Anti-inflammatory effectiveness of topically administered corticosteroids in the cornea without epithelium. ALLAN KUPFERMAN AND HOWARD M. LEHOWITZ.

The present studies demonstrate that modification of the derivative of a given steroid base alters its anti-inflammatory potential as measured by suppression of leukocyte invasion of the cornea.

A comparison of each drug's corneal bioavailability with its anti-inflammatory effectiveness shows the acetate derivative of prednisolone to be a more potent anti-inflammatory agent than the phosphate derivative. Similarly, the free alcohol derivative of dexamethasone proved to be more potent than the phosphate derivative. Increasing the concentration of prednisolone acetate from 0.125 per cent to 1.0 per cent results in a significant increase in its anti-inflammatory effectiveness in the cornea following topical administration. The same increase in prednisolone phosphate concentration does not produce a significant increase in its ability to suppress polymorphonuclear leukocyte infiltration of the cornea. When the epithelium of the inflamed cornea is intact, prednisolone acetate, 1.0 per cent ophthalmic suspension, is the most effective of the corticosteroid preparations studied. In the absence of an intact epithelium, prednisolone acetate, 1.0 per cent ophthalmic suspension, again produces the greatest mean reduction in polymorphonuclear leukocyte infiltration of the cornea although here one cannot demonstrate a statistically significant difference from the anti-inflammatory effect produced by prednisolone phosphate, 1.0 per cent ophthalmic solution, or dexamethasone alcohol, 0.1 per cent ophthalmic suspension. Overall, therefore, prednisolone acetate 1.0 per cent is the most effective of the topical agents studied for suppression of corneal inflammation.

Commercially available ophthalmic corticosteroid formulations differ in their ability to suppress corneal inflammation.1, 2 Among the preparations studied to date, prednisolone acetate, 1.0 per cent ophthalmic suspension, most effectively suppressed a corneal inflammatory response when the epithelium of the involved cornea was intact.2 However, variations in the differential solubility of these corticosteroids and the lipophilic nature of the corneal epithelial barrier raise the possibility that the relative anti-inflammatory effectiveness of these formulations might be different when the corneal epithelium is absent. The results of our investigation of this possibility are reported here.

Methods. With the exception of epithelial debridement prior to treatment, the methodology used in these experiments was identical to that reported previously.2, 4 Six commercial ophthalmic corticosteroid preparations were studied. They were (1) prednisolone acetate, 0.125 per cent ophthalmic suspension; (2) prednisolone acetate, 1.0 per cent ophthalmic suspension; (3) prednisolone phosphate, 0.125 per cent ophthalmic solution; (4) prednisolone phosphate, 1.0 per cent ophthalmic solution; (5) dexamethasone alcohol, 0.1 per cent ophthalmic suspension; and (6) dexamethasone phosphate, 0.1 per cent ophthalmic solution. The ophthalmic suspensions were placed in a shaker for 15 minutes immediately prior to ocular administration to ensure an even distribu-

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Results. The characteristics of the corneal inflammatory response produced by the interlamellar inoculation of clove oil have been described elsewhere. Each of the steroid preparations studied diminished this inflammatory activity in the cornea (as measured by a decrease in radiolabeled polymorphonuclear leukocytes) under at least some of the experimental conditions studied. In Group I animals prednisolone phosphate 0.125 per cent and dexamethasone phosphate 0.1 per cent did not produce significant inhibition of the initiation of the corneal inflammatory response. Each of the other drugs tested was effective. In Group II animals, where the therapeutic protocol was also designed to test each drug's ability to suppress the initial phases of the response to an inflammatory stimulus, all of the drugs tested demonstrated significant anti-inflammatory effects. In Group III animals, where a substantial inflammatory response was present when therapy was initiated, all of the drugs tested again produced a significant reduction in radiolabeled polymorphonuclear leukocytes infiltrating the cornea.

Among the prednisolone derivatives, prednisolone acetate 0.125 per cent was significantly more effective in reducing corneal inflammation than was prednisolone phosphate 0.125 per cent only during the first six hours of therapy (Group I); no significant difference could be demonstrated between the two preparations thereafter (Groups II and III). The identical pattern was observed at the higher 1.0 per cent concentration. In contrast, dexamethasone alcohol, 0.1 per cent ophthalmic suspension, demonstrated a significantly greater corneal anti-inflammatory effect than dexamethasone phosphate, 0.1 per cent ophthalmic solution, in all three experimental protocols. Comparison of the anti-inflammatory effectiveness of the four most potent compounds studied (prednisolone acetate, 1.0 per cent, prednisolone phosphate 1.0 per cent, dexamethasone alcohol 0.1 per cent, and dexamethasone phosphate 0.1 per cent) in the situation where the inflammatory response was most severe (Group III) indicated no significant difference among prednisolone acetate 1.0 per cent, prednisolone phosphate 1.0 per cent, and dexamethasone alcohol 0.1 per cent. However, all three of these compounds were significantly more effective than dexamethasone phosphate 0.1 per cent (p < 0.05).

An increase in the concentration of the acetate derivative of prednisolone from 0.125 per cent to 1.0 per cent resulted in a significant increase in the anti-inflammatory effectiveness of the drug (p < 0.05) in all three of the experimental protocols studied. The identical increase in concentration (0.125 per cent to 1.0 per cent) of prednisolone phosphate was accompanied by a significant increase in anti-inflammatory effectiveness only in Group I animals (where the lower concentration was ineffective). The data on anti-inflammatory effectiveness is summarized in Table I.

Topical application of each of the steroid preparations studied caused a reduction in the total white cell count per unit volume of peripheral blood and a simultaneous increase in the percentage of polymorphonuclear leukocytes. The net effect of these hematologic changes was an increase in the total number of polymorphonuclear leukocytes per unit volume of blood (Table II), presumably making a greater number of these cells available to participate in the corneal inflammatory response. The data presented in Table I were corrected to reflect these changes. The corrections assume a linear relationship between the total number of polymorphonuclear leukocytes per unit volume of blood and the total number of these cells available to invade the cornea. Correction of the data in this manner imparts a greater anti-inflammatory effectiveness to each preparation than do the uncorrected values from which the data were derived but does not alter the relative order of effectiveness among these drugs.

Discussion. The present studies demonstrate that modification of the derivative of a given steroid base alters its anti-inflammatory potential as measured by suppression of leukocyte invasion. Removal of the lipophilic corneal epithelium permits the water-soluble phosphate derivative of prednisolone to penetrate into the hydrophilic stroma in much higher concentration (16,538 µg-min/mm3) than the acetate (4574 µg-min/mm3). Nonetheless, the acetate produced a 53 per cent reduction in radiolabeled polymorphonuclear leukocytes invading the inflamed cornea while the phosphate reduced these cells by 47 per cent. (The difference is not statistically significant.) Thus, it required a 3.6 times greater tissue concentration of the phosphate derivative of prednisolone than of the acetate derivative to produce essentially the same effect, strongly suggesting a greater anti-inflammatory potency for the acetate. The evidence is even stronger relative to dexamethasone. Dexamethasone alcohol 0.1 per cent is a more effective anti-inflammatory agent in the cornea than is dexamethasone phosphate 0.1 per cent (both in the presence and in the absence of the epithelium) despite the fact that the phosphate achieves a higher concentration in the cornea in both instances.

Additional evidence that a change in the derivative of a steroid base alters its pharmacologic behavior as an anti-inflammatory agent can be obtained from a comparison of the data derived from the study of varying concentrations of the same steroid base. An increase in the concentration of prednisolone acetate from 0.125 per cent to 1.0 per cent produces a significant increase in both...
Table I. Mean decrease in corneal inflammatory activity following topical corticosteroid therapy

<table>
<thead>
<tr>
<th>Treatment protocol</th>
<th>Prednisolone acetate 0.125% suspension</th>
<th>Prednisolone acetate 1.0% suspension</th>
<th>Prednisolone phosphate 0.125% solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>21.2 ± 3.1, §</td>
<td>32.3 ± 3.6, §</td>
<td>6.1 ± 3.0, 1, §</td>
</tr>
<tr>
<td>(0.05 ml q1h × 6 immediately after induction of inflammation)</td>
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<tr>
<td>Group II</td>
<td>26.2 ± 4.11</td>
<td>46.6 ± 5.6</td>
<td>31.6 ± 6.1</td>
</tr>
<tr>
<td>(0.05 ml q1h × 6 immediately after induction of inflammation, then lapse of 18 hours, then repeat 0.05 ml q1h × 7)</td>
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<tr>
<td>Group III</td>
<td>29.7 ± 4.31</td>
<td>53.1 ± 6.2</td>
<td>42.3 ± 7.0</td>
</tr>
<tr>
<td>(0.05 ml q1h × 24 beginning 24 hours after induction of inflammation, then lapse of 18 hours, then 0.05 ml q1h × 7)</td>
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*Table entries are the arithmetic mean ± standard error of data derived from the study of 12 eyes (six rabbits). Values are control eyes (six rabbits).

†Indicates no significant difference from simultaneously run controls.

‡Indicates significant difference between 0.125 per cent and 1.0 per cent of the same derivative of prednisolone in the same treatment group.

§Indicates significant difference between acetate and phosphate prednisolone derivative of the same concentration in the same treatment group.

Table II. Percentage increase in blood polymorphonuclear leukocytes following topical administration of ophthalmic steroids

<table>
<thead>
<tr>
<th></th>
<th>Prednisolone acetate 0.125%</th>
<th>Prednisolone acetate 1.0%</th>
<th>Prednisolone phosphate 0.125%</th>
<th>Dexamethasone alcohol 0.1%</th>
<th>Dexamethasone phosphate 0.1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>32.0 ± 2.8</td>
<td>39.2 ± 3.4</td>
<td>35.1 ± 2.9</td>
<td>21.6 ± 3.0</td>
<td>24.8 ± 2.6</td>
</tr>
<tr>
<td>Group II</td>
<td>42.1 ± 4.1</td>
<td>47.8 ± 2.9</td>
<td>33.4 ± 3.8</td>
<td>45.6 ± 5.1</td>
<td>17.2 ± 3.0</td>
</tr>
<tr>
<td>Group III</td>
<td>43.6 ± 4.8</td>
<td>42.8 ± 4.1</td>
<td>42.2 ± 4.0</td>
<td>49.4 ± 3.1</td>
<td>36.2 ± 2.7</td>
</tr>
</tbody>
</table>

*Table entries are the arithmetic mean ± standard error of the mean of data derived from the study of at least six animals.

The corneal concentration of the drug and in its anti-inflammatory effectiveness, irrespective of whether the epithelium is intact or absent. Under the same conditions the identical increase in prednisolone phosphate concentration results only in an increase in the quantity of drug measurable in the cornea. No comparable increase in the ability of the phosphate derivative to suppress polymorphonuclear leukocytic infiltration of the cornea could be documented. Clearly, the two derivatives are not equivalent in their anti-inflammatory properties.

The phosphate derivative of both of the steroid bases studied (dexamethasone and prednisolone) appears to be a less effective anti-inflammatory agent following topical administration to the eye than other derivatives of the same steroid base. The reason for this is obscure. However, in several instances the phosphate produces a higher corneal concentration than other derivatives of the same steroid base. Since toxicity generally increases with the amount of drug present, the greater tissue concentration produced by the phosphate derivative in these circumstances may represent a greater toxic potential despite its relative ineffectiveness.

When the epithelium of the inflamed cornea is intact, prednisolone acetate, 1.0 per cent ophthalmic suspension, is the most effective of the corticosteroid preparations studied to date, and its anti-inflammatory superiority can be verified statistically. In the absence of an intact epithelium, prednisolone acetate, 1.0 per cent ophthalmic suspension, again produces the greatest mean reduction in polymorphonuclear leukocytic infiltration of the cornea, although here one cannot demonstrate a statistically significant difference from the anti-inflammatory effect produced by prednisolone phosphate, 1.0 per cent ophthalmic suspension, or dexamethasone alcohol, 0.1 per cent ophthalmic suspension. Overall, therefore, prednisolone acetate 1.0 per cent is the most effective of the agents studied for suppression of corneal inflammation.

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<table>
<thead>
<tr>
<th>Prednisolone phosphate 10% solution</th>
<th>Dexamethasone alcohol 0.1% suspension</th>
<th>Dexamethasone phosphate 0.1% solution</th>
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<tbody>
<tr>
<td>18.2 ± 7.1</td>
<td>30.9 ± 3.8</td>
<td>3.8 ± 2.21</td>
</tr>
<tr>
<td>33.1 ± 7.2</td>
<td>30.0 ± 6.1</td>
<td>14.1 ± 4.1</td>
</tr>
<tr>
<td>46.6 ± 8.1</td>
<td>41.8 ± 7.1</td>
<td>22.4 ± 6.1</td>
</tr>
</tbody>
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expressed as percent difference from the mean of 12 untreated treatment protocol (p < 0.05).

treatment protocol (p < 0.05).

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Key words: cornea, corneal inflammation, corticosteroid, steroid, prednisolone, dexamethasone, polymorphonuclear leukocytes, cornea.

REFERENCES


The effect of tear substitutes on tear film break-up time. MICHAEL A. LEMP, MICHAEL GOLDBERG, AND MARGARET R. ROODY.

Twelve commercial artificial tear solutions and a newly developed one were evaluated as to their effect on tear film breakup time (BUT) in ten normal subjects. Instillation of one drop of these solutions altered the BUT in such a way that serial BUT measurements could be used as an index of retention time. Results demonstrated significantly longer retention time for three related products (Adapt, Adaptec, and Adsortate) and a newly developed product (Alcon 0413 [Tears Naturale (Alcon T*)]). This method appears to be an accurate nonirritative way of assessing retention time of tear substitute/vehicles and demonstrate values much longer than previously reported by other methods.

Artificial tear solutions are used as replacement therapy in dry eye states: virtually identical formulations form the vehicles for the delivery of locally instilled medications to the eye. These solutions contain water-soluble polymers incorporated with the intent of prolonging retention in the conjunctival sac. In practice, however, their efficacy is limited by their short duration. Studies attempting to assess their stay in the conjunctival sac (retention time, contact time) have employed visible markers, e.g., argyrol, nickel chloride, and have shown retention times of about 3 to 10 minutes; alternatively, excretion of instilled solutions through the nasolacrinial duct has been shown. Other studies have measured intracorneal penetration of dyes and uptake of radioactive substances, but are only an indirect indication of relative efficacy of different solutions in facilitating incorporation of substances into the cornea.

If normal blinking is prevented, the precorneal tear film will break up, i.e., develop random dry spots. The interval between the last complete blink and the appearance of the first dry spot—breakup time (BUT)—has been found to be abnormally rapid in dry eye states. This is a reflection of decreased tear film stability. As part of a larger study of BUT in normal subjects, it was noted that after instillation of an artificial