Basal Tear Turnover and Topical Timolol in Glaucoma Patients and Healthy Controls by Fluorophotometry

Esmeralda V. M. J. Kuppens, Thorsten R. Stolwijk, Rob J. W. de Keizer, and Jaap A. van Best

To assess the effect of glaucoma and timolol on tear secretion, basal tear turnover was measured with fluorophotometry in 13 open-angle glaucoma patients not using any ophthalmic medication, 24 patients using timolol medication daily, and 41 healthy control subjects. Basal tear turnover is defined as the tear turnover at the lowest level of reflex lacrimation possible under physiologic conditions. Tear turnover was calculated from the decay of the tear fluorescence after instillation of fluorescein. Minimal influence of reflex lacrimation was obtained by instilling 1 μl of 2% fluorescein without touching the eye and by discarding measurements performed in the first 5 min. Minimization was confirmed by a monophasic decay of tear fluorescence. The values of patients who used timolol and those who did not use timolol were significantly lower than those of healthy control subjects (mean values in percent/minute ± standard deviation: 10.1 ± 3.2, 12.3 ± 4.1, and 15.6 ± 5.4, respectively; Student’s t-test: \( P < 0.02 \)). The values of patients who used timolol were significantly lower compared to those of patients who did not use timolol \( (P = 0.03) \). The tear film break up time values of patients who used timolol were significantly shorter than those of patients who did not use timolol and healthy control subjects (Fisher exact test: \( P < 0.03 \)). The results indicate that: (1) basal tear production does not change with age in healthy control subjects and open-angle glaucoma patients; (2) open-angle glaucoma has a decreasing effect on basal tear turnover; and (3) daily instillation of timolol in these patients causes an additional decrease in tear turnover and a destabilization of the pre-corneal tear film.

Timolol (Merck, Sharp & Dohme, Timoptol, Paris, France; or Timoptic, Rahway, NJ) was introduced in 1978 and its topical ocular administration is prescribed frequently to lower intraocular pressure (IOP) in glaucoma patients. These patients often complain of burning and dry eye sensations when they use drops of timolol daily, which suggests inadequate tear production.\(^1\)\(^2\)

Tear production often is clinically assessed with the Schirmer test even though several authors have criticized the test for its lack of precision.\(^3\)\(^6\) The integrity of the pre-corneal tear film is evaluated by the tear film break up time (BUT). Both tests have been used in glaucoma patients, and normal\(^7\)\(^8\)\(^9\) and decreased tear production\(^10\)\(^11\)\(^12\) have been reported during timolol medication.

The fluorophotometric determination of the fluorescein decay in tear film can be used as an indirect quantitative assessment of tear production.\(^13\)\(^14\) Without topical anesthesia, two phases of tear production have been shown: a fast initial phase of 2–5 min caused by reflex lacrimation followed by a slow phase caused by physiologic or basal lacrimation.\(^15\)

Tear turnover is the percent decrease of fluorescein concentration in tear film per minute after instillation of fluorescein. The basal tear turnover is defined as the tear turnover at the lowest level of reflex tear production possible under physiologic conditions. In the present study, the basal tear turnover in open-angle glaucoma patients not using ocular medication and in patients using timolol was determined and compared to that of healthy control subjects to investigate a possible effect of glaucoma or daily instillation of timolol on tear production.

**Material and Methods**

The data of the open-angle glaucoma patients who did not use ophthalmic medication, those who used timolol medication, and healthy control subjects are presented in Table 1. Patients were recruited from the...
Table 1. Patient and healthy control data

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Range</th>
<th>Age (yr) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients* using timolol</td>
<td>24</td>
<td>40–80</td>
<td>63 ± 10</td>
</tr>
<tr>
<td>Healthy controls†</td>
<td>27</td>
<td>40–80</td>
<td>58 ± 11</td>
</tr>
<tr>
<td>Patients* not using timolol</td>
<td>13</td>
<td>30–70</td>
<td>54 ± 10</td>
</tr>
<tr>
<td>Healthy controls‡</td>
<td>28</td>
<td>30–70</td>
<td>51 ± 11</td>
</tr>
<tr>
<td>Healthy controls‡</td>
<td>41</td>
<td>20–80</td>
<td>48 ± 18</td>
</tr>
</tbody>
</table>

* Open angle glaucoma patients. † For comparison with patients. ‡ For determination of age dependency.

Outpatient Department of the University Hospital Eye Clinic (Leiden, The Netherlands) and healthy control subjects were recruited from coworkers of the clinic and their relatives.

Inclusion Criteria

All participants had normal aspect of all corneal layers upon slit-lamp biomicroscopic examination without fluorescein (this would have interfered with fluorophotometry). All patients had had open-angle glaucoma for at least 6 mo with intraocular pressure lower than 30 mmHg.

Patients using timolol were administered one drop of a 0.25 or 0.50% timolol containing the preservative benzalkonium chloride (BAK; 0.01%) in each eye at 12 hr intervals for at least 6 mo.

Exclusion Criteria

All subjects who wore contact lens, who were receiving topical ophthalmic medication (except for timolol), or who had had systemic anti-glaucoma treatment or beta-blocking medication any time before fluorophotometry were excluded. Candidates with a history of ophthalmic disease were excluded from healthy control group.

The study was approved by the Medical Ethical Committee of the Leiden University Hospital, and informed consent was obtained from each subject after verbal and written explanation of the nature of the procedure had been provided.

Fluorophotometric measurements were carried out with the Fluorotron Master (Coherent Radiation Inc., Palo Alto, CA) fitted with a special lens (anterior segment adapter) for detailed scanning of the anterior segment of the eye along the optical axis. The scanning duration was reduced to 8 sec to prevent blinking during scanning by modifying the commercial software. In patients who used timolol, fluorophotometric measurements were performed 5 hr after timolol was instilled.

Measurement Procedure

Four fluorophotometric scans of each cornea were carried out to determine corneal autofluorescence. Then, 1 μl of a 2% disodium fluorescein solution in nonstabilized saline was instilled into the lateral part of the lower conjunctival fornix of each eye via a micropipette. Contact with the eye was avoided to minimize induction of reflex tear production. After instillation, the patient was asked to blink several times so a homogeneous distribution of fluorescein in the tear film could be obtained. Then, both eyes were scanned alternately every 1.5 min for about 30 min. During this time, the subjects were instructed to avoid rubbing their eyes and to do nothing that might stimulate reflex lacrimation. After the fluorophotometric measurements, the cornea was examined by slit-lamp biomicroscopy with fluorescein, and the BUT was determined. Schirmer’s test was performed after topical anesthesia using a slightly moistened fluorescein strip. The IOP was determined with applanation tonometry in all patients and in 10 of the healthy controls. The reproducibility of tear turnover measurements was assessed in three volunteers by repeating measurements at the same time of day after a 1 mo interval.

Calculations

The basal tear turnover, $T_t(t_0)$, at $t_0$ min after fluorescein instillation, expressed as percent decrease fluorescein concentration in tears per minute, is defined as:

$$T_t(t_0) = 100 \frac{[C_t(t_0) - C_t(t_0 + 1)]}{C_t(t_0)} \text{ percent/min.} \ (1)$$

where $C_t(t)$ = concentration of fluorescein in tear film at time $t$ (min).

Assuming a monophasic decay of the tear fluorescein starting 5 min after instillation (Fig. 1, left panel) with a decay time constant $\alpha$ (min$^{-1}$):

$$C_t(t) = C_t(0) \cdot e^{-\alpha t} \text{ ng/ml} \ (2)$$

the following is obtained:

$$T_t(t_0) = 100 \cdot (1 - e^{-\alpha t}) \text{ percent/min} \ (3)$$

If the tear film fluorescence measured is assumed to be proportional to the fluorescein concentration in the tear film, $\alpha$ can be calculated from the fluorescence decay curve by exponential regression to the data points.

Consideration of Sources of Errors

Several error sources in the measurement of basal tear turnover were considered.
Reflex lacrimation: To avoid interference by reflex lacrimation resulting from fluorescein instillation, fluorophotometric scans performed during the first 5 min were discarded. Reflex lacrimation causes a fast decay of tear fluorescein with a halftime of less than 3 min. This would result in a double exponential fluorescence decay curve. After 5 min, a monophasic fluorescence decay was seen in all subjects, indicating the absence of reflex lacrimation.

Changes in tear film thickness: The fluorescence measured with the fluorophotometer depends not only on the concentration of fluorescein in tear film, but also on tear film thickness (7 μm) because of the limited spatial resolution (depth of the measuring window, 0.5 mm). Tear film thickness at the moment of fluorescein measurement was assumed constant because tear fluorescence was measured at a fixed time (2 sec) after blinking. The initial 14% increase in tear volume resulting from instillation of 1 μl fluorescein solution into 7 μl of tear volume did not affect tear turnover measurements because scans performed during the first 5 min were discarded and the increase was supposed to disappear after a few blinks.

Corneal fluorescein: As a result of the limited spatial resolution, fluorescence of fluorescein in tear film is measured with fluorescein diffused into corneal stroma. Fluorescence from corneal stroma was supposed to not contribute significantly to the fluorescence signal as long as tear fluorescein values were much larger than stromal values. Interference of tear turnover measurements by corneal fluorescein can be detected by the presence of a fluorescence decay component with a decay halftime greater than 60 min (decay halftime of basal tear fluorescein, 5–15 min). This interference was minimized by discarding fluorophotometric scans performed more than 30 min after fluorescein instillation.

Corneal autofluorescence: Tear film fluorescence values were corrected for corneal autofluorescence because of the limited spatial resolution of the fluorophotometer.

In addition, tear fluorescence values below three times corneal autofluorescence were discarded to minimize errors of inaccurate corneal autofluorescence determination.

Tear Volume

Fluorophotometric measurements of fluorescein concentration in tears are reliable provided that fluorescein is homogeneously distributed in the tear fluid over the corneal surface, because fluorescence is measured in the central part of the corneal surface only (area of 2 × 0.1 mm). This homogeneity was verified by calculating the tear volume from the fluorescein concentration measured with the fluorophotometer.
immediately after a drop of fluorescein of known volume and concentration was instilled. A nonhomogeneous distribution of tear fluorescein would result in nonreproducible tear volume values.

Straightforward derivation of the tear volume, \( V_t \) (\( \mu l \)), yields:

\[
V_t = (C_d \cdot C_m^{-1} \cdot k^{-1} - 1) \cdot V_d
\]

where \( C_d \) = fluorescein concentration in the drop (ng/ml), \( C_m \) = fluorescein concentration measured with the fluorophotometer directly after instillation (ng/ml), \( k \) = correction factor (\( k = 250 \)) for the limited spatial resolution of the fluorophotometer, and \( V_d \) = drop volume (\( \mu l \)).

The value of \( C_m \) was obtained by a double exponential regression procedure to all fluorophotometric data points (Fig. 1). The value of \( k \) was obtained by measuring the fluorescein concentration with the fluorophotometer in a 7 \( \mu m \)-thick layer.18

### Statistical Analysis

The normality of tear turnover value distribution was assessed in the patient groups and the healthy controls, using D’Agostino’s test for departure from normality.19 Student’s t-test was used for evaluating the significance of differences. Forty one healthy controls were used to determine a possible correlation between age and basal tear turnover (age range, 20–80 yr). The patients who used timolol and the patients who did not were compared to healthy controls with corresponding ranges of age.

The Fisher exact test was used to analyze the BUT and Schirmer’s values. BUT values shorter than 10 sec and Schirmer’s test values smaller than 10 mm were considered not normal.20,21

### Results

#### Basal Tear Turnover

An example of the fluorescein decay in tear film after 1 \( \mu l \) of 2% fluorescein is instilled in a glaucoma patient is shown in Figure 1, right panel. Almost no reflex lacrimation was observed in this patient, in contrast to the decay of the healthy control in Figure 1, left panel. All participants in the study showed a single exponential fluorescence decay 5–30 min after fluorescein instillation. Reproducibility of tear turnover measurements was assessed by repeating the measurements after 1 mo at the same time of the day in three patients. Percent deviation was +8.9%, -3.0% and -6.0%, respectively.

The mean of the basal tear turnover values of the right and the left eye was calculated in each patient who did not use timolol, in each patient who used timolol, and in each healthy control because the values of the left and right eyes were found to be significantly correlated in the three groups (linear correlation coefficient: \( r = 0.8, P < 0.0001 \); \( r = 0.6, P < 0.01 \); and \( r = 0.8, P < 0.001 \), respectively). The mean tear turnover value was calculated in each group because the values were found to be normally distributed (D’Agostino’s test, \( P < 0.01 \)).

The mean tear turnover values and IOPs of patients who did not use timolol, patients who used timolol, and healthy controls are presented in Table 2. The mean tear turnover value of patients who did not use timolol was significantly lower than that of healthy controls (\( P = 0.02 \)), and the mean value of patients who used timolol was significantly lower than that of healthy controls and patients who did not use timolol (Student’s t-test, \( P < 0.001 \) and \( P = 0.03 \), respectively).

The mean tear turnover values did not correlate with age in healthy controls, patients who did not use timolol, and those who used timolol (\( r = 0.03, P = 0.8 \); \( r = 0.2, P = 0.5 \); \( r = -0.4, P = 0.04 \); and \( r = 0.2, P = 0.5 \), respectively; Figs. 2 and 3). The tear turnover
Fig. 3. Basal tear turnover expressed in percent per minute versus age in open-angle glaucoma patients not using ocular medication (closed symbols) and in patients using timolol (open symbols). The solid line and the broken lines indicate the mean value and 95% probability interval for healthy controls.

values in both patient groups did not correlate significantly with the IOP (r < 0.1).

A significant difference between the tear turnover values of the 14 patients who used 0.25% timolol solution and the values of the 10 patients who used 0.50% timolol solution was not found (mean value in percent/min ± standard deviation, 9.5 ± 3.1 and 11.1 ± 3.3, respectively; P > 0.2).

The BUT and Schirmer's test values of each group are presented in Table 3. The BUT values of patients who used timolol were significantly shorter than those of the patients who did not use timolol and the healthy controls (Fisher exact test, P = 0.004). The BUT values of patients who did not use timolol did not differ significantly from the values of the healthy controls (P = 1). Schirmer's tests of patients who used timolol and patients who did not use timolol were significantly lower than those of the healthy controls (P < 0.001). No significant difference was found between both patient groups (P = 0.8).

A superficial punctate keratopathy was seen on final slit-lamp biomicroscopic examination in four patients who used timolol, in one patient who did not use timolol, and in none of the healthy controls.

The mean IOP value in the 10 healthy controls was 14.7 ± 2.9 mmHg and did not differ from that reported in the literature (15.4 ± 2.5 mmHg; all values <21 mmHg).22

The tear volume values of each eye of five healthy controls and five patients, calculated with the use of Equation 4, are shown in Table 4. No significant difference between the mean values of both groups was found (9.2 ± 1.8 µl and 8.9 ± 2.4, respectively; Student's t-test, P > 0.7).

Discussion

To our knowledge, this is the first study that reports a decreased tear production of 22% in open-angle glaucoma patients not using opthalmic medication compared to healthy controls. Furthermore, open-angle glaucoma patients who used timolol opthalmic solution (0.25 or 0.50%) had a significant additional 18% decrease in tear production compared to patients who did not use timolol. Schirmer's test, performed after topical anesthesia and which has been suggested to reflect basal tear production,23 could not establish the significant decrease in tear production between both patient groups found by fluorophotometry.

Any explanation for the pathogenesis of the decreased tear secretion found in the glaucoma patients who did not use timolol remains speculative. The additional decrease in patients who used timolol might be attributed to the blocking properties of timolol on the β-receptors in the lacrimal gland, because timolol was proven to be present in active concentrations in the lacrimal gland of rabbits after topical instillation.24 In addition, it has been suggested that the local anesthetic properties of timolol can reduce the human

Table 4. Calculated tear volume

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (yr)</th>
<th>Right eye (µl)</th>
<th>Left eye (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy controls</td>
<td></td>
<td>7.4</td>
<td>9.2</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>9.2</td>
<td>7.7</td>
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<tr>
<td></td>
<td>36</td>
<td>10.6</td>
<td>10.3</td>
</tr>
<tr>
<td></td>
<td>66</td>
<td>8.3</td>
<td>13.0</td>
</tr>
<tr>
<td></td>
<td>67</td>
<td>9.1</td>
<td>7.0</td>
</tr>
<tr>
<td>Glaucoma patients</td>
<td></td>
<td>10.6</td>
<td>12.2</td>
</tr>
<tr>
<td></td>
<td>67</td>
<td>10.2</td>
<td>8.8</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>9.8</td>
<td>11.0</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>8.7</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>83</td>
<td>7.5</td>
<td>5.4</td>
</tr>
</tbody>
</table>

* Calculated using the value of fluorescein concentration in tears directly after instillation (equation 4).
corneal sensitivity, resulting in a reduced reflex demand for tears. However, several studies could not confirm this suggestion. One of the latter studies did not find a correlation between time of last medication and corneal sensitivity, but suggested there might be reduced sensitivity in older people, especially those older than 65 yr.

In our study, tear production did not decrease with age in the healthy controls or in both patient groups. The absence of age-dependent tear production in healthy controls contrasts with previous studies that used Schirmer’s test for assessing tear production. This finding corroborates a previous study in which no correlation was found between Schirmer’s test and tear turnover. A possible explanation for the age dependency of tear production found with Schirmer’s test might be that insertion of the filter paper induces reflex tear production, even after instillation of a local anesthetic, and that reflex, but not basal tear production decreases with age in healthy controls.

It should be noted that in the fluorophotometric assessment of tear production by tear turnover, a monophasic decay of fluorescein in the tear film was seen in all subjects 5 min after instillation, which indicates the absence of reflex tear production.

Tear volume was calculated in 10 eyes of healthy control subjects on the assumption that fluorescein is distributed homogeneously in tears. The values calculated corroborate this assumption because they were found in accordance with a previous study (mean volumes: this study, 9.2 ± 1.8 μl; previous study, 7.0 ± 2.0 μl). Similar tear volume values were obtained in 10 eyes of glaucoma patients, indicating that in these patients a decrease in tear turnover is associated with a decrease in tear flow.

The BUT values in patients who used timolol were significantly decreased compared to patients who did not use timolol and healthy controls, indicating instability of the pre-corneal tear film. This finding agrees with previous studies that demonstrated the preservative BAK (0.01%; present in timolol ophthalmic solution) can reduce the tear film BUT by approximately 50%. The toxic effects of BAK on the corneal epithelium also may explain the superficial punctate keratopathy found in 17% of the patients who used timolol.

In the present study, the tear production was found to be decreased in open-angle glaucoma patients compared to healthy control subjects. The tear production of these patients was even more decreased by the daily use of timolol ophthalmic solution and was accompanied by impairment of the stability of the precorneal tear film. These quantitative and qualitative impairments of tear film with the toxic effects of timolol solution on the corneal epithelium may explain the burning and dry eye sensations that open-angle glaucoma patients often complain about when they use timolol ophthalmic solution. Our results advocate careful use of timolol solution by patients predisposed to dry eyes.

Key words: age dependency, fluorophotometry, glaucoma, tear turnover, timolol

Acknowledgments

The authors are indebted to Prof. Dr. J. A. Oosterhuis, Department of Ophthalmology, University Hospital Leiden, The Netherlands, for his contribution to the design of this study.

References


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