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Comparison of Mucin Levels at the Ocular Surface of Postmenopausal Women With and Without a History of Dry Eye

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Abstract

Purpose—Determine 1) if levels of the glycocalyx membrane mucins, MUC1 and MUC16, and the secreted goblet cell mucin MUC5AC are altered in conjunctival cells and tears of postmenopausal women presenting with a history of non-Sjögren's dry eye, and 2) if mucin levels correlate with dry eye clinical diagnostic data.

Methods—Eighty-four postmenopausal women with a history of non-Sjögren's dry eye and 30 normal subjects were recruited for this study. Impression cytology samples were collected for mucin mRNA and protein analysis. Tears were collected for mucin protein assay. qPCR, western blot, and ELISA assays were used to quantitate MUC1, MUC16 and MUC5AC levels.

Results—Postmenopausal women with a history of dry eye displayed significantly increased MUC1 mRNA expression and cellular protein compared to normal subjects ($P<0.001$ and $P<0.0l$, respectively). Similarly, cellular MUC16 protein levels were significantly higher ($P<0.001$). Mucin levels were found to be correlated with the clinical characterization of the subjects, including staining and symptoms. Although cellular MUC5AC protein levels were increased in symptomatic subjects, the increase did not reach statistical significance.

Conclusion—Elevation in MUC1 and MUC16 mRNA and/or protein levels in postmenopausal non-Sjögren's dry eye patients with a history of dry eye may be a compensatory response to irritation and inflammation associated with the disease. Understanding the pattern of mucin expression associated with dry eye pathology may clarify factors involved in the progression of the disease and enhance the development of targeted therapies.

Keywords

Dry Eye; Ocular Surface Mucins; MUC1; MUC16; MUC5AC

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Introduction

Dry eye is a multifactorial disease of the ocular surface, prevalent in postmenopausal women, that results in damage to the ocular surface epithelium.[1] The apical membrane surface of the corneal and conjunctival epithelium is covered by a glycocalyx that forms the interface with the epithelium and the tear fluid. The glycocalyx is rich in membrane-tethered mucins including MUC1, MUC4 and MUC16,[2] the ectodomains of which are constituitively released into the tear film.[3] Also within the tear fluid, the goblet cell-derived, secreted mucin MUC5AC is present. Both mucin types play a role in epithelial surface protection. Secreted mucins clear the ocular surface of debris, and membrane mucins form a glycocalyx barrier to pathogen and dye penetrance.[2,4] Since dry eye disease is prevalent in postmenopausal women and, given the protective role of mucins at the ocular surface, this study focused on the comparison of mucin levels in postmenopausal women with or without significant symptoms of dry eye.

Ocular surface damage in dry eye is detected by a battery of tests including ocular surface staining using the dyes rose bengal, lissamine green and/or sodium fluorescein; measurement of tear film breakup time (TBUT); tear secretion (e.g., using the Schirmer test); and symptom assessment. To date, the only insight regarding the correlation of mucin expression with such clinical endpoints is the in vitro finding that the membrane mucin MUC16, along with its O-glycans, plays an important role in the barrier to rose bengal penetrance.[4,5]

Alterations in ocular surface mucins in dry eye have been previously reported. A decrease in goblet cell-derived MUC5AC in tears and impression cytology samples from Sjögren's patients, as compared to age-matched controls, has been demonstrated,[6] and a decrease in tear MUC5AC, as measured by immunolocalization of MUC5AC antibody to tears collected by Schirmer strip in undifferentiated keratoconjunctivitis sicca (KCS) has been reported.[7] Alteration in membrane mucins in dry eye has also been reported. Several immunohistochemical studies of membrane mucin distribution on apical conjunctival cells obtained by impression cytology have shown alterations in localization patterns with dry eye (e.g., see [8-10]). Binding of an antibody to a carbohydrate epitope on MUC16 to apical surfaces of conjunctiva (using antibody H185) has been shown to be reduced in non-Sjögren's dry eye patients and is correlated to disease severity.[9] An increase in the frequency of expression of a shorter, secreted form of MUC1, the result of a gene splice variant, has also been reported for dry eye.[11,12] These studies are, however, limited in that they provide only semiquantitative immunohistochemical data and/or relatively few patients.

Taken together, these studies implicate alterations in epithelial surface and/or goblet cell derived mucins in the pathophysiology of dry eye. To date, there has not been a study employing sufficient numbers of subjects in which mucin mRNA and mucin protein (both epithelial cell-associated mucins and soluble tear mucins) content is quantified and correlated to disease status. Such a study would provide valuable information on both changes in expression with disease, as well as release of mucins into the tears.

Thus, the purpose of this study was to 1) compare membrane-bound (MUCs 1 and 16) and soluble (MUC5AC) mucin levels and mucin mRNA expression in a group of postmenopausal women with a history of dry eye symptoms to that of age-matched postmenopausal women, and to 2) correlate mucin level data with clinical diagnostic data obtained from the subjects.

MATERIALS AND METHODS

Subject Selection

This study was conducted in compliance with good clinical practice, institutional review board regulations, informed consent regulations, and the tenets of the Declaration of Helsinki. Women with natural menopause and non-Sjögren's dry eye, ≥ 45 years-of-age and of any race, were recruited from a single center. Subjects were considered postmenopausal if they had had no menses for at least 12 months. Subjects were considered as having a history of dry eye based on the following: 1) documented diagnosis in medical charts made by a medical care provider ≥ 6 months prior to study visit and 2) a documented history for ≥ 3 months of complaints of ocular discomfort consistent with dry eyes (burning, stinging, blurring, gritty, dry, etc.). In addition to these two criteria, subjects included into the dry eye group must have answered, “yes” to the question, “currently using or have desire to use artificial tears, ocular lubricants or re-wetting drops.” Subjects with no medical diagnosis of dry eye, no ocular discomfort consistent with dry eye, no use of artificial tears, lubricants or re-wetting drops, and no autoimmune disorders were classified as normal (non-dry eye) subjects.

Exclusion criteria for all subjects included < 45 years-of-age, childbearing potential or menses within the last 12 months, surgical removal of ovaries with or without fallopian tube, removal of uterus or endometrial ablation, a medical diagnosis of Diabetes and/or autoimmune connective tissue disease (including Lupus Erythematosis, mixed connective tissue, Scleroderma, Rheumatoid Arthritis and/or Sjögren's Syndrome), keratorefractive ocular laser procedures, use of topical ocular medications, corneal surgery or other surgery to the corneal surface, punctal cauterization, or current punctal plugs. A history of plug placement was allowed if there was documentation in the medical chart of dislodgement or removal of the plug ≥ 30 days prior to the study visit. Also excluded were history of contact lens wear within the past 6 months or intraocular laser procedures within 1 year of the study visit.

Clinical Assessment

Once enrolled in the study, on the day of collection, subjects responded to dry eye symptom questionnaire and underwent routine clinical workup to further characterize disease presence and severity including assessment of tear breakup time (TBUT) and ocular surface staining, based on the NEI/Industry workshop method[13] (fluorescein and lissamine green); tear meniscus height, conjunctival redness, photophobia questionnaire (consisting of one question, “How often during the past ***24 hours*** were you noticeably more sensitive to light than normal for you?”; scaled from 0 (None) to 4 (Continuously)). In addition, symptoms were assessed using a dry eye symptom questionnaire (DESQ) consisting of the 7 following questions, scored 0 (Not at all) to 4 (6 or More Times). “1. How often during the last ***24 hours*** were your eyes noticeably ***burning*** more than normal for you? 2. How often during the last ***24 hours*** were your eyes noticeably ***stinging*** more than normal for you? 3. How often during the last ***24 hours*** were your eyes noticeably more blurry than normal for you? 4. How often during the last ***24 hours*** were your eyes noticeably more ***gritty*** than normal for you? 5. How often during the last ***24 hours*** were your eyes more ***dry*** than normal for you? 6. How often during the last ***24 hours*** did your eyes experience ***scratchiness***? 7. How often during the last ***24 hours*** did you experience ***foreign body sensation***?”

Sample Collection and Processing

Collection of tears with a capillary tube took place immediately after best-correct acuity was measured, and after slit lamp biomicroscopy. Collection of tears using a tear wash took

place a minimum of 30 minutes after capillary tear collection. Impression cytology was carried out immediately after tear wash.

Using a graduated disposable 5 μl microcapillary tube (Wiretol-Micropipettes, Drummond Scientific Co., Broomall, PA, USA) up to 5 μl of tears/eye were collected from the inferior temporal tear meniscus of each participant, without corneal anesthesia, taking care to ensure that the lid margin and corneal surface were not touched. Tears from both eyes were pooled. For tear wash collection, starting with the right eye, 60 μl of sterile, physiological saline (0.9% NaCl) (Minims, Chauvin Pharmaceuticals Ltd, Romford, Essex, UK) was applied to the superior bulbar region of the unanesthetized ocular surface using a sterile micropipette. Participants were asked to rotate their eyes without blinking, then the wash was collected from the inferior fornix using the micropipette. The same procedure was repeated with the left eye. Both tear washes were pooled together. All tear samples were immediately placed on dry ice until transfer to −80°C for storage.

Conjunctival epithelial cells were collected by impression cytology from each eye using sterile Millipore, MF membranes, (pore size 0.45 μM). Two drops of a topical anesthetic (Alcaine®, Alcon), dosed 60 seconds apart, were applied to the right eye. Fifteen seconds after the second drop of anesthetic, the subject was instructed to hold their gaze down to expose the superior conjunctiva. One piece of filter paper was placed on the superior region of the conjunctiva then removed with blunt forceps and placed in a sterile pre-labeled 2 ml capped polypropylene centrifuge tube containing 1 mL of RLT® RNA Isolation Buffer (Qiagen, MA, USA) containing 0.01% β-mercaptoethanol. The same procedure then took place on the temporal conjunctiva and the filter paper was placed in the same tube as the superior sample. Anesthesia and impression cytology of the left eye then took place as described for the right eye, with the exception that the two filter papers were placed in an empty sterile 2 ml capped polypropylene centrifuge tube, for subsequent protein extraction. All samples were immediately placed on dry ice, then transferred to −80°C for storage until processing.

RNA Isolation from Conjunctival Impression Cytology Samples and Reverse Transcription

Tubes containing 1 mL of RLT® buffer (Qiagen) and two impression cytology samples were allowed to thaw at room temperature, then vortexed for 30 seconds. Inpression cytology membranes were removed using a 21 gauge needle and samples were passed through a 21 gauge needle 10 times. Extraction of total RNA proceeded according to manufacturer's directions (RNeasy® Minikit, Qiagen). The DNase step, as recommended, was performed. The final isolation step was conducted with 40 μL of RNAse free water.

Protein Isolation from Conjunctival Impression Cytology Samples

Left eye impression cytology samples were used to isolate protein. Filter papers were placed cell side up on small glass plates and 5 μL of extraction buffer (2% SDS; 1X Complete™ protease inhibitor cocktail (Roche, Mannheim, Germany)) was placed on each. Using a steel scalpel blade, each membrane was cut into small pieces, which were placed in 600 μL capped polypropylene centrifuge tubes and covered with an additional 50 μL of extraction buffer. Tubes were vortexed then heated at 95°C for 10 minutes. Tubes were centrifuged at 12,000 g for 6 minutes and the protein extract was collected and transferred to a fresh, capped polypropylene centrifuge tube. Twenty μL of extraction buffer was added to the pelleted filter paper. Following vortexing and centrifugation, the wash was collected and added to the first protein aliquot.

Protein concentration in impression cytology samples was determined using the DC Protein Assay Kit®, and tear protein concentration was determined by Pierce Micro BCA assay following manufacture's instructions.

Mucin mRNA Quantitation

MUC1 and -16 mRNAs were assayed on reverse transcribed RNA isolated from the impression cytology samples using real-time PCR, as previously described.[14] Briefly, cDNA was reverse transcribed from the RNA using random hexamer primers with Superscript™ III First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA) according to the manufacturer's instruction. Relative expression of MUC1 and MUC16 was determined by multiplex real-time PCR using target (300 nM) and endogenous control (100 nM) oligonucleotide primers in the presence of gene-specific dye-labeled TaqMan probes (100 nM) and TaqMan® Universal PCR Master Mix (Applied Biosystems, Foster City, CA). Primers and probes used were as published,[14] except with the addition of MUC1 – Forward: CTGGTCTGTGTTCTGGTTGC; Reverse: CCACTGCTGGGTTTGTGTAA; TaqMan Probe: GAAAGAACTACGGGCAGCTG (Applied Biosystems). Data were collected on duplicate samples and fold-expression changes were calculated using the comparative ΔΔCt method as previously described.[15]

Protein Quantitation

Two types of assay (western blot and ELISA) were performed to optimize quantitation of multiple mucins from a single individual and to accommodate variable sample yield, which often precluded the ability to quantify all targets due to insufficient sample.

Western blot—For assay of MUC1 and MUC16 protein, protein from tear washes (MUC1), capillary tears (MUC16), or isolated impression cytology samples (50 μg for MUC1; 10 μg for MUC16) were denatured in non-reducing Laemmli sample buffer, separated in a horizontal 1% (w/v) SDS-agarose gel, and vacuum transferred to nitrocellulose membrane for western blot, as previously described.[3] Due to the large number of samples to be tested, each gel contained an aliquot of a single reference sample to normalize data between blots and allow semiquantitative comparisons of mucin levels. The reference sample for the western blots was protein isolated from an immortalized human corneal limbal epithelial (HCLE) cell line known to produce MUCs 1 and 16.[15] MUC1 protein content was detected with the MUC1-specific antibody 214D4 (Upstate; Lake Placid, NY) and MUC16 protein with the MUC16-specific antibody OC125 (Dako Corp.; Carpenteria, CA). Detection of antibody binding was performed by reaction of blots with Pierce West Pico Chemiluminescent Substrate, followed by exposure to Hyperfilm ECL (Amersham; Buckhamshire, UK). Densitometry was performed on blots photographed with a Kodak Digital Science Camera, using ID Image Analysis Software, Version 2.02 (Eastman Kodak, Co.; Rochester, NY), as described.[3] Data were normalized to the reference standard and expressed relative to the mean of the normal subjects.

ELISA—For assay of MUC16 glycosylation, the H185 antibody, which recognizes a carbohydrate epitope on MUC16,[16-18] was used in an ELISA on protein from tear wash (1 μg) and impression cytology samples (125 ng), as previously described.[18] MUC5AC content was determined on 0.5 μg of neuraminidase-treated protein from tear wash and impression cytology samples, using the MUC5AC-specific antibody 791, as previously described.[6] Proteins pooled from multiple tear wash samples from a single individual were loaded in each ELISA plate as a standard reference to control for variation between assays. Data were normalized to the reference sample and expressed relative to the mean of the normal subjects.

Statistical Analysis

The range and mean values of each diagnostic test and the scores on the symptom and photophobia questionnaires were compared between the dry eye patients and normal subject groups using Kruskal-Wallis tests in Instat3 statistical software (Graph Pad Software; San Diego, CA). The statistical significance of differences in mucin mRNA and protein levels in dry eye compared to normal subjects were determined using the Mann-Whitney test. Correlations between mucin data and clinical parameters of all subjects, both normal subjects and dry eye patients, were determined using the Spearman Rank Correlation test in Instat3.

RESULTS

Eighty-four postmenopausal patients with dry eye were enrolled into this study, with selection based on the inclusion criteria of a recent diagnosis (signs and symptoms) of dry eye in addition to the need or desire to use artificial tears or lubricants. Thirty normal, non-dry eye postmenopausal women, not using artificial tears or lubricants, were recruited as described in the Methods.

Ranges and mean values of the diagnostic tests and the symptom and photophobia questionnaires are shown in Table 1. Based on general guidelines provided in the DEWS report (1), all dry eye patients were found to fall into the mild-to-moderate dry eye category. However, it is clear from review of Table 1 that subjects not enrolled as dry eye presented with some signs of dry eye, but were not symptomatic based on questionnaire scores. The comparison of subjects is based on designation as symptomatic dry eye, as supported by a statistically significant difference in symptoms between the two groups as measured in symptom questionnaires and the need/desire of the dry eye patients to use a dry eye treatment.

Tear wash samples harvested prior to impression cytology yielded 10 to 70 μl for normal subjects (N) and 1 to 85 μl for dry eye patients (DE), with protein concentrations that ranged from 0.36 to 3.80 μg/μl (N) and 0.46 to 5.94 μg/μl (DE). Preliminary studies were performed to compare the two tear collection techniques, and similar results were found for mucin quantification (data not shown), albeit, tear wash collections yielded more protein than did capillary tear collection.

In some samples, there was insufficient protein to perform all mucin assays. The MUC1 assays require at least 5 times the amount of protein than assays of the other mucins, thus the number of data points for MUC1 tear assays was less (N=16 for normal tear samples and N=35 for dry eye tear samples) compared to other analyses.

Mucin Levels

For best accuracy of mucin mRNA and protein assay, assays that had previously been validated for quantitation of mucin gene expression and protein levels were used.[3,6,15] Any differences in starting amount of total RNA between samples was accounted for by normalization to expression of an endogenous control gene (GAPDH), and for variation between immunoblots and ELISA, data was normalized to a mucin reference included in each assay. The amount of MUC1 mRNA and cellular protein from impression cytology samples of dry eye patients was significantly higher relative to normal subjects (Fig. 1A). The amount of released MUC1 ectodomain in the tear washes did not, however, significantly differ between the two groups (Fig. 1A).

Similarly, the amount of MUC16 cellular protein from impression cytology samples of dry eye patients was significantly higher than that from normal subject samples (Fig. 1B). The

MUC16 mRNA levels, although increased in dry eye samples compared to normal subjects, did not reach the level of significant difference. The amount of MUC16 ectodomain in tears, as with MUC1, did not differ between normal and dry eye populations (Fig. 1B). Binding of the H185 antibody, which recognizes an O-acetylated sialic acid epitope on MUC16, to proteins in tear washes from dry eye patients, was significantly increased over normal subject values, whereas no differences were found in binding to impression cytology samples (Fig. 1B). In fact, a decrease, albeit not significant, was observed in amount of H185 binding to the cellular samples from dry eye patients (Fig. 1B). These data using the H185 antibody suggest that there is no change in glycosylation of MUC16 in the dry eye patients.

The amount of goblet cell-derived MUC5AC in cellular or tear samples did not differ significantly between normal and dry eye samples (Fig. 1C). There was, however, a trend of increased cellular MUC5AC in these mild-to-moderate stage dry eye patients.

Correlations Between Mucin Content and Dry Eye Diagnostic Tests

Clinical assay data obtained from both normal patients and dry eye subjects at the time of sample collection were ranked and correlated to the mucin data. Correlations of mucin data with dry eye diagnostic test data are shown in Table 2. A significant, positive correlation between MUC1 expression and lissamine green staining, and dry eye symptom questionnaire responses was found.

Similarly, MUC16 expression and protein levels showed several correlations to clinical parameters indicative of dry eye. MUC16 mRNA was negatively correlated to tear meniscus height. MUC16 cellular protein levels were positively correlated to multiple clinical data sets, including photophobia, sodium fluorescein (NaFl) staining, lissamine green staining, and scoring from the dry eye symptom questionnaire (Table 2). No correlations between H185 and clinical data were found. MUC5AC cellular protein negatively correlated to NaFl staining, whereas the amount of the MUC5AC in tears was positively correlated to lissamine green staining.

DISCUSSION

Data from this study demonstrate an increase in expression of membrane-spanning mucins MUC1 and MUC16 in the ocular surface epithelia of postmenopausal women with a history of and symptomatology associated with mild-to-moderate, non-Sjögren's dry eye. The increase is documented by measurement of both RNA and protein levels. Although not enrolled on the basis of clinical signs as in previous studies, the subjects recruited for this study are derived from a relatively large study population (N=84 dry eye and 30 normal) that demonstrated a mild correlation to several lines of clinical test results. The lack of clear clinical separation between the two groups in this study with respect to dry eye signs at the time of enrollment was not entirely surprising given the well-documented lack of reliability between dry eye diagnostic tests and/or the disconnect between signs and symptoms of dry eye.[19,20] It cannot be ruled out that some of the control, “normal” subjects may have early dry eye without typical symptoms. However, the large population size, inclusion criteria and demonstration of symptom separation between the two groups in this study, all suggest a role for elevated mucin expression in postmenopausal women presenting with mild-to-moderate symptoms of dry eye.

The increase in mucin levels associated with mild presentation of dry eye, associated more with symptoms as opposed to significant signs, may be a compensatory response to the irritation and early stage inflammatory characteristic of the disease. Furthermore, image analysis of the amount of immunohistochemically localized MUC1 on impression cytology

samples from patients with mild-to-moderate dry eye showed similar results, in that an increase in the membrane mucin was observed in the mild disease state.[10] As in this study, MUC16 was shown to be upregulated in conjunctival epithelial samples from patients with atopic keratoconjunctivitis.[21] Recently, MUC16 expression has been shown to be upregulated by the secretory form of phospholipase A2 group 2a (sPLA2-IIA) in human conjunctival epithelial cells in vitro.[22] Levels of sPLA2-IIA, a normal component of tears, have recently been demonstrated to be increased in the tears of dry eye patients.[23] This increase in sPLA2-IIA may be responsible for the increased expression of MUC16 in dry eye patients' conjunctival epithelium seen in our study.

By comparison, Caffery et al. did not detect an upregulation of MUC16 in a study of 25 KCS patients, but did find increased expression and protein levels of MUC16 ectodomain in tears of 25 Sjögren's Syndrome dry eye patients[14] The KCS patients were recruited on the basis of symptoms (requiring at least 6 out of a possible 10) and signs (Schirmer score of less than or equal to 10 mm in 5 minutes). Without additional information, it is difficult to determine the clinical similarity in the KCS group studied by Caffery in comparison to the group in this work and, thus, it is difficult to explain the difference in MUC16 findings between the two studies.

The assays used in our study include both the assay of mucin mRNA and mucin protein levels in the cells of the conjunctiva and the amount of released mucin ectodomain protein in tears. As such, there is no direct measurement of mucin levels on the apical surfaces of the conjunctiva. It is difficult, therefore, to correlate findings of this study to those of previous work demonstrating a decrease in amount of binding of the H185 antibody, which recognizes the H185 carbohydrate epitope on MUC16, to apical surfaces of conjunctival cells in non-Sjögren's dry eye patients. There was in these studies a correlation of decreased binding of H185 antibody to apical surfaces of the conjunctiva with severity of disease.[9]

Perhaps the loss of MUC16 from small, discrete apical areas on the ocular surface, as indicated by both rose bengal and lissamine green staining, is a local phenomenon that is not within the range of detection of the methods employed in the current study. The fact that the stained areas in dry eye lack MUC16 or its glycans is suggested by recent demonstration that abrogation of MUC16 expression or O-glycan expression in human corneal epithelium in vitro allows rose bengal entrance into the epithelium.[4,5] Furthermore, inflammatory mediators present in tears of dry eye patients, which include IFN-γ, TNF-α, and matrix metalloproteinases (MMPs) 7 and 9, have been demonstrated to have the ability to induce MUC16 ectodomain release, and, in the case of TNF-α, MUC1 ectodomain release as well.[24,25] These inflammatory mediators may be released locally and induce small areas of dye penetrance through the release of the ectodomain of MUC16.

Data obtained in this study of mild-to-moderate postmenopausal, non-Sjögren's dry eye patients showed no significant differences in MUC5AC cellular protein or tear levels as compared to the control population. These data differ from studies of patients with Sjögren's dry eye in which a significant decrease in MUC5AC expression levels and tear protein levels was demonstrated.[6] The data also differ from that of Zhao et al.,[7] who studied MUC5AC tear/cellular protein levels in non-Sjögren's dry eye patients, as determined by election of proteins from Schirmer strips that collect both tears and adherent conjunctival epithelial cells. The differences between the data reported herein may be due to differences in severity of disease in the study populations or differences in the measurement protocols used by Zhao et al.[7] Goblet cell numbers have been demonstrated to be significantly decreased in non-Sjögren's dry eye and in Sjögren's dry eye;[26] however, the patients within the study were reported to have moderate-to-severe disease. Perhaps changes in goblet cell numbers, thus, MUC5AC production, do not occur until later stages of disease. Thus, the

mild-to-moderate disease population in this study shows no significant difference in MUC5AC protein levels.

The level of mucins in subjects correlated to several lines of clinical test results, including surface staining and dry eye symptom questionnaire response. Of the clinical parameters examined, only dry eye symptom and photophobia questionnaires' results were significantly different between the normal subjects and dry eye patients, which was supported by the inclusion criteria of use, or lack there of, of artificial tears. Significant correlations were, however, found between the mucin assays and several of the clinical parameters when all the data were compared using the Spearman Rank Correlation Test. For example, as scores for photophobia, NaFl and lissamine green staining increased, so did the amount of MUC16 cellular protein. Conversely, as NaFl staining decreased, so did MUC5AC cellular protein. Further work is required to investigate whether quantitation of mucins would provide valuable insight and/or serve as an alternative sign to characterize dry eye severity and progression.

In summary, data from this study of postmenopausal women, symptomatic for mild-to-moderate dry eye, demonstrate that there is an increase in expression and protein levels of the membrane mucins MUC1 and MUC16, and that this upregulation correlates positively with several diagnostic tests. The upregulation in expression of the mucins may be a compensatory response to repair local areas of mucin loss on apical surfaces due to ectodomain release potentially induced by inflammatory mediators.[24] Additional studies are needed to gain insight into the association between mucin expression and dry eye, defined both by symptoms and other diagnostic endpoints. Such information would provide useful guidance into pathophysiological mechanisms of dry eye as well as potential treatment modalities, including anti-inflammatory agents and agents that influence mucin expression.

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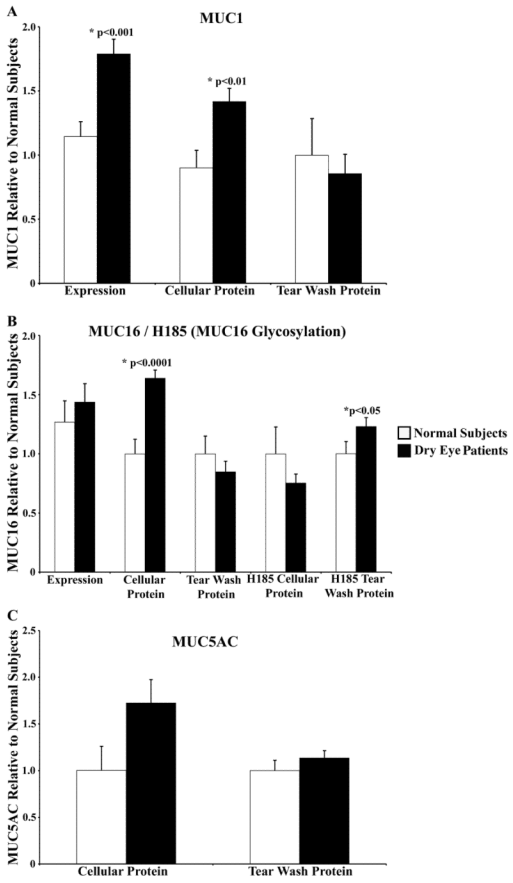

FIGURE 1.

Quantitative results obtained for assay of mucin mRNA expression and cellular and tear wash mucin protein amount in normal subjects and dry eye patients. Data are expressed relative to the mean of the normal control subjects and represent the mean ± SEM. Significant differences between normal subjects and dry eye patients are shown by an asterisk (*). *P* values are indicated on the graphs. (A) MUC1 expression and protein levels in conjunctival cells and tear washes; (B) MUC16 expression and protein as well as amount of the terminal O-acetylated sialic acid epitope on MUC16 that is detected with the H185 antibody; (C) MUC5AC cellular and tear wash protein. Sample sizes for all assays were N=29-30 for normal subjects and N=78-84 for dry eye patients, except for the tear wash assays for MUC1 (Normal N=16; Dry Eye N=35) and MUC16 (Normal N=26; Dry Eye N=65).

Table 1

Summary of Results of Diagnostic Tests

	Normal Subjects		Dry Eye Patients		
Diagnostic Test	**Range**	**Mean**	**Range**	**Mean**	**P Values**
Age (years)	45–80	59	45–84	64	ns P>0.05
Photophobia (0-4)	0–3	0.2	0–4	1.4	*** P<0.001
TBUT (seconds)	1.2–17.1	4.3	1.4–8.5	3.4	ns P>0.05
Tear Meniscus (mm)	0.1–0.3	0.2	0.1–0.5	0.2	ns P>0.05
NaFl Stain (0-4)	0–17	8.0	1.5–19.5	9.2	ns P>0.05
Lissamine Green Stain (0-4)	8–15	11.4	7.5–16.5	12.3	ns P>0.05
Conjunctival Redness (0-4)	0–3	1.1	0–3	1.4	ns P>0.05
DESQ	0–2	0.1	0–23	6.4	*** P<0.001

On the scale of 1-4, 4 represents more severe conditions. NaFL and lissamine green staining values are the sum of 5 areas of the ocular surface.

DESQ = Dry Eye Symptom Questionnaire. Values are the sum of the responses to the 7 questions on the questionnaire (0-4 scale for each).

*** Significant difference between normal subjects and dry eye patients using Kruskal-Wallis Test, $P<0.01$; ns = not significant.

Table 2

Spearman Rank Correlations of Mucin and Clinical Assays

Mucin	Significant Correlations with Clinical Data	Correlation Coefficient (r value)
MUC1 mRNA	Lissamine Green Stain	0.28
	DESQ	0.29
MUC1 Cellular	None	
MUC1 Tears	None	
MUC16 mRNA	Tear Meniscus	−0.33
MUC16 Cellular	Photophobia	0.25
	NaFl Staining	0.26
	Lissamine Green Stain	0.36
	DESQ	0.39
MUC16 Tears	None	
MUC5AC Cellular	NaFl Staining	−0.24
MUC5AC Tears	Lissamine Green Stain	0.37

*Significance for correlations were determined using Spearman Rank Correlation Test at P<0.01.

CLINICAL DATA COLLECTED: Age, TBUT, Photophobia, Tear Meniscus, NaFl Stain, Lissamine Green Stain, Conjunctival Redness, Dry Eye Symptom Questionnaire (DESQ).

All subjects were postmenopausal women. 114 Subjects: N=30, DE=84 (Mild/Moderate).