Lysozyme tear level in patients with herpes simplex virus eye infection. E. Eylän, D. Ronen, A. Romano, and O. Smetana.

The lysozyme level in tears of patients with HSV eye infection was examined and correlated with the clinical findings and presence of virus. The concentration of the enzyme in tears of patients during acute attack was 2.83 mg./ml. This value was significantly lower than that in tears from healthy controls (6.1 mg./ml.) and in tears from the patient’s healthy eye (4.46 mg./ml.). After termination of treatment with either IUDR or poly I:C, the lysozyme level rose to an average of 5.34 mg./ml., but it remained lower than in healthy subjects who had never suffered from HSV eye infection. This may be an indicator of possible future recurrences.

Lysozyme, a substance found in human granulocytes, monocytes, and macrophages, has been found to be of great value in diagnosis of certain diseases; it has been shown that the level of serum lysozyme is low in chronic lymphatic leukemia, acute lymphoblastic leukemia of childhood, blast cell leukemia, and Waldenström’s macroglobulinemia. Pick and Yeshurun found abnormally elevated serum and urinary lysozyme in five patients with acute monocytic leukemia and in one patient with chronic monocytic leukemia.

Tears, however, are one of the body fluids containing a high level of lysozyme in the healthy individual. Although it is generally believed that lysozyme in tears acts as a protective agent against bacterial infection, its role in eye disease is still unknown. Meyer et al., in 1948, first suggested that the concentration of lysozyme in tears is decreased in keratoconjunctivitis sicca and tends to vary with the severity of the disease. Van Bister-veld found a low concentration of lysozyme in the tears of 43 patients with Sjögren’s syndrome. Regan studied the tear lysozyme content in pathologic conditions and found that it tends to decrease in bacterial conjunctivitis, is markedly lower in the affected eye in corneal ulcers than in the nonaffected eye, and is either borderline or below normal in corneal dystrophy.

In the present work, the lysozyme content of tears was studied in patients suffering from herpes simplex virus (HSV) eye infection, during three stages of the disease: (1) acute attack, (2) after termination of treatment, and (3) at 3 months later, termed here the “latent period.” The lysozyme level was correlated with the clinical findings and presence of the virus.

Materials and methods

Collection of tears. The tears were collected from the lower fornix of the eye near the puncta lacrimalis or from the upper fornix near the lacrimal gland, by means of a Vitrex-microhematocrit capillary tube, nonheparinized, with a capacity of 75 µl (CHR Bardam, Birkerod, Denmark). The capillary tubes were then fixed in a minisal plate (Medidenta Co., Tel-Aviv); part of these were stored at -70° C. for isolation of HSV, and part at 4° C. for lysozyme determination.

Dilution of tear sample. The tear sample was later removed from the capillary tubes by a vacuum dropper to a parafilm paper. From this tear drop, 10 µl were removed by a micropipette and placed in a test tube containing 0.1 ml. of 0.15M phosphate buffer, pH 6.24, obtaining a final dilution of 1:10 of the tear sample. Lysozyme level was determined on this diluted tear sample.

Determination of lysozyme in tears. The lysozyme level was determined by the spectrophotometric method for quantitative determination of lysozyme in human tears as previously described.6 Collection of eye smears. Eye smear samples for HSV isolation were collected from both eyes during acute infection, after termination of treatment, and in the latent period. The eye smears were taken from the lower fornix of the conjunctiva with a sterile cotton swab moistened with M-199 and antibiotics and/or by scraping the lesion of the corneal epithelium. The swabs were dipped in a sterile tube containing M-199 with 5% fetal calf serum, penicillin, streptomycin, and mycostatin. The samples were stored at -70° C. Isolation and identification of HSV strains. The eye sample swabs were inoculated into primary rabbit kidney (PRK) tissue cultures and incubated at 37° C. until appearance of the cytopathogenic effect (CPE). After detection of CPE, specific neutralization was made with anti-HSV serum. All the HSV strains isolated from the eyes belonged to the type 1 HSV group. Isolation of HSV from patients’ infected eyes was a criterion for including patients in the investigated group.

Treatment. Nineteen patients were treated with 5-iodo-2-deoxyuridine (IUDR) (Virusan; Ika-pharm, Tel-Aviv, Israel) and 19 with polyinosinic-poly-cytidylic acid (poly I:C; Miles Chemical Co., Elkart, Ind.). The patients were treated during the acute period of the disease with two drops of Virusan, 10 times a day. In addition, Virusan ointment was applied to the lower fornix three times a day. Poly I:C was obtained as a lyophilized preparation. The patients were treated with poly I:C with DEAE-dextran (Pharmacia, Upp-
had disappeared, and no signs of the iritis re-

tained. Only some sensitivity of the eye with

epithelial damage was cured, the corneal edema

improved; this meant that the eye appeared to be completely normal clinically.

Viral isolation. Eye smears for virus isolation

were collected from patients throughout the three stages of the disease. During the acute attack, HSV was isolated from all cases. Patients treated with poly I:C were tested daily for the presence of the virus during the 5 to 8 days of treatment. The virus could be isolated for only 1 or 2 days immediately thereafter. The group of patients treated with IUDR could not be tested on a daily basis but only at 1 or 2 weeks after treatment began with IUDR, and at that time the virus could no longer be detected. During the latent period, the virus had completely disappeared.

Determination of lysozyme during three stages of the disease. Fig. 1 describes in the form of a histogram the lysozyme level in tears of patients suffering from HSV eye infection during three stages of the disease. These patients were divided into two groups according to treatment. One group consisted of 19 patients treated with IUDR, including two patients suffering from HSV in both eyes, thus making a total of 21 examinations. The second group contained 19 patients treated with poly I:C, only one of whom suffered bilaterally, making the total of examinations 20. The results in Fig. 1 are described for both the infected and noninfected eye in three columns (A, B, and C) according to stages of the disease. During the acute attack (A), the lysozyme level in tears from

Fig. 1. Lysozyme level (mg./ml. of egg-white lysozyme) in tears of patients with HSV eye infection. A, Acute attack. B, After termination of treatment. C, Latent period. ○, Patients treated with IUDR; ●, Patients treated with poly I:C; •, infected eye; ++, noninfected eye.

the infected eye is very low; it rises after treatment (B) and in most cases remains stable or continues to rise slightly in the latent period (C).

Table I shows a comparison of the mean values of lysozyme in patients’ tears during three stages of the disease with those of a control group of 60 normal subjects. The mean value of lysozyme during the attack was 2.8 mg./ml, increasing to 4.75 mg./ml. after treatment with IUDR and to 4.02 mg./ml. after treatment with poly I:C. Since there was no significant difference (p = 0.15) between these two posttreatment values, an average was calculated for the two—4.3 mg./ml. During the latent period the lysozyme level rose further still, to 5.34 mg./ml. There was a highly significant difference (p <0.001) between the lysozyme level in patients during attack and that of normal controls. This difference was maintained even following treatment (p <0.001) and fell to a slightly significant level during the latent period (p <0.05).
Table I. Comparison of lysozyme level in tears in HSV-infected patients

<table>
<thead>
<tr>
<th>Stage of the disease</th>
<th>N</th>
<th>Mean</th>
<th>S.D.</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>During attack</td>
<td>41</td>
<td>2.83</td>
<td>1.47</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Both groups after treatment</td>
<td>33</td>
<td>4.34</td>
<td>1.47</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Latent period</td>
<td>23</td>
<td>5.34</td>
<td>1.6</td>
<td>p &lt; 0.05</td>
</tr>
</tbody>
</table>

Table II. Application of Student's test (for paired observations) to the difference in lysozyme level in the eyes of the same patient

<table>
<thead>
<tr>
<th>Stage of the disease</th>
<th>N</th>
<th>Infected eye Mean</th>
<th>S.D.</th>
<th>Noninfected eye Mean</th>
<th>S.D.</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>During attack</td>
<td>25</td>
<td>2.64</td>
<td>1.38</td>
<td>4.46</td>
<td>1.6</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>After treatment</td>
<td>21</td>
<td>4.47</td>
<td>1.55</td>
<td>5.29</td>
<td>1.76</td>
<td>p = 0.011</td>
</tr>
<tr>
<td>Latent period</td>
<td>16</td>
<td>4.55</td>
<td>1.18</td>
<td>4.99</td>
<td>1.56</td>
<td>p = 0.166</td>
</tr>
</tbody>
</table>

Table III. Index of improvement according to the differences in lysozyme levels in the same eye during the three stages of the disease

<table>
<thead>
<tr>
<th>Stages of the disease compared</th>
<th>N</th>
<th>Mean</th>
<th>S.D.</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>During attack</td>
<td>32</td>
<td>2.89</td>
<td>1.52</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>After treatment</td>
<td>28</td>
<td>4.29</td>
<td>1.46</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Latent period</td>
<td>28</td>
<td>2.78</td>
<td>1.42</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>After treatment</td>
<td>28</td>
<td>5.27</td>
<td>1.59</td>
<td>p &lt; 0.001</td>
</tr>
</tbody>
</table>

Application of Student's t test (for paired observations) to the differences between each patient's infected eye and the healthy one (Table II) showed that during the attack, the infected eye had a significantly lower level of lysozyme than the normal eye (p < 0.001). After treatment the difference was less significant (p = 0.011), and during the latent period there was no significant difference between the two eyes.

The differences during the three stages of the disease in the same eye were also analyzed with Student's t test for paired observations (Table III). There was a highly significant difference between the enzyme level in the infected eye during attack and after treatment (p < 0.001). There was a highly significant difference between the eye during attack and in the latent period (p < 0.001). A less significant difference, indicating improvement, was observed in lysozyme level between the period right after treatment and the latent period (p = 0.016).

Discussion. The mean lysozyme level in tears of patients suffering from HSV eye infection (2.83 mg./ml.) was significantly lower than in tears from healthy persons (6.1 mg./ml.). The level was even significantly lower than that found in tears of the patient's healthy eye. In the latent period of the disease when the clinical situation had improved and the virus disappeared, the lysozyme level in tears in both eyes became equal. However, it was still lower (4.75 mg./ml.) than in healthy patients (6.1 mg./ml.) who had never suffered from HSV eye infection. It is possible that the low concentration of lysozyme found in the latent period is a characteristic of this entire group and is an indicator for possible recurrences.

A comparison of lysozyme level in tears of the infected eye during the three periods of the disease showed an increase as the virus disappeared and the clinical situation improved, reaching the level of the respective patient's normal eye. The lysozyme level of tears of patients with recurrent HSV eye infection should therefore be measured against the level in the healthy eye of the patient.

The phenomena of a low lysozyme level in tears of patients with HSV eye infections and the subsequent increase in level after treatment may be of clinical value. We know that the virus disappeared after 1 to 2 days of treatment with poly I:C, but the clinical examination showed improvement only on the sixth day of treatment. The lysozyme tear level, followed daily during treatment with poly I:C, may provide one of the criteria for determining the exact length of time necessary for treatment.

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Effect of imidazole on ionophore-induced ocular hypertension. STEVEN M. Podos.

The effect of intraperitoneal injections of imidazole on the elevation of intraocular pressure produced by the topical application of the cation ionophores A23187 or X537A was studied in rabbits. Pretreatment with 200 mg./kg. imidazole completely inhibited the ocular hypertension induced by 1.0 percent A23187. The elevation of intraocular pressure produced by 0.5 percent X537A was not blocked by pretreatment with imidazole.

The ionophores are agents that facilitate the transport of cations across biological membranes. The carboxylic ionophores include X537A, which complexes most cations, and A23187, which is selective for divalent cations. These ionophores stimulate many calcium-dependent cellular reactions. When applied to the rabbit eye, A23187 and X537A produce an elevation of intraocular pressure. This elevation of pressure is not accompanied by a rise of aqueous humor protein, nor is it blocked by pretreatment with indomethacin or prednisolone.

Imidazole is a compound that induces hypocalcemia in rats. It also has broad anti-inflammatory actions. Intraperitoneal administration of imidazoles inhibits the elevations of intraocular pressure and aqueous humor protein in rabbit eyes treated with prostaglandin E and nitrogen mustard, a nonprostaglandin-mediated irritant. Imidazole also inhibits thromboxane formation and could be of value in treating thromboembolic disorders.

A study was carried out of the effect of pretreatment with systemically administered imidazole on the elevation of intraocular pressure induced by the ionophores A23187 and X537A in rabbits.

Materials and methods. Ionophores A23187 and X537A were prepared by adding 10 mg. to a diluent of 0.1 ml. of dimethyl sulfoxide and 0.9 ml. of water. This 1.0 percent suspension was utilized in full concentration or diluted with water to produce 0.2 and 0.5 percent concentrations of ionophore. Equivalent diluents were prepared for each concentration.

Awake albino rabbits, 2 to 3 kg., were restrained in canvas wraps. Baseline intraocular pressure was measured with an Alcon applanation pneumotonometer, manometrically calibrated. Topical 0.5 percent prparacaine anesthesia was employed. In each experiment, 200 mg./kg. imidazole was injected intraperitoneally in half of the rabbits and an equal volume of the diluent for the imidazole in the control rabbits. One hour later intraocular pressure was remeasured. One eye of each rabbit then received topical application of 0.05 ml. of 1.0 percent A23187, and the fellow eye its diluent. Equal numbers of right and left eyes were treated in each group. Intraocular pressure measurements were repeated at 30, 60, 90, and 240 min. after application of the drugs. In similar experiments 0.2 percent A23187 or 0.5 percent X537A were used. External examinations were carried out on all animals.

Statistical analyses employed the paired t test or Student t test. Differences of p < 0.02 were considered significant.

Results. Pretreatment of rabbits with imidazole, 200 mg./kg. intraperitoneally, completely blocked the intraocular pressure elevation produced by the topical application of A23187 in concentrations of 0.2 and 1.0 percent (Table I). In control animals injected with buffer, the eyes receiving A23187 demonstrated a higher intraocular pressure that was significant for the 0.2 percent concentration (p < 0.02) at 30 min. after application and for the 1.0 percent concentration (p < 0.005) at 30 and 60 min. after instillation, as compared to the fellow eyes receiving topical ionophore diluent. At 60 min., the mean intraocular pressure of the eyes receiving 1.0 percent A23187 of animals that were pretreated with imidazole was significantly...