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The effect of rebamipide ophthalmic suspension on ocular surface mucins in soft contact lens wearers

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ABSTRACT

Purpose: To evaluate the changes in ocular surface mucins with 2%rebamipide ophthalmic suspension treatment in soft contact lens (SCL) wearers. Rebamipide suspension is a mucin secretagogue approved for the treatment of dry eye syndrome in Japan. In this study, the fluorescence intensity of wheat germ agglutinin conjugate of fluorescein (F-WGA) was used as a marker of membrane-associated mucins, and sialic acid concentration in tear fluids as a marker of secreted mucins.

Methods: Thirty-two eyes of 16 SCL wearers with discomfort were treated with rebamipide suspension at a dose of one drop in each eye four times daily for two weeks. The parameters of clinical efficacy were tear break-up time, fluorescein staining scores for the cornea and conjunctiva, and Schirmer test values. Fluorescence intensities in the central cornea were measured by fluorophotometry after the application of 5% F-WGA solution. Tears collected by Schirmer test strips were analyzed by high-performance liquid chromatography, and the concentrations of sialic acid, total protein, and the four major tear proteins, namely secretory IgA, lactoferrin, lipocalin-1, and lysozyme were measured.

Results: Significant increases in F-WGA fluorescence intensities ($p < 0.005$) were seen in the corneal surfaces. Sialic acid concentrations increased over time; however, the differences were not statistically significant. Except for a slight increase in kerato-conjunctival staining scores ($p < 0.05$) and secretory IgA ($p < 0.05$), no other significant differences were seen among clinical parameters or tear proteins.

Conclusions: Topical application of rebamipide suspension significantly increased F-WGA intensity, a marker of membrane-associated mucins in SCL wearers.

1. Introduction

Soft contact lenses (SCLs) are widely known to induce high levels of discomfort in around 30 to 50% of wearers [1]. The main symptoms among contact lens wearers are dryness and irritation, sometimes with poor association between clinical signs. One of the major factors relating to these symptoms is the interaction of the tear film and ocular surface induced by wearing SCLs.

SCLs interact with the tear film and influence the ocular surface both physically and biochemically [2], thus inducing instability of the tear film. These physical and biochemical changes to the tear film and ocular surface induced by wearing SCLs leads to instability of the tear film, which is clinically characterized by shortening of the tear film break-up time (TBUT) and can result in dry eye symptoms.

One of the major causes of tear film instability seems to come from the decrease of wettability due to changes among the ocular surface mucins. The influence on ocular surface mucins, however, appears to differ among subjects, contact lens materials, and analytical methods. Most reports state that the majority of cases have reduced secreted mucins, while others suggest the opposite scenario or no change [3]. Sialic acid in tear fluids, which is mainly found in the sugar chains of mucins, is reported to be a quantitative indication of tear mucin [4,5], and has been used as a marker of secreted mucins [6]. The measurement of membrane-associated mucins on ocular surface epithelia is technically difficult; hence, indirect methods using a binding protein or measurements of the amount of shedding mucin in the tears have been adopted; however, there are few such reports. Ocular surface glycocalyx by measuring the fluorescence intensity of wheat germ agglutinin conjugate of fluorescein (F-WGA) was previously evaluated. Considering the fact that F-WGA could bind to various glycol-proteins, it is confirmed to bind to the glycocalyx of ocular surface cells by transmission electron microscopy, since F-WGA has a high affinity for apical

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cells of the corneal and conjunctival epithelium [7,8]. F-WGA is known to predominantly bind to the glycocalyx of the ocular surface epithelium, and it was recently reported that it is suitable for the quantitative evaluation of ocular surface glycocalyx *in vivo* as a marker of membrane-associated mucins [9,10].

Rebamipide ophthalmic suspension (2%; Mucosta ophthalmic suspension UD2%; Otsuka Pharmaceutical Co., Ltd, Tokyo, Japan) was approved for clinical use in dry eye patients [11] by the Ministry of Health, Labour and Welfare of Japan in 2011. It is a preservative-free ophthalmic suspension, which is known to activate the mucin secretagogue, packed in a unit dose. Rebamipide has a unique course in drug discovery and has long been used as a treatment for gastric ulcers. Several studies have reported its clinical efficacy in dry eye syndromes, including Sjögren's syndrome [12] and short TBUT-type dry eye [13].

Promotion of the secretion and content of mucins by rebamipide suspension has been demonstrated in animal models only, and the effects have not fully been investigated in dry eye patients to date. The aim of the current study was to prospectively determine the changes in ocular surface mucins via treatment with rebamipide suspension in SCL wearers. F-WGA in the central cornea and sialic acid concentrations in tear fluids before and after treatment were assessed. The former was used as a marker of membrane-associated mucins, and the latter as a marker of secreted mucins in tears.

2. Materials and methods

2.1. Subjects

This study followed the tenets of the World Medical Association Declaration of Helsinki. Written informed consent was obtained from each subject to participate in the current study. The protocol was approved by the Institutional Review Board of the National Tokyo Medical Centre (approval no.: R10-022) and registered in UMIN clinical trials registry (registration no.: UMIN000025752).

16 SCL wearers were enrolled in this prospective study. The recruits included 7 men and 9 women and were aged 26 to 51 years (mean age, 34.2 ± 7.5). Overall, 9 subjects were hydroxyethyl methacrylate (HEMA) SCL wearers and 7 were silicone hydrogel (SH) SCL wearers. All subjects wore their SCLs on a daily basis with a mean wear time of 15.8 ± 2.1 h/day (range, 12–18 h/day). Their SCL wear history was 14.3 ± 8.3 years (range, 1–26 years). The subjects had no history of eye disease or previous surgery (except for self-reported symptoms of CL discomfort) and none of them had received topical drug therapy. All subjects successfully completed the trial and the data from both eyes of each subject were included in the statistical analysis.

2.2. Clinical assessment

At the baseline visit, two of the authors (CS and MF) each performed a routine ocular examination on all subjects following the same order. Fluorescein solution (1%; 1 μL) was used to measure TBUT. An electric metronome was used to measure the TBUT for three times and the average was calculated. Corneal fluorescein staining with a 1% fluorescein solution was assessed using the scoring system described by Shimmura [14], where the cornea is divided into three sections and graded on a scale of 0 to 3 at each section to calculate the total score ranged from 0 to 9 points. The fluorescein staining score of the keratoconjunctivitis sicca was determined using a blue-free barrier filter [15] according to the modified grading system of van Bijsterveld [16], where each eye is divided into three sections (temporal conjunctiva, cornea, and nasal conjunctiva) and scored from 0 to 3 at each section to calculate the total score ranged from 0 to 9 points. Tears were collected by inserting a Schirmer test strip (Alcon, Inc., Fort Worth, TX, USA) in the lower temporal one-third of the lid margin without anesthesia. After 5 min, the strips were removed, the wetted volume was measured and each strip was immediately placed in a 1.5-mL Eppendorf tube and stored at −80 °C until assay.

The subjects continued to wear their SCLs and were instructed to apply rebamipide suspension at a dose of one drop in each eye four times a day for 2 weeks. No other topical drug were used during this study. The subjects returned to the hospital after 2 weeks and underwent the same examinations performed at the baseline visit. The subjects were instructed not to use rebamipide suspension 1 h prior to the examination.

2.3. F-WGA intensity measurement on the corneal surface

The procedure for the measurement of F-WGA intensity was performed following a similar procedure reported previously [10,17]. Briefly, A 5% F-WGA solution was prepared in sterile 0.067 M phosphate-buffered saline (PBS), pH 7.4. A slit-lamp fluorophotometer (Anterior Fluorometer FL-500, Kowa Co. Ltd., Tokyo, Japan) was used to quantify the fluorescence intensity on the corneal surface.

The measurement was taken at least 15 min after contact lens removal. The subjects were seated in front of the fluorophotometer. The instrument was focused on a 2-mm diameter circle on the central cornea and the background fluorescence intensity was measured. Five microliters of 5% F-WGA solution was applied to both eyes using an Eppendorf micropipette. Five minutes later, the fluorescence intensity of the central cornea was measured.

2.4. Sialic acid assay

The concentration of *N*-acetyl-neuraminic acid (Neu5Ac), a major sialic acid in humans, was measured. The procedures for analyzing sialic acids were based on the report of Yasueda et al. [5] and are described in the previous report [18–20]. In brief, Schirmer strips containing tear samples were soaked in PBS to elute tear proteins. Tear extract was mixed with acetic acid to release the sialic acid. A mixture of 1,2-diamino-4,5-methylenedioxybenzene (DMB) solution, coupling solution, and water (ratio 1:5.4) was added, and the mixture was kept in the dark to develop fluorescence labeling. The reaction mixture was cooled to stop the reaction before high-performance liquid chromatography (HPLC) analysis (Shimadzu Corporation, Kyoto, Japan). The final results are expressed as μg/mL.

2.5. Tear protein assay

Procedures for analyzing the tear proteins are described in the previous report [18–21]. In brief, the total concentrations of tear proteins were determined by the Bradford method. The four major tear proteins, namely secretory immunoglobulin A (sIgA), lactoferrin, lipocalin-1, and lysozyme, were determined by fractioning each tear protein extract on an HPLC system. The results are expressed as mg/mL.

The sIgA/lysozyme ratio was calculated as a parameter indicating the activity of the sIgA producing plasma cells in the lacrimal gland [22,23].

2.6. Statistical analysis

The results are presented as means and standard deviations (SD). The nonparametric Wilcoxon signed-rank test was used to compare pre- and post-treatment data of each eye. Data pertaining to both eyes of each subject were analyzed using StatView version 5.0 software (SAS Institute, Inc., Cary, NC, USA). A probability (p) value of < 0.05 was considered statistically significant.

3. Results

3.1. Clinical efficacy

The changes in clinical parameters following topical application of