The effect of corticosteroids on the resorption of partial hyphema in rabbit eyes

Steven M. Podos, Louis H. Fingerman, and Bernard Becker

Partial hyphemas were created in corticosteroid-treated and untreated rabbit eyes. The disappearance of the hyphema was complete within 48 hours in control animals. Resorption was delayed for 2 to 5 days in treated eyes. Pretreatment with corticosteroids was necessary for the altered resorption rate. Tonograms demonstrated no significant difference between the two groups. Systemic chloroquine was found to have a similar effect to steroids. Possible mechanisms for the above findings are discussed.

Corticosteroid therapy has been reported to delay erythrocyte resorption from the anterior chamber of rabbit eyes. These studies involved the use of systemic corticosteroids and coagulated blood. End points of complete resolution were prolonged to 3 or 4 weeks. In the following experiments topical corticosteroids and heparinized blood were used so as to delineate more sharply the effects of steroids on hyphema resorption. In addition, a possible relationship was sought to corticosteroid-induced glaucoma.

Materials and methods

Production of hyphema. One-half milliliter of autologous blood was withdrawn from the ear veins of 1 to 2 kilogram male New Zealand albino rabbits into heparin-coated disposable 25 gauge needle syringes. Intravenous barbiturate and topical anesthesia preceded the paracentesis and withdrawal of about 100 μl of aqueous humor. The blood was injected through the same needle tract in amounts to replace the withdrawn aqueous and to create a hyphema occupying 50 per cent of the anterior chamber. The same procedure was used in all control and treated animals.

Treatment. The created hyphemas were treated with various doses and under varying dosage schedules of each of the following: topical and parenteral betamethasone (Celestone ophthalmic drops provided by Schering Corporation), topical triamcinolone acetonide hemisuccinate (Aristo-drops provided by Lederle Laboratories), subconjunctival depot methylprednisolone acetate (Depomedrol and vehicle provided by the Upjohn Company), and intramuscular chloroquine (Aralen provided by Winthrop Laboratories). The betamethasone vehicle* was employed topically in otherwise untreated eyes (vehicle control) and in the betamethasone group of fellow eyes (the other eye of an animal treated in one eye). The fellow eyes of the methylprednisolone series received its vehicle† subconjunctivally. Treatment

*The betamethasone vehicle was composed of 10 per cent sodium sulfacetamide in methylcellulose.
†The methylprednisolone vehicle was composed of 30 mg. of polyethylene glycol, 9 mg. of sodium chloride, and 0.3 mg. of myristyl gamma picolinium chloride per milliliter.
schedules and doses are defined on the various figures. Systemic administration involved daily injections. Subconjunctival therapy was performed with one dose of depot medication 2 days prior to hyphema creation. Topical treatment was varied (b. i. d., q. d. refers to pretreatment twice a day followed by drops once a day after the creation of hyphema).

Follow-up. All hyphemas were measured (to the nearest 0.5 mm.) with a millimeter rule held perpendicular to the limbus at 6 o'clock. Graphs of mean millimeters of blood versus day post hyphema are presented. A statistical analysis of the results at 36 hours after hyphema creation was carried out (Table 1).

Plasmin assay. A fibrin plate assay as described by Astrup and modified by Fletcher was employed.

Miscellaneous. Tonography was performed on restrained animals with topical tetracaine anesthesia only. Pathologic studies were carried out on a representative sample of eyes.

Results

Topical therapy. Rabbits treated in both eyes with the vehicle alone had complete hyphema resolution in 48 hours. When rabbits were pretreated for 2 days and treated twice a day in one eye with 0.1 per cent betamethasone, the hyphema produced took 5 to 8 days to disappear. The fellow eye also demonstrated a delay of resorption of blood (Fig. 1). At 36 hours the mean hyphema level in eyes treated with topical betamethasone was 1.9 mm. as compared to 0.25 mm. in the eyes of control animals. This proved to be statistically significant (P < 0.01). There was no significant difference between fellow and treated eyes (Table 1).

Pretreatment for 2 days was necessary in order to demonstrate the above effect (Fig.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of eyes</th>
<th>Hyphema level (mm.) (mean ± S.E.)</th>
<th>P₁</th>
<th>P₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>0.25 ± 0.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Betamethasone (topical, pretreated)</td>
<td>10</td>
<td>1.9 ± 0.40</td>
<td>&lt; 0.01</td>
<td>&gt; 0.8</td>
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<tr>
<td>Fellow eye</td>
<td>5</td>
<td>2.0 ± 0.25</td>
<td>&lt; 0.01</td>
<td></td>
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<tr>
<td>Betamethasone (topical, not pretreated)</td>
<td>5</td>
<td>0.3 ± 0.21</td>
<td>&gt; 0.8</td>
<td>&gt; 0.4</td>
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<tr>
<td>Fellow eye</td>
<td>5</td>
<td>0.6 ± 0.26</td>
<td>&gt; 0.2</td>
<td></td>
</tr>
<tr>
<td>Triamcinolone (topical, low dose)</td>
<td>5</td>
<td>0.3 ± 0.27</td>
<td>&gt; 0.8</td>
<td>&gt; 0.8</td>
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<tr>
<td>Fellow eye</td>
<td>5</td>
<td>0.4 ± 0.26</td>
<td>&gt; 0.6</td>
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<tr>
<td>Triamcinolone (topical, high dose)</td>
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<td>2.0 ± 0.56</td>
<td>&lt; 0.02</td>
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<tr>
<td>Fellow eye</td>
<td>5</td>
<td>1.0 ± 0.40</td>
<td>&gt; 0.1</td>
<td></td>
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<tr>
<td>Betamethasone (intramuscular, pretreated)</td>
<td>8</td>
<td>1.8 ± 0.35</td>
<td>&lt; 0.01</td>
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<td>Methylprednisolone (subconjunctival, pretreated)</td>
<td>9</td>
<td>2.8 ± 0.55</td>
<td>&lt; 0.01</td>
<td>&lt; 0.1</td>
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<tr>
<td>Fellow eye (vehicle, subconjunctival)</td>
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<td>1.3 ± 0.40</td>
<td>&lt; 0.05</td>
<td></td>
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<tr>
<td>Chloroquine (intramuscular)</td>
<td>8</td>
<td>2.0 ± 0.38</td>
<td>&lt; 0.01</td>
<td></td>
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</tbody>
</table>

S.E., standard error of the mean.
P₁, significance of difference in means between the treated group and the control group at 36 hours.
P₂, significance of difference in means between the treated group and the fellow eye group at 36 hours.

At 36 hours the mean level of blood in the two triamcinolone dosage groups was different with a P < 0.05.
TOPICAL BETAMETHASONE, PRETREATED

0.1% Betamethasone b.i.d.
- Treated (10)
- Fellow (5)
- Vehicle Control (12)

Fig. 1. The mean blood level (measured in millimeters with a millimeter rule held perpendicular to the limbus at 6 o'clock) is graphed at various time intervals (days) for 12 vehicle control, 10 0.1 per cent betamethasone, and 5 fellow eyes pretreated 2 days and treated topically twice a day for the course of the experiment. Resorption of blood was delayed in steroid-treated eyes as compared to the control eyes.

TOPICAL BETAMETHASONE, NOT PRETREATED

0.1% Betamethasone b.i.d.
- Treated (5)
- Fellow (5)
- Vehicle Control (12)

Fig. 2. The mean blood level (measured in millimeters with a millimeter rule held perpendicular to the limbus at 6 o'clock) is graphed at various time intervals (days) for 5 0.1 per cent betamethasone and 5 fellow eyes treated topically twice a day but not treated prior to the production of hyphema. Hyphemas in eyes not pretreated resolved at a rate very similar to that of the vehicle control eyes.

2). At 36 hours the nonpretreated topical betamethasone group was not significantly different from the vehicle control group (P > 0.8).

Triamcinolone, 0.1 per cent, used topically twice a day during pretreatment for 2 days and thereafter once a day for the course of the experiment, produced a delaying effect on blood resorption. If the dose schedule for triamcinolone was reduced to the steroid given once a day for 2 days of pretreatment and thereafter, there was a much reduced response as compared to the greater dose schedule (P < 0.05) (Fig. 3). At 36 hours only the higher dose results were significantly different from those of the control group (Table I).

Other routes of administration. Betamethasone, 0.2 mg., given intramuscularly each day of the experiment, with 2 days of pretreatment, delayed blood resorption from the anterior chamber (Fig. 4).

A delay in the disappearance of hyphema also resulted from one dose of depot methylprednisolone, 4 mg., given subconjunctivally 2 days prior to the creation of hyphema (Fig. 5). The blood levels of both of the above showed a significant difference (P < 0.01) when compared to controls at 36 hours (Table I). The greatest difference between steroid-treated and fellow eyes (P < 0.1) was in the subconjunctival methylprednisolone series (Fig. 5).

Chloroquine. Parenteral chloroquine dihydrochloride given in doses of 50 mg. daily with pretreatment for 2 days was effective in delaying hyphema resolution (Fig. 6). These results were significantly different (P < 0.01) from the controls at 36 hours.

Plasmin studies. No difference was found in the amount of plasmin activation in corneal and scleral buttons or in aqueous samples taken from steroid and vehicle treated eyes.

Tonographic studies. The average pressure (Pp) of 8 control eyes was 16.1 ± 0.7 mm. Hg, with an average outflow facility (C) of 0.28 ± 0.2; 4 topical betamethasone
Resorption of partial hyphema

Fig. 3. The mean blood level (measured in millimeters with a millimeter rule held perpendicular to the limbus at 6 o'clock) is graphed at various time intervals (days) for 0.1 per cent triamcinolone and fellow eyes treated topically with varying schedules before for 2 days and after hyphema creation. The annotations are described in procedure. The number of eyes in each group is in parentheses. The effect of this steroid on hyphema resolution seems to be dose responsive.

eyes P, 17.8 ± 0.5, C 0.28 ± 0.01; 4 fellow eyes P, 16.8 ± 0.7, C 0.29 ± 0.01. All the above measurements were made on the third day after hyphema creation. There was no significant difference between the mean pressures of treated and control eyes (P > 0.1).

Pathology. Massive amounts of erythrocytes were seen in the chamber angle of all animals at 24 to 48 hours after hyphema creation, with little difference between groups.

Discussion

Studies show that while heparinized blood leaves the anterior chamber of rabbit eyes in as few as 12 hours, it takes clotted blood 3 weeks to disappear. The effect of steroids on this process is more discretely elucidated if one uses anticoagulated blood. The data presented illustrate a decided delaying effect of corticosteroids on blood resorption from the chamber of rabbit eyes. This seems to be dose responsive. It occurred no matter what route of administration was used. The fellow eye of uniocularly treated rabbits also responded, indicating probable systemic absorption of corticosteroid. A strict uniocular response as seen in steroid-induced glaucoma in human eyes could not be produced. Subconjunctival injection produced the greatest difference between treated and fellow eyes. In our data there is a bias. The difference between the 1 mm. and 3 mm. level of hyphema is greater if one converts the linear measurement into an equivalent anterior chamber volume. Thus, our graphs underestimate the true difference. Pretreatment was necessary, however, as in human steroid-induced glaucoma. Because of the longer course of prior experiments on coagulated blood, there undoubtedly was time for a steroid effect to manifest itself without pretreatment as compared to our studies.

Glaucoma does produce a reduced resorption rate for hyphema. No tonographic evidence of glaucoma was found in our animals. The average tension was about 1 mm. Hg higher in treated than in untreated animals, but this difference was
not statistically significant in this small series. No changes in outflow facility were noted.

The mechanisms for steroid-induced glaucoma in humans and delayed hyphema resolution in rabbits are both unknown but may be related. One must note here that the blood of steroid-treated animals, when injected into untreated eyes, resorbed quickly, while the blood of untreated animals injected into pretreated eyes was delayed in resorption. Thus, the steroid effect is probably not on the blood but on the eye itself. Moreover, the majority of heparinized red blood cells has been shown to leave the anterior chamber intact, by way of the rabbit chamber angle. This would suggest that a steroid-induced macrophage inhibition is not the explanation for the delayed resorption of blood. A mechanical block of exit pores is an attractive hypothesis to explain the delay of red blood cell egress. Neither light microscopic studies of these eyes nor tonography provided evidence to support this hypothesis. Steroid inhibition of plasmin activation with resulting fibrin accumulation could not be demonstrated. Much fibrin was seen by slit-lamp examination in the anterior chamber of all paracentesed eyes, but there was no difference between steroid-treated and untreated specimens. Another possibility centers upon mucopolysaccharides. This component of outflow channels could accumulate to block exit routes. On the other hand, the well-documented steroid inhibition of mucopolysaccharide synthesis could result in meshwork collapse. Either mechanism could cause mechanical block. Again, no changes in outflow facility were demonstrable by tonography. Pomerantz has described the prolonged collapse of ears with parenteral papain treatment in corticosteroid-treated rabbits. Purportedly, papain lyases mucopolysaccharide and steroids prevent its synthesis. Chloroquine, too, prolonged reconstitution of the floppy ears. Similarly, parenteral chloroquine was found to mimic steroid delay of hyphema resorption.

Preliminary experiments indicate that 11-desoxycorticosterone acetate (DOCA), 1 per cent given topically three times a day with 2 days of pretreatment, does not delay hyphema resolution. Although it has been demonstrated to enter the anterior
chamber fluids when administered topically to rabbit or human eyes, DOCA does not produce pressure elevation even in human eyes known to develop pressure elevations on betamethasone. However, in vitro, 11-desoxycorticosterone does inhibit mucopolysaccharide sulfation, as do corticosteroids and chloroquine.

Other anti-inflammatory and steroid components that affect mucopolysaccharide and fibrin metabolism are currently being investigated for their effect on hyphema resorption. Electron microscopic studies are also in progress.

REFERENCES
5. Fletcher, A.: Personal communication.

Discussion
Emily D. Maloney, Gainesville, Fla. The paper by Drs. Podos, Fingerman, and Becker is extremely interesting and appears clearly to document a corticosteroid effect on the resorption of blood from the anterior chamber. Any criticism of such a good paper would be minimal, but a few factors deserve comment. For example, it is difficult for us to measure substances in the anterior chamber with precision. When we know, or have a preconceived idea, of what can be expected, unconscious bias may slightly exaggerate the results and direction of the expected results. This has certainly been a factor in experiments from some laboratories, and, although probably not important in this case, it might be a little bit more precise to have made all measurements of residual hyphema on a double-blind basis not knowing, whenever possible, which eyes were treated and which were not. It is clear that if bias is unconsciously introduced, statistical analysis would not eliminate it.

It is difficult to understand the apparent steroid effect on the second eye after topical or subconjunctival administration. Although it may be that corticosteroids were absorbed from topical administration, the finding by these authors in their own studies of, what appears to be, an appreciable dose-effect suggests that even if some systemic absorption occurred, the concentration differences between the two eyes should be enormous. In fact, it is difficult to believe that enough systemic absorption could occur to affect the other eye with a concentration anywhere near the same order of magnitude as that administered to the treated eye. This observation in the other eye suggests that some artifact may have been induced in study, but it is difficult for us to be certain of its nature.

We would be interested in the authors’ comments on the possibility that heparin itself may have had some local anti-inflammatory effect when used in concentrations sufficient to anticoagulate effectively the blood. Similarly, if the effect of corticosteroid is on the eye rather than the blood, why should pretreatment be required? Perhaps the observed effect is to reduce the amount of plasmoid aqueous formed by anterior chamber aspiration and thus decrease the blockage of the trabeculum by fibrin. If so, there might be no direct effect on hyphema absorption or on the trabeculum, but rather some effect on the preceding “traumatic iritis.”

This is an excellent study. We hope that further clarification of the corticosteroid effect will be sought.

Reply to Dr. Maloney. Although we felt that the measurement of our results was clear-cut, their
validity certainly would be enhanced if a double-controlled protocol had been employed. This criticism is so well taken that the experiment utilizing topical betamethasone was repeated in such a fashion. The results were verified. A significant difference between control and treated eyes at the 1 per cent level was found at 36 hours. Resorption curves were similar to those aforementioned. The fellow eyes were less significantly different from controls. In some of the fellow eyes, hyphema resorption was delayed in a range similar to that of the treated eyes.

It is difficult to explain the fellow eye effect. Systemic absorption with penetration into an inflamed eye in which the blood aqueous barrier has broken down can account for an effect, especially where high doses of potent glucocorticoids have been used. However, if one postulates systemic absorption, less should get into the fellow eye. At some lower dose the treated eye alone should react. We could not demonstrate this, although there was a differential between treated and fellow eyes. There are two other possibilities upon which one could speculate. The active principle could be produced by systemically absorbed steroid acting on an end organ. Here, locally acting drug would not be important and both eyes equally affected. Second, there is some evidence of a direct route from one eye to another.

Heparin did markedly reduce fibrin formation in animal eyes that we studied by slit-lamp microscopy after saline paracentesis. This must be a factor in the quicker hyphema resolution rates seen in both our treated and untreated eyes as compared to prior studies with clotted blood.

Pretreatment may be necessary to yield higher systemic levels, to inhibit or induce enzymes responsible for the delay effect, or to inhibit enough polysaccharide synthesis to affect the meshwork. The steroid effect could be one of anti-inflammation with decreasing vessel permeability. It could relate to a fibrin-filled anterior chamber blocking erythrocyte movement.

Many of the discussers provocative questions are currently being investigated.