Proteomic Profiling of Inflammatory Signaling Molecules in the Tears of Patients on Chronic Glaucoma Medication

Tina T. Wong,1,2,3 Lei Zhou,1,2 Jing Li,1,2 Louis Tong,1,3,4 Shao Zhen Zhao,5 Xiao Rong Li,5 Shang Juan Yu,1 Siew Kwan Kob,1 and Roger W. Beuerman1,2,6

PURPOSE. To identify the tear proteins associated with the long-term use of glaucoma medication by using proteomic analysis and to compare these proteins to those previously reported in primary dry eye disease.

METHODS. Eighteen patients treated with topical antiglaucoma medications and 10 normal age-matched subjects with no prior topical treatment were recruited for the study. Tears were collected by using Schirmer’s strip and analyzed by iTRAQ (isobaric tag for relative and absolute quantitation) for tear proteins by mass spectrometry. Conjunctival samples were collected and RNA expression determined by PCR.

RESULTS. Of the 124 identified tear proteins (99% confidence, ProtScore ≥ 2.0), we found that the tear levels of S100-A8, S100-A9, mammaglobin B, and 14-3-3 ζ/δ were significantly increased in the medicated group compared with levels in the nonmedicated group (P < 0.05). For S100-A9, mammaglobin B, and 14-3-3 ζ/δ, use of topical medication for less than 1 year did not reach statistical significance compared with that in the nonmedicated group. Eyes on topical medication for less than 1 year showed a decrease in proline-rich 4 protein tear level (P = 0.0049) compared to nonmedicated group. The tear proteins detected in the medicated group differed from those in the primary dry eye group.

CONCLUSIONS. Treatment with topical antiglaucoma medications for longer than 1 year may start to induce ocular surface inflammation. The inflammatory tear protein profile present in chronically medicated glaucomatous eyes appears to be different from that found in primary dry eye. Identification of tear proteins specific to medicated glaucomatous eyes will help to specifically develop targeted screening modalities and therapeutic agents different from current conventional dry eye management. (Invest Ophthalmol Vis Sci. 2011;52:7385–7391) DOI:10.1167/iovs.10-56532

From the 1Singapore Eye Research Institute, Singapore; the 2Department of Ophthalmology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; the 3Singapore National Eye Centre, Singapore; the 4Office of Clinical Sciences, and the 5Duke-NUS SRP Neuroscience and Behavioral Disorders, Duke-NUS Graduate Medical School, Singapore; and the 6Tianjin Medical University Eye Center, Tianjin, China.

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Corresponding author: Tina T. Wong, Glaucoma Service, Singapore National Eye Centre, 11 Third Hospital Avenue, Singapore 168751; tina.wong.t.l@snec.com.sg.
Current modalities for the accurate detection and quantitative evaluation of tear proteins are limited by the small volumes of tears collected in such eyes. With the use of proteomic technology, a quantitative global analysis of tear proteins can be achieved. In this study, we provide a quantitative description of the tear protein profile in glaucoma patients using topical antiglaucoma medication. The results were compared with those previously found in tears of patients with primary dry eye disease.

METHODS

Subject Selection

This study was performed in accordance with the Declaration of Helsinki and the SingleHealth Institutional Review Board. Informed written consent was obtained from all participants. Two groups of subjects were studied. The first group consisted of 18 patients with a diagnosis of glaucoma using topical antiglaucoma medication and scheduled for glaucoma filtration surgery. The second group comprised 10 normal subjects with no history of ocular surface disorders, ocular inflammation, eye surgery, or contact lens wear.

Sample Collection

Tear fluids were collected from all consenting subjects with a Schirmer's type I tear test, as in previous studies in our laboratory.11 In brief, a Schirmer's tear test strip was inserted in the lower fornix of the eye to collect tears without any application of topical anesthesia. After 5 minutes, the strip was removed from the eye and the length of the wetted area of the strip was measured. Study subjects in the control group with wetted areas of less than 5 mm in 5 minutes were excluded from the study, as they were considered to have undiagnosed dry eye. After collection, the Schirmer's strips were immediately frozen at −80°C for later analysis. The wetted portion of the Schirmer's strip was cut into small pieces and soaked in 150 μL of phosphate-buffered saline (PBS) for 3 hours to elute the tear proteins. The total tear protein concentration of each sample was measured in a protein assay (Micro BCA; Pierce Biotechnology, Inc., Rockford, IL). In addition, conjunctival samples were retrieved from the recruited patients at the time of trabeculectomy surgery. A small piece (2 × 4 mm) of conjunctiva was removed by the surgeon (TTW) during conjunctival dissection at the beginning of the surgical procedure, and placed immediately in RNA stabilizer (RNAlater, Ambion, Austin, TX) for subsequent analysis.

Quantitative Analysis of Tear Proteins

The iTRAQ (isobaric tag for relative and absolute quantitation, a technique used in proteomics to study quantitative changes in the proteome) study design is shown in Figure 1. The purpose of the iTRAQ reagent is to label in certain peptide fragments so that the levels can be compared between two samples, thus providing a ratiometric analysis of the peptide composition by mass spectrometry (QSTAR XL; ABI). A sample preparation procedure was followed as in previous studies and according to the protocol provided by the maker of an iTRAQ kit (Applied Biosystems, Inc., [ABI], Foster City, CA).11,12 After these preparatory steps, the samples were digested at 37°C overnight with trypsin (included in the iTRAQ kit). iTRAQ reagent 114 was added to the global control while iTRAQ reagents 115, 116, and 117 were added to the samples from medicated group. The samples were then incubated at room temperature for 3 hours. The contents of each iTRAQ reagent-labeled sample were combined and dried in a concentrator (SpeedVac; Thermo Fisher Scientific, Waltham, MA) after which 10 μL of loading buffer (0.1% formic acid, 2% acetonitrile in water) was added to reconstitute the sample before running the 2D nano-LC-nano-ESI-MS/MS analysis.

Analysis of Tear Fluid Using Mass Spectrometry and Two-Dimensional Liquid Chromatography

The separation step for the peptide mixture was performed with 2D nano-LC (Dionex, LC Packings, Sunnyvale, CA) and then analyzed for the peptide composition by mass spectrometry (QSTAR XL; ABI). As in previous studies, 2D LC separation of peptides was performed using a strong cation exchange (SCX) followed by reversed-phase (RP) chromatography.13–15 Elution of the peptide mixture was performed using 10 steps of salt plugging (20 μL injection of 10, 20, 50, 40, 50, 75, 100, 250, 500, and 1000 mM ammonium acetate) all at a flow rate of 50 μL/min. The RP column used in the second dimension was a 10 cm × 75-mm ID microcapillary LC column (self-packed from a PicoFrit; 360 μm OD, 75 μm ID, 50 cm; New Objectives, Woburn, MA). The capillary column had an integrated spray tip (15 μm opening) that could be directly coupled with the nanospray interface (Protana, Odense, Denmark) into the mass spectrometer (QSTAR XL; ABI). Using a solvent delivery system (Ultimate; Dionex, LC Packings), a linear gradient of acetonitrile (0.1% formic acid) from 20% to 95% over 85 minutes at a flow rate of ~300 μL/min was used to analyze the tryptic digests. Key parameter settings for the nanospray and other instrumentation were as follows: ionspray voltage (IS), 2200 V; curtain gas (CUR), 20; declustering potential (DP), 60 V; focusing potential (FP), 265 V; collision gas setting (CAD), 5 for nitrogen gas; and DP2, 15. All data were acquired using the information-dependent acquisition (IDA) mode (Analyst Q3 software; ABI). The enhance-all function was used in the IDA experiments. Switching criteria were set to ions greater than m/z 350 and smaller than m/z 1200 with a charge state of 2 to 4 and an abundance threshold of >20 counts. Former target ions were excluded for 60 seconds. IDA collision energy (CE) parameters script was used for automatically controlling the CE.

Western Blot Analysis

Total tear protein (25 μg) was loaded onto the 10% SDS PAGE and transferred onto a PVDF membrane (Bio-Rad, Hercules, CA). The membrane was then blocked with 5% milk for 1 hour before incubating with the following antibodies overnight at 4°C: rabbit anti-human 14-3-3 ε/ζ primary antibody (9639; Cell Signaling, Beverly, MA), mouse anti-human S100A8 antibody (BM4028, 1 μg/mL final concentration; Acris Antibodies, San Diego, CA) and mouse anti-human S100A9 antibody (H00006280-M01, 2 μg/mL final concentration; Abnova, Walnut, CA) overnight at 4°C. The membrane was then washed and incubated with appropriate secondary antibody for 1 hour at room temperature. The resulting immune complex was visualized using chemiluminescent substrates (SuperSignal; Pierce Biotechnology) and exposed to x-ray film. The intensity of the specific bands was quantitated using densitometry analysis (Image Station 4000R Pro; Eastman Kodak, Rochester, NY).

Reverse Transcription and Quantitative PCR Analysis

Conjunctival epithelium was disrupted by brief ultrasonication and total RNA was extracted (Trizol) reagent combined in a column (PureLink; both from Invitrogen). The concentration and the purity of

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**Figure 1.** Design of the iTRAQ experiments.
RNA were evaluated by spectrophotometry (NanoDrop, Wilmington, DE). Two hundred fifty micrograms of RNA was reversed transcribed to cDNA (RTIII reverse transcriptase; Invitrogen). Five tenths of a microliter of the diluted cDNA was used for SYBR-green based quantitative (q)PCR analysis. Primer sequences for 14-3-3ζ mRNA were as follows: forward, 5′ GTCCCGAGGCGCATCCCGG3′, and reverse, 5′ GCTCGGGGGAAGCGGTCCTA 3′. SYBR-green real-time master mix was purchased from Roche, and the qPCR reaction was performed on a 2% agarose gel to confirm the size of the end product. β-Actin mRNA was used as the internal control to ensure the reliability of the results. ΔCp was calculated as Cp(sample) minus Cp(actin). For each cDNA sample, qPCR was conducted in triplicate wells.

**Data Processing and Statistical Analysis**

The data output from the mass spectrometer (QSTAR XL; Applied Systems) were processed and searched against the International Protein Index (IPI, Human, version 3.54; using ProteinPilot, ver. 2.0.1; ABI/MDS Sciex). All protein identification was based on the criteria of Unused ProtScore ≥ 2.0 (99% confidence). Other settings in this software for data analysis were as follows: sample type, iTRAQ 4-plex (peptide labeled); cyd alkylation, MMTS; digestion, trypsin; instrument, ESI (QSTAR; ABI); special factors, none; quantitate tab, checked; ID focus, biological modifications; and search effort, thorough ID. The results suggested that the conjunctival epithelial cells are the likely source of the 14-3-3ζ protein.

**Results**

Patient characteristics including age, sex, and duration and types of antiglaucoma medication are illustrated in Table 1. Most patients were on two or more medications at the time of surgery.

**Table 1. Patient Demographics**

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample, n</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>Female</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Age, y</td>
<td>72 ± 7</td>
<td>64 ± 8</td>
</tr>
<tr>
<td>Duration on antiglaucoma medications, mean months (range)</td>
<td>31 (2–149)</td>
<td>N/A</td>
</tr>
<tr>
<td>Number of antiglaucoma medications, mean (range)</td>
<td>2.1 (1–3)</td>
<td>N/A</td>
</tr>
</tbody>
</table>

|                        |       |          |
|                       |       |          |
|                       |       |          |

**Table 2. iTRAQ Ratios of Up- or Downregulated Tear Proteins Comparing the Medicated Group with the Nonmedicated Control Group**

<table>
<thead>
<tr>
<th>Name of Protein</th>
<th>Average Ratio†</th>
<th>SD</th>
<th>t-Test (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall average (n = 18)</td>
<td>1.448</td>
<td>1.458</td>
<td>0.0001†</td>
</tr>
<tr>
<td>Mammaglobin B</td>
<td>1.290</td>
<td>1.624</td>
<td>0.0147†</td>
</tr>
<tr>
<td>S100-A9</td>
<td>1.195</td>
<td>1.575</td>
<td>0.0249†</td>
</tr>
<tr>
<td>14–3-3ζ (n = 12)</td>
<td>1.198</td>
<td>1.581</td>
<td>0.0276†</td>
</tr>
<tr>
<td>Proline-rich 4</td>
<td>0.757</td>
<td>1.461</td>
<td>0.0010†</td>
</tr>
<tr>
<td>Duration of topical treatment &lt;1 year (n = 6)</td>
<td>1.217</td>
<td>1.256</td>
<td>0.0256†</td>
</tr>
<tr>
<td>Mammaglobin B</td>
<td>1.251</td>
<td>1.840</td>
<td>0.2227</td>
</tr>
<tr>
<td>S100-A9</td>
<td>0.972</td>
<td>1.271</td>
<td>0.9610</td>
</tr>
<tr>
<td>14–3-3ζ (n = 5)</td>
<td>0.902</td>
<td>1.455</td>
<td>0.6652</td>
</tr>
<tr>
<td>Proline-rich 4</td>
<td>0.698</td>
<td>1.384</td>
<td>0.0049†</td>
</tr>
<tr>
<td>Duration of topical treatment &gt;1 year (n = 12)</td>
<td>1.579</td>
<td>1.513</td>
<td>0.0044†</td>
</tr>
<tr>
<td>Mammaglobin B</td>
<td>1.310</td>
<td>1.554</td>
<td>0.0118†</td>
</tr>
<tr>
<td>S100-A9</td>
<td>1.322</td>
<td>1.663</td>
<td>0.0152†</td>
</tr>
<tr>
<td>14–3-3ζ (n = 7)</td>
<td>1.466</td>
<td>1.523</td>
<td>0.0034†</td>
</tr>
<tr>
<td>Proline-rich 4</td>
<td>0.789</td>
<td>1.508</td>
<td>0.1264</td>
</tr>
</tbody>
</table>

* The true average ratio (medical/nonmedical) is expected to be found in the range of average ratio/SD to average ratio × SD.
† P < 0.05, significant change between the medicated group and the nonmedicated group.

Patients, 15 (83%) were on a prostaglandin analog. All 18 were on two or more medications at the time of surgery.

**Tear Protein Profiling and Protein Expression in Medicated Eyes**

Similar to the results of our study of dry eye patients, 124 tear proteins were observed (99% confidence, ProtScore ≥ 2.0) from six sets of iTRAQ experiments (list of the identified tear proteins are provided in Supplementary Table S1 (http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.10-6532/-/DCSupplemental). Among those 124 tear proteins, we detected 5 with iTRAQ ratios that showed a significant change between the medicated and control groups (P < 0.05), Table 2A. Specifically, the levels of four tear proteins, S100-A8, S100-A9, mammaglobin B, and 14–3-3ζ protein, were increased, whereas proline-rich 4 protein was found to be diminished in the medicated samples relative to levels in the nonmedicated controls. Further analysis of the data showed that if the duration on medication exceeded 1 year, a general increase in tear protein levels was observed compared with eyes on medication for less than 1 year (Tables 2B, 2C).

Figures 2 and 3 provide the identification and relative quantitation using representative mass spectra for 14-3-3ζ protein and S100-A8 protein. (iTRAQ reporter ions 114, 115, 116, and 117). The increased protein levels of 14-3-3ζ and S100-A9 was further corroborated by Western blot analysis (Figs. 4, 5). Western blot analysis of 14-3-3ζ protein in the tear samples of medicated and control subjects further confirmed the findings by iTRAQ (Fig. 4A). Since conjunctival epithelial cells are known to express 14-3-3ζ mRNA, we further analyzed the levels of 14-3-3ζ mRNA in conjunctival epithelial cells of both groups. The results suggest that the conjunctival epithelial cells are the likely source of the 14-3-3ζ protein detected in the tears. Increased expression of S100-A8 and -A9 proteins in the tears was also confirmed by Western blot (Fig. 5).

Correlation analysis was performed between the duration of use of topical glaucoma medications and S100-A8, S100-A9,
14-3-3ζ/δ, mammaglobin B, and proline-rich protein 4 (Table 3). We only found S100-A8 (r = 0.582) and 14-3-3ζ/δ (r = 0.541) to display some correlation with the duration of use of topical medication. A larger number of samples are required for more rigorous statistical validation.

Differences in Tear Protein Profile between Medicated Glaucoma Patients and Patients with Dry Eye

Previous reports suggest that long-term use of topical antiglaucoma medication induces dry eye symptoms.14,15 We compared the results with previously published data from this laboratory that were processed in the same fashion as the data in the present study and using the same mass spectrometer. In the previous study, several potential biomarkers for dry eye, as well as the severity of dry eye, were identified.11 The nine potential tear protein biomarkers used for comparison were α-enolase, S100-A4, S100-A11, lysozyme C, lactoferrin, prolactin-inducible protein, lipocalin-1, mammaglobin B, and proline-rich protein 4 (Fig. 6). The data from 23 patients with known dry eye and 18 controls (from Tianjin Medical University Eye Center, China, by SZZ and XRL) were used to compare with the data from our medicated group. We found that the levels of S100-A8 and -A9 proteins were profoundly elevated in both medicated and primary dry eye groups compared with the unmedicated, non–dry eye controls. Interestingly, further analysis using PCA clustering showed that the medicated group clearly discriminated from the primary dry eye group if the biomarker panels were combined from the two groups (nine tear proteins; Fig. 6). Differences in the dry eye and medicated groups were also apparent.

**Figure 2.** (A) MS/MS spectrum of one quadruply charged peptide ion (EKETELRICNDVLSLEKFLIPNASQESK at m/z = 1134.9269; confidence = 99%) originating from 14-3-3ζ/δ protein. (B) Relative quantification for 14-3-3ζ/δ protein between pooled global nonmedicated control samples (labeled by iTRAQ 114) and individual medicated samples (labeled by iTRAQ 115, 116, and 117).

**Figure 3.** (A) MS/MS spectrum of one quadruply charged peptide ion (MLTELEKALNSSIDVYHKYSLIK at m/z = 825.2397; confidence = 99%) originating from S100-A8 calcium-binding protein and (B) relative quantification for S100-A8 calcium-binding protein between pooled global nonmedicated control samples (labeled by iTRAQ 114) and individual medicated samples (labeled by iTRAQ 115, 116, and 117).
DISCUSSION

The quality of the tears covering the ocular surface can reflect and direct the health of the epithelial cells as well as affect vision. Sampling this extracellular fluid for identification of the molecules that characterize the state of the ocular surface can be achieved by standard clinical procedures such as the Schirmer’s type I tear test. Recent work in this field has demonstrated that tear proteomics is a valuable technology for identifying molecular biomarkers for OSDs such as dry eye, as well as for monitoring other OSDs and conditions.

Dry eye forms the basis for a large number of clinical presentations. Comparison of age-specific data on the prevalence of dry eye from large epidemiologic studies reveals a range of approximately 5% to over 35% at various ages, with recent studies in China and Japan yielding much higher prevalences than in the West. Since dry eye is increasingly prevalent in the aging population, the burden of dry eye will certainly increase in the future. OSD is characterized by the presence of the following clinical signs: unstable tear film, inadequate tear volume, and ocular surface decompensation. Patients describe a range of classic symptoms such as ocular irritation or grittiness, a burning sensation, sensation of dryness, tired feeling, tearing, photophobia and decrease in visual acuity. The properties of the tears have been shown to be centrally involved in the induction of dry eye. Moreover, tears from dry eyes contain increased amounts of inflammatory peptides and cytokines compared with tears from normal eyes.

The tear fluid is a complex fluid mixture of proteins, lipids, salts, mucin, and other small organic molecules. Tear proteins have an important role in the maintenance of the ocular surface, and changes in the quality and quantity of tear components reflect changes in the health of the ocular surface. In this study, we used iTRAQ chemical tagging technology coupled with nano-liquid chromatography and mass spectrometry to quantitatively compare tear protein profiles between medicated and unmedicated control groups. iTRAQ reagents allow simultaneous identification and quantitation of proteins in four different samples, one control and three medicated, in this study. This platform is a powerful tool for biomarker discovery by comparing diseased samples with normal controls or differential expression analysis by comparing a treated group with a nontreated group in an animal model and time-course studies. Individual control, pooled global control or internal pure protein standards can be used in the analysis for relative or absolute quantitation. In this study, a pooled global control (nonmedicated group) was used as a common reference to compare with samples from a medicated group.

The advantage of using a pooled control is that it can reduce variability as evidenced in our previous report on dry eye biomarkers. This approach uses pooled global control for normalization and compares samples across the board. For protein identification, we used the most stringent cutoff value—99% confidence (or ProtScore ≥ 2). According to ProteinPilot software, a 95% confidence level is widely used as the cutoff value for protein identification.
The 14-3-3 proteins are a family of abundant 28- to 33-kDa acidic polypeptides found in all eukaryotic organisms. In mammals, there are seven members: β, γ, ε, σ, ζ, θ, and η. 14-3-3 α, δ, and γ are the phosphorylated forms of β, γ, and θ, respectively. 14-3-3 proteins regulate diverse biological processes, such as cell cycle regulation, metabolism control, apoptosis, and control of gene transcription. In this study, we found the level of one of the isoforms, 14-3-3 ζ/δ, to be upregulated in the medicated group. Conjunctival epithelial cells are known to express all seven 14-3-3 isoforms. The peptide fragments originating from 14-3-3 are present in tear fluid. Mammaglobin B, which is also known as human secretoglobin (SCGB) 2A1 (lacryglobin, lipophilin C), is a small protein that forms heterodimers with secretoglobin 1D1 (lipophilin A) in tears. Mammaglobin B is known to be normally expressed in salivary gland, mammary gland, human endometrium, pituitary, testis, trachea, and ovary. On the ocular surface, besides its high abundance in tear film, it is expressed in lacrimal gland, glands of Moll, and meibomian glands of the eyelid, as well as in the epithelium of the conjunctiva. Similar to tear lipocalin, mammaglobin B also tends to bind hydrophobic ligands on the ocular surface and is believed to contribute to the low surface tension of tear fluid, which is critical for the stability of the tear film.

The only downregulated tear protein we found to be associated with tears from the medicated group is proline-rich 4 protein. Proline-rich 4 is an abundant tear protein made by the lacrimal gland. Five different proline-rich proteins were identified from our previous study. The role of proline-rich proteins in tear fluid is still largely unknown, but it seems that they may be involved in the defense mechanism of the ocular surface, as suggested by similar proline-rich proteins in the oral mucosal surface. Further investigation is needed to verify the significance of this finding.

Ocular surface changes, such as dry eye, have recently been found to be associated with the long-term administration of topical antiglaucoma medications. Pisella et al. reported that 25% of patients using preserved antiglaucoma eye drops developed dry eye symptoms. A recent report investigating the prevalence of OSD in glaucoma patients in the United States showed that for every additional bottle of topical glaucoma medication, the likelihood of the development of OSD increases. Glaucoma patients often require at least one but more commonly two drugs to effectively reduce the IOP to a sufficiently low target pressure that will reduce the rate of disease progression. Furthermore, the authors go on to report that more than 50% of primary open-angle glaucoma and ocular hypertensive patients experience ocular discomfort and significant ocular surface irritation from the chronic instillation of topical glaucoma medications to control their IOP. This aspect of medication-induced dry eye is similar to that of primary dry eye.

We compared the tear protein profiles between an antiglaucoma medication group and a primary dry eye group. Interestingly, we report for the first time different tear protein patterns in these two groups, as revealed by PCA. The only common proteins that were significantly elevated in both the medicated and dry eye groups, as revealed by PCA, are S100-A8 and -A9. S100-A8 and -A9 are secreted proteins that often function as heterodimers. Together with S100-A12, they are the major proinflammatory and stress-related proteins of the S100 family. Elevated levels of S100-A8 and -A9 proteins have been reported in several inflammatory disorders such as rheumatoid arthritis. Although most abundantly expressed in neutrophils, both proteins are also normally found expressed in conjunctival and limbal epithelial cells. Recently, elevated levels of both S100-A8 and -A9 were detected in tears of patients with dry eye and pterygium.

Mammaglobin B, which is also known as human secretoglobin (SCGB) 2A1 (lacryglobin, lipophilin C), is a small protein that forms heterodimers with secretoglobin 1D1 (lipophilin A) in tears. Mammaglobin B is known to be normally expressed in salivary gland, mammary gland, human endometrium, pituitary, testis, trachea, and ovary. On the ocular surface, besides its high abundance in tear film, it is expressed in lacrimal gland, glands of Moll, and meibomian glands of the eyelid, as well as in the epithelium of the conjunctiva. Similar to tear lipocalin, mammaglobin B also tends to bind hydrophobic ligands on the ocular surface and is believed to contribute to the low surface tension of tear fluid, which is critical for the stability of the tear film.

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The tear protein profile found in eyes receiving glaucoma medications for prolonged periods have both similarities and differences from that identified in tears from primary dry eye patients. Although, a common inflammatory presence is indicated, the differences may suggest the presence of different...
mechanisms leading to the common clinical features of tear dysfunction in the two conditions.

In conclusion, our study is the first to our knowledge to demonstrate a difference in the tear protein profile in patients receiving long-term glaucoma medication and in those with primary dry eye. Our data suggest that duration of use of topical antimיגיעה medications longer than 1 year may start to induce changes in ocular surface inflammation, as indicated by the significant increase in inflammation-associated proteins detected in tears. The identification of tear biomarkers unique to medicated eyes would not only be a useful diagnostic tool to identify individuals with preexisting ocular surface disorders, but also highlight certain molecules as potential therapeutic anti-inflammatory targets.

References


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