Dexamethasone inhibition of experimental endothelial cell proliferation in retinal venules. JOHN T. HEFFERNAN, SIDNEY FUTTERMAN, AND ROBERT E. KALINA.

The observation that endothelial cell proliferation in retinal blood vessels is induced by ocular trauma in rats has been extended to mice. Indomethacin, 10 mg/kg/day, failed to block incorporation of tritiated thymidine into nuclei of venular endothelial cells in rat retinas observed 40 hr after puncturing the lens, but dexamethasone effectively suppressed tritiated thymidine incorporation, with 50% inhibition obtained with 0.2 mg/kg/day. The prostaglandin pathway does not appear essential to the activation of endothelial cell proliferation in this system.

Needle puncture of the lens in rats has been found to produce an increase of 30- to 70-fold in the frequency of tritiated thymidine labeling by retinal venular endothelial cells. Thymidine incorporation peaked 40 hr after ocular trauma. At the same time, an inflammatory process was observed, characterized by the presence of marginated leukocytes within the lumina of retinal vessels.1

Experimental ocular inflammatory processes, their relationship to prostaglandin biosynthesis and leukocytic infiltration, and their suppression by anti-inflammatory agents have been extensively studied.2-5 The present studies were carried out to determine whether the stimulation of endothelial cell proliferation, a presumptive early step in neovascularization of the retina, was unique to the rat and possibly prostaglandin dependent.

Methods. The retinas of 30 200 gm male Sprague-Dawley rats were exposed to tritiated thymidine, specific activity 20 Ci/mmol (New England Nuclear Corp. Boston, Mass.), by injecting 3 μCi of tritiated thymidine through the pars plana into the vitreous of each eye; subsequently the retinas were digested with trypsin, and the resulting vascular networks were exposed to autoradiographic emulsion, developed, and counterstained with hematoxylin so that labeled cell nuclei could be observed and counted. The techniques for intravitreal injection, trypsin digestion of the retina, and autoradiography have been described previously.1

Labeling of the retinal vasculature was examined in a control group of eight 30 gm male CBA mice after exposure to tritiated thymidine, 10 μCi/gm administered intraperitoneally, and in an experimental group of eight mice previously subjected to ocular trauma.

References
Fig. 1A. Tritium labeling of cell nuclei of retinal vessels of mouse when retinas were isolated 1 hr following administration of tritiated thymidine 40 hr after lens puncture. (×100.)

Ocular trauma, characterized by capsular and cortical lens damage, was produced by passing a 30-gauge needle through the sclera 2 mm posterior to the corneal-scleral limbus and into the lenses of ether-anesthetized rats and mice. Tritiated thymidine was administered 40 hr following injury of the lens, and the animals were sacrificed 1 hr later.

Groups of rats received anti-inflammatory agents as follows. (1) Indomethacin, 5 mg/kg, was administered intraperitoneally twice daily as a suspension in 0.25 ml of 1% carboxymethyl cellulose, starting 8 hr before puncturing the lens and continuing throughout the experimental period. (2) Dexamethasone, 0.4 mg/kg, was administered as above. (3) Control groups received no injury or treatment, injury with no treatment, or injury with 0.25 ml of 1% carboxymethyl cellulose solution injected as above.

In a second experiment, dexamethasone in varying amounts was administered in 0.25 ml of carboxymethyl cellulose.

Results

Thymidine uptake by mouse retinal venules. The pattern of tritiated thymidine uptake and labeling of cells in the vasculature of mouse retinas obtained 40 hr after lens puncture (Fig. 1A) appeared to be identical in distribution and frequency to that observed in the rat (Fig. 1B). Labeling occurred primarily in postcapillary venules. Polymorphonuclear leukocytes could be observed within the lumina of veins in the preparations from mice previously subjected to lens puncture. In control mice the number of labeled venular endothelial cells counted was 0.57 ± 0.34 per thousand cells counted, whereas following lens puncture 59.5 ± 15.4 labeled cells were present.

Effect of anti-inflammatory agents. The anticipated increase in uptake of tritiated thymidine into venular endothelial cell nuclei of rats following ocular trauma was observed (Table I). The reductions in cell labeling found for the carboxymethyl cellulose and indomethacin-treated groups were not statistically significant (p > 0.05). However, dexamethasone treatment reduced cell labeling to the level generally observed for control rats that had not been subjected to ocular trauma (p < 0.01).

The capacity of dexamethasone to block the ef-
Fig. 1B. Tritium labeling of cell nuclei of retinal vessels of rat under same conditions as for Fig. 1A. (×100.)

Effect of ocular trauma was dose dependent (Fig. 2), with a half-maximal effect seen with a dose of approximately 0.1 mg/kg.

Discussion. In the mouse retina, as previously observed in rat retina,1 activation and subsequent incorporation of tritiated thymidine following ocular trauma occurred most frequently in the venules. Under similar experimental conditions the relative number of venular endothelial cells entering the S phase of the cell cycle was about the same for both species, and a strikingly similar pattern was seen, with labeled nuclei often concentrated within one vein or its tributaries. The results suggest that similar mechanisms are operative in both species.

Indomethacin administration at a level comparable to that in this study has been reported to suppress arachidonic acid–induced elevation of intraocular pressure by inhibiting prostaglandin biosynthesis from arachidonic acid.5 The latter can be released by hydrolysis of phospholipids of cells damaged by trauma or ischemia. The inability of indomethacin to suppress the induction by ocular trauma of proliferation of venular endothelial cells in the rat retina suggests that prostaglandins are not an essential mediator in this system. In contrast, dexamethasone in dosage comparable to that recommended for the treatment of collagen-vascular diseases effectively inhibited endothelial cell proliferation.6 It also effectively suppressed recruitment of leukocytes into the retinal vasculature of the traumatized eyes, an effect not observed with indomethacin. The possible role of the leukocyte response

Table I. Effect of indomethacin and dexamethasone on incorporation of intravitreal tritiated thymidine into endothelial cell nuclei of rat retinal venules subsequent to ocular trauma

<table>
<thead>
<tr>
<th>Insult</th>
<th>Treatment</th>
<th>Labeled cells*</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>4.6 ± 1.5</td>
</tr>
<tr>
<td>Lens puncture</td>
<td>None</td>
<td>56.6 ± 11.8</td>
</tr>
<tr>
<td>Lens puncture</td>
<td>Carboxymethyl cellulose</td>
<td>37.7 ± 7.8</td>
</tr>
<tr>
<td>Lens puncture</td>
<td>Indomethacin</td>
<td>30.4 ± 13.9</td>
</tr>
<tr>
<td>Lens puncture</td>
<td>Dexamethasone</td>
<td>2.7 ± 1.0</td>
</tr>
</tbody>
</table>

*Number of labeled cells per 1,000 venular cells counted; each value represents the mean ±S.E. for a group of 6 rats.

Tritiated thymidine was injected 40 hours following lens puncture.
Fig. 2. Dosage-dependent reduction by dexamethasone of labeling of endothelial cells by tritiated thymidine in rat retinal venules following lens puncture. Each value represents mean and S.E. from four experimental animals (eight eyes) that received dexamethasone intraperitoneally twice daily at the indicated dosage.

and the mechanism by which dexamethasone inhibits endothelial cell proliferation following ocular trauma remain to be determined.

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Key words: neovascularization of retina, retinal vessels, dexamethasone

REFERENCES


Synthesis of gamma crystallin by a cloned cell line from Nakano mouse lens. P. RUSSELL, D. A. CARPER, AND J. H. KINOSHITA

A cloned cell line was derived from a culture of Nakano mouse lens epithelial cells. The cloned cells grew vigorously and produced large numbers of lentoid bodies. Sodium dodecyl sulfate (SDS) and non-SDS slab-gel electrophoresis of the soluble proteins from the cultured cells revealed protein bands identical in pattern to those of purified gamma crystallin. Antibody to mouse gamma crystallin reacted to the soluble protein fraction of the cultured cells, indicating the synthesis in culture of gamma crystallin by this cloned cell line.

One method of obtaining homogeneity of tissue culture cells is the technique of cloning. By isolating and subsequently culturing single cells, cell lines with genetic uniformity are established. These cloned cell lines can be further studied for various properties. We have previously reported on the culture of lens epithelium from normal and Nakano mice.1 The Nakano mouse develops a hereditary cataract and offers an excellent model for the study of human congenital cataracts.2,3 In this study, a clone from a cell line of Nakano lens epithelium was selected and checked for the presence of gamma crystallin. The synthesis of this protein has been reported to occur in the lens fibers and not in the epithelium.4 Thus the presence of gamma crystallin in cultured cells is a possible indication that differentiation of the lens cells has taken place. The techniques of slab-gel electrophoresis and double immunodiffusion were utilized to determine the presence of this crystallin in the cloned cell line.