Preretinal Neovascularization Induced by Experimental Retinal Vein Occlusion in Albino Rats

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Retinal ischemia and neovascularization have been demonstrated in several animal models. To determine 1) whether the retinal or preretinal neovascularization can be induced in albino rats by retinal vein occlusion and 2) the type and rate of occurrence of neovascularization, we occluded retinal veins in albino rats by photodynamic thrombosis. After anesthesia, each of 36 rats received an injection of rose bengal photosensitive dye, and their veins underwent argon green laser treatment. Half or all the major retinal veins were occluded in 12 eyes and in 24 eyes, respectively. Ten control rats underwent the same procedures but the laser beam was directed between major retinal vessels. In 46 control eyes, rose bengal dye was seen to have perfused without laser treatment. Retinal detachment developed in most vein-occluded eyes within one day of venous occlusion, which was confirmed by fluorescein angiography. On follow-up at two weeks, only four of 24 eyes (16.7%) had undergone occlusion of all retinal veins showed new preretinal vessels on the optic disc. In these four eyes, severe disturbance of both retinal arterial and venous blood flow was observed, but no other eyes showed such severe combined disturbance. These data suggest that preretinal neovascularization in albino rats can be induced by this minimally traumatic method and that venous occlusion is severe enough to compromise arterial blood flow for a certain threshold period, thus inducing the development of preretinal neovascularization.

Key words: retinal vein occlusion, neovascularization, animal model, rose bengal, laser photoocoagulation

INTRODUCTION

In many vascular diseases such as retinal vein occlusion and diabetic retinopathy, retinal or preretinal neovascularization secondary to ischemia is one of the leading causes of visual loss. To elucidate its pathogenesis, animal models are needed, but it is well known that in many animals, retinal vein occlusion much more frequently

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induced iridic than retinal or preretinal neovascularizations.1-6 According to the literature, the latter has been successfully induced in pigs and pigmented rats.7-9

The most popular current occlusive procedure in pigmented animals is laser photoocoagulation combined with photosensitive dye, but the disadvantage of this method is that in addition to retinal vascular occlusion, there may be thermal damage to retinal pigment epithelium, choroid, and the sensory retina; such damage does not occur in actual vascular diseases. To minimize this problem, we used easily available albino rats, minimal laser
photoocoagulation and photosensitive intravascular dye. The purpose of this study was to investigate the incidence and patterns of retinal neovascularization and other concomitant findings in this animal model.

**MATERIALS AND METHODS**

**Animals**

All experiments were performed in accordance with the ARVO resolution on the Use of Animals in Research. Sprague Dawley albino rats (male or female, weighing 200-250 g) were anesthetized by intramuscular injection of ketamine hydrochloride (80 mg/Kg of weight) and xylazine hydrochloride (8 mg/Kg of weight). The pupil was dilated by 1.0% tropicamide. Prior to experiments, animals were biomicroscopically examined, and these that were judged to be normal were selected for experimental use.

**Venous occlusion groups and control groups**

In two groups of 12 and 24 rats, respectively, one eye of each animal underwent laser treatment. In the group of 12, treatment was restricted to half the major retinal veins, while in the other group, all such veins were treated. The control group consisted of ten rats, in each, the laser beam was directed linearly between major retinal vessels, and in one eye. The 46 contralateral eyes of the two venous occlusion groups and the control group were used as a rose bengal control group. Six eyes of three rats which underwent no procedures were used as a negative control group.

**Venous occlusion methods**

One minute before laser treatment, anesthetized rats were intravenously injected with 0.2 ml of rose bengal (40 mg/Kg; tetrachloro-tetraido-fluorescein sodium, certified purity, 90%; Sigma, St. Louis, MO, U.S.A.). Using a slide cover glass, they were subjected to argon green laser (wave length: 514.5 nm), and 2% methylhydroxypryoplycellulose (Methocel™, CIBA Vision Ltd, Switzerland) was applied over the cornea. During laser treatment, the aiming beam was at minimal intensity but the illumination beam was not used. Laser parameters were 0.1 second, 50 μm, and 150 mW. Twenty laser impulses were applied to each major retinal vein adjacent to the optic nerve head, for approximately ten burn lengths. Just before fluorescein angiography, one hour later, photographs of the fundus were taken (camera model TRC 50IA, Topcon, Japan). For angiography, 0.3 ml of 10% sodium fluorescein dye was injected rapidly into a tail vein and angiography was performed using a fundus camera.

**Postoperative follow-up**

One day after laser treatment and then weekly, the rats were examined by indirect ophthalmoscopy or slit-lamp biomicroscopy. In selected animals, more photographs of the fundus were taken and fluorescein angiography was performed.

The follow-up period was one week in 12 rats which underwent occlusion of half the retinal veins and two weeks in 24 rats in which all retinal veins were occluded. In the laser treatment control group (10 rats), the follow-up period was one week, and in the rose bengal control group, the period was one to two weeks.

**Histological examination**

After follow-up, rats were sacrificed by intraperitoneally injected overdose of pentobarbital. The eyeballs were enucleated and stab wounds were made at the scleroconneal junction with sharp blades. They were then fixed overnight with 4% paraformaldehyde – 2% glutaraldehyde in 0.1 M phosphate buffered solution, pH 7.4. On the following day, the cornea, lens and vitreous were removed, and the eyeball was cut into two halves which were re-fixed for 24 hours in the same fixation solution. After regular dehydration and paraffin embedding, semi-thin sections (6 μm) were stained with hematoxylin-eosin for light microscopy.

**RESULTS**

**Venous occlusion groups**

Retinal venous dilation and some peripheral retinal hemorrhage occurred after laser treatment of major retinal veins; complete venous occlusion and
relatively normal arterial blood flow were confirmed by fluorescein angiography performed one hour after treatment. On fluorescein angiography, no hyper- or hypofluorescent retinal lesions were seen, except at laser treated sites. Varying degrees of retinal detachment developed in 35 eyes within 24 hours, but this gradually subsided (Fig. 1). In one eye which underwent occlusion of half the retinal veins, retinal detachment did not occur.

During follow-up at one week, no retinal or preretinal neovascularization was seen in 12 eyes which had undergone occlusion of half the retinal veins. In ten eyes, retinal detachment still remained, but arterial blood flow was normal.

Fluorescein angiography performed two weeks after occlusion showed that in four of 24 eyes which had undergone occlusion (16.7%), preretinal neovascularization had developed on the optic disc; abnormal vessels were present and gradual, profuse leakage of dye was noted (Fig. 2). New vessels were detected at one week in one eye, and at two weeks in three eyes. In these four eyes, disturbance of arterial and venous blood flow, and a pale and reattached retina were observed during the follow-up period (Fig. 3). Other eyes without new vessels did not show such disturbance of retinal blood flow.

Two weeks after laser treatments, retinal detachments was still seen in three eyes, but in four eyes with new vessels, it had subsided.

Fig. 2. Preretinal new vessels on the optic disc induced by occlusion of all retinal veins, two weeks after laser treatment (upper panel). Fluorescein angiography revealed vascular complex on the optic disc in the early phase (middle panel) and profuse dye leakage in the late phase (lower panel).

Control groups

Arterial disturbance, retinal detachment and neovascularization did not develop in any eyes of the laser treatment or rose bengal control group.
DISCUSSION

Retinal ischemia induces retinal or preretinal neovascularization in a number of vision-threatening diseases such as retinal vein occlusion and diabetic retinopathy. For further investigation of its pathogenesis and treatment, suitable animal models are needed, but it is well known that in contrast with rubeosis iridis in monkeys, cats and rabbits, retinal or preretinal neovascularization is not easily and reliably induced in animals.1-6

Three groups researchers recently reported that using photocoagulation or photodynamic thrombosis, preretinal neovascularization has been successfully induced in domestic pigs, miniature pigs and pigmented rats.7-9 In one report,7 rose bengal dye and argon green laser with parameter settings of 10-30 seconds duration, 100 µm spot size and 100-180 mW intensity were used; two major branch veins or one major and several smaller veins in miniature pigs underwent laser treatment. In another report,9 fluorescein dye and argon blue-green laser with parameter settings of 1 second, 50 µm spot size and 50-100 mW intensity were used. Using the 78 diopter lens, the effective retinal spot size was approximately twice the venous diameter. Using Long Evans pigmented rats, all retinal main branch veins were occluded, and an average six shots was applied to each vein, followed by an average of four shots to the same vein.

Albino rats, used in this experiment, are less expensive and easier to obtain than pigs. In addition, it was thought that expected that photocoagulation-induced thermal damage would be less in albino than in pigmented rats; the former have far less melanin pigment in retinal pigment epithelium and choroidal melanocytes.

We have previously occluded retinal veins using this method but with different laser parameter settings; these were 0.1-1 second duration, 50 µm spot size, and 50-150 mW intensity. At that time, histologic examinations revealed more extensive and severe retinal damages in eyes which had undergone higher energy treatment (in press), and we believed that to minimize this unwanted damages, so that the artificial damage could be confined to retinal veins, we therefore used for

**Fig. 3.** One of the four eyes which developed preretinal neovascularization one week after laser treatment. Severe disturbance of arterial and venous blood flow was detected in all retinal areas, and two weeks after treatment, new vessels were found. The photographs are of the superior half (upper panel) and inferior half (lower panel) of the fundus.

**Histological examination**

On histological examination, varying degrees of inner and outer retinal damage was seen in the venous occlusion groups. Most eyes which underwent occlusion of all retinal major veins revealed paper-thin like retinal atrophy. Histological examination revealed no preretinal new vessels in addition to those already anigographically demonstrated in four eyes. Only laser-induced localized retinal damage was seen in the laser treatment control group; there was no any evidence of retinal detachment or neovascularization. There were no differences in histological findings between the rose bengal control groups and those of normal rat eyes.
lower energy laser treatment than previous two experiments, and minimal laser spot size without a magnifying lens.

In this study, retinal neovascularization and detachment were due to retinal vascular occlusion, not to the effects of laser treatment on the retina itself or the effects of rose bengal perfusion. These results are consistent with those of other reports\textsuperscript{9,10} and retinal detachment is not thought to be the direct cause of neovascularization.\textsuperscript{9}

Eyes which underwent occlusion of half the retinal veins showed no neovascularization, but the follow-up period was relatively short. In one previous report,\textsuperscript{3} new vessels developed up to two weeks after venous occlusion in rats. Thus, it is still unclear whether new vessels develop under these circumstances, though in this model, new vessels can be induced by the occlusion of all retinal veins.

The incidence of preretinal neovascularization in this model was far less than that of previous report; this might be due to less thermal and photodynamic injury to retinal veins, which in turn induced venous occlusion of more variable and shorter durations.

Interestingly, four eyes which developed neovascularization invariably showed temporary, severe disturbances of arterial and venous blood flow during the follow-up period. This suggests that retinal ischemia may be due to arterial insufficiency secondary to venous occlusion and if this ischemia persists for more than a certain threshold period, retinal or preretinal neovascularization ensues. These disturbances were not seen in other eyes which did not develop neovascularization, which suggests that in those eyes, retinal arterial insufficiency was minimal or present for a shorter time. We do not know which factors control the severity and duration of arterial insufficiency.

In this study, all new vessels on the optic disc were the preretinal type. A previous report, described the development of many forms of new vessels, including retinal new vessels elsewhere (NVE),\textsuperscript{9} inner limiting membrane damage due to heavy laser treatment or more severe retinal ischemia might induce these different types of neovascularization.

In conclusion, we occluded all retinal veins in albino rats, using photodynamic thrombosis, and preretinal neovascularization developed in four eyes. If the factors which control the severity of arterial insufficiency or duration are fully understood, this animal model can be used in future studies of retinal neovascularization.

REFERENCES


