Corticosteroid Treatment of Laser Retinal Damage Affects Prostaglandin E₂ Response

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The current study investigated the effect of steroid treatment of eyes subjected to a single retinal argon laser lesion on vitreal accumulation of both prostaglandin E₂ (PGE₂) and protein and their relationship with the amounts of PGE₂ released from the retina–choroid. Laser exposure resulted in an elevation in the amounts of PGE₂ released by the retina–choroid of laser-treated eyes: there was an initial peak on day 1, followed by a higher peak on day 7 (13.0 ± 3.9 ng/mg protein and 32.9 ± 4.9 ng/mg protein, respectively) after which levels progressively declined. Steroid treatment prevented the initial peak, but did not prevent the enhanced PGE₂ amounts released during the second week. Thus, on day 7 the amounts released were lower by 32% than in the untreated group (21.0 ± 8.6 ng/mg protein vs 32.9 ± 4.9 ng/mg protein, P = 0.000); by day 14, however, peak values in the treated group were higher than in the untreated group (32.2 ± 12.4 ng/mg protein and 10.6 ± 4.5 ng/mg protein, respectively). In steroid-treated eyes, vitreal PGE₂ concentration remained unchanged from baseline over a 2-week follow-up, whereas in the untreated laser group, levels peaked on day 7 to 10.7 ± 3.6 ng/ml, exceeding baseline levels of 5.8 ± 1.7 ng/ml (P = 0.0002). Laser exposure was also associated with a biphasic elevation in vitreal protein concentrations on days 3 and 14 (0.68 ± 0.16 mg/ml and 0.79 ± 0.13 mg/ml, respectively); these were significantly higher than the baseline value of 0.43 ± 0.12 mg/ml (P = 0.03 and P = 0.004, respectively). Steroid treatment resulted in a single elevation of vitreal protein concentration, occurring on day 7, when values reached 1.00 ± 0.31 mg/ml. Our study demonstrated that steroid treatment of laser-induced retinal damage transiently reduced the amounts of PGE₂ released from the laser exposed retina–choroid. This inhibition occurred during the initial phase after exposure; at the later phase, augmented release was resumed. In addition, this treatment affected vitreal PGE₂ and protein concentrations selectively; the accumulation of PGE₂ above baseline was completely prevented while that of protein was lowered only partially. Invest Ophthalmol Vis Sci 31:9-13, 1990

Argon laser-induced retinal lesions have been associated with an inflammatory reaction which is characterized by extensive retinal and choroidal damage and polymorphonuclear infiltration as well as with protein leakage and fluorescein leakage into the vitreous, which is indicative of a disruption of the blood–retinal barrier (BRB). Iris exposure to various laser modalities is related to elevated aqueous protein and prostaglandin E₂ (PGE₂) levels, the latter known for its mediatory role in the inflammatory reaction, and its effect on the integrity of ocular barriers. The effect of retinal laser exposure on changes in the levels of PGE₂ in the posterior segment have not been studied.

The inhibitory effect of corticosteroids on prostaglandin (PG) production is well established and is partially responsible for their antiinflammatory activity. This suppression of PGE₂ production is mediated through reduction of the availability of arachidonic acid, a substrate for PG formation.

In the current study, we investigated the effect of corticosteroid treatment of argon laser-induced retinal injury on vitreal levels of both PGE₂ and protein and their relationship with the amounts of PGE₂ released in vitro by the retina–choroid.

Material and Methods

The study consisted of three groups of age- and sex-matched rabbits, raised in alternating 12-hr periods of light and dark, using a regular fluorescent light source. All experiments complied with the provisions of the ARVO Resolution on the Use of Animals in Research.

1. Control group: 28 rabbits that were not exposed to either laser irradiation or noncoherent light illumination and had no ocular pathology.

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Supported by the United States Army Medical Research and Development Command Contract DAMD 17-85-G-5013.

Submitted for publication: May 9, 1988; accepted June 12, 1989.

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2. Untreated argon laser-exposed group: 60 rabbits with a single argon laser-induced retinal burn, that were divided into four subgroups: 20, 18, 12, and 20 rabbits in which PGE2 and protein were determined 1, 3, 7, and 14 days, respectively, after exposure.

3. Corticosteroid treated argon laser-exposed group: 57 rabbits, with a single argon laser-induced retinal burn that were treated with corticosteroids according to the procedure described below. This group was also divided into 4 subgroups: 12, 22, 11, and 12 rabbits in which PGE2 and protein were determined 1, 3, 7, and 14 days, respectively, after exposure.

Thirty minutes before the laser procedure all rabbits had been anesthetized by 35 mg/kg ketamine (Vetalar; Parke Davis, Morris Plains, NY) and 5 mg/kg xylazine hydrochloride 2% (Rompun, Bayer, Leverkusen, Germany) injected intramuscularly; this was followed by pupil dilatation with 0.5% tropicamide 1% (Mydramide 1%; Fischer, Tel Aviv, Israel) and local anesthesia with 0.1% benoxinate hydrochloride 0.4% (Localin; Fischer, Tel Aviv, Israel).

**Argon Laser Exposure**

A continuous wave green–blue argon laser was used (265 Excitor; Lasertek). The laser beam was focused on the retina of the right eye only of each rabbit, through a Goldman coated lens. Laser exposure consisted of a single burn at a power setting of 300 mW, a spot size of 500 μm, and a duration of 0.5 sec, aimed 2 disc diameters below the optic disc, and resulting in an ophthalmoscopically visible burn. During the procedure, the animals were naturally exposed to both the laser irradiation and to the noncoherent light of the slit lamp used for illumination, required for a precise laser burn application.

**Sample Preparation**

After enucleation, the cornea was cut at the limbus, and the lens and iris were removed and discarded, while the vitreous was separated as described, and put into another vial. Likewise, a retina–choroid preparation consisting of the whole retina attached to the choroid was separated, and put into another vial.

**Prostaglandin E2 Determination**

The retina–choroid preparation was incubated in 0.6 ml Kreb's Ringer's bicarbonate hepes buffer, pH 7.4, in a slow shaking bath at 37°C for 15 min. At the end of the incubation period, the tissue was removed, and samples from the incubation media were withdrawn for PGE2 determination. The incubation medium was extracted twice with two volumes of ether at pH 3.8, and the aqueous phase was assayed for PGE2 with the radioimmunoassay technique as described. After their removal from the incubation media, the retina and choroid underwent homogenization in 1.0 ml of ice-cold 50 mM Tris–HCl buffer, pH 7.0, for 30 sec at 4°C, and then underwent centrifugation at 3000 rpm for 5 min. The supernatant was used for protein determination as described.15

The vitreous body of each eye was incubated similarly, in 1.0 ml of the same buffer; on completion of the incubation period, a sample was withdrawn from the media for PGE2 determination.

PGE2 was measured using the radioimmunoassay technique with a specific antibody for PGE2 (Miles-Yeda, Rehovot) having a 3% cross reactivity with PGE1. The cross reaction of this antiserum with other prostaglandins was less than 3%. Day-to-day reproducibility and in-run precision of the analytical procedure was examined by processing, in each assay, samples of vitreous and buffer containing known amounts of PGE2. To determine PGE2 recovery rate, the appropriate tritiated prostaglandin was added to the medium, which was then cooled to 4°C and acidified to pH 4 with sodium acetate buffer (1 M, pH 3.8), and extracted as described. Part of the dissolved aqueous phase was used to assess procedural loss, and the remainder was used for the radioimmunoassay.

**Protein Determination**

Protein was measured in the vitreous body using the modified Roseborough-Lowry method.

**Steroid Treatment Procedure**

Steroid treatment with a dose of Dexamethasone sodium phosphate (0.5 mg/kg weight, intramuscularly; Dexacort; Ikapharm, Tel Aviv, Israel) was started during the 1st hr after laser exposure and was repeated daily.

**Results**

PGE2 Release from Retina–Choroid (Table 1)

In the control group, the mean amount of PGE2 released by the retina–choroid into the incubation medium was 6.0 ± 2.3 ng/mg protein, which was considered baseline value.

In the untreated laser-exposed eyes, PGE2 release (during a 2-week period) was twice elevated above baseline levels: a transitory initial peak on day 1 was followed by a higher peak on day 7 (13.0 ± 3.9 ng/mg protein and 32.9 ± 4.9 ng/mg protein, P = 0.02 and P = 0.003, respectively), after which the levels progressively declined. On day 14, however, the levels were still significantly higher than baseline (10.6 ± 4.5 ng/mg protein, P = 0.003).

In the steroid-treated laser-exposed eyes, PGE2 release by the retina–choroid exceeded baseline on day...
Table 1. Prostaglandin E2 release by the retina–choroid of argon laser-exposed eyes: The effect of steroid treatment

<table>
<thead>
<tr>
<th>Time after exposure (days)</th>
<th>Prostaglandin E2 levels (ng/mg protein; mean ± SD)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated laser group</td>
<td>Steroid-treated laser group</td>
</tr>
<tr>
<td>1</td>
<td>13.0 ± 3.9</td>
<td>4.4 ± 2.1</td>
</tr>
<tr>
<td>3</td>
<td>7.5 ± 3.9</td>
<td>6.6 ± 2.7</td>
</tr>
<tr>
<td>7</td>
<td>32.9 ± 4.9</td>
<td>21.0 ± 8.6</td>
</tr>
<tr>
<td>14</td>
<td>10.6 ± 4.5</td>
<td>32.2 ± 12.4</td>
</tr>
<tr>
<td>Baseline</td>
<td>6.0 ± 2.3</td>
<td></td>
</tr>
</tbody>
</table>

* P value for student t-test comparing the untreated laser-exposed eyes at each time interval with the corresponding steroid-treated group.
† n: number of eyes involved in each group.
‡ NS: not statistically significant.

7, when levels were lower than in the corresponding untreated laser group (21.0 ± 8.6 ng/mg protein and 32.9 ± 4.9 ng/mg protein, respectively). However, on day 14 the amounts released by the treated retina–choroid were further enhanced, while in the corresponding untreated group, levels were already declining at this time (32.2 ± 12.4 ng/mg protein and 10.6 ± 4.5 ng/mg protein, P = 0.000, respectively).

Vitreal PGE2 Levels (Table 2)

In the control group, the mean vitreal PGE2 level was 5.8 ± 1.7 ng/ml and was considered baseline. In the untreated laser-exposed group vitreal PGE2 levels peaked only once (day 7) above baseline (10.7 ± 3.6 ng/ml, P = 0.0002), as compared with the steroid-treated group, in which vitreal PGE2 levels remained unchanged from baseline throughout the observation period. Thus, steroid treatment prevented the accumulation of vitreal PGE2 to above baseline.

Vitreal Protein Levels (Table 3)

In the control group, the mean vitreal protein level was 0.43 ± 0.12 ng/ml and was considered baseline. In the untreated laser-exposed group, vitreal protein content was elevated twice during a 2-week period; on day 3 and 14 it reached values of 0.68 ± 0.16 ng/ml and 0.79 ± 0.13 ng/ml, respectively, which were significantly higher than baseline levels (P = 0.03 and P = 0.004, respectively).

In the steroid-treated laser-exposed eyes, vitreal protein levels during the 2-week follow-up peaked only once (day 7) above baseline values, but reached values higher than those in the corresponding untreated group (1.00 ± 0.31 ng/ml vs 0.48 ± 0.13 ng/ml, P = 0.001, respectively). Thus, steroid treatment only partially prevented the leakage of protein in the vitreous body.

In the laser-exposed group, association between changes in vitreal PGE2 level and the amounts of PGE2 released by the retina–choroid was noted only during the second week after exposure. However, in the steroid-treated group (Table 3), no such correlation was observed: excessive PGE2 production was not reflected in the vitreal levels, which remained unchanged from baseline.

Changes in protein vitreal levels in both the untreated and the steroid treated groups (Table 3) did not coincide with changes of either vitreal PGE2 content or PGE2 release.

Table 2. Vitreal prostaglandin E2 levels after argon laser-induced retinal lesion: The effect of steroid treatment

<table>
<thead>
<tr>
<th>Time after exposure (days)</th>
<th>Prostaglandin E2 levels (ng/ml; mean ± SD)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated laser group</td>
<td>Steroid-treated laser group</td>
</tr>
<tr>
<td>1</td>
<td>3.64 ± 1.7</td>
<td>3.4 ± 1.2</td>
</tr>
<tr>
<td>n† = 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3.0 ± 4.5</td>
<td>5.9 ± 2.2</td>
</tr>
<tr>
<td>n = 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>10.7 ± 3.6</td>
<td>5.9 ± 2.2</td>
</tr>
<tr>
<td>n = 23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>3.6 ± 1.9</td>
<td>4.0 ± 1.5</td>
</tr>
<tr>
<td>n = 23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>5.8 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>n = 30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P value for student t-test comparing each of the untreated laser-exposed eyes at each time interval with the corresponding steroid-treated group.
† n: number of eyes involved in each group.
‡ NS: not statistically significant.

Table 3. Vitreal protein levels in argon laser-exposed lesion: The effect of steroid treatment

<table>
<thead>
<tr>
<th>Time after exposure (days)</th>
<th>Vitreal protein levels (mg/ml; mean ± SD)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated laser group</td>
<td>Steroid treated laser group</td>
</tr>
<tr>
<td>1</td>
<td>0.52 ± 0.15</td>
<td>0.41 ± 0.13</td>
</tr>
<tr>
<td>n† = 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.68 ± 0.16</td>
<td>0.41 ± 0.10</td>
</tr>
<tr>
<td>n = 22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.48 ± 0.13</td>
<td>1.00 ± 0.31</td>
</tr>
<tr>
<td>n = 22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>0.79 ± 0.13</td>
<td>0.47 ± 0.10</td>
</tr>
<tr>
<td>n = 23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.43 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>n = 32</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P value for student t-test comparing each of the laser untreated eyes at each of the time intervals with the corresponding steroid-treated group.
† n: number of eyes involved in each group.
‡ NS: not statistically significant.
Discussion

In eyes subjected to retinal argon laser exposure, a disruption of the BRB has been demonstrated\(^{13,14}\) and histopathologic evidence for a long-term inflammatory reaction has been obtained also.\(^{1,2}\) No data, however, is yet available on PGE\(_2\) involvement in the posterior-segment response after laser-induced retinal injury. In our investigation of retinal laser exposure, we studied the in vitro PGE\(_2\) release by the whole retina–choroid without separating the retina from the choroid, because this technique avoided trauma that may be induced during separation, and because a visible retinal lesion affects both choroid and retina.\(^{1,2}\)

Our study demonstrated an increase in the amounts of PGE\(_2\) released by the laser exposed retina–choroid; an initial transitory increase on day 1 was followed by a second peak on day 7, which extended through day 14. Laser exposure resulted also in an accumulation of vitreal PGE\(_2\) levels above baseline on day 7, while vitreal protein levels exceeded baseline twice, on days 3 and 14.

Steroid treatment prevented the initial elevation in PGE\(_2\) release by the retina–choroid, but not the development of a later peak on day 7, at which time the amounts released were only 32% lower than in the corresponding untreated group. By day 14, a further enhancement resulted in maximal peak values. Since PGE\(_2\) release by the retina–choroid was measured in vitro while its vitreal levels were determined in vivo, the applicability of these values might be relative.

Since the steroidal inhibitory effect on PGE\(_2\) production is well established,\(^{12-14}\) our observation of an inhibitory effect on PGE\(_2\) release by the retina–choroid during the initial phase after laser exposure is not unusual.

However, the demonstration of the loss of this inhibitory effect during the 2nd week is as yet unknown. It may be related to the reported lack of an inhibitory effect of long-term maternally administered dexamethasone on PGE\(_1\) synthesis in fetal rat lung.\(^{17,19}\)

Vitreal PGE\(_2\) content in our corticosteroid-treated eyes did not significantly exceed baseline levels during the 2-week observation period, despite the in vitro evidence for the excessive release of PGE\(_2\) by the retina–choroid. PGE\(_2\) levels in the vitreous are regulated, however, both by the rate of its release by the retina and by removal through active transport mechanisms located at the BRB.\(^{20-22}\)

Corticosteroid treatment only partially reduced the above-baseline elevation of vitreal protein levels, as seen in their single peak, as compared with a biphasic elevation in the untreated group. This finding is in accord with a study on endotoxin-induced uveitis,\(^{23}\) in which steroids had little effect on excessive vitreal protein levels but a significant effect in suppressing the clinical signs and in reducing the polymorphonuclear cellular infiltration. Elevation of protein levels in the vitreous to above baseline in our steroid-treated group might be indicative of persistent leakage or of inability of the BRB absorptive mechanisms to remove the excessive vitreal accumulation.

Elevated protein levels in our laser-exposed groups did not coincide with the increase in vitreal PGE\(_2\) concentration; this result is in accordance with a report that the laser-induced disruption of BRB is only partially mediated by PGs\(^{11}\) and with reports of a suggested nonPG-dependent breakdown of the ocular barrier.\(^{24-27}\)

Our finding of the transient nature of the steroidal inhibitory effect on PGE\(_2\) release must be considered in view of three factors: 1) Steroid treatment of various inflammatory conditions maintains its effectiveness over a long period of time. 2) The antiinflammatory effect of corticosteroids might be mediated through various mechanisms besides that of prostaglandins: by lysosomal and cell membrane stabilization;\(^{28}\) by inhibition of complement-induced granulocyte aggregation;\(^{29}\) or by prevention of the polymorphonuclear infiltration into the inflamed tissue or exudate.\(^{30-32}\) 3) Various prostaglandins suppress the inflammatory response when administered exogenously; the antiinflammatory effect of prostaglandins was observed with subcutaneous application of PGE\(_1\) and PGE\(_2\) in arthritis\(^{33,34}\) as well as in immunogenic vasculitis.\(^{35}\) Likewise, intravitreal administration of PGs in rabbits with bacterial endotoxin uveitis resulted in decreased vascular permeability,\(^{36}\) while application of PGE\(_1\) and prostaglandin F\(_2\) prior to the induction of corneal trauma reduced the resultant inflammatory response and the formation of endogenous PGs.\(^{37}\)

It has been suggested that excessive levels of PGE\(_2\) generated during the inflammatory reaction might act as suppressors of the inflammatory response as a result of secondary elevation in cyclic AMP.\(^{38}\) Therefore, the loss of the steroidal inhibitory effect on PGE\(_2\) synthesis that we observed during the later phase after laser exposure does not necessarily reflect a loss or reduction of the PGE\(_2\) antiinflammatory activity.

In summary, in our study, retinal laser exposure resulted in an elevation of the amounts of the in vitro PGE\(_2\) released by the retina–choroid, as well as in increased PGE\(_2\) and protein vitreal levels. Steroid treatment only partially prevented the increased PGE\(_2\) release by the retina–choroid since, as we observed, the inhibitory effect was not evident during the late phase after laser exposure. This observation that has not yet been reported may support the suggested antiinflammatory role of PGs when applied exogenously in low doses in various organs including
the eye.\textsuperscript{28-32} In addition, steroid treatment affected vitreal PGE\textsubscript{2} and protein levels differently; vitreal PGE\textsubscript{2} accumulation above baseline was completely prevented, while vitreal protein accumulation was only partially reduced.

**Key words:** laser, prostaglandin E\textsubscript{2}, retina-choroid, vitreal prostaglandin E\textsubscript{2}, corticosteroids

**References**


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