Prostaglandin E in Rabbit Aqueous Humor After Nd-YAG Laser Photodisruption of Iris and the Effect of Topical Indomethacin Pretreatment

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Ocular changes (prostaglandin E, protein, pupil diameter, and intraocular pressure) induced by photodisruption of pigmented rabbit iris with neodymium-yttrium aluminum garnet (Nd-YAG) laser and the effect of topical indomethacin upon those changes were examined. Concentrations of prostaglandin E in laser-treated eyes (1, 3, 5, 10, and 20 lesions) were substantially greater than those in normal eyes and were associated with an initial hypertensive response. This finding was particularly striking in the case of 20 lesions. In that case, concentrations of prostaglandin E increased 50-fold, from 99 pg/ml of control level to 5049 pg/ml 60 min after irradiation. Disruption of blood aqueous barrier measured by protein concentration, changes in intraocular pressure, and pupil diameter occurred at a similar dose range of laser application. Concentration of protein and changes in pupil diameter already were prominent at 15 min after laser treatment, and changes in intraocular pressure were prominent at 60 min. Indomethacin pretreatment abolished most of these responses, suggesting that acute reactions following photodisruption largely depended on prostaglandin synthesis in iris tissue. Invest Ophthalmol Vis Sci 33:1685-1689, 1992

Argon and neodymium-yttrium aluminum garnet (Nd-YAG) laser iridectomies are well established procedures for treating pupillary block glaucoma and narrow angle glaucoma.1,2,3 An acute ocular response occurs when the laser is used for photocoagulation of the iris,4 and it has provided a useful model in the study of reaction pattern of the injured eye.3 When the eye is traumatized, signs such as miosis, hyperemia of conjunctiva and iris, increase in intraocular pressure, and breakdown of the blood-aqueous barrier can be observed.5 Recent studies suggest that prostaglandins (PGs) and some neural factors might be involved in the mediation of iris response.6,7 PG-like activity appeared in the aqueous humor after ruby laser photocoagulation of the pigmented rabbit iris. A substantial amount of PGE2 could be identified by chromatography in the extract of iris tissue.5 In addition, the PGE level of aqueous humor was correlated with the number of lesions by argon laser.4

Indomethacin, a potent inhibitor of PG synthase, has been reported to be an effective blocker of many forms of ocular irritation.6,9-12 In this study, we used Nd-YAG laser for photodisruption of rabbit iris. The ocular response was monitored by measuring PGE and protein levels in aqueous humor after laser treatment. Changes in intraocular pressure and pupil diameter also were measured.

Materials and Methods

Animals and Experimental Protocol

Fifteen adult pigmented rabbits of either sex, weighing from 2.5–3.5 kg, were used. All eyes were examined with a slit lamp and only those without signs of inflammation were used. One eye of each rabbit was exposed to Nd-YAG laser; the other eye was used as an untreated control. For the measurement of PGE and protein levels, aqueous humor was obtained from both eyes by anterior chamber paracentesis. The intraocular pressure and pupil diameter were measured on untreated and laser-exposed eyes at different times just before the anterior chamber paracentesis. The first series of experiments were carried out with five rabbits, with the number of laser lesions (1, 3, 5, 10, and 20) varied in each rabbit. After 60 min, intraocular pressure and pupil diameter were measured. Aqueous humor was collected by paracentesis.

In the second series of experiments, five rabbits...
were exposed to 10 laser lesions and the intraocular pressure and pupil diameter were measured at 15, 30, 60, 180, and 360 min after the treatment. Aqueous humor was collected right after the measurements.

The effect of indomethacin pretreatment was tested in the final series of experiments. Two drops of 0.5% indomethacin ophthalmic solution (Senju Pharmaceutical Co., Osaka, Japan) were instilled in one eye of the five rabbits four times at 180, 120, 60, and 30 min before the laser treatment. Irradiation of laser and various determinations were performed as in the second series of experiments.

Laser Photodisruption

A mode-locked Nd-YAG laser (model OPL-4; Meditec, Tuttlingen, West Germany) was used for photodisruption of the iris. The rabbit was placed in front of a slit lamp (Model RO 2000 SY; Rodenstock, West Germany), which was attached to an articulated arm of the Nd-YAG laser. Ten minutes before laser irradiation, ketamine hydrochloride (20 mg/kg) was administered intramuscularly for anesthesia. In all experiments, the laser power setting was 3 mJ and three bursts (wavelength, 1064 nm; duration, 30 picoseconds per mode-locked pulse; spot size, 50 μm) were applied to produce a lesion. The laser beam was shot on aiming spots located along the circle at 1 mm from the pupillary margin. The spots were evenly distributed along the circumference, depending upon the number of lesions.

Paracentesis of Anterior Chamber

About 100 μl of aqueous humor were withdrawn carefully from the treated and untreated eyes at the designated times. Paracentesis was performed with a 27 G needle attached to a tuberculin syringe. Five min prior to the paracentesis, ketamine hydrochloride (20 mg/kg) solution was administered intramuscularly for anesthesia. Paracentesis was performed only once in each eye after the laser irradiation.

Determination of Intraocular Pressure and Pupil Diameter

An applanation tonometer (model R 900; Haag-Streit, Berne, Switzerland) attached to a slit lamp (model SL-3D; Topcon, Tokyo Japan) was used to measure intraocular pressure of rabbit eyes before and after the photodisruption. At the designated time, intraocular pressures of the laser-treated eyes were determined. The change in intraocular pressure was obtained from the differences in values of intraocular pressure between the pretreated eye and the laser-treated eye.

The pupil diameter was measured with a caliper (model E-2404; Storz, St. Louis, MO, USA) under a brightness of 150 lux. Brightness was checked with a digital lux tester (model 3422; Hioki, Nagano, Japan). The change in pupil diameter was calculated by subtracting the value of pupil diameter in the pre-treated eye from the value of pupil diameter in the laser-treated eye.

Radioimmunoassay of Prostaglandin E and Measurement of Protein

The PGE concentration in rabbit aqueous humor was determined with a radioimmunoassay kit purchased from Clinical Assays, Cambridge, MA. PGE in the sample was converted into PGB by adding 1 N NaOH, adjusting the pH to 12.5, and placing it in a boiling water bath for 5 min. The sample was cooled to room temperature and neutralized to pH 7.4 with 1 M acetic acid. Each sample in duplicate was added to an appropriate volume of 10 mM Tris-HCl buffer, pH 7.4, containing 0.1% gelatin (gel-tris buffer) to bring the total volume of the mixture to 0.6 ml in a polypropylene tube. [3H] PGB1 (7600 counts per minute, 50 μl) and rabbit anti-PGB, serum (50 μl, diluted as recommended) were added and mixed. After incubation for 60 min at 37°C, 100 μl of normal rabbit serum and 100 μl of goat anti-rabbit serum were added and incubated overnight at 4°C.

The tubes were centrifuged for 30 min at 1600 × g, and the resulting supernatant was carefully aspirated. One milliliter of 0.1 N NaOH was added to the remaining precipitate, and the solution was transferred to a glass vial. Ten milliliters of counting solution was added, and the radioactivity was measured with a liquid scintillation counter.

Protein level of aqueous humor was determined by the dye binding method of Bradford, using bovine serum albumin as the standard.

Results

Ocular Response Depends on the Number of Nd-YAG Laser Lesions

Various numbers of laser lesions (1–20) were produced on rabbit irises, and the changes of PGE and protein levels in aqueous humor were determined 60 min after irradiation (Fig. 1). Maximal response occurred when 20 lesions of laser photodisruption were produced. However, even with three lesions, almost half of the maximum PG synthesis was induced. The concentration of PGE in aqueous humor increased 50-fold from 98 pg/ml to 5041 pg/ml with 20 lesions.

Protein concentration increased with a pattern similar to PG. It increased from 0.54 mg/ml in untreated controls to 19 mg/ml in irradiated eyes with 20 lesions.

Intraocular pressure and pupil diameter were measured 60 min after laser irradiation and were com-
Fig. 1. Concentrations of prostaglandin E (●) and protein (○) in aqueous humor 60 min after photodisruption of the pigmented rabbit iris with 1, 3, 5, 10, and 20 lesions.

pared with the measurements of untreated control eyes (Fig. 2). Intraocular pressure increased, depending upon the number of lesions, while the pupil diameter decreased. Maximal changes in intraocular pressure and pupil diameter were 4 mmHg and -4 mm, respectively.

Time Courses of Ocular Response to Laser Irradiation

After 10 laser lesions, all determinations were performed at various times. The concentration of PGE in aqueous humor reached a peak 15 min after the photodisruption and decreased gradually during the subsequent experimental period (Fig. 3). PGE level at 15 min was 9,049 pg/ml, 92 times the control value of 98 pg/ml. After 6 hr, it returned almost to the control level. Indomethacin pretreatment completely inhibited the increase in PGE resulting from the photodisruption (Fig. 3).

Figure 4 shows time-dependent changes in protein level in aqueous humor of the eyes with 10 laser lesions. Two peaks, at 15 min (13.5 mg/ml) and 60 min (11.0 mg/ml), were observed. Then the concentration of protein decreased. The untreated control value was 0.54 mg/ml. The occurrence of both peaks was completely blocked by topical indomethacin pretreatment (Fig. 4).

According to the two-way analysis of variance, the difference between protein levels of aqueous humor in laser photodisrupted eyes with and without topical indomethacin pretreatment was statistically significant ($P < 0.05$).

When the changes in intraocular pressure were traced for up to 6 hr, maximal increase was observed at about 60 min after irradiation ($+4.3 \pm 1.53$ mmHg; Fig. 5).

Damaged eyes slowly regained their normal ocular pressures thereafter. Topical indomethacin clearly inhibited the increase in intraocular pressure (Fig. 5).

Fig. 2. The effect of laser photodisruption on the intraocular pressure (●) and pupil diameter (○). The increase of intraocular pressure (mm Hg) and the decrease of pupil diameter (mm) were calculated by subtracting the values of pretreatment eyes from those of the laser-treated eyes. Intraocular pressure and pupil diameter of both eyes were determined 60 min after laser irradiation.

Fig. 3. Concentration of prostaglandin E (ng/ml) in the aqueous humor samples obtained from eyes with 10 laser lesions (●) and contralateral untreated eyes (○). Another group of rabbits were pretreated topically with indomethacin, as described in the Materials and Methods, and their eyes were irradiated to produce 10 lesions (△). Anterior chamber paracenteses were performed at 15, 30, 60, 180, and 360 min after the irradiation.
Fig. 4. The effect of laser photodisruption on the concentration of protein in aqueous humor. Protein (mg/ml) was measured samples of aqueous from eyes with 10 laser lesions (●), contralateral untreated eyes (○), and eyes with 10 laser lesions after topical indomethacin pretreatment (∆).

The difference was significant at 30 min (P < 0.05) and at 60 min (P < 0.05), according to the unpaired t-test.

Fig. 5. The effect of laser photodisruption on intraocular pressure. Rabbits were treated as described in Figure 3 (●: irradiated eyes; ∆: indomethacin-pretreated irradiated eyes). Intraocular pressure (mean ± SE) was determined for laser-treated and pretreatment eyes at 15 (n = 5), 30 (n = 4), 60 (n = 3), 180 (n = 2), and 360 (n = 1) min after the irradiation, and the changes in intraocular pressure were calculated as described in Materials and Methods.

Miosis caused by the laser photodisruption was evident at 15 min (change of pupil diameter was -3.10 ± 0.82 mm) after irradiation and then gradually decreased (Fig. 6). Indomethacin pretreatment inhibited considerably the irradiation-induced constriction of pupil. The maximal decrease in pupil diameter in indomethacin pretreated eyes was -1.50 ± 0.58 mm. The difference was statistically significant at 15 min (P < 0.05), according to the unpaired t-test.

Discussion

Photodisruption with argon laser produced a transient rise in intraocular pressure and inflammatory reaction. The action of argon laser is thermal and causes burning and shrinkage of iris tissue, while Nd-YAG laser causes tissue disruption by optic breakdown and the resulting shock wave. Transparent and pigmented tissue can be cut easily with Nd-YAG laser. Thus the color of the iris is not important in Nd-YAG laser and penetration with Nd-YAG laser is much easier than with argon laser. However, the potential complications of this new laser have not been fully investigated. In this study, we examined the effects of Nd-YAG laser irradiation on the ocular responses in rabbits.

When photodisruption of iris is carried out with Nd-YAG laser, complications such as the rise in intraocular tension, iritis, iris pigment dispersion, and minimal "candle wax"-type bleeding may occur. These changes are known to be associated with the breakdown of blood-aqueous barrier, damage to iris tissue, and production of PGs.
Recently, Weinreb et al employed sensitive and specific radioimmunoassays to quantitate the prostanoids released into the aqueous humor after iris photocoagulation with an argon laser. \(^4\) PGE\(_2\) was found to be the major prostanoid, and its concentration increased rapidly following laser irradiation. Other prostanoids, including PGD\(_2\) and PGF\(_2\), also were identified in aqueous humor samples. The increase in PG within the iris tissue has been implicated in the production of various ocular responses such as increased intraocular pressure and constriction of pupil. In addition, it has been suggested that the breakdown of the blood-aqueous barrier in argon laser-irradiated iris is mediated by PG production. \(^5\)

Our study also confirmed these results. As shown in Figures 1, 3, and 4, PGE and protein contents of aqueous humor increased significantly after the laser treatment. Maximum increases in PGE level occurred within 15 min after irradiation (Fig. 3).

Two peaks of protein level, at 15 min and 60 min, were observed. Then the concentration of protein decreased. The first peak may be a result of the initial bleeding induced by the photodisruption of iris, while the second peak is a result of the breakdown of the blood-aqueous barrier. However, the appearance of both peaks was completely blocked by topical indomethacin pretreatment (Fig. 4). A transient rise in intraocular pressure and miosis also were observed (Figs. 2, 5, and 6).

Indomethacin has been widely used to inhibit PG synthesis. It alleviates postoperative inflammation following cataract surgery. \(^18,19,20\) In the present study, the increase in PGE and protein caused by photodisruption was inhibited completely by indomethacin pretreatment (Figs. 3 and 4). This implies that PGE is largely responsible for initiating the disruption of blood-aqueous barrier.

On the other hand, the increase in intraocular pressure and the decrease in pupil diameter could not be completely inhibited by indomethacin pretreatment. This suggests that another mediator, possibly of cholinergic origin, also may contribute to the production of the ocular response to trauma. \(^14\)

Key words: indomethacin, intraocular pressure, neodymium-yttrium aluminum garnet, prostaglandin, radioimmunoassay

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References