Group II PLA₂ Content of Tears in Normal Subjects

K. Matti Saari,¹ Valterri V. Abo,¹ Ville Paavilainen,¹ and Timo J. Nevalainen²

PURPOSE. To determine the concentration of group II phospholipase (PL) A₂, an antimicrobial molecule, in tears of normal subjects in different age and sex groups.

METHODS. PLA₂ content of tears was measured in 122 healthy volunteers with ages ranging from 20 to 89 years (mean, 49.5 years) by a time-resolved fluoroimmunoassay using a polyclonal rabbit antibody to recombinant human PLA₂.

RESULTS. The mean concentration of PLA₂ in tears was 54.5 ± 53.9 μg/ml. It was highest in the age group 20 to 29 years (81.6 ± 32.0 μg/ml), and a decrease of concentration occurred with an increase of age. PLA₂ values were statistically significantly lower in the age group 60 to 69 years (P = 0.0013) and 70 years or more (P = 0.0001) than in the age group 20 to 29 years. There were no statistically significant differences in PLA₂ content of tears between the genders in any age group (P = 0.798).

CONCLUSIONS. The results indicate that tears contain a high concentration of PLA₂ and that PLA₂ levels decrease with an increase of age and/or reflex tear component of the sample analyzed.

Tears play an important role in the nonspecific defense against bacterial and fungal invasion by washing away the microbes from the surface of the eye.¹ Tears contain several proteins with antimicrobial properties.¹⁻³ Alexander Fleming reported the presence of lysozyme in tears and described its ability to lyse leisedeiticys.⁴ Lysozyme is a bacteriolytic enzyme that hydrolyzes β 1–4 linkages between N-acetylmuramic acid and N-acetylgalactosamine that stabilize bacterial peptidoglycans, especially in Gram-positive bacteria.⁵ The mean lysozyme content of tears in normal subjects is 1700 μg/ml.⁶ Besides lysozyme, the antimicrobial properties of tears have been explained by their high concentration of lactoferrin, betalysin, complement, and secretory immunoglobulin A.¹⁻³⁻⁸ Nevalainen et al.⁹ were the first to report the presence of phospholipase (PL) A₂ in human main lacrimal gland and tears. We observed two specialized cell types in the main and accessory lacrimal glands, one synthesizing group II PLA₂ and the other synthesizing lysozyme.¹⁰ Lysozyme was present in the secretory granules of most acini, whereas PLA₂ was seen in a minority of acinar cells, primarily in the central parts of lobules in the main and accessory lacrimal glands.¹⁰

PLA₂ is a lipolytic enzyme that catalyzes the hydrolysis of the acyl ester bond at the sn-2 position of phosphoglycerides.¹¹ In humans, several enzyme proteins have been identified, including an 85,000 mol wt intracellular PLA₂ and two 14,000 mol wt secretory PLA₂.¹²⁻¹⁴ The secretory PLA₂ are divided into two subgroups on the basis of the amino acid sequence.¹⁵ Group I PLA₂ is present in the pancreas, and group II PLA₂ in platelets and different fluids and tissues, for example, in Paneth cells, chondrocytes, and synovial fluid and in prostatic gland cells and seminal plasma.¹⁶⁻¹⁷ In tears, group II PLA₂ is princi-

the concentration of PLA₂ in tears was highest in the age group 20 to 29 years and that a decrease of the PLA₂ concentration occurred with an increase in age and/or with a reflex component of the sample.

METHODS

Subjects

The concentration of PLA₂ in tears was studied in 122 healthy volunteers, 60 men and 62 women with ages ranging from 20 to 89 years (mean, 49.5 years; Table 1). None of the subjects wore contact lenses. The values for PLA₂ were assessed in tear samples from one eye of each healthy subject. The principles of the World Medical Association Declaration of Helsinki were followed. Informed consent was obtained from each subject giving the tear specimen. The study protocol was approved by the Ethics Committee of the University of Turku and the Turku University Hospital.

Collection of Tears

Nonstimulated tears were collected using disposable 5-μl microcapillaries (Microcaps 5 μl, Drummond Scientific Co., Broomall, PA) or 5- or 10-μl microcapillaries (Duran Ringcaps; Hirschmann Laborgeräte, Eberstadt, Germany) under a Haag-Streit 900 biomicroscope. The samples were gathered from the marginal tear strip of the lower lid near the lateral canthus, with care being taken not to irritate the conjunctiva, cornea, or lid margin. Usually (97 subjects), a 5-μl tear sample was taken using a microcapillary, but if the tear production was exceptionally scarce (1.0–4.9 μl in 15 subjects) or plentiful (5.1–7.0 μl in 8 subjects and 20 μl in 2 subjects), an additional calibrating scale was used beside the microcapillary. In all cases the collection time was limited to 5 minutes. The tear samples were diluted from 1:300 to 1:1100 with physiological saline and kept frozen at −70°C until assayed.

PLA₂ Assay

The concentration of PLA₂ in tear fluid was measured by a time-resolved fluoroimmunoassay using a polyclonal rabbit antibody to recombinant human group II PLA₂.¹⁸ The results are expressed as micrograms per milliliter (μg/ml).

Statistical Analysis

PLA₂ values in different age and sex groups were compared using a two-way analysis of variance. After overall test, all six age groups were compared pairwise. The comparisons were made within sex groups as well as for all subjects. Because several comparisons were done simultaneously, Tukey–Kramer multiple comparison method was used to adjust the P values. All P values lower than 0.05 were considered statistically significant. The results are expressed as means ± SD.

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RESULTS

The mean concentration of PLA2 in tears of normal subjects was $54.5 \pm 33.9$ μg/ml in the total material. Analysis using the General Linear Models Procedure showed that the PLA2 values in different age groups were not similar ($P = 0.0025$). However, there were no differences in the PLA2 values between the genders in different age groups and in the total material ($P = 0.798$).

The PLA2 content of tears in normal subjects was highest in the age group 20 to 29 years both in men ($79.6 \pm 29.6$ μg/ml), in women ($83.7 \pm 35.8$ μg/ml), and in all subjects ($81.6 \pm 32.0$ μg/ml). A decrease in the concentration of PLA2 occurred with an increase of age from 30 to 70 years or more (Fig. 1). When compared with the PLA2 values of tears in the age group 20 to 29 years, the PLA2 values of tears were statistically significantly lower in men in the age group 70 years or more ($P = 0.046$), in women in the age groups 60 to 69 years ($P = 0.028$) and 70 years or more ($P = 0.013$), and in the total material in the age groups 60 to 69 years ($P = 0.0013$) and 70 years or more ($P = 0.0001$; Table 2).

DISCUSSION

The results of this study showed that in normal human tears the mean concentration of PLA2 was $54.5 \pm 33.9$ μg/ml, which is consistent with the normal values ($36.7 \pm 2.99$ μg/ml, mean ± SEM) reported by Qu and Lehrer. It is important to notice that the concentration of PLA2 in tears ($54.5$ μg/ml) exceeded the levels found in normal serum ($3.7$ μg/ml) by four orders of magnitude and also markedly exceeded the concentration of PLA2 in the seminal plasma ($13.4 \pm 12.7$ μg/ml). The present results showed that there were no significant differences in the PLA2 content of tears between the genders as similarly reported earlier on lysozyme secretion.

The present results showed that the PLA2 content of tears was highest in the age group of 20 to 29 years and that decreasing values with an increase of age from 30 to 70 years or more were seen both in men, women and in the total material. Similarly, the concentration of lysozyme in tears has been shown to be highest in the age group 21 to 40 years, and a decrease of lysozyme concentration occurred with an increase in age from 30 to 40 years. Thus, both the PLA2 and lysozyme contents of tears decreased with an increase of age.

In this study the flow rate of tears varied markedly between the subjects, and the volume of tears collected varied between 1 and 20 μl. In this connection, the decrease in the PLA2 content of tears could also be explained by a decrease in reflex tearing with age. However, we found no statistically significant differences in the PLA2 content of nonstimulated tears in subjects with slow secretion compared with subjects with fast secretion ($P = 0.82$).

PLA2 is principally responsible for the ability of tears to kill a broad spectrum of Gram-positive bacteria. Only 1.1 ng/ml of PLA2 sufficed to kill *Listeria monocytogenes* and 15 to 80 ng/ml of PLA2 killed *Staphylococcus aureus*. Micrococcus luteus strain was killed by 13 μg/ml of lysozyme and by 0.3 μg/ml of PLA2. These concentrations were 130-fold lower than the concentration of 1768 μg/ml of lysozyme in tears and 180-fold lower than the concentrations of PLA2 in normal tears (Table 2). The present results (Table 2) show that even for the older subjects (70 years or older) the levels of PLA2 in tears are considerably above the level required for effective killing of Gram-positive bacteria.

In summary, we found that the mean PLA2 content of tears was $54.5 \pm 33.9$ μg/ml. It was highest in the age group of 20 to 29 years, and a decrease of PLA2 concentration occurred with an increase in age from 30 to 70 years or more. These findings are consistent with previous evidence that PLA2 plays a substantial antibacterial role in tears.

**Table 1.** Distribution of Number of Normal Subjects According to Age and Sex

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>No. of Men</th>
<th>Mean Age</th>
<th>No. of Women</th>
<th>Mean Age</th>
<th>Total No.</th>
<th>Mean Age</th>
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<tbody>
<tr>
<td>20–29</td>
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<td>23.3</td>
<td>10</td>
<td>22.9</td>
<td>20</td>
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<td>30–39</td>
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<td>34.6</td>
<td>12</td>
<td>35.6</td>
<td>22</td>
<td>35.1</td>
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<tr>
<td>40–49</td>
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<td>10</td>
<td>45.7</td>
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<td>53.2</td>
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<td>10</td>
<td>64.5</td>
<td>20</td>
<td>65.0</td>
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<tr>
<td>≥70</td>
<td>10</td>
<td>77.0</td>
<td>10</td>
<td>76.8</td>
<td>20</td>
<td>76.9</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>49.9</td>
<td>62</td>
<td>49.2</td>
<td>122</td>
<td>49.5</td>
</tr>
</tbody>
</table>

**Fig. 1.** PLA2 concentration (mean ± SD) of tears in different age groups.

**Table 2.** Mean Group II PLA2 Content in Tear Fluid of Healthy Subjects in Different Age Groups

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>20–29</td>
<td>79.6 ± 29.6</td>
<td>83.7 ± 35.8</td>
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<tr>
<td>30–39</td>
<td>55.3 ± 21.5</td>
<td>58.9 ± 28.9</td>
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<td>40–49</td>
<td>53.2 ± 33.5</td>
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<td>50–59</td>
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<td>60–69</td>
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<td>54.2 ± 28.4</td>
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<tr>
<td>70–</td>
<td>32.4 ± 27.8*</td>
<td>30.8 ± 25.6†</td>
</tr>
<tr>
<td>Total</td>
<td>53.6 ± 32.8</td>
<td>55.2 ± 35.2</td>
</tr>
</tbody>
</table>

Values are means ± SD, expressed as μg/ml.

* $P = 0.046$ vs. 20–29 years; † $P = 0.028$ vs. 20–29 years; ‡ $P = 0.015$ vs. 20–29 years.
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References