Fundus and Histopathological Study of Radial Optic Neurotomy in the Normal Miniature Pig Eye

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Objective: To demonstrate the fundus and histopathological changes in the normal miniature pig eye after radial optic neurotomy.

Methods: Ophthalmoscopic examinations, fundus photography, and fluorescein angiography were performed on 12 eyes that underwent radial optic neurotomy, 5 normal eyes, and 7 eyes that underwent vitrectomy (from 12 pigs in total) preoperatively and 1, 7, 14, 30, and 90 days postoperatively. These eyes were enucleated 120 days postoperatively. Sixteen eyes (8 pigs in total) that underwent radial optic neurotomy were enucleated 1, 3, 7, and 48 days (2 pigs each time) postoperatively. The enucleated eye sections were stained with hematoxylin-eosin, Masson trichrome, and Luxol fast blue.

Results: The retina radial to the site of radial optic neurotomy darkened gradually with the increasing curvature of the major retinal arteries. The filling time intervals from the retinal artery to the retinal vein were prolonged. At the incision site, there was a loss of nerve fibers, which were subsequently replaced by collagenous tissue. No anastomotic vessels formed by the end of the study.

Conclusions: Postoperatively, the retinal circulation seemed somewhat sluggish compared with that seen preoperatively. Segmental retinal nerve atrophy eventually formed. The procedure itself may not be the sole factor for the formation of shunt vessels.

Clinical Relevance: To date, radial optic neurotomy is controversial and persuasive animal studies are lacking. In combining fluorescein angiography with histopathological examination, this study may be somewhat helpful.

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CENTRAL RETINAL VEIN OCCLUSION (CRVO) is a common retinal vascular cause of visual loss.1 Its pathogenesis is not well known, though some evidence supports central retinal vein thrombosis at the level of the lamina cribrosa as the cause.2 There is no definitely proven, effective management to date.3 Radial optic neurotomy (RON) was designed to alleviate constriction of the central retinal vein at the level of the lamina cribrosa, and 73% of patients who underwent RON experienced an improvement in visual acuity.4

However, the subsequent reports of RON were not as dramatically encouraging.5-7 Some studies implied that the choriotinal anastomosis formation may be an alternative mechanism of action.8-11 To our knowledge, there was only 1 in vivo animal report about RON, but the follow-up period was only 3 weeks and no imaging techniques were used.12

One study13 suggested that miniature pigs can replace monkeys for arteriovenous filling time investigations. The porcine retina is holangiotic and the major retinal venules lie centrally in the optic disc.14 The lamina cribrosa of the pig is very strong and embeds the retinal blood vessels with a thickness of 0.4 to 0.6 mm.12 Since the retinal hemorrhage resulting from CRVO may make it difficult to identify whether the fundus changes after surgery were caused by RON or CRVO, we chose the pig eye without CRVO as an experimental model.

METHODS

ANIMALS

Miniature pigs weighing 10 to 12 kg were obtained from the Department of Animal Sci-
ence, Beijing Agricultural University, Beijing, China, and were housed by the Department of Animal Studies, People’s Hospital, Peking University, Beijing. They were treated following the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. A total of 20 pigs were divided into 2 groups: in group 1 (12 pigs), 12 randomly chosen unilateral eyes underwent RON and 7 of the contralateral eyes underwent axis vitrectomy, with no procedures performed on the other 5 eyes. At 120 days after surgery, the eyes were enucleated. In group 2 (8 pigs), both eyes of each pig underwent RON and were enucleated at 1, 3, 7, and 48 days (4 eyes of 2 pigs each time) after surgery.

PREPARATION
Animals were systemically anesthetized with intramuscular injections of ketamine hydrochloride (15 mg/kg) and xylazine hydrochloride (15 mg/kg) followed by a retrobulbar injection of 3 mL of 2% lidocaine hydrochloride. Pupils were dilated using 0.5% phenylephrine hydrochloride and 0.5% tropicamide eyedrops. Before enucleation was performed, excessive pentobarbital sodium was injected to kill the animal.

VITRECTOMY AND RON
Under systemic anesthesia, a standard 3-port pars plana axis vitrectomy was performed without posterior vitreous detachment. With a 20-gauge microvitreoretinal blade, a single-stab radial neurotomy was performed starting at the edge of the optic disc. To protect the major retinal vessels and bimaculars in the pig eyes, the superior or inferior side was chosen. The blade was then directed posteriorly into the optic nerve and the depth of the incision placed the blade just beyond the widest portion of the diamond-shaped tip.

OPHTHALMIC PROCEDURE
Ophthalmic examinations including slitlamp examination, indirect ophthalmoscopy, and fluorescein angiography were performed on group 1 eyes preoperatively and at 1, 7, 14, 30, and 90 days after surgery. Photography was accomplished with a standard Topcon TRC-50EX fundus camera (Topcon, Tokyo, Japan). Color slides as well as red-free photographs were recorded. Two milliliters of 10% fluorescein sodium solution (Guangzhou Mingxing, Guangzhou, China) was injected into 1 of the marginal ear veins manually and the timer was simultaneously started. Photography began prior to the appearance of fluorescein in the choroidal vessels and was continued with intervals shorter than 1 second during the first 40 seconds. Thereafter, the frequency of recording decreased and the late phases were taken after about 5 minutes.

HISTOPATHOLOGICAL PREPARATION
The enucleated eyes remained immersed in 4% formaldehyde for at least 24 hours at 4°C. All of the tissues were embedded in paraffin, and 4-µm sections were stained with hematoxylin-eosin, Masson trichrome, and Luxol fast blue.  

RESULTS
FUNDUS CHANGE
Except for severe vitreous hemorrhage in 1 eye and subretinal hemorrhage beside the RON site in 1 eye, no hemorrhage or only mild hemorrhage was observed at the RON site in the remaining eyes. No other significant complications were encountered during surgery. After surgery, the retina radial to the RON site darkened gradually and no shunt vessels appeared at the RON site until 90 days postoperatively (Figure 1). The retinal arteries, especially the artery near the RON site, became more tortuous in 7 eyes that underwent RON (Figure 2) from 7 days postoperatively until the end of the study. After the vitreous hemorrhage was completely absorbed, both mean filling time intervals from the choroid to the retinal artery and from the retinal artery to the retinal vein were prolonged when compared with the preoperative mean filling time intervals (Table). No significant change could be observed postoperatively in the pig eyes that underwent axis vitrectomy.

HISTOPATHOLOGICAL RESULTS
Owing to the demyelination of myelinated nerve fibers, the balloon-like change of the hematoxylin-eosinstained section and weak staining by Luxol fast blue ap-

![Figure 1. A, Fundus photograph of a pig retina 90 days after radial optic neurotomy. The retina radial to the incision darkened owing to the loss of the retinal nerve fiber layer. B, At the late venous phase of fluorescein angiography, no shunt vessels appeared at the incision site.](image-url)
peared at the RON site with foci of hemorrhage, interstitial edema, and a few inflammatory cells at 1 day after surgery. Local hemorrhage infiltrated into the circumambience and backside (Figure 3). At 3 days postoperatively, the balloon-like changes were aggravated, with a dividing line between normal and abnormal tissue (Figure 4). At 7 days postoperatively, the fibroblasts accumulated at the incision site with hyperplastic neuroglial cells and dispersed pigmented granules. At 48 days postoperatively, a collagen component filled in the incision at the optic disc (Figure 5) and neuroglial cells accumulated at the rear optic nerve behind the incision.

Table. Filling Time Intervals of Fluorescein Angiography Before and After Radial Optic Neurotomy in 12 Pig Eyes*

<table>
<thead>
<tr>
<th>Location</th>
<th>Preoperative</th>
<th>At 14 Days</th>
<th>At 30 Days</th>
<th>At 90 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choroid to retinal artery</td>
<td>2.1 (0.8)</td>
<td>2.6 (0.9)</td>
<td>2.8 (1.4)</td>
<td>2.7 (0.8)</td>
</tr>
<tr>
<td>Retinal artery to retinal vein</td>
<td>4.4 (0.8)</td>
<td>5.3 (1.3)</td>
<td>5.2 (1.2)</td>
<td>4.7 (0.9)</td>
</tr>
</tbody>
</table>

*Values are expressed as mean (SD).
At 120 days postoperatively, myelinated nerve fibers disappeared and were replaced by the collag-
enous tissue with hyperplasia of capillaries (Figure 7).

**COMMENT**

Radial optic neurotomy is a controversial management modality that emerged recently. Despite the promising result, controlled clinical trials and animal studies are still necessary to test it. The mechanism of action in some successfully treated cases is a topic of discussion. In the article by Opremcak et al, the concept of compression or increased pressure from the scleral outlet compartment is the basis of RON. However, the subsequent reports showed that the chorioretinal anastomosis developed at the RON site, which may have occurred earlier than in the natural course. The videoangiography study implied that the retinal circulation improvement may be correlated with RON.

In the pig eyes without CRVO in our study, both the fluorescein angiography and histopathological study showed that no anastomosis formed. Though expected to improve retinal circulation, single decompression of the central retinal vein at the level of the lamina cribrosa did not lead to a shortened retinal circulation time (Table).

In our study, the changes of the shape of major retinal arteries were observed in the normal eyes (Figure 2), which had not been reported in the clinical reports of eyes with CRVO. It would be difficult to identify whether RON or CRVO itself caused this in the eyes with CRVO. This occurred as early as 7 days postoperatively, so we may exclude the possibility that the mechanical traction force of fibrous membrane formation was the cause. The intravenous pressure that increased after surgery may explain this, though we cannot provide much more evidence besides the mildly prolonged retinal circulation time. In fact, the method in our experiment to record retinal circulation times by fluorescein angiography was not accurate enough to make a conclusion regarding the changes of retinal blood flow after surgery. We used fluorescein angiography mostly as an imaging method and considered its data to be a preliminary clue. Further study applying more accurate quantification methods is necessary.

Because RON is a traumatic procedure performed on delicate and critical structures, safety should be considered before effectiveness. Whereas we tried to avoid the injury of major vessels during surgery, severe vitreous hemorrhage was encountered and may have been caused by the laceration of vessels beneath the optic nerve head. Previous histopathological findings of RON demonstrated complete axonal nerve fiber loss distal to the neurotomy site at 3 weeks after surgery. Furthermore, with the same findings at the early stage of follow-up, we found that the localized collagenous tissue replaced optic nerve fibers at the incision site at 4 months postoperatively. In our study, the nerve fiber layer of the retina radial to the incision site attenuated gradually (Figure 1A), which may explain the postoperative segmental visual field loss that was reported by Williamson et al.

![Figure 5. Photomicrograph of a pig optic disc 48 days after radial optic neurotomy. The incision healed with collagen tissue (arrow) (hematoxylin-eosin, original magnification ×80).](image1)

![Figure 6. Photomicrograph of a pig optic nerve 48 days after radial optic neurotomy (hematoxylin-eosin, original magnification ×80). Inset, The density of neuroglial cells increased, as shown by weak staining by Luxol fast blue.](image2)

![Figure 7. Photomicrograph of a pig optic nerve 120 days after radial optic neurotomy. The myelinated nerve fibers (flat rosiness) of the incision site disappeared and were replaced by collagenous tissue (green) with hyperplasia of the capillaries (Masson trichrome, original magnification ×80).](image3)
In summary, localized optic nerve atrophy eventually developed at the incision site and no improvement of retinal circulation with a lack of anastomotic vessel formation was found after the central retinal vein was decompressed by RON. Controlled clinical trials are necessary, but further animal studies are also needed to understand the mechanism of action of RON and to make it a safer procedure.

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REFERENCES


Correction

Error in Author Name. In the book review titled “Glaucoma: A Colour Manual of Diagnosis and Treatment, Third Edition,” published in the May issue of the ARCHIVES (2005;123:712-713), the author’s name should have appeared as follows: Teresa C. Chen, MD. The ARCHIVES regrets the error.