A Link between Tear Instability and Hyperosmolarity in Dry Eye

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PURPOSE. Tear film instability and tear hyperosmolarity are considered core mechanisms in the development of dry eye. The authors hypothesize that evaporation and instability produce transient shifts in tear hyperosmolarity that lead to chronic epithelial stress, inflammation, and symptoms of ocular irritation. The purpose of this study was to provide indirect evidence of short-term hyperosmolar conditions during tear instability and to test whether the corneal epithelium responds to transient hyperosmolar stress.

METHODS. Five subjects kept one eye open as long as possible, and overall discomfort and sensations associated with tear break-up were scaled. Later, the same subjects used the same scales to report discomfort sensations after instillation of NaCl and sucrose hyperosmolar drops (300–1000 mOsM/kg). A two-alternative, forced-choice experiment was used to estimate osmolarity thresholds. In the second experiment, primary cultured bovine corneal epithelial cells were transiently stressed with the same range of hyperosmolar culture medium, and proinflammatory mitogen-activated protein kinase (MAPK) pathway and cytokine production were measured by Western blot analysis.

RESULTS. Tear instability led to an average discomfort rating of 6.13 and sensations of burning and stinging. These sensations also occurred with hyperosmolar solutions (thresholds, 450–460 mOsM/kg) that required 800 to 900 mOsM/kg to generate the same discomfort levels reported during tear break-up. MAPK was activated at 600 mOsM/kg of transient hyperosmolar stress.

CONCLUSIONS. These experiments provide a link between hyperosmolarity and tear instability, suggesting that hyperosmolar levels in the tear film may transiently spike during tear instability, resulting in corneal inflammation and triggering sensory neurons. (Invest Ophthalmol Vis Sci. 2009;50:3671–3679) DOI:10.1167/iovs.08-2689

Dry eye, a common condition affecting millions,1–5 is characterized by symptoms of ocular discomfort, dryness, irritation, and visual disturbance.6–5 The 2007 DEWS (Dry Eye Workshop) report considered tear instability and hyperosmolarity to be core mechanisms of dry eye, suggesting that tear instability leads to hyperosmolar conditions and inflammation, resulting in dry eye symptoms of ocular irritation.6 However, conditions within the thin tear film over the corneal surface are difficult to measure. Thus, the underlying connection between tear instability and hyperosmolarity, which is considered central to the dry eye condition, remains theoretical.7,8

Tear osmolarity is currently measured from the lower meniscus and ranges from approximately 300 to 310 mOsM/kg in normal eyes.9,10 The current cutoff for a dry eye diagnosis is 316 mOsM/kg.11 Although tear hyperosmolarity in individual patients with dry eye may reach as high as 360 mOsM/kg,12 however, most animal and in vitro studies of hyperosmolarity in dry eye require long-term exposure to higher levels (400–600 mOsM/kg) to demonstrate an inflammatory response of the ocular surface via activation of the mitogen-activated protein kinase (MAPK) pathway and cytokine production.13,14 Therefore, the minor increase in chronic levels of lower meniscus tear osmolarity measured from patients with dry eye may be insuffcient to generate the inflammatory response hypothesized to underlie the corneal damage/sensations associated with dry eye.

However, tear film osmolarity measured from the lower meniscus may not fully reflect the rapid spatial and temporal changes that occur over the corneal surface during the interblink interval. The corneal compartment of the tear film is separated from the meniscus by “black lines”15,16 and can be subject to rapid thinning due to evaporation and tear break-up,17,18 especially among patients with dry eye19 or when blinking is slowed during tasks requiring concentration.20 Thus, the osmolarity of the corneal tear film compartment may change rapidly during the interblink interval.15 In support of this idea, subjects in previous studies in our laboratory used the descriptors burning and stinging to describe sensations during periods of tear break-up, which supports the hypothesis that hyperosmolarity increases in the tear film with increasing tear instability.21 However, the amount of change required to produce these sensations is unknown, as is the response of the corneal epithelium to very transient shifts in hyperosmolarity.

Thus, although current theories predict transient increases in corneal tear film hyperosmolarity during tear instability,1 the amount of the increase and its possible effects on the underlying epithelium and nerves have not been investigated. Therefore, we designed this study to estimate the levels of hyperosmolarity in the unstable corneal tear film by comparing the corneal psychophysical response to instilled hyperosmolar drops and irritative sensations during tear instability (experiment 1). We then determined whether transient exposure to these levels of hyperosmolarity induces a proinflammatory response from the in vitro corneal epithelium (experiment 2).
METHODS

Experiment 1: Sensory Response to Instability and Hyperosmolar Solutions

Five subjects with no history of ocular disease were selected for the study. All subjects had participated in previous psychophysical experiments or agreed to be trained to detect small differences in corneal sensation before beginning the study. Informed consent was obtained from all participants. The study was approved by the Indiana University Human Subject Committee and was conducted in accordance with the tenets of the Declaration of Helsinki.

Sensory Response to Tear Instability. We used staring tear break-up dynamics (S-TBUD), a method previously developed in our laboratory, to study the rate and repeatability of tear break-up (TBU) in normal and dry eye subjects. We selected the VAS descriptors of burning, stinging, irritation, pricking, and cooling based on data from our previous S-TBUD studies. This procedure was repeated three times with 5-minute intervals between testing.

As described previously, we quantified the dry area growth rate (DAGR) for tear break-up from S-TBUD trials by extracting video frames and calculating the percentage of the area of break-up (AB) within the exposed corneal surface for each. The maximum blink interval (MBI), which was the length of time that the subject kept the eye open during the trial, and tear break-up time (TBUT) were also measured.

Sensory Response to Hyperosmolar Solutions. In this experiment, we determined the threshold for detection and the suprathreshold sensory response to hyperosmolar solutions by psychophysical methods. We used the same five subjects as in the previous experiment to ensure that scaling of sensations was consistent. All subjects were trained to distinguish small differences in solution osmolarity before beginning the project.

Both NaCl and sucrose isosmolar and hyperosmolar solutions were tested. Although NaCl could be expected to contribute significantly to tear film osmolarity, sodium ions can also interact with ion channels and nociceptive nerve endings while sucrose should not. Sucrose was used as a control to determine whether the sensory response we measured was due to the effect of hyperosmolarity or Na⁺ or Cl⁻ ions.

Isosmolar and hyperosmolar solutions were prepared by adding sucrose or NaCl to phosphate-buffered saline (PBS), and the osmolarity was adjusted by measurement with a vapor pressure osmometer (Wescor, Inc., Logan, UT). A sterile pipette was used to instill 40 μL of test solution onto the upper cornea while the lid was slightly lifted by the experimenter and the subject was looking down. The drop was allowed to flow down and bathe the cornea before the subject blinked, so that the subject could assess the level of corneal discomfort from the drop.

The threshold for detection of hyperosmolar NaCl and sucrose solutions was determined using a two-alternative, forced-choice (2AFC) paradigm. Two drops were instilled, one into each eye. One eye received an isosmolar reference drop (300 mOsM/kg) and the other a test drop, ranging from 400 to 600 mOsM/kg. This range was established by a pilot experiment, which showed that >600 mOsM/kg could be easily detected by subjects, whereas <400 mOsM/kg was undetectable. Test drops were given in 25 mOsM/kg steps, with each level of osmolarity tested 15 times, for a total of 135 sets per type of hyperosmolar solution or 270 repetitions in total. The eyes and the order of solutions were chosen at random. The two drops were instilled one after another (within 10 seconds), and subjects were asked to choose which eye felt more discomfort. There was a minimum 10-minute period between instillation of pairs of drops, which was established as an effective washout period by a separate set of pilot experiments. The percentage of trials in which the eye receiving the hyperosmolar solution felt more discomfort was plotted for each level of osmolarity. To obtain the detection threshold for hyperosmolar solutions, data were fitted with Quick’s version of the Weibull function, which was used to interpolate the sensory threshold for hyperosmolarity (osmolarity necessary for 75% correct performance).

To determine the suprathreshold ocular sensations caused by hyperosmolar drops of NaCl and sucrose, we tested 300 to 1000 mOsM/kg solutions in 100-mOsM/kg steps, with each level of osmolarity tested three times, for a total of 24 test drops per type of solution. We stopped at an upper limit of 1000 mOsM/kg because preliminary testing showed high levels of discomfort over that level. In these experiments, only one eye was tested at a time, alternating between left and right. The order of solutions was randomized, with at least 10 minutes between drops. After each drop, the subject reported the overall intensity of discomfort and scaled the five different sensations on the VAS, as described in the preceding TBU experiment. All data were fit by the following Stevens’s law power function:

\[ \psi(I) = kI^\alpha \]

where \( I \) is the stimulus magnitude, \( \psi(I) \) is the psychophysical function encompassing the subject’s sensation in response to the stimulus, \( \alpha \) is the exponent dependent on the type of stimulation, and \( k \) is a proportionality constant that is determined by the type of stimulation and the units used.

Experiment 2: Corneal Epithelial Response to Hyperosmolar Solutions

In the second portion of the study, we determined whether the in vitro corneal epithelium responds to short-term hyperosmolar stress (using levels of osmolarity suggested from experiment 1) by activating the MAPK pathway.

Table 1. Dry Eye Symptoms Measured in Each Subject

<table>
<thead>
<tr>
<th>Subject</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB, %</td>
<td>8.11 ± 6.08</td>
<td>0.73 ± 0.35</td>
<td>28.62 ± 16.31</td>
<td>9.03 ± 3.89</td>
<td>19.89 ± 5.30</td>
</tr>
<tr>
<td>MBI, s</td>
<td>90.22 ± 18.05</td>
<td>34.55 ± 10.71</td>
<td>14.46 ± 4.72</td>
<td>10.81 ± 10.64</td>
<td>13.11 ± 2.65</td>
</tr>
<tr>
<td>AB/MBI (DAGR)</td>
<td>0.0010 ± 0.0007</td>
<td>0.0002 ± 0.0001</td>
<td>0.0190 ± 0.0059</td>
<td>0.0153 ± 0.0116</td>
<td>0.0152 ± 0.0028</td>
</tr>
<tr>
<td>TBUT, s</td>
<td>25.00 ± 21.79</td>
<td>18.35 ± 6.66</td>
<td>4.33 ± 0.58</td>
<td>4.00 ± 0.00</td>
<td>4.17 ± 0.76</td>
</tr>
</tbody>
</table>

Data are the mean ± SD of results in three trials.
Corneal Epithelial Cell Culture. Bovine eyes were obtained from a local abattoir. Corneal epithelial–stromal patches were excised and soaked in 1× dispase solution for 1 hour to loosen the epithelial–stromal junction. The epithelial layers were then carefully peeled off and soaked in 0.25% trypsin to separate the cells. The cell suspension was cultured in Dulbecco’s modified Eagle’s medium/nutrient mixture F-12 (DMEM/F12; Invitrogen-Gibco, Grand Island, NY) containing 5 ng/mL EGF, 50 μg/mL insulin, 50 μg/mL gentamicin, 1.25 μg/mL amphotericin B, and 6% fetal bovine serum (FBS), at 37°C under 5% CO₂ and 95% humidity. The medium was renewed every 2 to 3 days until the cells were confluent. The cells were then subcultured into 35-mm Petri dishes, under the same culture conditions. The epithelial phenotype of these cultured cells was confirmed by epithelial morphology and immunofluorescent staining with cytokeratin antibodies (AE-5).

Hyperosmolarity Exposure. Confluent cultures were washed three times with PBS and switched to a serum-free medium (DMEM-F12 without FBS) for 24 hours. The cultures were then treated with 700-mOsm/kg DMEM-F12 solution (osmolarity was adjusted by adding sucrose or NaCl) for 10 and 30 seconds and 2, 5, 10, 15, and 30 minutes, and washed with cold PBS three times. A 10-second exposure time period was the shortest that could be reliably delivered in our experimental setup.

We also varied the level of osmolarity, within the range suggested by the results in experiment 1. Confluent cultures were treated with 300, 400, 500, 600, 700, 800, 1000, and 1200 mOsm/kg DMEM-F12 solutions (osmolarity was adjusted by adding sucrose or NaCl) for 30 seconds. The 30-second time period was chosen because the time course experiments suggested it produced a marked activation of at least some MAPK pathways.

Western Blot. Adherent cells were lysed in a nondenaturing cell lysis buffer (Cell Signaling Technology, Beverly, MA) containing 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na2EDTA, 1 mM EDTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na3VO4, 1 μg/mL leupeptin, 1 mM PMSF, and protease inhibitor cocktail. The cells were scraped and transferred to microcentrifuge tubes, and sonicated for 10–15 seconds. The cell extracts were centrifuged at 14,000 g for 10 minutes at 4°C, and the supernatants were stored at 80°C before use. The total protein concentrations of the conditioned media and cell extracts were determined by a bicinchoninic acid (BCA) protein assay (Pierce Biotechnology, Rockford, IL).

Scales of sensation caused by TBU

Figure 2. Sensations reported by each subject during S-TBUD trials (average of three trials). Black bar: mean of all five subjects.
Cell extract samples with equal amount of protein in each lane were mixed with 6 \%/H11003 SDS reducing sample buffer and boiled for 5 minutes before loading. The proteins were separated by SDS polyacrylamide gel electrophoresis (PAGE) and transferred to NC membranes for 1 hour, which were blocked with 5% nonfat milk in TBST (50 mM Tris [pH 7.5], 0.9% NaCl, and 0.1% Tween-20) for 1 hour at room temperature, and then incubated overnight at 4°C with a rabbit anti-body against JNK (1:1000), p-JNK (1:1000), ERK (1:1000), p-ERK (1:1000), P38 (1:1000), or p-P38 (1:1000). The membranes were washed with TBST three times and then incubated for 1 hour at RT with horseradish-peroxidase–conjugated goat anti-rabbit IgG (1:2000 dilu-
tion). After the membranes were washed four times, the signals were detected with an enhanced chemiluminescence reagent (ECL; Pierce Biotechnology). Data were analyzed using gel analysis software (Un-
scan-it; Silk Scientific, Orem, UT).

RESULTS

Experiment 1: Sensory Response to TBU and Hyperosmolar Solutions

The average age of the five subjects was 31.4 \( \pm \) 11.9 years (range, 21–52), and two subjects were men. According to the DEQ, the subjects reported a range of relatively mild dry eye symptoms, from none to rare or infrequent symptoms of discomfort, dryness, grittiness, tired eyes, burning, and grittiness. Only one male subject had been diagnosed with mild dry eye, but none was using drops for dry eye. The Schirmer I test results (without anesthetic) in all five subjects averaged 17 \( \pm \) 3 mm (range, 14–22) and TIBUT averaged 11 \( \pm \) 10 seconds (range, 4–25 seconds).

Sensory Response to TBU. All subjects showed areas of TBU or tear thinning at the end of each S-TBUD trial, but the AB and MBI varied greatly among subjects. Table 1 shows average results from the three trials of each subject. Three subjects (3, 4, and 5) showed relatively rapid TBU in a short period (high DAGR) that approached dry eye subjects in a previous study.\(^{19}\) The other two subjects were able to keep their eyes open longer and showed slow thinning of the tear film.

TBU and sensation levels reported from individual trials of two subjects are shown in Figure 1. Figure 1a shows the final fluorescein image, just before the blink, from trial 3 of subject 4. Figure 1b shows the DAGR graph for the trial, with associated sensations in Figure 1c. This subject, who reported mild to moderate dry eye symptoms on the DEQ and was diagnosed with dry eye, indicated high levels of discomfort, irritation, burning, and stinging, with lower levels of pricking and cooling during the trial. Figure 1d shows a similar data set from a subject with more stable tear film, longer MBI, and less TBU.

![FIGURE 3. Detection threshold for NaCl (a) and sucrose (b). Each curve represents a Weibull fitting of one individual subject's data. Dashed lines: the average threshold (75% correct).](image-url)

![FIGURE 4. Overall discomfort grade for suprathreshold NaCl and sucrose hyperosmolar solutions. Dashed lines: the osmolarity corresponding to TBU.](image-url)
same y-axis. Each data point represents an average of the three trials for each subject and the bar shows the mean. The grade of overall discomfort averaged at 6.1 ± 1.1 in all five subjects, with a range of 5.0 to 7.5. There was no significant difference between reported levels of irritation, stinging, burning, and pricking, but cooling was significantly less than the others (ANOVA with post hoc, P < 0.05).

**Sensory Response to Hyperosmolar Solutions.** Figures 3a and 3b show the individual psychometric functions in all five subjects and from the 2AFC hyperosmolarity detection task. The average detection threshold for sucrose was 460 ± 16 mOsM/kg (range, 442–476 mOsM/kg), which was slightly higher than the NaCl threshold of 454 ± 14 mOsM/kg (range, 438–474 mOsM/kg). The difference between the thresholds was not significant (P = 0.12, paired t-test).

Average overall discomfort scores associated with different levels of suprathreshold sucrose and NaCl hyperosmolar solutions are shown in Figure 4. The average grade of overall discomfort increased monotonically as the osmolarity stimulus increased, reaching significance at 600 mOsM/kg for sucrose and 500 mOsM/kg for NaCl (ANOVA with post hoc, P < 0.05). Generally, for the same osmolarity, subjects reported a slightly higher overall discomfort with NaCl than with sucrose, but the difference was not statistically significant (P = 0.066, repeated-measures ANOVA). Best fit power curves for discomfort data yielded similar exponents (α) for NaCl and sucrose (0.8857 and 0.8848, respectively). Values for the scalar constant (β) were also similar between the two solutions (0.025 and 0.021, respectively). Within the ranges tested, the responses to increasingly hyperosmolar sucrose solutions showed an almost linear relationship to dosage, whereas responses to NaCl appeared to begin to level off at approximately 900 mOsM/kg.

Figure 5 shows the VAS scores of irritation, burning, stinging, pricking, and cooling sensations for NaCl (Fig. 5a) and sucrose (Fig. 5b) hyperosmolar solutions. Each data point represents the average response from all five subjects for each sensation, and aggregate data were fitted with power functions. Power-fitting formulas for each sensation and best fit correlations are listed in Table 2. Exponents of power fitting ranged from 0.9514 to 1.0160 for NaCl and 0.9184 to 1.4000 for sucrose, reflecting the almost linear relation between the stimulus and reported sensation within the range tested. Correlations between the stimulus and VAS sensation were very high and were statistically significant (P < 0.0001, ANOVA). As Figure 5 shows, three of the response curves (irritation, burning and stinging) were so similar that they overlapped each other for both NaCl and sucrose. Pricking was reported at slightly lower levels, and cooling was reported least.

**Estimation of the Level of Hyperosmolarity during Tear Instability.** The overall purpose of the first experiment in the study was to estimate the levels of hyperosmolarity that

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**Table 2. The Power-Fitting Formulas, Correlation, and Corresponding TBU Discomfort Comparison Osmolarities for Dry Eye Sensations**

<table>
<thead>
<tr>
<th>NaCl</th>
<th>Power-Fitting Formula</th>
<th>r</th>
<th>TBU Comparison (mOsM/kg)</th>
<th>Sucrose</th>
<th>Power-Fitting Formula</th>
<th>r</th>
<th>TBU Comparison (mOsM/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irritation</td>
<td>y = 0.01345x^{0.9745}</td>
<td>0.984</td>
<td>775 (670–966)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stinging</td>
<td>y = 0.01154x^{0.9940}</td>
<td>0.980</td>
<td>809 (559–989)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burning</td>
<td>y = 0.01499x^{0.9514}</td>
<td>0.964</td>
<td>753 (612–1009)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pricking</td>
<td>y = 0.00725x^{0.1060}</td>
<td>0.968</td>
<td>854 (578–1261)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cooling</td>
<td>y = 0.00430x^{0.0964}</td>
<td>0.938</td>
<td>810 (357–1519)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irritation</td>
<td>y = 0.01577x^{0.9384}</td>
<td>0.99</td>
<td>882 (747–1133)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stinging</td>
<td>y = 0.00878x^{0.0503}</td>
<td>0.98</td>
<td>931 (624–1054)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burning</td>
<td>y = 0.00748x^{0.0510}</td>
<td>0.99</td>
<td>855 (693–1138)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pricking</td>
<td>y = 0.00222x^{1.1700}</td>
<td>0.99</td>
<td>931 (638–1315)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cooling</td>
<td>y = 0.00018x^{1.4000}</td>
<td>0.99</td>
<td>1080 (466–1742)</td>
<td></td>
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</table>
may occur during tear instability by making comparisons between discomfort experienced during S-TBUD trials and exposure to hyperosmolar solutions. Figure 4 shows that the average grade of discomfort during the S-TBUD testing was 6.1, which was also generated by an osmolarity of 809 mOsm/kg for NaCl and 914 mOsm/kg for sucrose. The full range of discomfort scores for all trials of S-TBUD testing was 5.0 to 7.5, which corresponds to an estimated range of 696 to 972 mOsm/kg of hyperosmolarity due to NaCl and 785 to 1125 mOsm/kg for sucrose. Table 2 lists similar TBU discomfort comparisons for each individual sensation on VAS scales.

**Experiment 2: Epithelial Response to Hyperosmolar Solutions**

**Time Course Experiments.** Figure 6 shows a representative blot of the time course of MAPK activation by 700 mOsm/kg sucrose (Fig. 6a) and NaCl (Fig. 6b) hyperosmolar conditions, with the ratios of p-MAPK/MAPK graphed at the bottom of the figure. The sucrose hyperosmolar stress activated both JNK2 and p38 phosphorylation after as little as 10 seconds of exposure, and NaCl after 30 seconds of exposure. The phosphorylation level increased as the exposure time

![Figure 6. Western blot analysis of MAPK phosphorylation after exposure to 700 mOsm/kg sucrose (left) and NaCl (right) hyperosmotic stress for 10 and 30 seconds and 2, 5, 10, 15, and 30 minutes. Data are the ratio of phosphorylated MAPK to total MAPK for JNK, p58, and ERK-1 and -2.](image)

![Figure 7. Western blot analysis of MAPK phosphorylation after 30 seconds exposure to 300, 400, 500, 600, 700, 800, 1000, and 1200 mOsm/kg sucrose (left) and NaCl (right) hyperosmotic stress. Data are the ratio of phosphorylated MAPK to total MAPK for JNK, p58, and ERK-1 and -2.](image)
tear pH due to decreases in [CO2] during eye opening could provide another chemical stimulus. The major stabilizer of tear film pH is the bicarbonate buffer system, which exists as a gradient of CO2 levels from the anterior chamber to 0 in air.

Thus, rapid changes in tear film thickness during tear instability would not be expected to markedly influence tear film pH, although some tear film proteins may also contribute to pH. The sensation of irritation is less specific to stimulus type and could be associated with a mechanical, chemical, or high-temperature stimulus. A cooling sensation was reported by some subjects and may occur when evaporation associated with tear thinning leads to stimulation of cold receptors.

Thus, these results strongly suggest that transient increases in tear film hyperosmolarity during tear instability caused the sensations noted by subjects during the S-TBUD trials. Even though sucrose is physiologically unrealistic, we included it in the investigation to serve as a control for the effect of hyperosmolarity. The absence of significant differences between NaCl and sucrose solutions suggests that the sensory response to these solutions is due to hyperosmolarity, rather than to the chemical effect of Na⁺ or Cl⁻ ions. However, suprathreshold responses to NaCl began to level off at 800 mOsM/kg, which may indicate saturation produced by Na⁺ ions interacting with nerve terminals. If we had expanded our range of testing above 1000 mOsM/kg, it is possible that a marked saturation effect would have emerged.

Threshold levels for detection of hyperosmolar solutions in this experiment, approximately 450 mOsM/kg, were well above the highest levels measured from the lower meniscus, reportedly 360 mOsM/kg. Thus, the symptom of burning, often reported by patients with dry eye, may be caused by levels of hyperosmolarity substantially above threshold in the dry eye tear film. In addition, the tear film osmolarity over the corneal surface is likely to be grossly underestimated by measurement from the lower meniscus, as this fluid is a mix of newly secreted tears and older tears wiped from the ocular surface by the upper lid during the blink.

The psychophysics portion of this experiment was based on the concept that corneal nerves are able to sense changes in osmolarity in the corneal compartment of the tear film. Indeed, the range of detection thresholds among subjects was surprisingly small, considering the differences in age and sex among subjects, suggesting that thresholds were highly characteristic of the human cornea.

Our results from psychophysical experiments suggest that corneal sensory neurons respond to increased tear film hyperosmolarity, ranging upward from thresholds near 450 mOsM/kg. Previous studies have demonstrated that corneal epithelial cells respond to these levels of hyperosmolarity with inflammation and apoptosis. In this experiment, we showed that exposure times as brief as 10 to 30 seconds of 700 mOsM/kg...
NaCl were adequate to activate the MAPK pathway, specifically JNK and p-38. ERK was not activated in our experiments, but less robust ERK expression with hyperosmolar stress has also been found in other cell types.57 Thus, our results provide strong support for the hypothesis that the corneal epithelium reacts to very short-term hyperosmolar stress that may occur within areas of TBU or thinning. Although we did not measure resultant cytokine release or apoptosis, others have shown that these downstream events are likely with hyperosmolar activation of the MAPK pathway in corneal epithelium.13,14 We did not test for apoptosis in this study, and so cell cultures that appeared viable by live/dead assay staining may have included apoptotic cells.

We used an unusual study design, combining very different psychophysical and cell biology techniques to test our hypothesis concerning the effects of fluctuations of hyperosmolality on the corneal surface. Our data demonstrate surprising agreement between the two cell types likely to be affected by these fluctuations: corneal epithelial cells and sensory neurons. Both cell types responded at approximately 600 to 700 mOsM/kg, neurons with a pain response and epithelial cells with a proinflammatory signal. Because the purpose of corneal neurons is to protect the epithelial tissue from damage or injury, these results suggest that hyperosmolar stress at that level is potentially injurious to the epithelium and thus is protected by the pain response. The common symptom of burning and stinging among patients with dry eye also implies that tear film fluctuations over 450 mOsM/kg commonly occur.

Events within areas of TBU and thinning over the corneal surface are unknown. Within areas of tear thinning, it is often assumed that tear film hyperosmolality increases,6,7 but TBU remains a poorly understood phenomenon. Most agree that the aqueous component of the tear film is absent within areas of break-up,39,40 leaving the underlying bound mucin layer exposed. However, the mucin layer is highly hydrated and should be isosmotic with the tear film and cornea. It is possible that the retracting aqueous layer and subsequent exposure of the bound mucin layer during TBU also leads to local hyperosmotic conditions. We plan to study this phenomenon further in future studies by real-time tracking of corneal sensations and the emergence of TBU or thinning.

Our results suggest that the ocular surface may frequently be exposed to hyperosmolar conditions when local tear film thinning or break-up occur. Blinking and reflex secretion of tears are natural processes that should tend to mitigate prolonged local hyperosmotic conditions. The ocular surface is a mucous membrane that requires proper wetting for good vision and cellular function, thus protective signaling of stress and damage is critical. Fluctuating hyperosmolality may provide this signal, most likely triggering a blink, which should act to rewet the surface and restore isosmotic conditions.6

Tear deficiency6 or slowing of the blink during a concentrating task may result in local or global spikes in hyperosmolality of the tear film, which stress the corneal epithelium and underlying nerves. The resultant inflammation and irritation of underlying nociceptors may account for the symptoms of ocular irritation noted among patients with dry eye.37 Our results also suggest that, while transient spikes in hyperosmolality are stressful to corneal surface cells, the outcome may not necessarily be cellular death. This may provide one explanation for the often cited poor correlation between dry eye symptoms and clinically demonstrable cellular tissue damage.38,49

References


