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Inflammatory Effects of Continuous-Wave Neodymium: Yttrium Aluminum Garnet Laser Cyclophotocoagulation

Mark P. Nasisse,* M. Christine McGahan,* M. Bruce Shields,† David Echelman,† and Lloyd N. Fleisher*

The uveal inflammatory response was studied in 31 rabbits treated unilaterally with neodymium: yttrium aluminum garnet (Nd:YAG) cyclophotocoagulation. Fifteen applications of 3.5-J energy were delivered to the dorsal and ventral perilimbal sclera using a contact continuous-wave system. On days 1, 3, 8, and 15, the inflammatory effects were assessed. Peak levels of aqueous humor protein (11 ± 3 mg/ml), prostaglandin E2 (8.9 ± 3.0 ng/ml), leukocytes (205 ± 113/μl), and iris-ciliary body myeloperoxidase activity (6.32 ± 1.4 U/mg protein) occurred on day 3 and rapidly decreased between days 7 and 15. Vitreal protein levels also peaked at day 3 but remained elevated through day 15 (3.8 ± 1.3 mg/ml). By contrast, aqueous erythrocytes were most numerous (22,614 ± 10,517/μl) on day 8. Levels of leukotriene B4 remained low in all eyes at all intervals. Correlative histologic changes were ciliary coagulation necrosis, severe vascular congestion, and a predominantly mononuclear inflammatory cell infiltrate. These data suggest that Nd:YAG cyclophotocoagulation in rabbits induces a relatively mild inflammatory response that is associated with significant vascular compromise. Although these observations may not be analogous to the situation in the human eye, they may provide a model with which to compare the relative effects of different treatment parameters to help establish the optimum protocol.

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Transscleral neodymium: yttrium aluminum garnet (Nd:YAG) laser cyclophotocoagulation commonly is used to treat refractory glaucoma.1-7 The popularity of this technique is related in part to the assumption that the inflammation-related adverse effects attributed to conventional cyclocryosurgery, including effusive retinal detachment, phthisis bulbi, and postoperative pain,8-13 are less severe after this procedure. The magnitude and effects, however, of Nd:YAG laser-induced inflammation are controversial. In several studies, the comparative response in rabbits to Nd:YAG cyclophotocoagulation and cryocycosurgery were described as similar when the clinical parameters of aqueous cells and flare were used as indicators of inflammation.14,15 Evidence also has been presented to suggest that uveal inflammation may be important in the pressure-lowering effects of Nd:YAG cyclophotocoagulation by either reducing aqueous humor production or increasing uveoscleral outflow.16 Inflammation, therefore, may play both beneficial and harmful roles after this procedure. The laser mode (pulsed versus continuous wave) and delivery system (contact versus noncontact) also influence the type of tissue insult produced17-21 and potentially the degree of postoperative inflammation. We tried to characterize the inflammatory response of the normal rabbit eye to continuous-wave Nd:YAG cyclophotocoagulation by quantifying changes in protein, prostaglandin (PG), and leukotriene (LT) levels in intraocular fluids and myeloperoxidase activity in uveal tissue. We correlated these changes with the histologic effects of treatment.

Materials and Methods

Animals and Laser Treatment

Thirty-one 2-kg Dutch belted rabbits were treated unilaterally with 30 applications of Nd:YAG laser energy divided between the dorsal and ventral sclera. Laser surgery was done under general anesthesia (ketamine hydrochloride 35 mg/kg; Fort Dodge Laboratories, Fort Dodge, IA; xylazine 5 mg/kg; Mobay, Shawnee, KS) in accordance with the ARVO Resolution on the Use of Animals in Research and with the approval of the Laboratory Animal Use Committee, North Carolina State University. Energy was delivered through a 600-μm diameter silica fiber posi-
tioned perpendicular to, and in contact with, the conjunctiva and 1 mm posterior to the limbus. A commercial continuous-wave system (Microruptor 3; Lasag, Thun, Switzerland) was used to deliver 3.5 J of energy (0.7 sec and 5 W) per application. Seven animals were killed on day 1, and eight animals were killed on days 3, 8, and 15 by barbiturate overdose. At each of these intervals, the eyes of six animals were used for the calculation of inflammatory cell type and number, PG and LT levels, and uveal myeloperoxidase activity; the globes of the other animals were used for gross and light and electron microscopic examination. The untreated eye served as the control.

Clinical Evaluation

The animals were evaluated immediately before being killed by slit-lamp biomicroscopy, and the intensity of the aqueous protein (flare) and iris vascular response was graded from 0-3+ (for flare, 0 = no Tyndall effect, with 1, 2, and 3, representing mild, moderate, and severe Tyndall effects, respectively; for iris vascular response, 0 = no abnormalities visible, 1 = mild hyperemia, 2 = hyperemia and mild engorgement of vessels, and 3 = hyperemia and severe engorgement and/or iris hemorrhage).

Quantitation and Identification of Inflammatory Cells

Immediately after death, aliquots of aqueous humor were collected in heparinized syringes, stained with modified Wright-Giemsa stain, and differential leukocyte counts done under light microscopy at 400X magnification. Total leukocyte and erythrocyte numbers in the aqueous humor were determined by the hemacytometer method.

Quantitation of Protein

Aliquots of aqueous and vitreous humor were assayed for protein by an earlier method.22

Measurement of Myeloperoxidase Activity

Myeloperoxidase activity, a marker for neutrophil content in uveal tissue, was assayed by the method previously described.23 Iris and ciliary body tissues were homogenized; the homogenate was frozen and thawed three times, sonicated, and centrifuged at 40,000 X g. The myeloperoxidase activity was assayed spectrophotometrically by measuring the change in absorbance at 460 nm of a mixture of tissue homogenate, buffer, o-diansidine dihydrochloride, and hydrogen peroxide. The activity was expressed as units of peroxidase degradation per minute of reaction.

PG and LT Levels

Aqueous and vitreous humor were acidified to pH 3.5 with 5 μl of H3PO4 (0.49 mol/l), extracted into six volumes of ethyl acetate (twice), dried under vacuum in a Speed-Vac concentrator (Savant, Hicksville, NY), and reconstituted into 500 μl of methanol. Aliquots of the methanol reconstitute were dried under a vacuum, reconstituted in 300 μl of a modified Krebs-Henseleit buffer (pH 7.4), and assayed for PGE2 and LTB4. Details of the PGE2 assay have been described previously.24 The LTB4 levels were measured using a highly specific radioimmunoassay kit (Amersham, Arlington Heights, IL).

Histopathologic Assessment

The globes were sectioned transversely at the equator and immediately immersed in chilled McDowell’s and Trump’s fixative. After a minimum of 4 hr of fixation, the anterior segments were sectioned sagitally. One half of the tissue was processed routinely, and 6-μm sections were cut and stained with hematoxylin and eosin for light microscopy. Tissues for transmission electron microscopy were rinsed twice in 0.1 mol/l phosphate buffer and postfixed in osmium tetroxide 2%. After two rinses in distilled water, the tissues were dehydrated in a graded ethanol series and embedded in Spurr resin. Semithin sections (0.5 μm) were cut with glass knives and stained with toluidine blue 1%. Thin sections (70 nm) were cut with a diamond knife and stained with alcoholic uranyl acetate and lead citrate.

Statistical Methods

Numbers of inflammatory cells, protein levels, PGE2 and LTB4 levels, and myeloperoxidase activity were compared between treated and untreated eyes by paired student t-tests. The same parameters were compared for different posttreatment intervals by analysis of variance, and significant differences were determined by Tukey’s method. Differences were considered significant at P < 0.05.

Results

Clinical and Macroscopic Effects

Laser applications were associated with the focal coagulation of conjunctival tissue at the point of fiber contact and, less commonly, by a popping sound. If the fiber tip was pressed firmly to the conjunctiva, scleral cavitation (represented by deep burns) was seen. Mean scores for flare on posttreatment days 1, 3, 8, and 15 were 1.3, 1, 1.3, and 0.78, respectively. Mean scores for iris vascular response were 1, 1, 0.75,
and 0 at the same intervals. Macroscopic examination of treated ciliary bodies revealed focal white areas of apparent tissue coagulation in the ciliary processes and pars plana, occasionally associated with complete disruption of the ciliary epithelium. Ciliary lesions were sometimes associated with small amounts of organized fibrin and hemorrhage.

**Erythrocytes and Inflammatory Cells in Aqueous Humor**

Erythrocytes were present in large numbers in the aqueous humor at all evaluation intervals, with significantly higher levels occurring on day 8 (mean, 22,614 ± 10,517/μl; Fig. 1). Leukocytes, by contrast, although present in low numbers, were significantly more numerous on day 3 (mean, 205 ± 254/μl). The predominant inflammatory cell was the monocyte, representing 73.6% and 64.2% of all leukocytes on days 3 and 8, respectively (Fig. 2). There were no significant differences between intraocular cell counts from untreated eyes at different intervals.

**Protein Levels in Intraocular Fluids**

In treated eyes, protein levels were significantly higher (mean, 11.0 ± 7.0 mg/ml) in aqueous humor on day 3 compared with other intervals; they declined rapidly between days 8 and 15 (Fig. 3). Vitreal protein levels, however, were greatest on day 8 (mean, 3.9 ± 1.5 mg/ml) and remained unchanged at day 15. Protein levels in the control eyes were not significantly different between evaluation intervals.

**Uveal Myeloperoxidase Activity**

Although generally higher than in control uvea, myeloperoxidase activity was only significantly greater than in control eyes on day 3 (mean, 6.32 ± 3.1 units of activity/mg protein, Fig. 4).

**PG and LT Levels**

Aqueous humor PGE₂ levels were significantly higher (mean, 8.9 ± 6.9 ng/ml) on day 3 than at other intervals and rapidly decreased between days 8 and 15 (Fig. 5). Significant differences were not found in vitreal PGE₂ or in aqueous or vitreal LTB₄ levels in treated versus untreated eyes.

**Histologic Changes**

The consistent pathologic response to cyclophotocoagulation was coagulation necrosis of the ciliary processes and epithelium accompanied by mild-to-severe vascular congestion. At all intervals, deep scleral burns were seen adjacent to areas of ciliary necrosis, which was characterized by disruption of both pigmented and nonpigmented epithelial cell layers and dispersion of melanin pigment. On day 1, low-to-moderate numbers of polymorphonuclear leukocytes were observed in the subepithelial ciliary stroma and adjacent to the arterioles. By day 8, however, mononuclear inflammatory cells predominated, extending into the iris in the more severely affected eyes.

Effects of Nd:YAG cyclophotocoagulation attributable to vascular compromise were seen at all intervals. At day 1, loss of normal mural architecture was seen in ciliary arterioles (Fig. 6), and there were large quan-
Fig. 3. Protein levels (±SD) in aqueous and vitreous humor after transscleral Nd:YAG cyclophotocoagulation.

Fig. 4. Myeloperoxidase activity (±SD) in uveal tissue after transscleral Nd:YAG cyclophotocoagulation.

Fig. 5. Prostaglandin levels (±SD) in aqueous humor after transscleral Nd:YAG cyclophotocoagulation.

Ultrastructural Changes

The most significant ultrastructural changes observed involved the ciliary vasculature and epithelium. Although specimens from all periods were affected, those from day 1 were the most severe. Vascular endothelial necrosis was prominent and characterized by rupture of plasma membranes with loss of identifiable intracellular organelles (Fig. 7). In both the pigmented and nonpigmented ciliary epithelium, there was severe cytoplasmic vesiculation with intercellular separation.

Discussion

The inflammatory sequence initiated by traumatic or noxious stimuli begins with damage to cells and connective tissue and the subsequent release of a diverse group of inflammatory mediators that include lysosomal enzymes, histamine, bradykinin, arachidonic acid metabolites, oxygen metabolites and free radicals, cytokines, chemotactic factors, and products of complement activation. Vascular permeability is changed, constituents of plasma leak through damaged interendothelial cell junctions, and the migration of neutrophils and eventually monocytes is stimulated. The features and severity, however, of the ensuing inflammatory process are variable and depend largely on the nature of the underlying insult.

For Nd:YAG cyclophotocoagulation, vascular compromise is a particularly prominent feature, evidenced by both the elevated levels of aqueous and vitreal protein, and particularly by the numbers of erythrocytes found in aqueous humor and extravascular uveal tissues. Because the escape of erythrocytes from the vascular compartment is a passive process that occurs in proportion to the extent of the vascular injury, this effect (when seen after laser exposure) must be considered severe. Although vascular perme-
ability may be altered by inflammatory cell mediators such as histamine, bradykinin, and prostaglandins, the duration of protein leakage, the magnitude of erythrocyte extravasation, and the histologic changes seen in this study are most compatible with direct vascular injury. This conclusion agrees with the histologic findings of previously reported rabbit studies, in which acute effects of vessel thrombosis and endothelial cell lysis and persistence of vessel changes as long as 9 months after treatment were described. The drop in aqueous protein levels by day 15, however, implies rapid restoration of the bloodocular barrier.

The second prominent feature of Nd:YAG cyclophotocoagulation is the mild inflammatory response, as indicated by the relatively low aqueous inflammatory cell counts, aqueous protein, and PGE2 levels, and particularly the uveal myeloperoxidase activity. This low uveal enzyme activity was consistent with the number of neutrophils seen in the histologic sections.

Because the rabbits' irritative ocular response is considerably greater than that of humans and because of significant differences in anatomy of ciliary vasculature, the applicability of these results to humans is subject to some speculation. Histologic features of

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**Fig. 6.** (A) Photomicrograph showing mural damage in ciliary arteriole 1 day after cyclophotocoagulation (H&E, ×870). (B) Photomicrograph showing extensive hemorrhage in ciliary process 1 day after cyclophotocoagulation. Mononuclear inflammatory cells are prominent (H&E, ×500).
Mechanisms proposed to account for the pressure-lowering effects of Nd:YAG cyclophotocoagulation are (1) direct destruction of aqueous secreting ciliary epithelium,\(^3^8\) (2) indirect ciliary epithelial cell destruction through ischemia of supporting vasculature or secondary to inflammation,\(^1^6\) (3) increased uveoscleral outflow, and (4) creation of alternate drainage routes, such as transscleral flow.\(^3^9\) If inflammation plays a significant role in lowering intraocular pressure after transscleral cyclophotocoagulation, it may be advantageous not to inhibit this operative effect. However, if ciliary vascular compromise is a crucial component to effective lowering of intraocular pressure, procedures that affect the vasculature more selectively may be appropriate. Recent studies show that continuous-wave transscleral cyclophotocoagulation seen in the dog, a species whose ocular inflammatory response is considered intermediate between that of humans and rabbits, were found to be similar to those we reported.\(^3^6\) In the only study describing the acute effects of Nd:YAG cyclophotocoagulation in humans, the consistent finding was disruption of the ciliary epithelium and separation of the epithelial layers from the underlying stroma by fibrin and a low number of leukocytes.\(^3^7\) Although the magnitude of the response we found was considerably greater, it is conceivable that the results observed in human and rabbit eyes share a common mechanism of direct vascular and ciliary insult, with leakage of protein and a mild inflammatory response.
pulsed lasers produce an explosive effect in ciliary tissue; continuous-wave application produces more of a coagulative effect. The tissue effects of transscleral Nd:YAG laser energy also vary significantly between contact and noncontact systems. Given that these variables are likely to affect the relative extent of vascular damage, understanding the interaction of vascular compromise and inflammation would appear to be crucial to the development of optimum systems for transscleral cyclophotocoagulation.

In conclusion, continuous-wave Nd:YAG cyclophotocoagulation in the rabbit produces a profound vascular insult that is accompanied by a relatively mild inflammatory response. Information gained in this study can provide a useful basis for future studies to identify the parameters associated with the most effective pressure reduction and the least adverse effects.

Key words: neodymium: YAG laser, cyclophotocoagulation, inflammation, myeloperoxidase, prostaglandin

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References


