REFLEX LACRIMATION IN PATIENTS WITH GLAUCOMA AND HEALTHY CONTROL SUBJECTS BY FLUOROPHOTOMETRY


PURPOSE. Steady state tear turnover (TTO), defined as TTO under normal physiological conditions, is significantly lower in patients with untreated glaucoma than in healthy control subjects. To obtain more information on the effect of glaucoma on lacrimation, a method for quantification of reflex lacrimation was developed and applied to patients with glaucoma or ocular hypertension and healthy control subjects.

METHODS. After instillation of 2 μl of fluorescein (2%), the decay of fluorescein concentration in tears was measured by fluorophotometry over 10 minutes to determine steady state TTO. Then, reflex lacrimation was induced by stimulating the trigeminal nerve with ethanol vapor via the nostrils. Thereafter, the decay of fluorescein and corresponding steady state TTO were determined again. An index of reflex lacrimation, defined as the percentage decrease in fluorescein concentration as a result of stimulation, was calculated by forward and backward extrapolation of the steady state decay of the fluorescein concentration in tears, relative to the time of stimulation.

RESULTS. The index of reflex lacrimation was determined in 16 patients with newly discovered but not yet treated glaucoma, 16 patients with untreated ocular hypertension, and 16 healthy control subjects. The values did not differ between groups (mean ± SD, 67.0% ± 17.7%, 63.5% ± 21.3%, and 70.4% ± 19.6%, respectively; ANOVA, P > 0.25). Surprisingly, the steady state TTO after stimulation was lower than that before stimulation in each group (ratio, 0.62 ± 0.46; paired t-test, P < 0.04).

CONCLUSIONS. The method developed is appropriate for the quantification of reflex lacrimation. Reflex lacrimation is not influenced significantly by glaucoma or ocular hypertension. The decreased steady state TTO after reflex stimulation may be caused by exhaustion of the lacrimal glands after excessive reflex lacrimation, indicating that normal lacrimation probably also contains reflex tears. (Invest Ophthalmol Vis Sci. 2000;41:709–714)

Although the subject of intense discussion, human lacrimation is generally considered to consist of two components: basal tear flow, which is produced constantly, and reflex or stimulated tear flow, which is evoked by external stimuli, such as cold wind, sneezing, irritating gas, or a foreign body in the eye, that activate a reflex arc.1 Tears are secreted by the main lacrimal gland, which is situated in the superior lateral corner of the orbit behind the orbital rim, and by the accessory lacrimal glands, which are located in the upper fornix and in the conjunctiva of the upper eyelid. Although Jones2 attributed reflex lacrimation to the main lacrimal gland and basal secretion to the accessory lacrimal glands, other authors3,4 consider that both glands contribute, to different extents, to basal and stimulated tear secretion.

Because the definition of basal secretion is also subject to discussion,3 within the context of this article, basal tear secretion is defined as that secretion which occurs continuously and which is not evoked by any kind of stimulation of the pathways leading to activation of the reflex arc. Furthermore, we define a “steady state tear flow” as the tear flow under normal physiological conditions. Because steady state tear flow can also contain some reflex secretion, it will be equal to or larger than basal tear secretion. Tear flow can be estimated quantitatively by fluorophotometric measurement of tear turnover (TTO). TTO is the percentage decrease in fluorescein concentration in tears per minute after instillation of fluorescein. Steady state TTO is the TTO for steady state tears and will be equal to or larger than TTO caused by basal secretion only. Steady state TTO has been previously referred to as basal TTO,5,6 suggesting that only basal, noninnervated lacrimation is involved. We introduce the term steady state TTO because the latter suggestion may not be correct.

Steady state TTO is lower in patients with primary open-angle glaucoma (POAG) or ocular hypertension (OHT) than in age-matched healthy control subjects and is correlated with the vertical or horizontal cup/disc ratios.7–9 This diminished TTO may be explained by dysfunction of the parasympathetic (pterygopalatine) pathway, which supplies the autonomic innervation to the accessory lacrimal glands, or by damage to the autonomic nerve fibers after glaucomatous cupping of the optic nerve head, resulting in malfunction of the autonomic plexus that influences steady state tear secretion.
Several groups in Europe, Japan and the United States have attempted to design reliable methods to generate and measure reflex flow, but results are not yet optimal. To our knowledge, quantitative data concerning reflex lacrimation in patients with glaucoma are not available. Because such data may provide information about the mechanism of reflex lacri-
mation and its relation to glaucoma, we designed a standardized method to measure reflex lacrimation in patients with untreated POAG or OHT and in healthy control subjects.

**MATERIALS AND METHODS**

**Patients and Control Subjects**

Patients with POAG or OHT were recruited from the outpatient ophthalmic department of the Leiden University Medical Center. Healthy volunteers were recruited among visitors and clinical staff and their relatives. The patients were selected according to the following criteria: newly detected untreated POAG or untreated OHT, open angle by gonioscopy, and intraocular pressure above 21 mm Hg, measured three times or more by applanation tonometry on separate occasions. Patients with POAG also had to meet the following criteria: glaucomatous visual field defects on Humphrey Field Analyzer 30-2 threshold test and glaucomatous optic disc cupping as evidenced by indirect ophthalmoscopy. Patients and healthy control subjects who used medication known to influence lacrimation, who showed disorders of the lacrimal system or corneal abnormalities on slit-lamp biomicroscopic examination, or who wore contact lenses were excluded from the study. The study followed the tenets of the Declaration of Helsinki, and informed consent was obtained after the nature and possible consequences of the study were fully explained. The institutional human experimentation committee of the Leiden University Medical Center approved the study.

**Instrumentation**

A scanning fluorophotometer (Fluorotron Master; Ocumetrics, Mountain View, CA) equipped with a special lens (Anterior Segment Adaptor) for detailed scanning of the anterior segment was used. Reflex lacrimation was induced by stimulation of the nasal mucosa with ethanol vapor, which activates the sensory afferent pathway for reflex tear flow by stimulating the nerve endings of the first and second branches of the trigeminal nerve. These trigeminal fibers reach the lacrimal nucleus in the pons. The saturated ethanol vapor was administered through two nostril adapters via a flow regulator that provided a constant nitrogen flow of 8 l min⁻¹. The water container of the flow regulator was filled with ethanol 70%. The flow regulator was connected to a nitrogen cylinder equipped with a pressure-reducing valve so that pressure was held constant at

**TABLE 1. Reproducibility of the Determination of Steady State Tear Turnover and Index of Reflex Lacrimation**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Day</th>
<th>Volunteer 1</th>
<th>Volunteer 2</th>
<th>Volunteer 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>25</td>
<td>41</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>First tear turnover (% min⁻¹)</td>
<td>1</td>
<td>11.4</td>
<td>7.09</td>
<td>13.8</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>12.8</td>
<td>10.3</td>
<td>9.08</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>9.29</td>
<td>10.6</td>
<td>12.7</td>
</tr>
<tr>
<td>Mean ± SD (% min⁻¹) All</td>
<td>11.2 ± 1.8</td>
<td>9.3 ± 1.9</td>
<td>11.9 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>Second tear turnover (% Min⁻¹)</td>
<td>1</td>
<td>8.53</td>
<td>6.41</td>
<td>6.86</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.85</td>
<td>7.16</td>
<td>8.90</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6.03</td>
<td>8.49</td>
<td>7.63</td>
</tr>
<tr>
<td>Mean ± SD (% Min⁻¹) All</td>
<td>5.1 ± 3.9</td>
<td>7.4 ± 0.9</td>
<td>7.8 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>Index of reflex lacrimation (%)*</td>
<td>1</td>
<td>55.1</td>
<td>85.8</td>
<td>66.7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>50.1</td>
<td>71.1</td>
<td>72.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>88.0</td>
<td>87.9</td>
<td>70.9</td>
</tr>
<tr>
<td>Mean ± SD (% decrease) All</td>
<td>64.4 ± 20.6</td>
<td>81.6 ± 9.2</td>
<td>69.9 ± 2.8</td>
<td></td>
</tr>
</tbody>
</table>

*Percentage decrease in tear fluorescein concentration by reflex tear stimulation.
1.1 bar. An electronic shutter with an open time of 5 seconds was placed between the cylinder and the flow regulator.

**Measurement Procedure and Calculations**

Four fluorophotometric scans of one randomly selected eye were performed before fluorescein instillation to determine the average corneal autofluorescence. Because corneal autofluorescence is measured together with the fluorescence of fluorescein in the tear film, because of the limited spatial resolution of the fluorophotometer, corneal autofluorescence must be determined and subsequently subtracted from the fluorescence measured after fluorescein instillation.

Two microliters of fluorescein (2%) was instilled without anesthesia into the temporal side of the lower fornix by means of a capillary tube. The time of instillation was registered, and the subject was asked to blink without squeezing to distribute the fluorescein homogeneously in the tearfilm. Three or four series of three fluorophotometric scans were made over 15 minutes to measure the decrease in fluorescein concentration in the tearfilm (Fig. 1). The first scan used in the calculations was made 5 minutes after the fluorescein instillation to avoid possible interference by reflex tears induced by the instillation. The fluorescein decay curve was used to calculate the first steady state TTO value. Then, reflex lacrimation was induced by applying ethanol vapor via the nostrils for 5 seconds. After 5 minutes, to avoid interference by stimulated reflex tear flow, a second steady state fluorescein decay curve was recorded by making three to four series of three fluorophotometric scans over 10 minutes, and the second steady state TTO value was calculated. Values below 4× corneal autofluorescence were not used in the calculations because these values are not reliable owing to the inaccuracy of the determination of corneal autofluorescence (10% or more), which has to be subtracted from the signal measured, and because of the decreasing signal-to-noise ratio at lower fluorescence intensities. The percentage decrease in fluorescein concentration in tears at the moment of reflex stimulation (time s in Fig. 1) was calculated by forward and backward extrapolation of the first and second decay curves (thick arrow in the figure). This percentage was assumed to be proportional to the reflex lacrimation caused by nasal stimulation and was used as an index of reflex lacrimation.

**Figure 4.** Index of reflex lacrimation versus steady state TTO before stimulation (left) and after stimulation (right) in patients with glaucoma (■), patients with ocular hypertension (○), and healthy control subjects (△). Solid lines were obtained by a linear regression procedure applied to all data points; dotted lines represent the 95% confidence limits for the solid lines; and broken lines, the 95% probability range for the data points.

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (y)</th>
<th>IOP (mm Hg)</th>
<th>First TTO (%·min⁻¹)¹</th>
<th>Index of Reflex Lacrimation (%)†</th>
<th>Second TTO (%·min⁻¹)¹</th>
<th>Ratio between Second and First TTO*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with untreated POAG</td>
<td>61.8 ± 14.0</td>
<td>28.6 ± 7.3</td>
<td>12.5 ± 7.7</td>
<td>67.0 ± 17.7</td>
<td>5.9 ± 4.3</td>
<td>0.63 ± 0.61</td>
</tr>
<tr>
<td>(n = 16)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with untreated ocular hypertension (n = 16)</td>
<td>57.1 ± 10.0</td>
<td>24.3 ± 3.6</td>
<td>14.2 ± 6.3</td>
<td>63.5 ± 21.3</td>
<td>6.9 ± 3.3</td>
<td>0.62 ± 0.39</td>
</tr>
<tr>
<td>Healthy Controls (n = 16)</td>
<td>55.8 ± 9.9</td>
<td>16.4 ± 5.1</td>
<td>13.3 ± 6.5</td>
<td>70.4 ± 19.6</td>
<td>6.3 ± 3.3</td>
<td>0.61 ± 0.38</td>
</tr>
</tbody>
</table>

Values are means ± SD. IOP, intraocular pressure.

¹Steady state tear turnover before (First TTO) or after (Second TTO) reflex tear stimulation.

†Percentage decrease in tear fluorescein concentration by reflex tear stimulation.
This method of quantification of reflex lacrimation takes the steady state tear flow before and after nasal stimulation into consideration, which allows calculation of the part of tear fluorescein decay that can be attributed exclusively to evoked reflex tears. Note that no fluorophotometric measurements were performed for a few minutes before nasal stimulation (because of patient and gas adjustments) and thereafter (because reflex tears were still present). The fluorescein instillation itself could not be used as a stimulus for inducing reflex lacrimation because this stimulus was found to be uncontrollable.

All data processing and calculations were performed with the use of specially developed software (program “Reflex”, author: J. A. Van Best).

Determination of Stimulus Duration and Reproducibility

To determine the effect of stimulus (ethanol vapor) duration on the index of reflex lacrimation, different stimulus durations (0.5, 1, 2, 5, and 10 seconds, respectively) were used to elicit reflex lacrimation in three healthy control subjects on another occasion.

To investigate the reproducibility of the method, the index of reflex lacrimation was measured in three healthy volunteers (aged 25, 41, and 61 years) on 3 separate days.

Statistical Analysis

The normality of the distribution of the values was assessed for each parameter and each group with D’Agostino’s test for departure of normality. For normal distributions, a single factor analysis of variance (ANOVA) testing the null hypothesis that the values did not differ among the three groups, was performed, and if necessary, the Turkey multiple comparison procedure was applied for each pair of groups. The correlation between two parameters was assessed using the Pearson correlation coefficient. The null hypothesis $H_0$: slope $= 0$ was tested against $H_a$: slope $\neq 0$ using Student’s t-statistic.

RESULTS

Two typical examples of the decay of tear fluorescein concentration in healthy control subjects, aged 38 and 62 years, during the determination of reflex lacrimation are presented in Figures 1 and 2, respectively. The steady state TTO after the stimulation of reflex tear flow was about equal to that before the stimulation in Figure 1 (10.8% · min$^{-1}$ and 10.1% · min$^{-1}$, respectively) but not in Figure 2 (21.0% · min$^{-1}$ and 5.9% · min$^{-1}$, respectively). Reflex lacrimation was about equal in both controls (index of reflex lacrimation: 68.7% and 64.3%, respectively).

The duration of the ethanol vapor stimulus was varied in three healthy volunteers between 0.5 and 10 seconds to determine its effect on the reflex lacrimation index (Fig. 3). In all cases maximal reflex lacrimation was obtained after approximately 4 seconds. For this reason, standard stimulus duration of 5 seconds was chosen.

The reproducibility of this method for determination of reflex lacrimation was evaluated by repeated measurements in three volunteers (Table 1). The mean SD of the reflex lacrimation index was 15.7%.

Information regarding age, IOP, first TTO, reflex lacrimation index, and second TTO of each group (patients with glaucoma, patients with ocular hypertension, and healthy control subjects) are presented in Table 2. All parameters were normally distributed in each group and in all three groups together. The reflex lacrimation index and the first and second TTO did not differ significantly between the three groups (ANOVA, $P > 0.25$). No correlation was found between the cup-disc ratio of the optic disc and the reflex lacrimation index ($r = -0.14, P = 0.35$). The three groups did not differ significantly in age (ANOVA, $P > 0.25$). The reflex lacrimation index was not correlated with age ($r = -0.13, P = 0.34$).

In each of the three groups, the steady state TTO was significantly lower after reflex stimulation than before stimulation ($0.52 \pm 0.46$; mean $\pm$ SD; paired t-test, $P < 0.04$). Taking all patients and control subjects together, a weak but significant negative correlation was found between the reflex lacrimation index and the first TTO ($r = -0.43, P = 0.0026$; Fig. 4, left panel) and between the reflex lacrimation index and the second TTO ($r = -0.44, P = 0.0016$; Fig. 4, right panel).

DISCUSSION

We developed a method to quantify reflex lacrimation reliably, based on the standardized stimulation of the nasal mucosa by ethanol vapor. The mean SD of repeated measurements of the reflex lacrimation index in three healthy volunteers was 15.7%. This rather high value can be attributed to a deviating value for one volunteer (volunteer 1 in Table 1). This high value could be due to various factors, such as atmospheric conditions, sneezing, cold, ventilation, and the psychological state of the volunteer.

We found that the reflex lacrimation index of patients with untreated POAG or OHT patients was not different from that of healthy control subjects and did not correlate with the cup/disc ratio. This indicates that reflex lacrimation is not affected by glaucoma or ocular hypertension. In contrast, steady state lacrimation is decreased in these patients and correlates with the cup/disc ratio.9 This discrepancy may be due to a greater vulnerability of the nerves innervating the lacrimal glands active during steady state lacrimation in comparison with those active during reflex lacrimation.

An unexpected finding was that the mean steady state TTO after reflex stimulation was approximately half that before stimulation. This difference probably cannot be attributed to an increase in reflex lacrimation during the first TTO measurement in response to fluorescein instillation, because the steady state TTO values corresponded to steady state TTO values found in previous studies (mean values $\pm$ SD in % · min$^{-1}$: 11.4 ± 3.1, 14.7 ± 3.0, and 15 ± 5.3 in patients with untreated glaucoma, patients with untreated ocular hypertension, and healthy control subjects, respectively). An explanation for the lower TTO after stimulation could be that tears originating from the main lacrimal gland (“reflex tears”) are part of normal (steady state) lacrimation and that induction of strong reflex lacrimation by external stimulation exhausts the tear production by that gland (see Fig. 3), resulting in a temporarily lower steady state tear production. Such exhaustion may originate, for instance, from a mechanism involving temporary tachyphylaxis to neuromediators released in the lacrimal gland during
the reflex response. This explanation is supported by the negative correlation found between the first TTO and reflex lacrimation. Thus, a high steady state lacrimation before stimulation may partly involve reflex tears, leaving less tear fluid available for (further) stimulated lacrimation. Analogously, a large evoked-reflex lacrimation response would temporarily exhaust the lacrimal glands, leaving less tear fluid available for the subsequent steady state tear flow, thus resulting in a low second TTO. These results suggest that, before stimulation, the steady state tear flow is probably composed of secretions from both noninnervated and innervated lacrimal glands. After stimulation, the lacrimal glands are exhausted and only a low steady state tear secretion remains. In other words, the steady state tear secretion measured under normal physiological conditions is partly under neural control. These considerations are supported by the lower Schirmer I values found previously in healthy volunteers after nasal mucosal anesthesia,4 indicating participation of sensory stimulation of the nasal mucosa in normal lacrimation.

The fluorescein decay curves used to calculate the first TTO were recorded over 10 minutes compared with 30 minutes in previous studies,9 because we measured three parameters (first TTO, index of reflex lacrimation, and second TTO) after instillation of fluorescein instead of one. This may cause inaccuracies in the calculated TTO values, which may explain why the difference between TTO values of glaucoma patients and healthy control subjects did not reach significance in this study.

In conclusion, the method for measurement of reflex lacrimation was found to be reliable and suitable for use in patients and healthy control subjects. The results show that glaucoma or ocular hypertension does not significantly affect reflex lacrimation and that normal physiological lacrimation probably consists at least partly of reflex tears.

References