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Decreased mucin concentrations in tear fluids of contact lens wearers

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Abstract

Characteristics of tear-film may be influenced by contact lens wear, because contact lenses present the habitual, direct rubbing action of the lids upon the covered ocular surface and may cause changes of tear-film. In the present paper, influence of contact lens on proteins in tear samples was studied using carbohydrates attached to the protein as a marker. We found that *N*-acetylneuraminic acid (Neu5Ac) was significantly decreased in tear samples of volunteers wearing contact lens (wearing, 86.1 ± 57.7 nmol/ml; normal, 190.2 ± 121.9 nmol/ml). Analysis by polyacrylamide gel electrophoresis demonstrated that the amounts of major proteins in tear fluids, such as lactoferrin and secretory immunoglobulin A were not changed upon wearing contact lenses. In contrast, cellulose acetate membrane electrophoresis revealed that mucin band in tear samples from contact lens wearers showed significant decrease as examined by lectin staining.

Keywords: Tears; Mucins; Contact lenses; Sialic acids; Electrophoresis

1. Introduction

Mucins occupy an important part of the tear-film, and play various important roles, such as keeping viscosity and lubrication to retard fluid evaporation and anchor the aqueous tear-film to the underlying cornea and conjunctival surface [1]. In addition, mucins in tear fluid also show protection against pathogens by interaction with various microbes due to the densely glycosylated surface of the mucins [2]. Mucins in tear-film belong to a family of glycoproteins that contain a large amount of *O*-glycosidically linked carbohydrate chains through hydroxyl groups of Ser/Thr residues [3]. Many studies have been reported on identification of the mucins expressed in tear-film. Two types of mucins, gel-forming mucins of high molecular masses, such as MUC2, MUC5AC, MUC5B and MUC6 and soluble mucins of low molecular masses, such as MUC7 and MUC9 have been reported [4,5]. One of the major secretory mucins, MUC5AC is secreted into tear fluid mainly via goblet cells, which are located in the conjunctiva. The lacrimal gland provides tear fluid, electrolytes, anti-microbial proteins and other protective molecules for protection of ocular surface. In addition, the lacrimal gland secretes small amount of the gel-forming mucin, MUC5B and the small soluble mucin, MUC7 [6]. Three membrane associated mucins, MUC1, MUC4 and MUC16 have been demonstrated to be expressed by ocular surface epithelia [7–10]. Shedding of membrane-associated mucins from the underlying epithelial cells may also contribute to the mucin component of tear fluid [11,12]. MUC4 has also been found in tear samples [8]. It is not clear whether a secreted version of MUC1 gene product is present in tear fluid [13]. Lactoferrin, lysozyme, tear specific pre-albumin, albumin, transferrin, secretory immunoglobulin A (sIgA) and IgG are also common components in tear fluid [14]. These proteins are related to the gel-forming and lubrication for albumin [15], protection for sIgA [16] and serve as a surfactant in tears for lactoferrin [17].

Several evidences suggested that conjunctival mucin secretion may be impaired in various disorders affecting the ocular surface including dry eye disease, hypovitaminosis A, ocular pemphigoid, Stevens-Johnson syndrome, Sjogren's

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syndrome and other types of ocular surface trauma [18,19]. Patients with dry eye from Sjogren's syndrome showed a significant decrease of MUC5AC in mRNA levels, correlating with a significant decrease in MUC5AC mucin in tear samples [20]. Chronic ocular surface involvement, such as dry eye disease showed subclinical inflammation [21]. Inflammation of the conjunctiva in chronic diseases, keratoconjunctivitis sicca and ocular rosacea is also significantly associated with a decrease in goblet cell density and mucin production [22]. These results indicate that inflammation of ocular surface is profoundly related to impairment of mucin secretion.

Contact lens wearing, in terms of comfort and clinical tolerance, is strongly linked with the pre-ocular tear-film. Adapted wearers of contact lenses present elevated tear osmolarity [23]. The lipid layer is compromised by the presence of contact lens [24,25], leading to increased evaporation [26]. Subclinical inflammation of the conjunctival epithelium is generally involved in the process of chronic aggression. It is well established that the wearing of contact lenses increases the risk of ocular surface inflammation and infection [27]. Moreover, rigid contact lenses induce tear-film instability and are associated with damage to the ocular-surface epithelium and mucin layer [28]. A report suggested that mucus production may be increased by non-goblet cells in the tarsal conjunctiva in response to contact lens wear [29]. On the other hand, tear albumin, lysozyme and lactoferrin show constant levels upon contact lens wearing [23].

Recently, we developed a few methods for the determination of mucins in digestive organs [30,31] and showed cytoprotective reaction of gastric mucins released by protease-activated receptor-2 agonist [32]. As reported in these papers, sialic acids are a good marker for monitoring mucous proteins in biological samples, because the non-reducing termini of mucous carbohydrate chains are often occupied with sialic acids. In the course of the analysis of sialic acids in tear samples, we found that tear samples collected from contact lens-wearing subjects obviously contained smaller amount of sialic acids, and these finding prompted us to investigate the influence of contact lenses on the production of mucous proteins in tear samples.

2. Experimental

2.1. Materials

1,2-Diamino-4,5-methylenedioxybenzene (DMB) and 3,3′-diaminobenzidine tetrahydrochloride (DAB) were obtained from Dojin (Kamimashiki-gun, Kumamoto, Japan). Samples of *N*-acetylneuraminic acid (Neu5Ac), *N*-glycolylneuraminic acid (Neu5Gc) and 2-keto-3-deoxy-D-glycero-D-galactononoic acid (KDN) were kindly supplied by Dr. Ohta of Marukin-Bio (Uji, Kyoto, Japan). Cellulose acetate membrane (Seleca-V) was purchased from Advantec Toyo Roshi Kaisha (Taito-ku, Tokyo, Japan). Silver nitrate and trisodium citrate were obtained from Kishida Chemical (Chuo-ku, Osaka, Japan). Tween 20, horseradish peroxidase-conjugated streptavidin (HRP–streptavidin), albumin (human), lactoferrin (human) and sIgA (human) were obtained from Sigma–Aldrich (Shinagawa-ku, Tokyo, Japan). Iron(II) sulfate heptahydrate, veronal buffer, trichloroacetic acid, sulfosalicylic acid were obtained from Wako Chemical (Chuo-ku, Osaka, Japan). Biotin-labeled *Agaricus bisporus* lectin (ABA) was obtained from Seikagaku Kogyo (Chuo-ku, Tokyo, Japan). All other reagents and solvents were of the highest grade commercially available or HPLC grade. Water was purified with a Milli-Q purification system (Millipore, Tokyo, Japan) after deionization and distillation.

2.2. Tear samples

Collection of tear samples was performed by similar procedure reported previously [33]. Briefly, saline (100 μl) warmed to 35 °C was instilled in the nasal side of one eye by a micropipette without anesthesia. After several forced blinks, a 50 μl portion of diluted tear fluid was collected by another micropipette from the temporal side of the palpebral fissure. Collected tear samples from each volunteer were stored at −80 °C until assay. Tear samples were obtained with the permission of the Ethics Committee in the university and used in accordance with the tenets of the Declaration of Helsinki.

2.3. Protein estimation

Protein concentrations were detected by the method of Lowry et al. using bovine serum albumin as a standard [34].

2.4. Analysis of sialic acids in tear fluid

The procedure for derivatization of sialic acid with DMB was described previously [30,31]. Briefly, a tear sample (10 μl) was mixed with 4 M aqueous acetic acid solution (10 μl), and the mixture was kept at 80 °C for 3 h to release sialic acids. After cooling the mixture, 0.7 M DMB solution in 1.4 M aqueous acetic acid (100 μl) containing 0.018 M sodium hydrosulfite and 0.75 M β-mercaptoethanol was added, and the mixture was kept at 50 °C for 150 min. After cooling the mixture, a portion (10 μl) was analyzed by HPLC.

2.5. High-performance liquid chromatography

Sialic acids after fluorescent labeling with DMB were analyzed on an octadecyl-silica column (Cosmosil 5C18AR-II, 4.6 mm i.d. × 150 mm, Nakarai Tesque, Nakagyo-ku, Kyoto, Japan) using a Tosoh 8020 HPLC apparatus (Tosoh, Minato-Ku, Tokyo, Japan) with a Shimadzu RF 535 fluoromonitor (Shimadzu, Nakagyo-ku, Kyoto, Japan). Peaks were detected at 448 nm with irradiating a 375 nm light. Elution was carried out at a flow-rate of 0.9 ml/min at 40 °C using a mixture

of acetonitrile–methanol–water (2:14:84, %, v/v) as mobile phase.

2.6. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Tear sample (1 μl) was mixed with sample-loading buffer (20% glycerol; 0.25 M Tris–HCl; pH 6.8, 4.6% SDS and 10% β-mercaptoethanol, 9 μl) and kept in the boiling water bath for 5 min. The mixture was applied and separated by SDS-PAGE (9% T, 2.6% C) according to Lammli's method [35]. Gels were stained by Coomassie Blue staining [36] or silver staining [37].

Spots detected by Coomassie Blue staining were excised to determine sialic acids. The spots were kept in acetonitrile (200 μl). The shrunk gels were collected and reswollen with 2 M aqueous acetic acid (100 μl) and kept at 80 °C for 3 h to release sialic acids. A portion of the supernatant containing released sialic acids (20 μl) was analyzed by HPLC after fluorescent derivatization with DMB as described above.

2.7. Electrophoresis on cellulose acetate membrane

Electrophoresis of tear samples (1–4 μg as protein) on cellulose acetate membrane was performed in two steps. At the initial step, electrophoresis was performed at 0.125 mA/cm per membrane for 20 min. This step removed inorganic ions and caused better resolutions of proteins. Then, the current was changed to 0.75 mA/cm and continued for further 40 min. A 0.06 M veronal buffer (pH 8.6; ionic strength, 0.06) was used as the running buffer. After fixing proteins by keeping the membrane in 0.4 M trichloroacetic acid–0.03 M sulfosalicylic acid for 5 min, the membrane was immersed in 1% acetic acid for 5 min and stained with freshly prepared colloidal silver solution for 20 min with shaking. Finally, the membranes were washed with water for 10 min.

Colloidal silver solution was prepared according to the method reported by Matsuda et al. [38]. Briefly, an aqueous solution (5 ml) of 0.28 M trisodium citrate dihydrate was mixed with a solution (12 ml) containing 0.25% Tween 20 and an aqueous solution (15 ml) of 0.028 M iron(II) sulfate heptahydrate. An aqueous solution (1 ml) of 0.35 M silver nitrate was added and vigorously shaken to present flocculation of silver particles. To the mixture, an aqueous solution (2 ml) of 15% acetic acid was added, mixed and used for staining immediately after preparation.

We used a portion of the membrane for silver staining, and other portions were used for determination of sialic acids. The bands showing spots on silver staining were excised and kept in 2 M aqueous acetic acid (100 μl) at 80 °C for 3 h to release sialic acids. A portion of the supernatant (20 μl) was analyzed by HPLC after fluorescent derivatization with DMB.

We also examined the bands detected by silver staining on cellulose acetate membrane using SDS-PAGE. The bands showing the same mobility with those detected by silver staining were excised and kept in water (1 ml) and agitated by vortex mixer for 30 min. A portion (500 μl) of the supernatant was collected and dried by a centrifuged evaporator. The residue was dissolved in sample-loading buffer (20% glycerol; 0.25 M Tris–HCl; pH 6.8; 4.6% SDS and 10% β-mercaptoethanol, 10 μl) and kept in the boiling water bath for 5 min. The mixture was applied and separated by SDS-PAGE (9% T, 2.6% C) according to Lammli's method [35]. Gels were stained by silver staining [37].

2.8. Lectin affinity staining of mucin proteins on cellulose acetate membrane

After cellulose acetate membrane electrophoresis of tear samples, the proteins were fixed in 5% sulfosalicylic acid for 5 min and washed with T-PBS (0.05% Tween 20 in PBS) for 5 min. The washing procedure was repeated again. Then, the membrane was kept in 2 M aqueous acetic acid solution at 37 °C for 24 h to remove sialic acids from sugar chains and washed two times with T-PBS for 5 min. The membrane was kept in biotin-labeled ABA solution (3 μg/ml in T-PBS) overnight at room temperature, and washed two times in T-PBS buffer for 5 min. The membrane was then kept in HRP–streptavidin solution (1 μg/ml in T-PBS) for 1 h and washed three times in T-PBS for 15 min. Finally, the membrane was visualized by keeping in 0.1 M Tris–HCl buffer (pH 7.4) containing 0.05% DAB and 0.31% H_2O_2 for 5 min.

2.9. Statistical analysis

Statistical comparisons between each group were done using an unpaired *t*-test. A *p*-value of 0.05 was retained for significance. The calculation was performed PRISM (Version 4.0) for Windows (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Analysis of sialic acids in tear fluids

We analyzed bound sialic acids in tear samples from 13 volunteers. The samples were collected from contact lens wearers (CW, 7 people, 44 samples) and non-wearing subjects (NW, 6 people, 40 samples). The samples were also collected 12 h after taking off contact lenses from two volunteers who were included in the contact lens wearers (TO, two people, five samples) (Table 1). The volunteers were composed of eight men and five women, whose average age was 24.2 years. These volunteers had no topical drug history, and no ocular disease served as controls.

Sialic acids were determined as their DMB derivatives after hydrolysis in 2 M acetic acid followed by fluorescent derivatization. The results are summarized in Table 1. Neu5Ac was abundantly present in all tear samples, but Neu5Gc and KDN that are also common sialic acids in various mammals, were not detected in all tear samples.

Table 1
Analysis of sialic acids in tear fluids

Volunteer no.	Gender	Age	Contact lenses	Neu5Ac (nmol/ml)	Protein (mg/ml)	Number of samples
1	Male	30	NW	181.0 ± 165.7[a]	0.91 ± 0.84	6
2	Male	23	NW	95.2 ± 77.2	0.66 ± 0.24	7
3	Male	22	NW	238.9 ± 84.3	1.36 ± 0.42	12
4	Male	34	NW	131.3 ± 60.5	1.49 ± 0.95	5
5	Male	21	NW	298.5 ± 257.8	1.67 ± 1.15	4
6	Female	23	NW	226.2 ± 123.9	1.03 ± 0.39	6
	Male 5 (83%) Female 1 (17%)	25.5 ± 5.2 Range 21–34	NW	190.2 ± 121.9	1.14 ± 0.64	40
7	Male	23	CW	145.7 ± 13.9	1.54 ± 0.01	4
8	Male	24	CW	132.3 ± 64.4	1.05 ± 0.41	10
9	Male	23	CW	77.6 ± 58.5	0.66 ± 0.30	13
10	Female	24	CW	52.9 ± 24.1	0.55 ± 0.13	5
11	Female	22	CW	60.8 ± 34.8	0.64 ± 0.10	3
12	Female	22	CW	34.9 ± 22.6	0.58 ± 0.18	4
13	Female	25	CW	81.4 ± 33.1	0.71 ± 0.23	5
	Male 3 (43%) Female 4 (57%)	23.3 ± 1.1 Range 22–25	CW	86.1 ± 57.7	0.78 ± 0.37	44
8	Male	23	TO	268.9 ± 215.2	1.16 ± 0.39	3
9	Male	22	TO	132.1 ± 123.4	0.88 ± 0.46	2
	Male 2 (100%) Female 0 (0%)	23.5 ± 0.7 Range 23–24	TO	214.2 ± 180.5	1.05 ± 0.39	5

NW, non-wearing subjects; CW, contact lens wearers; TO, 12 h after taking off contact lenses from contact lens wearers; Neu5Gc, KDN, not detected.
[a] Mean ± S.D.

Fig. 1 shows comparison of Neu5Ac and protein concentrations in tear samples between NW and CW groups.

Concentrations of Neu5Ac were 190.2 ± 121.9 nmol/ml for NW group and 86.1 ± 57.7 nmol/ml for CW group, respectively. The level of Neu5Ac in CW group showed significantly lower values than those observed for NW group ($p < 0.0001$; Fig. 1(A)). Interestingly, Neu5Ac concentrations were recovered to 214.2 ± 180.5 nmol/ml after taking off the contact lenses for 12 h (TO group). In contrast, NW group showed significantly higher protein concentrations than those in CW group ($p < 0.005$; Fig. 1(B)). Recovery of the protein concentration in TO group was also observed, but not significant.

We also observed the changes of Neu5Ac and protein concentrations in tear samples collected from single subject (volunteer 3) for ca. 6 months (from 1 August 2003 to 31 January 31 2004). Neu5Ac levels in tear samples from right and left eyes were 223.4 ± 76.9 nmol/ml (six samples) and 254.3 ± 95.7 nmol/ml (six samples), respectively, and protein concentrations in tear samples from right and left eyes were 1.43 ± 0.46 mg/ml (six samples) and 1.30 ± 0.40 mg/ml (six samples), respectively. The results indicated that Neu5Ac and protein showed constant levels during the course of test period. No significant differences for Neu5Ac and protein between right and left eyes were observed.

3.2. SDS-PAGE analysis

Tear samples from NW subjects (volunteers 2, 3 and 4) and CW subjects (volunteers 8, 9 and 12) were examined by SDS-PAGE. Three major bands at 28, 64 and 75 kDa were observed by Coomassie Blue staining (Fig. 2(A)). We

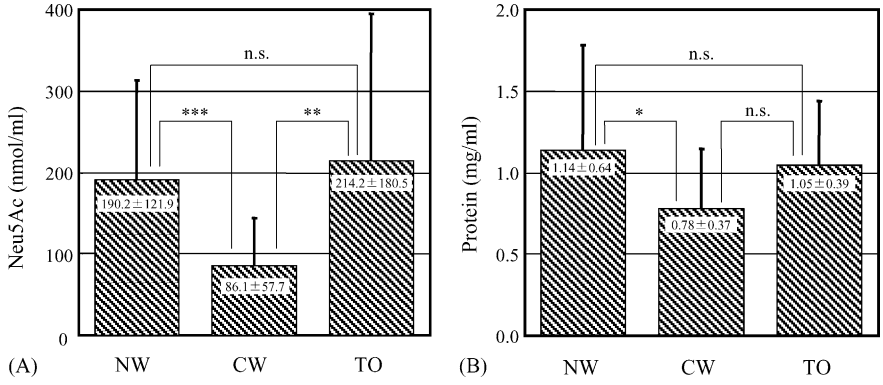

Fig. 1. Sialic acid level (A) and protein level (B) in tear samples of the NW, CW and TO groups; n.s., not significant; $^{*}p < 0.005$; $^{**}p < 0.001$; $^{***}p < 0.0001$.

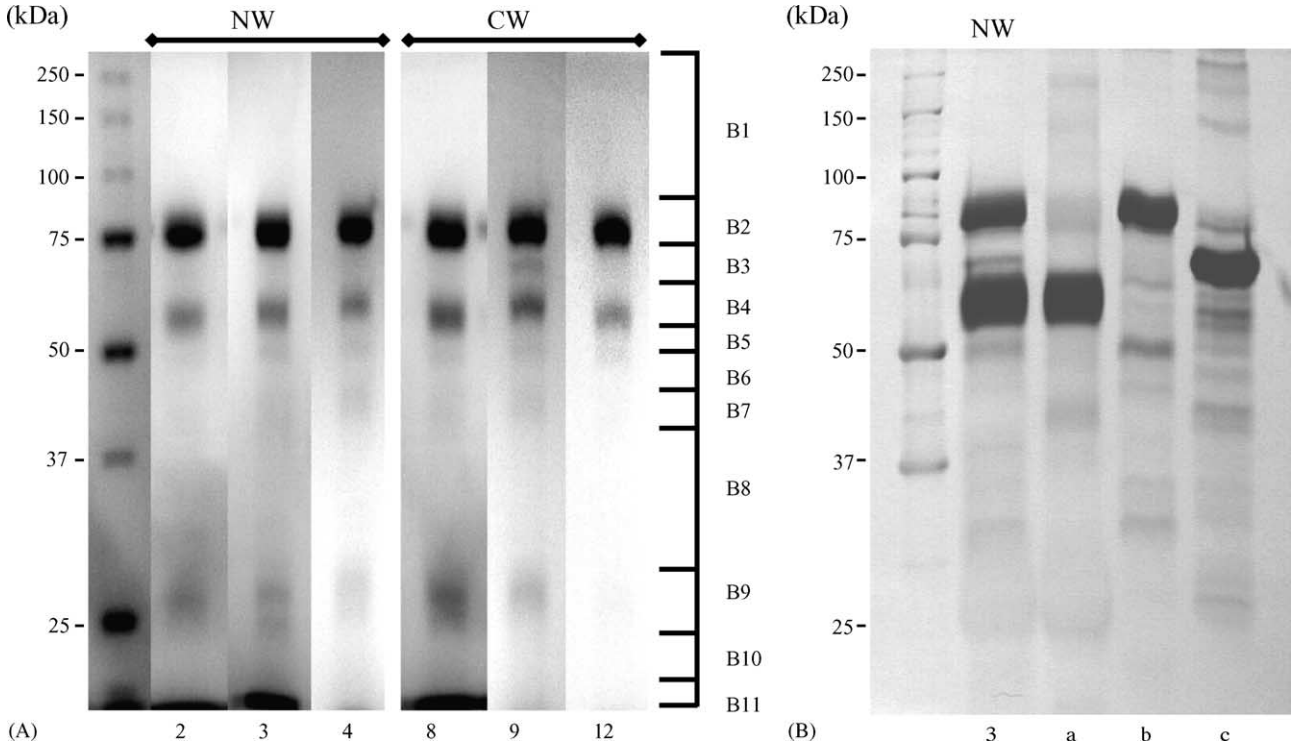

Fig. 2. SDS-PAGE analysis (9% T, 2.6% C). (A) Tear samples from the NW subjects (volunteers 2, 3 and 4) and the CW subjects (volunteers 8, 9 and 12) by Coomassie Blue staining. (B) Standard samples of: (a) sIgA; (b) lactoferrin; (c) albumin, and the tear sample of volunteer 3 by silver staining.

did not observe obvious difference between NW and CW groups.

These protein bands were identified as sIgA (light and heavy chain) and lactoferrin, respectively, by comparison with standard samples of sIgA and lactoferrin that were reported to be abundantly present in tear fluids (Fig. 2(B)) [39,40].

The gels after visualization with Coomassie Blue were cut off as shown Fig. 2(A) (right side) and sialic acids in the bands were analyzed. The bands 2 and 9 at 75 and 28 kDa, contained a large amount of Neu5Ac (5.8–9.6 nmol/ml). Lactoferrin at 75 kDa was reported to be a glycoprotein containing sialic acids [41]. sIgA light chain was observed at 28 kDa, but it does not contain glycans [42]. Therefore, the band at 28 kDa probably contains some unknown glycoproteins as well as sIgA light chain. It should be noted that recoveries of sialic acids for both NW and CW groups were quite low (15.7 and 32.8%, respectively). The reasons of these low recoveries were due to difficulties in analysis of mucin proteins by PAGE due to large molecular masses, because mucin proteins could not enter the gel. Based on these considerations, we examined tear samples by cellulose acetate membrane electrophoresis.

3.3. Electrophoresis on cellulose acetate membranes

Tear samples from NW subjects (volunteers 3 and 6) and CW subjects (volunteers 8 and 9) were analyzed by cellulose acetate membrane electrophoresis. A clear band at the same position with albumin (Fig. 3(B), part c) and a broad band at the similar position with those of sIgA and lactoferrin were observed in both NW and CW subjects (Fig. 3(A and B)).

Proteins containing *O*-glycans can be detected using ABA, which specifically recognizes T-antigen (Gal(1-3GalNAcα1-Ser/Thr) [43]. Fig. 3(C) shows the results on lectin affinity staining of tear samples using ABA after cellulose acetate membrane electrophoresis. Interestingly, the sample from NW subject (volunteer 3) showed a clear band at the similar position with that of bovine submaxillary mucin (BSM). In contrast, the sample from CW subject (volunteer 8) did not show the band at the position, where BSM was detected. Slow migration proteins observed in both NW and CW subjects were due to sIgA. Lactoferrin and albumin were not detected by the lectin affinity staining (Fig. 3(C and D)) [41].

We determined sialic acids in these bands after direct hydrolysis of the bands followed by DMB derivatization. As shown in Fig. 4, B3, B4 and B5 from both NW and CW subjects contained Neu5Ac.

B3 from NW subject contained a large amount of Neu5Ac, but the same zone from CW subject contained less amount of Neu5Ac. The results are summarized in Table 2. Data from three volunteers for each NW and CW subjects are shown.

The band 3, which was detected by lectin affinity staining contained Neu5Ac most abundantly. Neu5Ac in this band for NW subjects (146.3 nmol/ml) was five-fold abundantly present than that for CW subjects (26.5 nmol/ml). Although bands 4 and 5 also contained Neu5Ac, 65% of Neu5Ac were

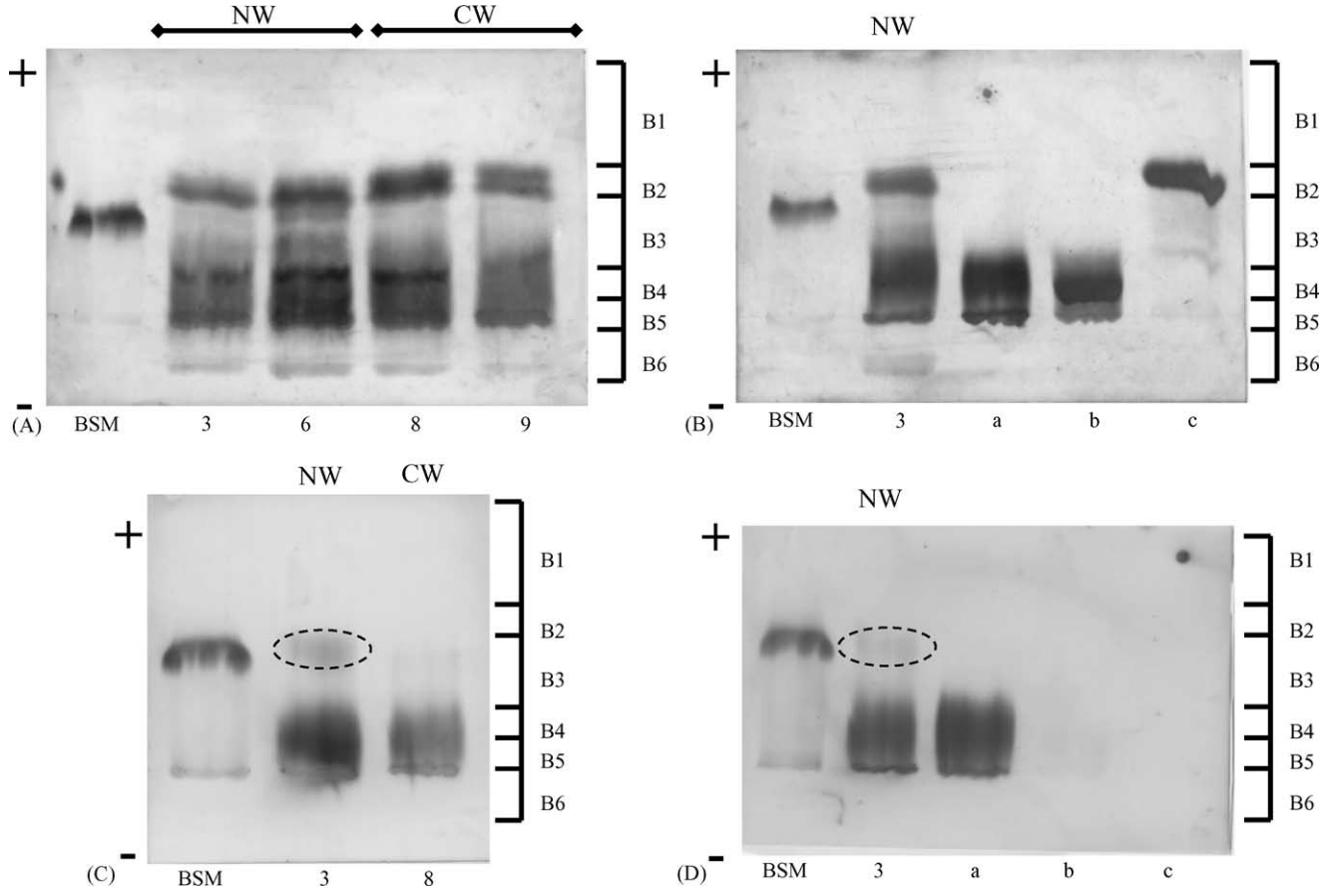

Fig. 3. Cellulose acetate membrane electrophoresis. (A) Tear samples from the NW subjects (volunteers 3 and 6), the CW subjects (volunteers 8 and 9) and bovine submaxillary mucin (BSM) by silver staining. (B) Standard samples of: (a) sIgA, (b) lactoferrin and (c) albumin. (C) Tear samples from the NW subject (volunteer 3), the CW subject (volunteer 8) and BSM. The bands were detected using ABA lectin. (D) Standard samples of: (a) sIgA, (b) lactoferrin and (c) albumin. The bands were detected using ABA lectin.

due to band 3 in NW subjects. On the contrary, 39% of Neu5Ac were due to band 3 in CW subjects. These data clearly indicate that decrease in sialic acids in CW subjects was mainly due to decrease of band 3 protein (i.e. mucin proteins). Propriety of the data was confirmed from the recovery data of 69.9 and 72.9% for NW and CW subjects, respectively.

Each band on cellulose acetate membrane was further examined by SDS-PAGE after extraction of proteins with water (Fig. 5).

As indicated above, tear samples showed two bands at 64 and 75 kDa derived from lactoferrin and sIgA as the major proteins when examined by silver staining. On cellulose acetate membrane electrophoresis, these proteins were observed at bands 4 and 5. Although the band 2 was not visualized in Fig. 5, B2 band was due to albumin, which was present as a minor protein in tear fluids. This was confirmed by SDS-PAGE analysis of the bands obtained by repetitive collections of B2 band in Fig. 4 followed by extraction with water (data not shown). In contrast, band 3 did not show obvious band by SDS-PAGE. The results indicated that macromolecular mucin protein did not enter the gel and could not be detected.

4. Discussion

There have been many reports on the determination of mucins in tear-film using impression cytology on conjunctival goblet cells [44,45], rose bengal staining on the epithelium of mucus membrane [46] and tear-film break-out time [47]. Although an accurate method has been needed for determination of secreted mucins in tear fluids, no standard methods have been developed. In the previous papers, we proposed that sialic acids are a good marker for monitoring mucin-like proteins in biological samples [30–32].

In the present study, we applied an HPLC method for the analysis of sialic acids after pre-column derivatization with DMB to determine secreted mucins in tear fluid. Neu5Ac levels in tear samples from 13 volunteers including CW and NW subjects varied from 34.9 to 298.5 nmol/ml and the average was 135.6 ± 106.9 nmol/ml. The results were well correlated with the previously reported values (119.9 ± 91.5 nmol/ml) in healthy human tear fluids [33]. In contrast, Neu5Gc and KDN were not detected in all tear samples [48].

Neu5Ac of CW group showed significantly lower level than that of NW group. We examined non-specific adhesion

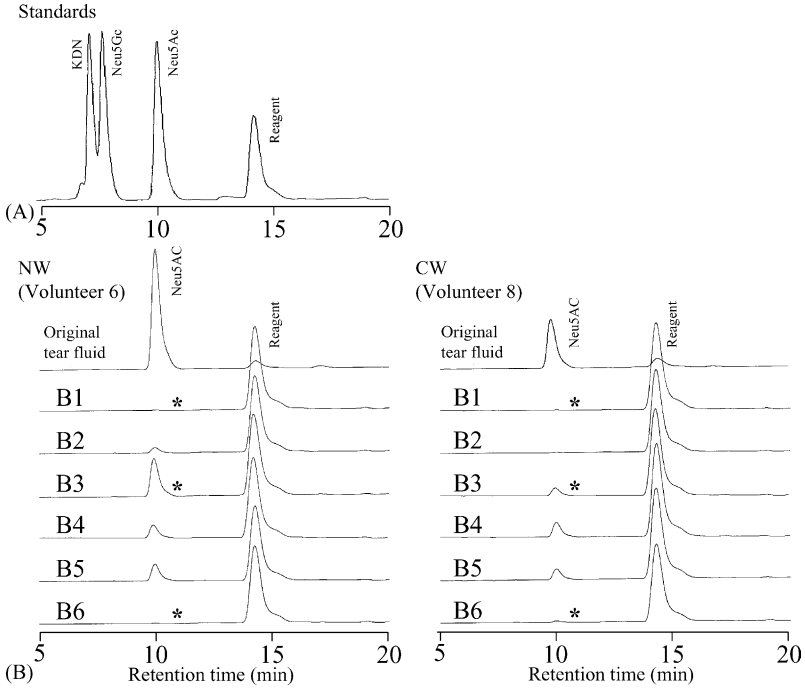

Fig. 4. Analysis of sialic acids after derivatization with 1,2-diamino-4,5-methylenedioxybenzene (DMB). (A) Standard solutions of Neu5Ac, Neu5Gc and KDN. (B) Sialic acids released from bands separated by electrophoresis of tear samples on cellulose acetate membrane. *Two-fold diluted.

of mucin proteins to contact lenses and found that sialic acids were not detected in lenses (data not shown). We also examined influence of contact lens type, such as soft contact lens and rigid contact lens. But significant changes were not observed (data not shown).

Tear samples were examined by electrophoresis using polyacrylamide gel and cellulose acetate membrane to confirm, which component was responsible for decrease of sialic acids. Although similar protein profiles were observed between NW and CW subjects as already reported by Claudon-Eyl et al. [49], cellulose acetate membrane electrophoresis clearly indicated that decrease of Neu5Ac in CW subjects was due to dramatically decreased level of mucin in tear fluids.

Many controversial data were reported on mucin secretion upon contact lens wearing. Mucin production might be increased by non-goblet cells in the conjunctiva in response to contact lens wear [29]. On the other hand, mucin production from goblet cells was decreased in contact lens wearers, although there was no involvement of the goblet cell population [50]. Protein levels of MUC5AC in the tear fluid of patients with Sjogren's syndrome were lower than normal individuals, in succession to reduction of the number of RNA transcripts for MUC5AC in the conjunctival epithelium [20,50]. A significant negative correlation was observed between MUC5AC levels of production involving decrease of goblet cell population and inflammatory markers ICAM-1 in ocular rosacea and dry eye patients [22]. Strong inflammation due to ocular surface diseases may decrease the goblet cell population as well as mucin production from goblet cells. The inflammation was higher in patients with ocular surface diseases than in the contact lens wearers, with maybe a minimum level of inflammation for goblet cell involvement, so that inflammation level could not be high enough to induce

Table 2
Analysis of Neu5Ac in the bands of cellulose acetate membrane electrophoresis

Band no.	NW (nmol/ml, $n = 3$)	CW (nmol/ml, $n = 3$)
1	1.1 ± 1.4[a] ($0.5 \pm 0.6\%$)	0.0 ± 0.0 ($0.0 \pm 0.0\%$)
2	13.1 ± 8.2 ($5.8 \pm 3.7\%$)	1.1 ± 0.9 ($1.6 \pm 1.3\%$)
3	146.3 ± 18.9 ($65.2 \pm 8.4\%$)	26.5 ± 5.7 ($38.6 \pm 8.3\%$)
4	24.8 ± 5.8 ($11.0 \pm 2.6\%$)	20.4 ± 3.2 ($29.7 \pm 4.6\%$)
5	37.8 ± 5.8 ($16.9 \pm 2.6\%$)	17.3 ± 2.3 ($25.2 \pm 3.3\%$)
6	1.5 ± 1.1 ($0.7 \pm 0.5\%$)	3.3 ± 1.4 ($4.8 \pm 2.1\%$)
Total	224.5 ± 34.6 ($100.0 \pm 15.4\%$)	68.5 ± 10.4 ($100.0 \pm 15.2\%$)
Original tear fluids	321.3 ± 61.2	94.0 ± 5.0
Recovery (%)	69.9	72.9

[a] Mean ± S.D.

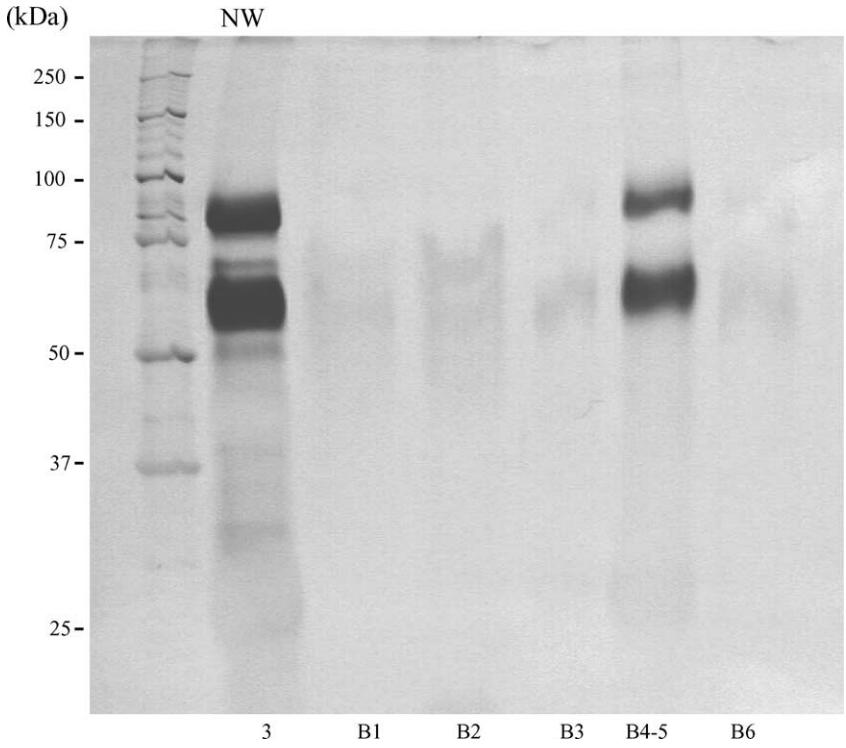

Fig. 5. SDS-PAGE analysis (9% T, 2.6% C) of proteins released from bands (B1–B6) separated by electrophoresis of tear samples (volunteer 3) on cellulose acetate membrane.

goblet cell loss but decrease mucin production [50]. Our results supported decrease of mucin production in contact lens wearers. We observed that Neu5Ac was recovered to normal level after taking off contact lenses for 12 h. Taking off contact lenses may cure the minimum level of inflammation, and mucin secretion from goblet cell is provably recovered to normal level.

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