Prostanoids in Rabbit Aqueous Humor: Effect of Laser Photocoagulation of the Iris

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The authors measured concentrations of prostanoids (prostaglandin-like substances) in aqueous humor from normal pigmented rabbit eyes and from those subjected to argon laser photocoagulation of the iris. The predominant prostanoids quantitatively were prostaglandin E₂ (PGE₂), PGF₂α, and PGD₂ with minor amounts of 6-keto-PGF₁α and thromboxane B₂. In all cases, concentrations of prostanoids in laser-treated eyes were substantially greater than those in normal eyes. This finding was particularly striking in the case of PGE₂ which increased 60-fold from 87 pg/ml to 5.5 ng/ml after irradiation. Concentrations of prostanoids following photocoagulation were related to the number of administered laser lesions and prostanoid release was associated with an initial hypertensive response and disruption of the blood-aqueous barrier. Invest Ophthalmol Vis Sci 26:1087-1092, 1985

When traumatized, the rabbit eye undergoes a pattern of changes consisting of miosis, conjunctival and iris hyperemia, increased intraocular pressure and breakdown of the blood-aqueous barrier. The pattern of ocular changes and the severity of the response depend on the species and type of injury. An acute ocular response occurs when the laser is used to photocoagulate the iris. Since prostaglandins (PGs) and neural elements are both involved in the mediation of this response, photocoagulation of the iris has provided a useful model to study the injury response. PG-like activity in aqueous humor has been previously measured using only a crude bioassay. After ruby laser photocoagulation of the pigmented rabbit iris, PG-like activity in aqueous extracts appeared to be of the E-type and chromatography of partially purified extracts of iris tissue indicated a predominance of prostaglandin E₂ (PGE₂). However, it is now recognized that many prostanoids (PG-like substances) have similar actions and may not be separated by the bioassay system. Hence, it is important to determine whether the prostanoids released in response to photocoagulation are PGE₂ or a combination of other prostanoids with PGE-like activity.

In the current investigation, we measured concentrations of specific prostanoids in aqueous humor from normal rabbit eyes and from those subjected to laser photocoagulation of the iris and have correlated their levels with the number of administered laser lesions.

Materials and Methods

Animals and Experimental Protocol

We used adult pigmented (Dutch) rabbits of either sex which weighed at least 2.5 kg. All eyes were initially examined with a slit lamp and only animals without signs of ocular inflammation were included in the study. We performed anterior chamber paracentesis to obtain samples of aqueous humor for the measurement of prostanoids and protein in normal rabbits and at timed intervals after iris photocoagulation in treated rabbits. These animal investigations conform to the ARVO Resolution on the Use of Animals in Research.

In the first series of experiments, we evaluated the prostanoids in aqueous humor pooled from six photocoagulated eyes, 60 min after 16 laser applications, and from six eyes of three normal rabbits. After identifying PGE₂ as quantitatively the major prostanoid in the aqueous humor samples from laser-irradiated eyes, we examined the influence of two, four, eight, and 16 burns on its release 15 or 60 min after laser treatment in individual aqueous samples.

After identifying PGE₂ as quantitatively the major prostanoid in the aqueous humor samples from laser-irradiated eyes, we examined the influence of two, four, eight, and 16 burns on its release 15 or 60 min after laser treatment in individual aqueous samples. PGE₂, PGD₂, and PGF₂α were then measured in individual samples of aqueous humor of laser-treated (both four and 16 burns) and contralateral untreated eyes at 15, 30, 90, 120, and 360 min after treatment.

In the final series of experiments, we attempted to correlate the concentrations of PGE₂ in individual
Table 1. Prostanoids in aqueous humor

<table>
<thead>
<tr>
<th>Prostanoid</th>
<th>Concentration of Prostanoid (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostaglandin E₂</td>
<td>5,549</td>
</tr>
<tr>
<td>Prostaglandin F₂α</td>
<td>1,580</td>
</tr>
<tr>
<td>Prostaglandin D₂</td>
<td>1,402</td>
</tr>
<tr>
<td>Thromboxane B₂</td>
<td>50</td>
</tr>
<tr>
<td>6-keto-prostaglandin F₁α</td>
<td>26</td>
</tr>
<tr>
<td>Prostaglandin F₆ metabolite↑</td>
<td>155</td>
</tr>
<tr>
<td>Prostaglandin E₆ metabolite↑</td>
<td>56</td>
</tr>
</tbody>
</table>

* Treated eyes had 16 argon laser lesions administered to the iris and aqueous humor withdrawn after 1 hr and pooled.
† 13,14-dihydro-15-keto-PGF₂α.
‡ 11-deoxy-13,14-dihydro-15-keto-11,16-cyclo-PGE₂.

Laser Photocoagulation

In all experiments, an argon (488 nm) laser was used to photocoagulate the surface of the iris of one eye of each rabbit. Under local anesthesia (0.5% proparacaine), a contact lens with a +66D planoconvex button was placed over the laser-treated eye to focus the laser light (100 μm diameter, 1 watt and 0.2 sec). Laser applications were 1 mm from the pupillary margin and spaced circumferentially at regular intervals in a counterclockwise direction over 360 deg. When 16 applications were used, eight were placed in each of two adjacent concentric circles. After treatment, the contact lens was removed.

Paracentesis

At designated time after irradiation about 100 μl of aqueous humor was withdrawn from both the treated and untreated eyes of each rabbit with a 27-gauge needle and a tuberculin syringe. Ten minutes prior to paracentesis, ketamine hydrochloride (20 mg/kg) and acepromazine (0.4 mg/kg) were administered intramuscularly for anesthesia. Anterior chamber paracentesis was performed only once in each eye and corresponded to a specific number of laser applications and a specific time interval.

Intraocular Pressure

Using a calibrated pneumatonometer, intraocular pressure was measured just prior to photocoagulation (baseline) and then at 15, 30, 60, 120, and 360 min post-treatment. The change in intraocular pressure in the laser-treated and control eye was calculated at each time interval by subtracting the baseline intraocular pressure from the measured intraocular pressure. The corrected change in intraocular pressure was then obtained by subtracting the change in intraocular pressure in the control eye from that in the laser-treated eye. Throughout the 6-hr postoperative period, the rabbits had access to food and water.

Prostanoid Assays

We measured PGE₂, PGF₂α, PGD₂, thromboxane B₂ (TXB₂), 6-keto-PGF₁α (the major degradation product of prostacyclin) and products of PGF₂α and PGE₂ metabolism (13, 14-dihydro-15-keto-prostaglandin F₂α and 11-deoxy-13, 14-dihydro-15-keto-11, 16-cyclo-prostaglandin E₂, respectively) by sensitive and specific radioimmunoassays. The radioimmunoassays employed have been described and validated in detail elsewhere. The cross-reactivities of the antisera with other major prostanoids are almost exclusively less than 1%.

Protein

A modification of the method of Lowry 15 was used to assay for protein from aliquots of the same aqueous samples used for prostanoid analysis.

Results

The predominant prostanoids found in the aqueous humor 60 min after iris photocoagulation were PGE₂, PGF₂α, and PGD₂ with minor amounts of 6-keto-PGF₁α and TXB₂ (Table 1). The concentrations of prostanoids in the laser-treated eyes were substantially greater than those in the control eyes. This finding was particularly striking in the case of PGE₂ (5.5 ng/ml after treatment), the concentration of which was increased by more than 60-fold after laser irradiation. This difference was less pronounced for PGF₂α (1.6 ng/ml) and PGD₂ (1.4 ng/ml), the concentrations of which increased by about four-fold and three-fold, respectively. There were also clear differences between the treated and control eyes in concentrations of 6-keto-PGF₁α (two-fold) and TXB₂ (four-fold); however, only low concentrations of these prostanoids were found. Although substantial amounts of products of both PGE₂ and PGF₂α, metabolism were detected, differences between laser-treated and control eyes were minimal.

After ascertaining that PGE₂ was the major prostanoid in the aqueous humor following iris photocoagulation, we evaluated the relationship between the number of laser lesions and PGE₂ release after 15 min and 60 min (Fig. 1). The concentration of PGE₂.
in aqueous humor of eyes treated with 16 lesions was more than three-fold greater than that in the aqueous humor of eyes with four lesions at both 16 and 60 min after irradiation. There were no apparent differences in the PGE2 levels in aqueous humor obtained from eyes with two and four lesions or eight and 16 lesions.

To further evaluate this relationship, we then assayed PGE2, PGD2 and PGF2α, the three major PG products released after iris photocoagulation, at given times after either 16 (Fig. 2) or four (Fig. 3) applications of laser light. At all time points examined, the levels of PGE2 exceeded those of PGD2 and PGF2α. PG concentrations were maximal within 30 min of laser treatment in most cases and within 90 min of treatment in all cases. The concentrations of prostanoids released after 16 lesions were greater than those released after four lesions at all measured time intervals. Small amounts of PGF2α were detected after 16 lesions and none could be detected after four laser lesions.

We then attempted to relate the amount of PGE2 released following the administration of either four or 16 laser lesions with the change in intraocular pressure and aqueous protein.

**Intraocular Pressure**

Within 15 min after photocoagulation, the intraocular pressure was increased in both groups of laser-treated eyes. Intraocular pressure reached a peak within 30 min and then decreased during the subsequent experimental period. In general, the corrected change in intraocular pressure was greater in the eyes with the greater number of laser lesions (Fig. 4). By two hours after treatment, intraocular pressure in the laser-treated eyes was reduced below baseline.
Fig. 4. The effect of laser photocoagulation of the pigmented rabbit iris on corrected change in intraocular pressure. The corrected change in intraocular pressure (mmHg) is calculated by subtracting the change in intraocular pressure from baseline in the contralateral untreated eye from that in the laser-treated eye. Intraocular pressure (mean ± SE) was determined for laser-treated and contralateral eyes at 15 (n = 15), 30 (n = 12), 60 (n = 9), 120 (n = 6), and 360 (n = 3) min, respectively, for 16 (•) and four (▲) laser lesions.

Protein

Compared with contralateral untreated eyes, the aqueous humor protein increased in eyes with either four or 16 laser lesions (Fig. 5). The concentration of protein began to decrease in laser-treated eyes after 2 hr and was generally higher in the aqueous humor of eyes with 16 laser lesions. Also, it remained elevated in these eyes for a longer period than in eyes with fewer lesions. Even after 6 hr, the concentration of protein was elevated in the aqueous humor of laser-treated eyes compared with that of untreated eyes.

PGE2

In the eyes receiving either four or 16 laser lesions, the concentrations of PGE2 in the aqueous humor were increased at 15 min compared with the contralateral untreated eyes (Fig. 6). In general, PGE2 levels in the aqueous humor began decreasing within 1 hr, although they were still raised after 6 hr.

Discussion

Previously, investigators used only a crude bioassay to measure PG-like activity in aqueous humor after ruby laser photocoagulation of the pigmented rabbit iris. In those studies, PG-like activity in aqueous extracts appeared to be of the E-type and chromatography of partially purified extracts of iris tissue indicated a predominance of PGE2. In the current investigation, we employed sensitive and specific radioim-
munoassays to quantitate the prostanoids released into the aqueous humor after iris photocoagulation with an argon laser. PGE2 was the major prostanoid found and its concentration increased rapidly following laser irradiation. Further, the magnitude of this increase was related to the number of administered laser lesions.

Besides PGE2, other prostanoids were identified in the aqueous humor samples including PGD2 and PGF2α. To our knowledge, these prostanoids have not been measured previously in such experimental circumstances. Their effects in the normal rabbit eye or in mediating the laser-induced injury response may well be different from those of PGE2 since these prostanoids have different and often opposing actions in other physiological and biochemical systems. For example, these three PGs have qualitatively and quantitatively differing actions on adenylate cyclase activity, guanylate cyclase activity and calcium mobilization and may have specific receptors.16

Our finding of PG metabolites in the aqueous humor of both photocoagulated and normal rabbit eyes may also be important. 15-Hydroxyprostaglandin-dehydrogenase (PGDH) is the main enzyme in prostanoid catabolism in most, if not all, tissues studied and its activity is very low in ocular tissues.17,18 Removal of endogenously released PGs from the intraocular fluids is now considered to occur by facilitated transport across the blood–ocular barrier maintained by the epithelium of the ciliary processes and possibly also the posterior iris and retinal pigment epithelium.18,19 Therefore, biologically effective amounts of PGs can be eliminated from the anterior chamber by specific uptake as well as bulk outflow of aqueous humor. The possibility that PGs can be metabolized by intraocular tissues, as has been shown in other systems,20 may influence the response of the eye to injury as well as the fate of exogenously administered PGs.

Extrapolation of this laser-induced response in the rabbit eye to the primate eye should be done with caution since responses to ocular trauma within different vertebrate species are known to vary considerably.1 For example, whereas rabbits exhibit an indomethacin-sensitive response to many forms of irritation which is presumably PG-mediated, responses to similar irritants in owl monkeys are generally indomethacin-resistant and manifested only by pupillary constriction.1,3 The explanations for these species differences have not been clearly ascertained although it is possible that anatomic differences in the tissues which comprise the blood–aqueous barrier play a role. Biochemical differences between species are also undoubtedly contributing. For example, cyclooxygenase activity is greater in the anterior uvea of the albino rabbit eye than in that of the human.21

In this investigation, we have demonstrated that there is substantial PG release following photocoagulation of the pigmented rabbit iris associated with an initial hypertensive response and an accompanying disruption of the blood–aqueous barrier. The tissue source of these PGs is likely to be the iris, although other tissues, particularly the ciliary body, cannot be excluded. We measured more that 13 ng/ml of PGE2 in the aqueous humor 30 min after the iris received 16 argon laser lesions. Introduction of 5 ng of PGE2 into the anterior chamber of rabbits also can induce a disruption of the blood–aqueous barrier.22 The concentration of PGE2 in the aqueous humor following such an injection is similar to the maximal concentration of endogenous PGE2 after laser irradiation assuming an aqueous humor volume of 300 μl. This reproducible experimental procedure, in which the response to the localized laser lesion is readily quantifiable, can be used to further evaluate the biosynthesis and action of prostanoids.

Key words: prostanoid, prostaglandin, laser, iris, photocoagulation

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References

8. Butler JM, Unger WG, and Cole DF: Alex reflex in ocular


