An Experimental Model of Ectropion Uveae and Iris Neovascularization in the Cat

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Neovascularization of the iris (NVI) is one of the most frequently studied intraocular vascular proliferations in animal models. Ectropion uveae has not been a consistent finding in these studies. In this study, a surgical model of ectropion uveae and iris neovascularization was developed that involved lensectomy, vitrectomy, bipolar cautery and transection of all three principal branch veins in the cat eye. Twelve of 14 eyes that received this procedure developed postoperative retinal detachments with a clinical picture of hemorrhagic retinopathy. These eyes progressed to a clinical picture of NVI within 1–7 wk. Eight eyes developed ectropion uveae for as much as 300°. At the light microscopic level, a fibrovascular membrane was apparent on the anterior iris stroma in 9 of 14 eyes and further involved the angle in six eyes. Invest Ophthalmol Vis Sci 33:1796–1803, 1992

Neovascularization of the iris (NVI) is a common clinical finding in human ocular disease related to posterior ischemia. Proliferative diabetic retinopathy, central retinal vein occlusion, and carotid artery stenosis represent the major clinical entities leading to neovascularization of the iris (NVI) and neovascular glaucoma. Several studies have appeared in the literature that describe animal models of neovascularization of the iris. These studies principally have used nonhuman primates1–7 and rabbits8, while one report described a model of iris neovascularization in the cat9. Most studies have employed lensectomy combined with retinal detachment or experimental vein occlusion to stimulate neovascularization of the iris. Ectropion uveae, an eversion of the pupillary margin, is frequently associated with neovascular glaucoma but has not been a consistent finding in any of the animal models of neovascularization of the iris.

The purpose of the present study was to establish a model of ectropion uveae and neovascularization of the iris that can be the starting point for more detailed studies of the histological and cell biological events that constitute these processes. After evaluating several different surgical protocols, a procedure involving lensectomy and vitrectomy through the pars plana, followed by complete closure of the three principal branch veins with bipolar cautery and transection, was chosen. Of the 14 animals subjected to this surgical protocol, 12 developed clinically apparent neovascularization of the iris within 1–7 wk of the surgery. Eight of these eyes developed ectropion uveae. Neovascularization was confirmed by light microscopy in nine cases. By light microscopy, neovascularization was confirmed in nine eyes, and six had evidence of fibrovascular involvement of the anterior chamber angle.

Materials and Methods

Animals

All animals were purchased from the Animal Resource Service of the University of California, Davis. The investigators adhered to the ARVO Resolution on the Use of Animals in Research. Animals were fed and given water ad libitum and were housed as a colony of 12 or fewer animals with environmental enhancement.

Surgery

Surgery was performed on the right eye of six-month-old cats under halothane anesthesia with endotracheal intubation. The pupil was dilated with 1% tropicamide, 1% atropine, and 2.5% phenylephrine. A lateral canthotomy and limbal peritomy were created in preparation for standard three port pars plana surgery. A phacemulsification lensectomy (Allergan Medical Optics Phacoemulsifier, Irvine, CA) was performed in all animals. In five animals, a phacoemulsi-
fication lensectomy was performed as a separate procedure 2–4 wk before the vitreoretinal surgery. In nine animals, the lensectomy was performed at the same time as the vitreoretinal surgical procedure. After lensectomy, a vitrectomy (Storz MVS 1000, St. Louis, MO), and air-fluid exchange were performed. Two bent 27 G needles were attached to bipolar cautery leads and were used to elevate, cauterize, and transect the three principal branch veins (Fig. 1). This created small retinal tears in the area of the transected veins. The sclerotomies, peritomy, and canthotomy were each sutured, and subconjunctival injections of 20 mg gentamicin and 2 mg dexamethasone were given. Bacitracin/neomycin/polymyxin hydrocortisone acetate ointment and 1% atropine sulfate were administered three times a day for one week.

Slit-lamp biomicroscopy and indirect ophthalmoscopy were performed the first postoperative day and then at weekly intervals.

Tissue Processing

Globes were enucleated under deep ketamine anesthesia and were immediately placed into freshly prepared 4% paraformaldehyde, 0.1 M phosphate buffer, pH 7.4. Animals were killed with intravenous injection of pentobarbital. A 1 cm incision was placed 5 mm posterior to the limbus, and the globes were immersion fixed for 24 hr. After fixation, the globes were hemisected at the ora serrata, and the anterior segment was divided into four quadrants. One quadrant was dehydrated through a graded series of alcohols and embedded in Immunobed (Polysciences, Inc., Warrington, PA), a glycol methacrylate resin, for use in light microscopy.

Light Microscopy

For routine evaluation of histology, 2–3-μm sections of tissue were cut from quadrants embedded in Immunobed. Sections were mounted on slides and stained with Gill's hematoxylin followed by aqueous eosin Y containing phloxin and acid fuchsins.

Results

Fourteen eyes of 14 cats underwent pars plana lensectomy, vitrectomy, air-fluid exchange, and retinal vein cautery and transection. The cats were examined weekly for evidence of NVI, ectropion uveae, angle closure, retinal detachment, and hemorrhagic retinopathy.

Vein cautery and transection (Fig. 1) resulted in a hemorrhagic retinopathy similar to that seen in retinal vein occlusion, and retinal detachment in twelve cases. Retinal detachments resulted from tears intentionally created during vein cautery and transection.

Twelve of the 14 eyes developed clinically apparent NVI within 1–7 wk after surgery (mean = 2.5 wk) as seen in Table 1. This was accompanied by ectropion uveae in eight eyes. Figure 2 illustrates the neovascularization seen clinically in a typical eye with a fine branching network of superficial blood vessels over the iris surface associated with ectropion uveae. All eyes with NVI had total retinal detachments with a
Table 1. Clinical and histopathologic findings

<table>
<thead>
<tr>
<th>Animal number</th>
<th>NVI (weeks) onset/regression</th>
<th>Retinal detachment</th>
<th>NVI membrane</th>
<th>Ectropion uvea</th>
<th>Angle NV</th>
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</table>

variable degree of hemorrhagic retinopathy. Two of the four eyes without NVI had attached or only partially detached retinas.

NVI was clinically noted to regress in four eyes 4–12 wk after surgery. In two of these eyes, however, ectropion uveae also was apparent, and a fibrovascular membrane on the anterior surface of the iris was verified by light microscopy. In the remaining two eyes that showed regression and in cat no. 291 there was no histological evidence of anterior membrane formation or neovascularization. In these eyes, the clinical finding of NVI probably represents an inflammatory vascular dilation rather than true neovascularization. Finally, five eyes with postoperative vitreous hemorrhage developed elevated intraocular pressure as demonstrated by buphthalmos, while none of the eyes with NVI and a clear vitreous showed evidence of elevated intraocular pressure.

At the light microscopic level, 9 of 14 eyes that had undergone the combined lensectomy, vitrectomy, and vein cautery/transection procedure exhibited a fibrovascular membrane on the anterior surface of the iris. Figure 3A demonstrates a typical fibrovascular membrane that extends from the pupillary margin to the anterior chamber angle of Descemet’s membrane. A normal cat iris is presented in Figure 3B. Inflammatory cells were noted within the iris stroma and on the surface of the cellular membrane. These types of changes also were noted adjacent to the iris epithelium in several cases. The degree of inflammation observed clinically or histologically, however, did not correlate with the presence or degree of development of the anterior cellular membrane. Congestion of the ciliary processes also was noted in most surgical eyes.

Figure 4A presents a higher magnification photomicrograph of a dense fibrovascular membrane. This membrane is anterior to the layer of melanocytes or iridiphores that demarcate the normal anterior border.
Fig. 3. Photomicrograph of a fibrovascular membrane on the anterior surface of the iris. Cat 092 developed iris neovascularization 1 week after experimental vein cautery and transection which persisted until the eye was enucleated at three months. Panel (A) is a 3-μm radial section through the iris, at a magnification of ×40. Arrows demarcate the fibrovascular membrane. Panel (B) is a photomicrograph of a similar section from a control eye, also at ×40.
of the cat iris. Figure 4B illustrates a normal cat iris.

Ectropion uveae was associated with the neovascular membrane in eight eyes. A typical example of this pathology is seen in Figure 5, where a fibrovascular membrane on the anterior surface of the iris extends onto the posterior pigment epithelium, resulting in ectropion uveae. One of the eyes with neovascularization did not develop ectropion uveae, but had histopathologic evidence of posterior synechiae binding the pupillary margin to remnants of the lens capsule.

Anterior chamber angle involvement was identified in six of the nine eyes with fibrovascular membranes. Figure 6 demonstrates a fibrovascular membrane extending from the anterior surface of the iris, across the angle, onto the endothelial surface of the cornea.

Discussion

The findings of this study indicate the surgical procedure involving lensectomy, vitrectomy, and branch...
Fig. 5. Photomicrograph of ectropion uveae. Cat 453 developed neovascularization of the iris 2 weeks after experimental vein cautery and transection, which persisted until enucleation of the globe at 15 weeks. Arrowhead indicates the fibrovascular membrane on the anterior surface of the iris. Arrows indicate iris epithelium which has moved to an anterior position due to contraction of the fibrovascular membrane. Asterisk indicates the iris sphincter muscle. Magnification is $\times125$.

vein closure by cautery and transection produce a consistent model of ectropion uveae and neovascularization of the iris in the cat eye. Clinically, neovascularization of the iris was apparent in 1-7 wk and usually persisted for several months. In all cases where neovascularization of the iris was observed, a retinal detachment also was noted. Because a previous model of neovascularization of the iris in the cat involved retinal detachment as a pathogenic procedure, this event undoubtedly contributed to the development of NVI in these eyes along with hemorrhagic retinopathy resulting from vein cautery and transection.

Histologically, the experimental eyes demonstrated the development of a cellular membrane with many fine vessels on the anterior surface of the iris stroma. Ectropion of the iris sphincter muscle and the iris epithelium could be demonstrated clinically and histologically. These findings support the notion that the cellular membrane on the anterior surface of the iris is contractile. In the one eye with NVI but no ectropion uveae, posterior synechiae between the iris and the lens capsule remnants could have prevented anterior rotation of the pupil margin and thus prevented demonstration of ectropion uveae despite the fibrovascular membrane.

In eyes with angle involvement, there was profuse cellular proliferation in the angle and/or synechial closure. Only one quadrant per eye was surveyed by light microscopy so the incidence of angle involvement may be higher than we have reported. There was no clear example of endothelialization of the angle or peripheral iris, a feature commonly found in the histopathology of neovascularization of the iris and neovascular glaucoma in the human eye.\textsuperscript{10-12}

Overall, many of the clinical and histopathological features of this surgical model of ectropion uveae and neovascularization of the iris in the cat parallel those found in human ocular disease. This model should be an excellent starting point for a more thorough examination of the temporal and cellular events that lead to anterior fibrovascular membrane formation on the iris.
Fig. 6. Photomicrograph of neovascularization within the anterior chamber angle. Cat 451 developed neovascularization of the iris 2 weeks after experimental vein cauterity and transection, which persisted until enucleation of the globe at 9 weeks. Panel (A) illustrates the closed anterior chamber angle with fibrovascular proliferation. The large arrow indicates the area of the normal anterior surface, now obscured by the fibrovascular membrane. The small arrow indicates a capillary within the fibrovascular membrane. The arrowhead indicates corneal endothelium. Magnification is ×120. Panel (B) is the anterior chamber angle of a control eye. Magnification is ×120.
**Key words:** iris neovascularization, ectropion uveae, animal model

**References**