm that the percentage of ruptured pollen was ≥95%. The solutions were immediately cooled using ice water and centrifuged at 11,000 × g and 4 °C for 3 min before removing the supernatant, which was stored at 4 °C as the post-rupture elution solution. To remove the remaining pollen residue, the solution was washed three times via centrifugation with artificial tears, disrupted with an ultrasonic disruptor Handy Sonic UR-20P (Tomy Seiko Co., Ltd., Tokyo, Japan) for 15 min, and centrifuged at 11,000 × g and 4 °C for 3 min before removing the supernatant, which was stored at 4 °C as the disrupted pollen residue solution. The post-rupture elution solution and the disrupted pollen residue solution were analyzed using Cry j 1 and Cry j 2 ELISA kits (Institute of Immunology Co., LTD) by a sandwich assay.

Statistical analysis

Rate tests were conducted to assess pollen rupture rates, and unpaired Student's t-tests were performed to compare antigenic protein levels. The level of significance for statistical analyses was 5% (two-sided).

Results

Effects of artificial tears, leporine lacrimal fluid, and eyewash on cedar pollen rupture rate

Micrographs of pollen rupture in artificial tear (pH 7.1) and eyewash (pH 6.0) solutions are shown in Figure 1. The changes observed in cedar pollen rupture rates in eyewash (pH 6.0), artificial tears (pH 7.1), and leporine lacrimal fluid (pH 7.8) over time are shown in Figure 2. The rupture rates at 5, 30, and 60 min were 44.6%, 81.6%, and 89.9%, respectively, in artificial tears; 68.5%, 80.8%, and 90.3%, respectively, in leporine lacrimal fluid; and only 1.6%, 4.0%, and 7.1%, respectively, in eyewash.

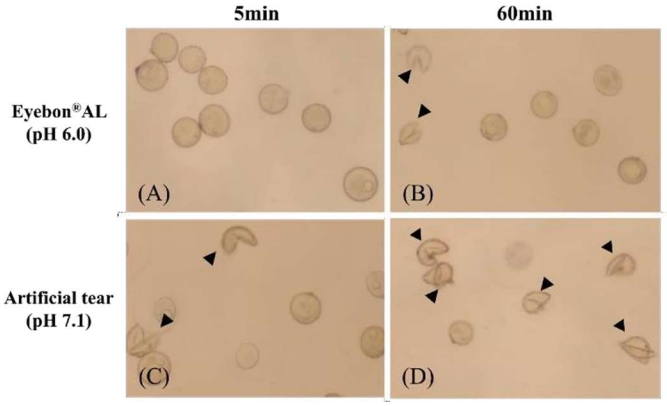

Figure 1.

Micrographs showing pollen in each solution.(A) Few pollen ruptures are observed after 5 min in eyewash solution (pH 6.0). (B) Limited pollen rupture is observed after 60 min in eyewash solution (pH 6.0). (C) Limited pollen rupture is observed after 5 min in artificial tear solution (pH 7.1). (D) Most of the pollens rupture after 60 min in artificial tear solution (pH 7.1).

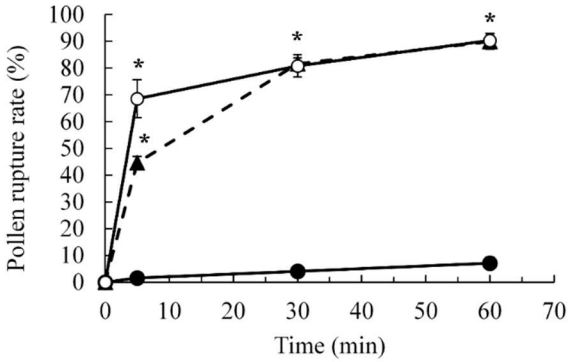

Figure 2. Change in cedar pollen rupture rates.The rupture rate is significantly lower in eyewash at pH 6.0 (●) between 5 and 60 min (especially, 60 min) compared with that in artificial tear solution at pH 7.1 (▲) and leporine lacrimal fluid at pH 7.8 (○); n = 3, *p<0.05

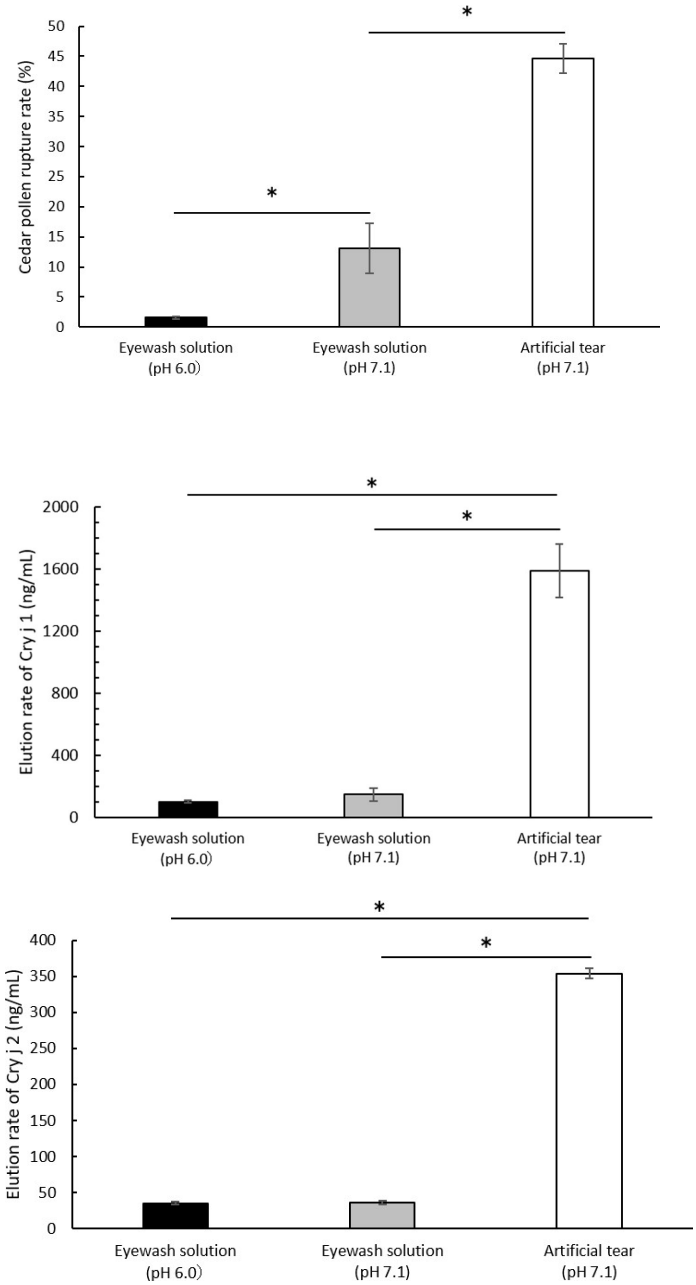

Figure 3. (A) Cedar pollen rupture rate in pH-adjusted test solutions. (B) Elution rate of Cry j 1 (C) Elution rate of Cry j 2. Cedar pollen rupture rates and antigenic protein elution are lower in eyewash solution with pH adjusted at the level of artificial tears (pH 7.1) than in non-pH-adjusted eyewash (pH 6.0); n = 3, *p < 0.05.

Effects of solution pH on cedar pollen rupture rate and antigen elution

The cedar pollen rupture rate after 5 min in eyewash solution with pH adjusted to 7.1 (solution 3), same as the pH in artificial tears, is shown in Figure 3 (A). In addition, the elution rates of antigenic proteins Cry j 1 and Cry j 2 are shown in Figure 3 (B and C, respectively). The elution densities of Cry j 1 and Cry j 2 were 1587.6 ng/mL and 354.1 ng/mL, respectively, in artificial tears. However, the densities were significantly lower in eyewash solution at pH 6.0 (99.7 ng/mL and 35.1 ng/mL, respectively for Cry j 1 and Cry j 2). In addition, after adjusting the pH of the eyewash solution to 7.1, the pollen rupture rate changed to 1.6% and the elution densities of Cry j 1 and Cry j 2 were 145.4 ng/mL and 35.7 ng/mL, respectively.

Effects of artificial tear and eyewash ingredients on cedar pollen rupture rate

The pollen rupture rates in the basic eyewash and basic boric acid solutions where other artificial tears and eyewash ingredients were added are shown in Figure 4. The pollen rupture rate in the basic eyewash solution at pH 7.1 (solution 3) was 14.0%. On the other hand, the pollen rupture rate was significantly higher at 40.2% and 67.4% in solutions containing potassium chloride (solution 4) and sodium chloride (solution 5), respectively. In addition, the rupture rate in the boric acid solution at pH 7.1 (solution 6) was 19.2% but was significantly higher at 46.1% in solution containing potassium chloride/sodium chloride (solution 7).

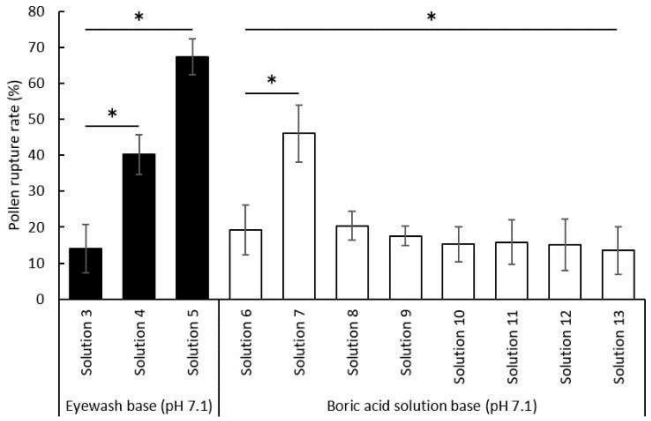

Figure 4. Effects of ingredients contained in artificial tears and eyewash on pollen rupture rates. The rupture rate is significantly higher in test solutions containing potassium chloride (solution 4) and sodium chloride (solution 5) (n = 3, *p < 0.05). In addition, the rupture rate is significantly lower in test solutions containing polysorbate, l-menthol, and d-borneol (solution 13) (n = 3).

The rupture rate was significantly lower (13.5%) in the solution containing polysorbate, l-menthol, and d-borneol (solution 13). No significant changes were observed in solutions containing dipotassium glycyrrhizinate, sodium chondroitin sulfate, chlorpheniramine maleate, epsilon aminocaproic acid, and polysorbate 80 (solutions 8 to 12).

Antigenic protein levels in pollen residue after rupture

The Cry j 1 and Cry j 2 levels in the post-rupture elution and disrupted pollen residue solutions for suspensions with ≥95% pollen rupture rates are shown in Figure 5. Cry j 1 and Cry j 2 levels were 2816.9 ng/mL and 569.9 ng/mL in the post-rupture elution solution, respectively, and 1409.2 ng/mL and 227.6 ng/mL in the disrupted pollen residue solution, respectively. Therefore, both Cry j 1 and Cry j 2 remained in the pollen residue.

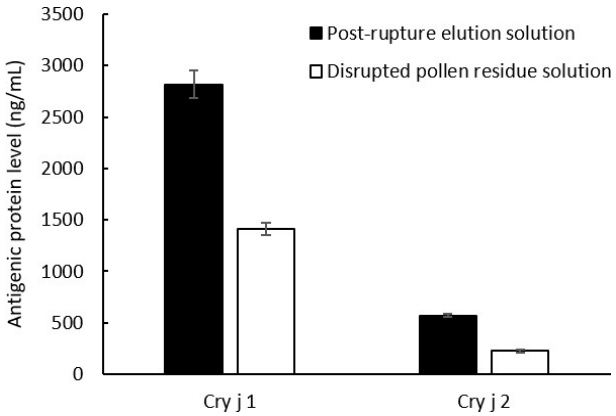

Figure 5. Antigenic protein levels in pollen residue. after rupture Cry j 1 and Cry j 2 also remain in the pollen residue (n = 3).

Discussion

Washing out airborne antigens from the ocular surface is a useful preventive measure against ocular allergic symptoms during the pollen dispersal period. Furthermore, the lacrimal fluid has self-washing functions; however, it ruptures the cedar pollen wall [4]. Therefore, an eyewash formulation that not only eliminates pollen, but also decreases pollen wall rupture is preferable during the pollen dispersal period.

In this study, the effects of eyewash products on pollen rupture and antigenic protein elution, as well as those of ingredients contained in eyewash products and artificial tears, were investigated in vitro. The pollen rupture rate after 5 min was lower in the eyewash solution than in the artificial tears and leporine lacrimal fluid. After 5 min, approximately 70% of the pollen had ruptured in the leporine lacrimal fluid. Therefore, it is important to wash out airborne pollen as soon as possible to prevent ocular allergic symptoms. A study of nasal discharge and cedar pollen rupture suggested that almost 80% of pollens ruptured within 5 min. The contributing factors include contact with mucin, a protein contained in nasal discharge, lysozyme activity, and physicochemical influences including pH and temperature [6]. In lacrimal fluid, pollen rupture is caused by alkali conditions as well as the presence of lysozymes, proteins, andmucins.

In this study, we were also able to demonstrate that eyewash suppressed the pollen rupture rate to approximately 7% after 60 min. Considering that pollen rupture is promoted by alkali conditions [7], an eyewash solution with the same pH as that of artificial tears was analyzed. The pollen rupture rate and elution of Cry j 1 and Cry j 2 were significantly decreased even at this pH, suggesting that there may be ingredients that could suppress pollen rupture, in addition to the effect of pH level. Next, the rupture rates were investigated in pH-adjusted test solutions containing potassium chloride, sodium chloride, and other ingredients contained in eyewash. The addition of potassium chloride and sodium chloride increased the pollen rupture rate, suggesting the involvement of chloride, sodium, and potassium ions in pollen rupture, and that pollen rupture was reduced by eyewash that did not contain these ions.

In a study involving mock rainfall [8], Cry j 1 elution increased with increasing ion concentrations, suggesting the importance of ion concentrations in eyewash and eyedrop solutions. However, most ingredients contained in eyewash had little influence. In the current study, polysorbate, l-menthol, and d-borneol were the only ingredients that suppressed pollen rupture, which are oily ingredients, and considering that salts promoted rupture, we concluded that the ingredients with lower ion concentrations would suppress pollen rupture.

Furthermore, almost half of the eluted antigenic proteins (Cry j 1 and Cry j 2) released by pollen rupture remained in the residue. Cry j 1 was contained in the pollen wall while Cry j 2 was contained in the inner membrane and starch granules in the cellular sac [9,10]. Cry j 1 may therefore elute regardless of rupture, whereas Cry j 2 elutes only after rupture [6]. However, this study indicated that not all antigens elute after rupture and that pollen residue may cause ocular allergic symptoms after rupture. Therefore, to prevent ocular allergic symptoms, it is important not only to wash out the pollen right away, but also to remove the pollen residue after rupture sometime later.

Our results indicate that eye washing during the pollen dispersal period may wash out antigens. It potentially suppresses allergic reactions by suppressing pollen rupture and eliminating pollen, depending on the contents and properties of the eyewash. In addition, to adequately eliminate pollen, it is preferable to wash eyes immediately before pollen rupture. It is also important to wash eyes after pollen rupture to prevent ocular allergic symptoms.

Acknowledgements

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Conflict of Interest

Miho Tanaka, Takafumi Saeki, Manabu Nozaki, and Hiroko Yano are employees of Kobayashi Pharmaceutical Co., Ltd. The rest of authors declare no conflicts of interest associated with this manuscript.

Authors' contributions

Miho Tanaka, Takafumi Saeki, Hiroko Yano, Kazumi Fukagawa, and Hiroshi Fujishima developed the research concept and designed the study. Miho Tanaka contributed to data collection. Miho Tanaka, Hiroko Yano, Kazumi Fukagawa, and Hiroshi Fujishima performed the statistical analysis and interpreted the results. Miho Tanaka wrote the original draft of the manuscript, which was revised by Takafumi Saeki, Miho Tanaka, Hiroko Yano, and Manabu Nozaki. All authors have read and approved the final version of the manuscript.

Abbreviations:

OTC: over-the-counter, ELISA: enzyme-linked immunosorbent assay.

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