Effect of hydrocortisone on the mobilisation of leucocytes in corneal wounds

P. K. BASU, M. AVARIA, AND R. JANKIE
From the Department of Ophthalmology, University of Toronto, Canada

SUMMARY We have studied in rabbits the effect of subconjunctivally injected hydrocortisone on the polymorphonuclear leucocyte invasion of corneal wounds at different times after an injury. One group of rabbits was treated with the steroid (hydrocortisone group) and the other not (control group). After making nonpenetrating trephine incisions on the cornea we obtained cellular samples by the impression technique at a given postoperative period (2, 4, or 6 hours), and then the animal was killed. The cornea was processed for histological study of the infiltrating cells. At any postoperative period the number of polymorphonuclear leucocytes in the corneal wounds of the hydrocortisone group was significantly less than the number in the identical wounds of the control group (p<0.01 to 0.001).

Glucocorticosteroids are frequently applied locally to reduce inflammatory reactions of the eye. However, the exact mechanisms by which they act as a local anti-inflammatory agent have not yet been fully elucidated. We wished to study the effect of subconjunctivally injected hydrocortisone on the kinetics of the polymorphonuclear cells (PMNs) migrating into corneal wounds during the early cellular phase of inflammation.1

Materials and methods

Thirty-six albino male New Zealand rabbits (4 to 5 kg) with normal eyes were divided into 2 equal groups, (1) the control group, and (2) the hydrocortisone group. The animals were anaesthetised intravenously with sodium pentobarbital (Nembutal, Abbot Laboratories Ltd, Montreal, Quebec, 30 mg/kg). The animals of the hydrocortisone group were injected in each eye subconjunctivally with 0.25 ml of a 2.5% solution of hydrocortisone sodium succinate (Sigma Chemical Company, St Louis, Mo, USA). The site of injection was in the 12 o'clock meridian, about 5 mm from the limbus. On each cornea of the 36 animals one central and one peripheral nonpenetrating circular incision (4 mm in diameter and 0.1 mm in depth) were made with a Castroviejo corneal trephine (Storz Instrument Company, St Louis, Mo, USA). The centre of the peripheral incision was placed 5 mm away from the limbus—the right being placed on the 10.30 o'clock meridian and the left on the 1.30 o'clock meridian. The injured portions of the cornea were kept covered by the eye lids.

After 2, 4, or 6 hours following the corneal trephination cellular samples were collected from the traumatic lesions (6 animals being used for each period in each group) by gently pressing a plastic cover-slip (Thermanox, cat. no. 5408, lot no. 128324, Lux Scientific Corp., Newbury Park, California, USA) over the entire trephine wound (impression technique).2 The materials attached to the cover-slip were dried and stained with a rapidly acting stain similar to Wright-Giemsa stain (Diff-Quick, Harleco, Gibbstown, NJ, USA).2 The PMNs were then counted under a microscope (fitted with a grid for counting purposes) in a total surface area of 1.00 square mm per cover-slip from at least 4 randomly selected sites under a 100 times magnification (Fig. 1a). Immediately after the cover-slip samples were taken each animal was killed with an overdose of pentobarbital (120–250 mg/kg). Both corneas with a scleral rim were excised from each rabbit, fixed with 10% formalin, sectioned in paraffin, and stained with Giemsa stain. Under 450 times magnification the number of PMNs were counted in a total area of 1.00 square mm per section (including both sides of each
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Fig. 1a Cellular impression on a plastic cover-slip (Thermanox) and stained with Diff-Quick. (× 31). Insert shows cells at a higher magnification. (× 464).

corneal incision) from at least 4 different corneal sections, 50–100 μm apart (Fig. 1b).

Blood samples of the living animals treated and not treated with steroid were taken for total and differential blood counts.

Results

With regard to the PMN response the 2 eyes of an animal in each group behaved similarly. In each group the numbers of PMNs in the 2 central or 2 peripheral lesions in each rabbit were similar. In both groups the number of PMNs in the peripheral lesions in any animal was higher than that in the central lesions, irrespective of the sampling time or sampling technique.

The data for the central or peripheral lesions of both groups of animals with respect to a given

Fig. 1b Histology of a corneal wound showing leucocyte infiltration in the stroma Giemsa stain. (× 143). Insert shows cells at a higher magnification. (× 468).
Table 1. Comparison of leucocyte counts in the central and peripheral corneal wounds in the control and steroid-treated groups by the impression technique

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Control group lesion</th>
<th>Hydrocortisone group lesion</th>
<th>Significance of difference*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Central</td>
<td>Peripheral</td>
<td>Central</td>
</tr>
<tr>
<td>2 hr</td>
<td>177±7±17-1 (b)</td>
<td>139±9±6 (c)</td>
<td>44±3±7-9 (d)</td>
</tr>
<tr>
<td></td>
<td>302±2±29-3 (a+b)</td>
<td>83±6±17-5 (c+d)</td>
<td>166±8±24-9 (h)</td>
</tr>
<tr>
<td>4 hr</td>
<td>143±7±9±1 (e)</td>
<td>107±6±10±1 (g)</td>
<td>274±4±35±0 (g+h)</td>
</tr>
<tr>
<td></td>
<td>392±1±17-6 (e+f)</td>
<td>274±4±35±0 (g+h)</td>
<td>202±9±7±4 (f)</td>
</tr>
<tr>
<td>6 hr</td>
<td>405±2±11±9 (i)</td>
<td>131±2±7±2 (k)</td>
<td>334±1±14±6 (k+l)</td>
</tr>
<tr>
<td></td>
<td>919±5±31±6 (i+j)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mean±SE per sq mm. n=6. *Paired t test.

The number of cells counted by the impression and histological techniques ran parallel. The number of PMNs found in the centrally situated lesions of the control group were higher than the number of PMNs found in the centrally situated lesions of the hydrocortisone group. The number of PMNs in the lesions situated peripherally in both groups varied in a similar fashion. Consequently the total number of PMNs in the corneal lesions of the control group were significantly higher than that of the hydrocortisone group (impression technique, p<0.01 to 0.001; histology, p<0.01 to 0.001).

Our data thus showed that hydrocortisone injected subconjunctivally could significantly reduce the migration of polymorphonuclear cells in both the central and peripheral nonpenetrating incised corneal wounds, at least during the first 6 hours following the injury in rabbits. As shown in Figs. 2a and 2b, in contrast to the control group, in the hydrocortisone group the PMNs tended to remain within the lumen of the blood vessel, resulting in fewer PMNs in the extravascular tissue.

With regard to the total and differential blood counts there was no difference between the steroid treated and untreated groups.

Table 2. Comparison of leucocyte counts in the central and peripheral corneal wounds in the control and steroid-treated groups by the histological technique

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Control group lesion</th>
<th>Hydrocortisone group lesion</th>
<th>Significance of difference*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Central</td>
<td>Peripheral</td>
<td>Central</td>
</tr>
<tr>
<td>2 hr</td>
<td>74±2±30±0 (b)</td>
<td>11±0±3-1 (c)</td>
<td>24±4±5-1 (d)</td>
</tr>
<tr>
<td></td>
<td>107±7±4-9 (a+b)</td>
<td>35±4±8±2 (c+d)</td>
<td>45±1±3-5 (h)</td>
</tr>
<tr>
<td>4 hr</td>
<td>86±7±4-5 (f)</td>
<td>33±8±1-1 (g)</td>
<td>78±9±4-6 (g+h)</td>
</tr>
<tr>
<td></td>
<td>139±6±6±4 (e+f)</td>
<td>45±3±2-2 (k)</td>
<td>134±0±5±4 (k+l)</td>
</tr>
<tr>
<td>6 hr</td>
<td>86±6±1±4 (i)</td>
<td>45±3±2-2 (k)</td>
<td>134±0±5±4 (k+l)</td>
</tr>
<tr>
<td></td>
<td>231±5±5±6 (i+j)</td>
<td></td>
<td></td>
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</tbody>
</table>

*Mean±SE per sq mm. n=6. *Paired t test.

Discussion

Despite the fact that the anti-inflammatory effects of glucocorticosteroids have been well known for many years the exact mechanisms of the action is still not completely understood. Granulocytic reaction to an acute inflammatory stimulus entails a series of events, including production and release of these cells by the bone marrow, their delivery into the circulatory system, their adherence to the vascular endothelium at the stimulus site followed by diapedesis into the extravascular compartment, directional movement of the cells following a chemotactic gradient, and finally phagocytosis of offending agents by them. Ketchel and coworkers observed that certain steroids can affect the amoeboid movement of leucocytes. Ward suggested that corticosteroids by acting directly on the leucocytes can render them incapable of responding to chemotactic stimuli. He also indicated that a certain chemotactic factor of serum (an activated protein—protein complex of the fifth and sixth components of complement) is suppressed by hydrocortisone and methyl prednisolone. Gewurz et al. also demonstrated inhibition of serum complement by hydrocortisone. Cline and Melmon showed that hydrocortisone can inhibit kinins having leukotactic properties. Besides these the anti-
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Fig. 2a Histology of control group, 4 hours. The PMNs appear to emigrate freely out of the lumen of a limbal blood vessel and numerous leucocytes are seen in the tissue surrounding the blood vessel. (Giemsa stain, × 375.)

2b Histology of hydrocortisone group, 4 hours. The PMNs appear to stay within the lumen of a limbal blood vessel. There are fewer leucocytes in the extravascular tissue. (Giemsa stain, × 375.)

Inflammatory effects of hydrocortisone have also been related to other factors such as the inhibition of oedema, fibrin deposition, and capillary dilatation.5

In our investigation we were interested in studying the effect of hydrocortisone on the quantitative mobilisation of PMNs into corneal wounds. The selection of the impression technique in our work was based on the fact that it is a fast and easy way to obtain cells from corneal wounds.2 The histological data confirmed the results obtained by the impression technique.

The cornea is an avascular tissue, and haematological cells are rarely seen in the normal cornea. Hence it offers an excellent medium for studying the sequences of leucocyte migration.1 12 The PMNs observed in the corneal wounds came from the limbal and conjunctival blood vessels, either through the corneal tissue peripheral to the wound (intracorneal route) or via the tear film13 (extracorneal route) or both.14 Histological studies showed that the PMNs found in the corneal wounds in the first few hours came predominantly via the extracorneal route. Later both routes were involved. Hydrocortisone reduced the invasion of the corneal wounds by the PMNs during all periods up to 6 hours.

How the locally administered steroid reduces the PMN infiltration of the cornea is at present a matter of speculation. The dose of the steroid given conjunctivally was small. Hence it is unlikely that the subconjunctivally injected steroid had any significant effect on the release of granulocytes from the bone marrow, their delivery in the vascular system, and their shift from the marginal to the circulatory compartment.4 In fact we found that the steroid-treated and untreated animals had identical total and differential blood counts at all times. Our study showed that at least in the early stage of the corneal inflammatory reaction (up to 6 hours) hydrocortisone given locally inhibited the passage of PMNs from the intravascular to the extravascular compartment (Figs. 2a and 2b). The steroid might have brought this about locally by affecting one or more of the following factors: adherence of granulocytes to the vascular endothelium, their diapedesis into the extravascular space, and their chemotaxis.4 Other local factors as mentioned by Gilman et al.3 may also be involved. Further work is necessary to isolate and characterise these factors with respect to the PMN invasion of the eye tissues.

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References
P. K. Basu, M. Avaria, and R. Jankie