Lysozyme activity during adaptation to soft contact lenses

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Abstract
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LYSOZYME ACTIVITY DURING ADAPTATION

TO SOFT CONTACT LENSES

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David M. Perry

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Sponsored by the Oregon Optometric Association
ABSTRACT

Lysozyme previously has been shown to be reduced from normal levels following ocular pathology, irritation, or hard contact lens wear. Utilizing a spectrophotometric method to measure the activity of human tear lysozyme against a living bacterial substrate, we compared the activities in a group of new soft contact lens wearers during an adaptation period of two weeks with activities from a group of non-contact lens wearers. We found no significant difference in the tear lysozyme activities between the two groups but found a stabilizing effect of soft lens wear upon random activity variations.
INTRODUCTION

Lysozyme, an enzyme secreted by the lacrimal gland, comprises approximately 25% of the total protein in tears.\(^1\) It is selectively bactericidal due to its ability to hydrolyze chemical bonds within the cell walls of some species of bacteria.\(^2\)

Tear lysozyme can be measured either as a direct concentration or indirectly by its enzyme activity. Measurement of lysozyme by the different methods has resulted in substantial variability among published findings. The variability also depends on subject variability and tear collection techniques (reflex vs. basic tears).

When the anterior segment of the eye is subjected to mechanical irritation or is pathological, lysozyme levels may change. Reduced tear lysozyme levels have been associated with various pathologies having diverse etiologies such as lupus erythematosis,\(^3\) Sjogren's syndrome, blepharoconjunctivitis,\(^4\) and epidemic keratoconjunctivitis.\(^5\) Reduced lysozyme activity in human tears has also been attributed to corneal ulcers, corneal dystrophy,\(^4\) and smog;\(^6\) yet cigarette smokers appear to have normal lysozyme levels.\(^7\) Transient reductions in tear lysozyme, with subsequent return to normal, have been measured following induced cornea trauma in albino rabbits.\(^5\) Those pathologies which result in the greatest disturbance of normal corneal physiology typically produced the greatest reduction in lysozyme activity.\(^5\)

Contact lens wear may cause a sufficient disturbance of corneal physiology to be detected as a change in lysozyme levels. Hill found normal tear lysozyme concentrations following adaptation to hard contact lenses, but total protein concentration was reduced.\(^8\) Hathaway found no significant change in total protein concentration during adaptation to soft
contact lenses, but did report that lysozyme concentration increased approximately 13% when the cul-de-sacs of a normal subject were irritated with a cotton swab. Balik reported an increase in both lysozyme and total tear protein concentrations after only one hour of soft contact lens wear.

Previous investigations of tear lysozyme in contact lens wearers have measured concentration. However, when activity is measured for tear enzymes other than lysozyme (glycolytic enzymes), there is reduced activity after two weeks of soft lens wear which indicates a prolonged change.

These studies suggest that a change in lysozyme activity may be a sensitive indicator for changes in anterior segment physiology that occur in soft contact lens wearers. Therefore, we measured human tear lysozyme activity against a bacterial substrate by collecting tear samples from soft contact lens wearers during a two week period of adaptation, and from non-contact lens wearers during a similar two-week period.
METHODS

Two groups of subjects were selected from a university clinic patient population (Table 1). A group of fifteen subjects was selected to wear soft contact lenses using the following criteria:

(1) no current or previous contact lens wear within two months prior to the study.
(2) keratometric findings not greater than 1.25D difference between the two principle meridians in each eye.
(3) refractive error not greater than $\pm 6.00 \text{D.S.}$, and anisometropia not greater than $2.00 \text{D.S.}$
(4) acuity through soft contact lenses not greater than one Snellen line difference from best spectacle correction acuity.
(5) no ocular hyperemia, irritation or discomfort during lens wear.
(6) no current or recent ocular or systemic medications.
(7) good general and ocular health, including absence of allergies.

Fifteen subjects were selected to be the control group and were chosen to approximate the experimental group in age, refractive data, and health criteria. All potential subjects in both groups completed medical questionnaires and underwent biomicroscopy examinations prior to selection to eliminate those with pathological histories and those engaged in
pharmacological therapy.

Each subject in the contact lens group was fitted with Bausch and Lomb polymacon sof1ens in accordance with the Bausch and Lomb fitting method, which calls for complete corneal coverage and movement which does not produce excessive awareness. They were instructed to begin with four hours of wearing time on the first day of tear sampling, and to increase wearing time one hour a day, up to maximum desired wearing time. By the two week sample time, all soft lens wearers were using their lenses twelve hours or more daily. Lens care was accomplished using heat disinfection, non-preserved saline, and preflext cleaner only; no other ocular or contact lens solutions were permitted.

A tear sample was taken from each subject eight times during the study. A 5ul tear sample was withdrawn from the lower fornix of each eye, using sterile single use glass micropipettes. Care was taken during sampling to minimize lid and conjunctival irritation in an attempt not to stimulate reflex tearing, a factor not addressed in previous studies, where variability was a problem. An initial sample was taken from subjects in both groups prior to soft lens wear in the experiment group, and further samples were taken from both groups at 30 minutes, 2 hours, 4 hours, 24 hours, 48 hours, 1 week, and 2 weeks following soft lens wear.

To measure the activity of the tear lysozyme, a substrate of Micrococcus lysodeikticus (ATCC #4698), a species highly susceptible to lysozyme hydrolysis, was prepared. The bacteria were rehydrated from lyophilized cultures and subcultured in tryptic soy broth at 30° C. for 48 hours prior to each sampling session. These broth cultures were centrifuged, resuspended in phosphate buffer (0.066M KH$_2$PO$_4$ 81.5% and 0.066M Na$_2$HPO$_4$ 18.5%), and diluted to an initial absorbance between 1.0 and 1.2
as measured with a Beckman DB-G grating spectrophotometer using 450mm in air as a reference.

Each 5ul tear sample was diluted to a 1:11 in phosphate buffer and 25ul of this diluted sample was then mixed with 3ml of the washed bacterial substrate in a 1cm pathlength cuvette. The absorbance was re-measured one and two minutes later. The resultant reduction in absorbance caused by hydrolysis of the bacteria is an indication of the activity of the lysozyme in the tear sample.

RESULTS

Absorbance readings were recorded at one and two minute intervals; the one minute absorbance changes were selected for detailed statistical analysis because they showed a smaller standard deviation and narrower range. The mean absorbance changes for each of the two groups at each sample time are shown in Table II.

A student's t-test for independent means showed no significant difference between lysozyme activity in the soft lens group as compared to the control group, for all sample times.

The mean absorbance changes and standard deviations are represented graphically in Figure I.

Dispersion of the data shows consistent and significant variability throughout both the soft lens group and the control group, with largest
standard deviation occurring at the twenty-four hour sample in the soft lens group.

A Pearson product correlation between left and right eyes of the control and soft lens group is compared in Table III. The mean correlation coefficient of the control group is .88 and the mean correlation coefficient for the soft lens group is .91.

DISCUSSION

We did not measure any significant difference in lysozyme activity between groups at any sample time. This means that using our experimental protocol soft lens wear did not affect the lysozyme activity during adaptation. However, any change in lysozyme activity during soft lens adaptation could have been subtle and below the threshold of sensitivity of the methods in this study.

Variability, as seen in the large standard deviations in both groups, may be masking the true changes in lysozyme activity. The greatest standard deviations for all data were found in the soft lens group at 30 minutes and 48 hours after initial insertion. Fluctuations in lysozyme activity, therefore, are greatest for soft lens wearers at these time intervals and merit further investigation. Several factors may account for the variability. The bacteria, since they were living organisms in a growth cycle, may not have the same lysozyme susceptibility at each testing time even though they were prepared using a standard protocol. Reflex tearing may have been stimulated even though great care was used to prevent this. Reflex tears have been shown to have different lysozyme activities than do basic tears and the change in osmolarity accompanying the reflex tear may also change activity.
A correlation between left and right eyes of subjects in both groups showed the greatest discrepancy in the control group at one half hour after their first sample. This may have been due to some transient mechanical irritation during sampling procedure, but was not found in the soft lens group. In fact, the distribution of correlation values showed the control group had standard deviations from mean sample time values almost twice (1.8) those found in the soft lens group. This indicates that the differences in lysozyme activity between left and right eyes were less subject to variation in the soft lens group than in the controls. This stabilizing effect upon apparently normal day to day fluctuations of enzyme activity may be due to a levelling or homeostatic effect upon corneal physiology over short periods of time. This creates some further potential use for soft contact lenses in the treatment of minor corneal abnormalities.

On the average, adaptation responses are lesser in soft lens wearers as compared with PMMA lens wearers. The fact that soft lens wearers have less pronounced adaptation responses does not mean these responses are not worthwhile to observe and quantify, but it does demand more sophistication in the assessment techniques.
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<thead>
<tr>
<th>Gender</th>
<th>Count</th>
<th>Age Range</th>
<th>Mean Age</th>
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<tbody>
<tr>
<td>Males</td>
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<td>16 - 33</td>
<td>25.5</td>
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<tr>
<td>Females</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>18 - 34</td>
<td>25.6</td>
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<tr>
<td></td>
<td>4</td>
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Soflens Group

Control Group

Table I: Subject group description
<table>
<thead>
<tr>
<th>Time</th>
<th>Mean Absorbance Changes (Soft Lens Group)</th>
<th>Mean Absorbance Changes (Control Group)</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>.557 ± .139 (X) .61 (Range)</td>
<td>.590 ± .122 (X) .54 (Range)</td>
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<td>30 min.</td>
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<td>.592 ± .144 (X) .61 (Range)</td>
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<td>2 HR</td>
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<td>4 HR</td>
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Table II: Mean absorbance changes per group after one minute.
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<th>Soft lens Group</th>
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<tr>
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<td>2 Hour</td>
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<td>2 Week</td>
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<td>.97</td>
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<tr>
<td>Mean</td>
<td>.88</td>
<td>.91</td>
</tr>
</tbody>
</table>

Table III: Pearson product moment correlation between left and right eye activity values for soft lens and control group.
Figure I: Mean absorbance changes and standard deviations after one minute.

\[ X \pm 1S \]

\( S \) = Soft Lens Group

\( C \) = Control Group
REFERENCES


