Radial Optic Neurotomy in the Porcine Eye Without Retinal Vein Occlusion

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Objective: To demonstrate the histopathologic changes in the porcine eye without retinal vein occlusion after radial optic neurotomy (RON).

Methods: A RON was performed in 14 normal eyes of 12 Yorkshire Cross pigs. One radial stab incision at the edge of the nasal optic nerve head was made using a 20-gauge microvitreoretinal blade (Visitec) while the intraocular pressure was elevated. Surgery was concluded when hemostasis was achieved. Weekly ophthalmoscopic examinations were performed. Group 1 eyes (4 eyes of 2 pigs) were enucleated at the end of surgery. Group 2 eyes (4 eyes of 4 pigs) were enucleated 1 week postoperatively, and group 3 eyes (4 eyes of 4 pigs) were enucleated 3 weeks postoperatively. In group 4 (2 eyes of 2 pigs), animals underwent vitrectomy and RON, and eyes were enucleated 3 weeks postoperatively.

Results: Ophthalmoscopic examination demonstrated engorged blood vessels at the RON site up to 3 weeks after surgery with minimal or no hemorrhage. Histological examination of the optic nerve demonstrated foci of hemorrhage, interstitial edema, reactive gliosis, and rare inflammatory cells. At 3 weeks, there was complete axonal nerve fiber loss distal to the neurotomy site.

Conclusions: After RON, marked gliosis and complete axonal nerve fiber loss occur at the neurotomy site. Although bleeding was rare intraoperatively in this porcine model, hemorrhage and interstitial edema were present within the optic nerve at the neurotomy site histologically.

Clinical Relevance: Radial optic neurotomy remains a controversial method of treatment for central retinal vein occlusion. To our knowledge, this is the first study in the literature describing the histopathologic findings after RON.

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The pigment is not only localized to the lamina cribrosa, but appears to involve all fibrovascular septa of the nerve. The pig retinal blood vessels pass to the retina through the papilla centrally or near its borders. There are 4 main arterial branches, consisting of a nasal and a temporal vessel in both the superior and inferior segments of the fundus. The veins follow a similar pattern, but the branches are less numerous. Our objective was to perform a RON in pigs and to examine histologically the optic nerve and adjacent tissues.

**METHODS**

**ANIMALS**

Twelve Yorkshire Cross pigs (14 eyes), aged 3 to 4 weeks, were treated in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. All eyes were included in the final analysis. Twelve eyes underwent RON and were randomly assigned to 3 study groups. In group 1 (4 eyes of 2 animals), eyes were enucleated at the end of surgery. In group 2 (4 eyes of 4 animals), eyes were enucleated 1 week postoperatively. In group 3 (4 eyes of 4 animals), eyes were enucleated 3 weeks postoperatively. In group 4 (2 eyes of 2 animals), eyes underwent pars plana vitrectomy and neurotomy and were enucleated 3 weeks postoperatively.

**PROCEDURE**

Animals underwent premedication with 0.1 mL of 1% atropine sulfate (Phoenix Pharmaceutical Inc, St Joseph, Mo) intramuscularly and then anesthetized with 10 to 40 mg/kg of intramuscular ketamine hydrochloride (Fort Dodge Laboratories, Fort Dodge, Iowa) and 2 to 3 mg/kg of intramuscular xylazine hydrochloride (Phoenix Pharmaceutical Inc). If additional anesthesia was needed during the procedure, 10 to 40 mg/kg of intramuscular ketamine hydrochloride was used. For pupillary dilation, 0.25% scopolamine hydrobromide (Alcon Laboratories Inc, Fort Worth, Tex), 1% atropine sulfate (Alcon Laboratories Inc), and 2.5% phenylephrine hydrochloride (Bausch & Lomb Pharmaceuticals Inc, Tampa, Fla) were used. Proparacaine hydrochloride (Bausch & Lomb Pharmaceuticals Inc) was the topical anesthetic. The conjunctival sac was irrigated with 5% povidone-iodine solution. All animals were initially examined with indirect ophthalmoscopy to exclude any preexisting vitreoretinal abnormalities. One or both eyes of each animal were subjected to proptosis, and 3 sclerotomies were created using a 19-gauge microvitreoretinal (MVR) blade for the introduction of the infusion cannula, a fiberoptic light probe, and a 20-gauge MVR blade. Infusion of lactated Ringer solution maintained intraocular pressure via the infusion line. The 20-gauge MVR blade was used to perform a radial incision at the nasal edge of the optic nerve head while the intraocular pressure was elevated (Figure 1). In group 4, eyes underwent complete vitrectomy with creation of a posterior vitreous detachment, followed by RON as described. Surgery was concluded when hemostasis was achieved, and the sclerotomies and conjunctiva were sutured with 7/0 polyglaclin 910 (Vicryl; Ethicon Inc, a division of Johnson & Johnson, Somerville, NJ) and 6/0 plain gut, respectively. In groups 2, 3, and 4, 20 mg of gentamicin sulfate (Abbott Laboratories, North Abbott Park, III) was injected into the subconjunctival space.

**POSTOPERATIVE EVALUATION**

The 4 eyes of the 2 animals killed on the day of surgery (group 1) were enucleated at that time and sent for histological evaluation. Eight of the 10 remaining animals were followed up for 1 week (group 2) or 3 weeks (groups 3 and 4), with examinations at 1, 2, and 3 weeks after surgery. At each time, the retina was examined by indirect ophthalmoscopy, and fundus photographs were obtained. After the final examination, animals were killed with intracardiac pentobarbital sodium, and the eye was enucleated for histological examination.

**HISTOPATHOLOGY**

After enucleation, the eyes underwent immediate sharp-razor penetration close to the pars plana to ensure rapid penetration of fixative. The eyes remained immersed in 4% paraformaldehyde for at least 24 hours at 4°C. All tissues were embedded in paraffin, and 5-µm sections were stained with hematoxylin-eosin. Masson trichrome histochemical staining was performed.

**IMMUNOHISTOCHEMISTRY**

Sections from the paraffin block were cut at 4 to 5 µm, placed on positively charged glass slides, deparaffinized in organic solvents, treated with methanolic hydrogen peroxide to quench endogenous peroxidase activity, and rehydrated. Sections were reacted with glial fibrillary acidic protein and neurofilament pro-
tein monoclonal antibodies (dilutions, 1:4000 and 1:100, respectively; DAKO, Carpinteria, Calif). Nonimmune rabbit immunoglobulins (DAKO) were used as negative control specimens, and appropriate positive tissue controls were also tested. Phosphate-buffered saline was used throughout the procedure. Incubation time for the primary reaction was 45 minutes at 40°C, and 20 minutes at 40°C for the secondary and tertiary reactions. The unlabeled, bound primary antibody was linked with biotinylated goat anti-rabbit IgG (1:300; Vector Laboratories, Inc, Burlingame, Calif) and detected with horseradish peroxidase–labeled streptavidin (1:800; Jackson ImmunoResearch Laboratories, Inc, West Grove, Pa). Immunoreactivity was visualized using diaminobenzidine as the chromogen, with Harris modified hematoxylin (Fisher Scientific Co, Pittsburgh, Pa) as the counterstain. In addition, the width of the MVR stab incision within the lamina cribrosa was measured using the 100-µm bar on the hematoxylin-eosin sections in all 4 group 1 eyes. The width was measured at the top, in the middle, and at the bottom of the lamina cribrosa.

RESULTS

No significant complications were encountered during surgery in any eye. The optic nerve remained unchanged postoperatively in all but 6 eyes in which engorged blood vessels developed near the neurotomy site immediately after RON and were present as long as 3 weeks postoperatively. In some cases, a small hemorrhage from the neurotomy site was observed at the time of surgery; however, no hemorrhage was present at 2 weeks postoperatively. The local loss of the nerve fibers adjacent to the neurotomy site was present for less than 1 hour (Figure 2). The loss of nerve fibers was most apparent 3 weeks after surgery.

PATHOLOGY RESULTS

Hematoxylin-eosin–stained sections of the optic nerve and posterior retina confirmed the presence of the neurotomy site. The optic nerve was characterized by foci of hemorrhage, interstitial edema, reactive gliosis, and rare inflammatory cells. Sections taken at the peripheral portion of the neurotomy site in proximity to retina demonstrated retinal tissue incarcerated in the sclera at the neurotomy site (Figure 3). Results of Masson trichrome histochemical staining showed early scarring at the neurotomy site within 1 week and confirmed the presence of a healed scar at the neurotomy site at 3 weeks (Figure 4). Immunohistochemical staining for glial fibrillary acidic protein demonstrated early reactive gliosis in group 1 eyes that were immediately enucleated, and the reactive gliosis was sustained and diffuse throughout the entire segment of optic nerve at 3 weeks (Figure 5). Neurofilament protein showed weak staining of the axons in the immediate vicinity of the neurotomy site at the time of immediate enucleation, and progressive absence of axons in the vicinity of the neurotomy site at 1 week. At 3 weeks, there was complete axonal nerve fiber loss distal to the neurotomy site along the entire segment of optic nerve that was sharply demarcated from adjacent intact nerve fibers (Figure 6). Light microscopic examination demonstrated no neovascularization or chorioretinal shunt formation at the neurotomy site at any time. The neurotomy wound had a mean width of 105.5 µm within the lamina cribrosa; however, the gap was filled with fibrosis at 1 week (Figure 7). There were no differences in the histological findings of the optic nerve in the vitrectomized and nonvitrectomized eyes.

COMMENT

Radial optic neurotomy remains a controversial treatment for eyes with CRVO. No treatment has proven successful in treatment of this disease thus far. Intravenous streptokinase and recombinant tissue plasminogen activator have been used to treat eyes with CRVO, but despite encouraging results, possible severe systemic adverse effects, including death, have prevented their widespread use. To decrease the risk of the systemic adverse effects of recombinant tissue plasminogen activator, Lahey and colleagues demonstrated that intravitreal administration did not lead to catastrophic hemorrhagic events in their nonrandomized study, and some eyes appeared to benefit from the therapy. Weiss demonstrated that it was possible to deliver recombinant tissue plasminogen activator directly into the retinal vein using a transvitreal approach, and some patients may benefit from this treatment. McAllister and colleagues demonstrated that the creation of a chorioretinal-venous
anastomosis using a high-intensity laser was beneficial in some eyes with CRVO. However, the potential for serious complications exists, such as hemorrhage, preretinal fibrosis, traction retinal detachment, vitreous hemorrhage, choroidal neovascularization, and choroidovitreal neovascularization.

Histological studies demonstrate that most CRVO cases are associated with a thrombus in the central retinal vein at the level of or just posterior to the lamina cribrosa. Studies by Vasco-Posada and Arciniegas suggested cutting the sclera around the optic nerve via an external approach. Despite promising results, this technique was not further developed. Opremcak and colleagues hypothesized that CRVO is a neurovascular compression syndrome resulting from increased pressure within the confined space of the scleral outlet. In their nonrandomized study, a RON was performed transvitreally with a 20-gauge MVR blade in an attempt to decompress the optic nerve. Eight (73%) of 11 patients with CRVO showed an improvement of visual acuity with a mean gain of 5 lines of vision. In cadaver eyes, an MVR blade could cut the cribriform plate, scleral rim, and adjacent sclera without ocular perforation. To our knowledge, no study in the literature describes the histological effects of RON in an animal eye. In the present study, no significant complications during or after neurotomy were observed; however, results of histological examination suggested that RON was a traumatic procedure. The neurotomy site was characterized by foci of hemorrhage, interstitial edema, and rare inflammatory cells. Fibrosis and a glial scar were evident within 1 week. Immunohistochemical staining of the optic nerve demonstrated diffuse reactive gliosis and complete axonal nerve fiber loss distal to the neurotomy site (Figure 5).

Because the mechanical constriction of the central retinal vein at the level of the lamina cribrosa is a possible pathoetiologic mechanism of CRVO, a surgical approach (such as RON) to relieve this compartment syndrome may be beneficial. In our histological study, RON resulted in a mean wound width of 105.5 µm; however, the neurotomy site was filled with fibrous tissue at 1 week.
Therefore, it is not clear how long the RON can relieve any mechanical forces on the retinal veins (Figure 7). Further animal studies such as the measurement of blood flow in the retinal veins after RON may provide some answers.

In a surgical technique study of cadaver human and porcine eyes, Lit and colleagues demonstrated that puncture of the lamina cribrosa with a specially designed lancet tip was possible without serious injury to the optic nerve. These authors speculated that their technique could be another approach to decompress the central retinal vein via an intravitreal approach. Although the authors performed additional in vivo experiments in rabbits, there was no information about the effectiveness of this procedure in humans. There are no data as to whether this technique is less traumatic than RON.

Another possible explanation of how RON improves the course of CRVO is that RON leads to chorioretinal shunt formation after neurotomy. In our study, we did not observe any new chorioretinal shunt formation on ophthalmoscopic or histological examinations. The lack of shunt development may be multifactorial. In our study, RON was performed in healthy, nonischemic pig eyes, the follow-up period was relatively short (3 weeks), and no imaging techniques that might be helpful in recognizing such shunts early, such as fluorescein angiography, were performed.

Because RON is a traumatic procedure, it remains a controversial method of treatment for CRVO. Although preliminary clinical reports of RON are encouraging, further study is necessary to establish RON as a standard treatment for CRVO.

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REFERENCES


Figure 7. A, A light micrograph of a pig optic nerve immediately after surgery. The gap (arrows) within the lamina cribrosa (arrowheads) is caused by the microvitreoretinal blade. B, A 1-week postoperative micrograph of a pig optic nerve. The fibrotic tissue fills the area where the cut was made (arrows) (original magnification x100).