4 percent pilocarpine did not differ significantly over the 24 hr. period with little variation suggesting systemic effect. Only did the 2 percent pilocarpine continue to significantly reduce IOP for the full 24 hr. period in both groups of dogs, but also 2 percent pilocarpine had significant pressure-lowering effects in the contralateral untreated eyes in glaucomatous and normotensive dogs. In the untreated eyes with 2 percent pilocarpine the IOP remained low over the 24 hr. period with little variation suggesting systemic effect. Maximal pupillary constriction preceded maximal reductions of IOP by several hours in most cases. Miosis was greatest between 45 min. and 2 hr., then slowly returned to pretreatment levels.

The response of decreasing IOP and miosis in the canine appears similar to man with regard to amount of response and time course. The mode of action of pilocarpine in the canine has not been reported and will be evaluated in future studies. The maximal response with 2 percent pilocarpine was not anticipated, since the maximal dose response in other species is usually much higher. This may be associated to the anatomical differences present in the canine iridocorneal angle, specifically the ciliary musculature and absence of a scleral spur, or to an increased sensitivity to cholinergic agents. Also, irritation at higher doses was considerable and may interfere with the action of pilocarpine. Other concentrations of pilocarpine will be evaluated to establish a dose-response for the dog. This study indicates the glaucomatous beagle is a useful model for the study of the effects of cholinergic agents.

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Key words: open-angle glaucoma, animal model, pilocarpine, cholinergic, dog, intraocular pressure.

REFERENCES


Lack of levamisole effect on experimental herpes keratitis. Herbert E. Kaufman and Emily D. Varnell.

Levamisole, which is an anthelminthic, can restore depressed cell-mediated immunity (CMI) under some circumstances. In a controlled trial of experimental herpetic keratitis in rabbits, levamisole was found to have no significant effect on acute herpetic keratitis or its recurrence rate. This is consistent with previous findings that other nonspecific CMI stimulation had no effect on recurrences of experimental herpes keratitis. Because of the known tendency of levamisole to produce agranulocytosis, we believe it should not be used in man unless proven effective in a carefully controlled double-blind study.

The role of cell-mediated immunity (CMI) in herpetic keratitis, both acute and recurrent, is uncertain. It seems unlikely that CMI plays a role.
major part in acute herpetic keratitis, but it has been postulated to be a crucial factor in recurrent herpetic keratitis.

In mice with depressed CMI levamisole, an anthelminthic, has been shown to be able to restore CMI to nondepressed cellular levels. There is some evidence that in cancer patients, depressed levels of CMI can be enhanced with levamisole, although further data would be desirable on the subject. On the other hand, there is good evidence that in normal persons or in situations in which CMI is approximately normal, levamisole seems to have no effect. Because of the possibility that levamisole might stimulate CMI, it seemed worth investigating in an experimental system. This paper reports the lack of effect of levamisole on acute herpetic keratitis as well as on recurrences of herpes.

Materials and methods. Two hundred New Zealand white rabbits weighing 3 to 5 lb. were infected in both eyes with McKrae strain herpesvirus by lightly traumatizing the epithelium and placing a drop of virus suspension into the cul-de-sac.

Three days later, 75 of the rabbits were used for the study on acute infection. Corneas were stained with fluorescein and examined by biomicroscopy for the presence of dendritic ulcers. Twenty-six animals were then treated with levamisole, 20 mg./kg., by intraperitoneal injection one time and with petrolatum ointment in both eyes six times a day for 5 days. Twenty-five rabbits were treated six times a day with 0.5% idoxuridine ointment (Stoxil) in both eyes, and 24 were treated with petrolatum ointment for 5 days.

On treatment days 3, 4, and 5, eyes were graded on a blind basis for severity of keratitis, the examiner having no knowledge of the treatment received by any animal. Severity of keratitis was graded on a 0 to 4 scale with 0 being normal cornea, 1 being ¼ the area of cornea ulcerated, and 4 being the entire cornea ulcerated.

Thirty days after infection all surviving animals were used for the recurrence study. Half of the animals originally treated with the levamisole plus another 16 animals were treated with 20 mg./kg. levamisole at weekly intervals for 1 month. The remaining animals were untreated.

Eyes were examined daily for 30 days for the presence of ulcers, and swabbings were cultured for the first 10 days of this recurrence phase for the presence of virus. Swabbings of the conjunctival cul-de-sac were placed on primary human embryonic kidney cultures and maintained at 37°C C. for at least 3 weeks or until cytopathogenic effect was noted.

Results. Levamisole, when given in a dose of 20 mg./kg. to rabbits with dendritic keratitis, did not significantly alter the acute infection (Table I). Higher concentrations of levamisole were toxic to normal rabbits. There were no statistically significant differences in the recurrence rates of rabbits treated with levamisole and those of untreated controls (Table II). There was a slight but not statistically significant effect of levamisole on the detection of herpesvirus in conjunctival swabbings (Table III).

Discussion. In this experiment, there is a suggestion that levamisole might have had some effect on reducing the recurrence rate for the first 2 weeks, but if anything, this was reversed during

### Table I. Levamisole treatment of acute experimental herpes keratitis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of eyes</th>
<th>Severity of keratitis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 3</td>
</tr>
<tr>
<td>Levamisole</td>
<td>44*</td>
<td>1.92</td>
</tr>
<tr>
<td>(20 mg./kg.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stoxil, 0.5%, 6 times daily</td>
<td>48*</td>
<td>1.85</td>
</tr>
<tr>
<td>Control</td>
<td>46</td>
<td>2.00</td>
</tr>
</tbody>
</table>

*Eyes from survivors for the entire period.

### Table II. Effect of levamisole on recurrence of experimental herpes keratitis

<table>
<thead>
<tr>
<th>Week</th>
<th>No. of animals</th>
<th>%</th>
<th>No. of animals</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levamisole given during recurrence phase only:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0 (24)*</td>
<td>7 (70)*</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2 (24)</td>
<td>8 (70)</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>8 (24)</td>
<td>14 (70)</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>12 (20)</td>
<td>20 (64)</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Levamisole given acutely plus during recurrence phase:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0 (10)</td>
<td>7 (70)</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0 (9)</td>
<td>14 (70)</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2 (9)</td>
<td>11 (64)</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>3 (6)</td>
<td>30 (64)</td>
<td>47</td>
<td></td>
</tr>
</tbody>
</table>

*Number of animals in group indicated in parentheses.

### Table III. Effect of levamisole in the secretion of herpesvirus in the rabbit

<table>
<thead>
<tr>
<th>Positive virus isolations</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
</tr>
<tr>
<td>Levamisole</td>
</tr>
</tbody>
</table>

*Number of animals in group indicated in parentheses.
the last 2 weeks of the study. All in all, there are no statistically significant differences between the levamisole-treated group and the control group in the incidence of clinical recurrences. Although the shedding of virus might be studied in greater detail, we considered that clinical recurrences were the most important factor to study.

Levamisole is not a harmless drug. It has produced agranulocytosis\(^7\) and other kinds of toxicity.\(^7\) Unless, therefore, some clear-cut effect is found, it would seem unjustified to give such a drug to man.

In the past, we have studied other methods of nonspecifically stimulating CMI.\(^6\) Our studies have included the use of *Staphylococcus* phage lysate and BCG stimulation to activate macrophages and lymphocytes and stimulate the reticuloendothelial system. These did not reduce recurrences of herpes. Drugs like levamisole have been shown to be active in vitro\(^9\) and in some other situations in which CMI has been depressed. As with the other nonspecific CMI stimulation, we did not find levamisole active in preventing recurrences in experimental herpetic keratitis.

One of the frustrating problems in reviewing anecdotal information on recurrent herpetic keratitis appears to be that patients tend to have a variable recurrence pattern. Sometimes people will get recurrences very frequently for a period of time, and then these will spontaneously decrease, seldom maintaining a constant incidence throughout life. If such patients are treated at the attack peak with any agent thought to possibly reduce recurrence rate, then it appears that this agent has therapeutic activity, if only because of the spontaneous decrease in recurrence rate—which might be expected if someone has already reached peak recurrence frequency.

Because of the known toxicity of levamisole, this study would suggest that levamisole is not active in recurrent herpetic keratitis and should not be used in random cases of recurrent herpes outside of a carefully controlled double-blind clinical trial which might definitively answer the question of its possible activity in man.


Key words: herpesvirus, keratitis, cell-mediated immunity, levamisole.

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Tear calcium and magnesium levels of normal subjects and patients with hypocalcemia or hypercalcemia. R. AVISAR, H. SAVIR, Y. SID, AND J. PINKHAS.

Tear calcium and magnesium levels were measured in eight patients with hypercalcemia and two patients with hypocalcemia and compared to that of 72 subjects with normal serum calcium and magnesium levels. No correlation was found between tear and serum calcium and magnesium levels. Tear calcium level has no diagnostic importance.

The tear level of several substances such as lysozyme\(^1\) and lactic acid dehydrogenase\(^2\) (LDH) have proved to be useful in the study of eye diseases. Uotila et al.\(^3\) found no apparent correlation between tear and serum calcium measurements. The aim of the present work was to study tear calcium levels in patients with a systemic disease such as sarcoidosis, hyperparathyroidism, or hypoparathyroidism which could alter the calcium excretion patterns. We examined whether calcium level in the tear fluid can be of more diagnostic importance than static calcium levels in blood.

Since calcium and magnesium transport are related to each other in various systems such as the intestinal epithelium or the renal tubule, it is of interest to measure with great accuracy both