Studies on intravitreal blood vessels. I. A new experimental model

Donald R. Sanders and Gholam A. Peyman

We produced an experimental animal model simulating intravitreal neovascularization by injecting 0.1 ml. of a solution containing 15 mg. of ammonium chloride (NH₄Cl) into the vitreous cavity of albino rabbits. This dosage causes exudative retinal detachment, destruction of the retina, liquefaction of the vitreous, contraction of the posterior hyaloid membrane, and only minimal retinal vessel damage. Contraction of the posterior hyaloid membrane draws the retinal vessels into the mid-vitreous cavity. The intravitreally displaced retinal vessels provide an excellent model for evaluation of treatment modalities for intravitreal neovascularization.

Key words: experimental model, intravitreal neovascularization, ammonium chloride, exudative retinal detachment.

Recently, there has been great progress in the treatment of diabetic and sickle cell retinopathies which cause retinal neovascularization and vitreous hemorrhage. Surface neovascularization can be treated by argon laser1–5 or xenon arc photocoagulation,6,7 but special problems are encountered when intravitreal neovascularization is present and requires treatment. Frequently, the energy absorbed by these blood vessels is not enough to cause occlusion.5 In addition, those vessels with a large caliber may, in fact, burst as a result of treatment and cause vitreous hemorrhage.6

With the use of photocoagulation in the treatment of intravitreal neovascularization, the problem of inadvertent damage to underlying tissue can also become significant when the neovascularization arises from the optic disk.8

New experimental treatment modalities, specifically radiofrequency probe9 and vitrectomy,10,11 have recently become available and show great promise in the treatment of intravitreal neovascularization. Previous models, notably injection of lactic acid in the vitreous by Imre12 and production of retrolental fibroplasia in kittens by Ashton and co-workers13–15 and Patz,16 produce intravitreal neovascularization in close proximity to the retina. These models are difficult to produce and seldom simulate the clinical picture found in the late stage of diabetic retinopathy, in which new vessels are drawn into the mid-vitreous cavity. As a result, new experimental treatment modalities, specifically radiofre-
frequency probe and vitrectomy cannot be properly evaluated before use in human retinopathies. We have, therefore, developed a new, easily reproduced model that simulates intravitreal neovascularization.

Methods and materials

Experiment I. Ten albino rabbits weighing 2 to 3 kilograms each were anesthetized by intravenous injection of 65 mg of sodium pentobarbital, and 2 drops of 1 per cent cyclopentolate dilated their pupils. The animals were divided into five groups of two each. Both eyes of each rabbit were injected with 0.1 ml of solutions containing either 5 mg, 10 mg, 15 mg, 20 mg, or 30 mg of ammonium chloride. A 27-gauge needle injected the solution through the pars plana at the superior temporal quadrant into the mid-vitreous cavity with the bevel of the needle directed toward the posterior surface of the lens. The injection was performed under direct observation of the needle and the fundus with an indirect ophthalmoscope and a 20-diopter condensing lens. To prevent an increase in intraocular pressure, an equivalent amount of fluid was released by anterior chamber paracentesis with a 27-gauge needle.

The animals were observed daily by direct and indirect ophthalmoscopy over periods ranging from one day to three months. Fundus photographs were taken to illustrate the various changes. The rabbits were then killed by an overdose of sodium pentobarbital, and the eyes were enucleated and fixed by immersion in a solution of 1 per cent gluteraldehyde and 1 per cent formaldehyde in phosphate buffer, pH 7.4. Sections, prepared by embedding in paraffin, cutting with an American Optical microtome, and staining with hematoxylin and eosin, were examined under a light microscope.

Experiment II. Based on the results of Experiment I, dosages of 15 mg (12 rabbits) and 20 mg (12 rabbits) of ammonium chloride in 0.1 ml of solution (osmolarities of 2,775 and 3,700 milliosmols per liter, respectively) were injected into both eyes of the experimental animals. Injection, observation, and histologic examination were done exactly as in Experiment I. In addition, fluorescein angiography was performed to evaluate the patency of the blood vessels, 2 ml of 10 per cent Na fluorescein was injected intravenously while the vessels were observed with an indirect ophthalmoscope and photographed with a Zeiss fundus camera.

Results

Experiment I. Immediately after injection, when the NH₄Cl came into contact with the surface of the retina, a localized whitish reaction occurred. Within 10 to 15 minutes after injection, a localized exudative detachment followed. No other immediate changes in the retina could be observed ophthalmoscopically. A posterior lens opacification appeared minutes after injection, but did not interfere significantly with retinal observation and cleared within 24 hours. Some scattered posterior subcapsular opacities persisted, however.

In all eyes there was progression of the exudative detachment one day after injection. In the area of detachment, whitish degenerated retinal fragments floated in the posterior vitreous (Fig. 1). At the 5 mg and 10 mg dosages, the retinal changes were localized to the inferior area of the fundus which, because of the technique of injection, received the greatest concentration of ammonium chloride. Eyes injected with 30 mg of NH₄Cl, in addition to showing large areas of exudative detachment, developed flame-shaped hemorrhages (Fig. 2) and segmentation of the blood column (Fig. 3) on both the temporal and nasal sides of the vascularized portion of the rabbit retina. These
Ftg. 2. Retinal hemorrhage (arrow) following intravitreal injection of 30 mg. of NH₄Cl.

Ftg. 3. Segmentation of retinal vessels (arrow) following intravitreal injection of 30 mg. of NH₄Cl.

Fig. 4. Optic disk (arrows) showing complete obliteration of retinal vessels following intravitreal injection of 30 mg. of NH₄Cl.

vessels became obliterated and disappeared within 12 to 16 days after injection (Fig. 4). The effect of intravitreal injection of 15 mg. and 20 mg. of ammonium chloride is described in Experiment II.

Experiment II. The immediate retinal changes in all eyes of this group were similar to those in Experiment I. Because the inferior nasal area was exposed to the greatest concentration of ammonium chloride, the retina in this area was the first to become white and develop an exudative detachment. Within one to three days after injection of the 20 mg. dose, the vessels of the nasal area of the vascularized rabbit retina became congested and showed localized flame-shaped hemorrhages as in Fig. 2. The vessels in the temporal portion showed congestion without hemorrhage. In the following four to five days the vitreous gel became liquefied, and the posterior hyaloid membrane detached from the retina but remained attached at the optic disc. With the detachment of the posterior hyaloid and its contraction, the vascularized portion of the rabbit retina along with the myelinated nerve fibers separated from the rest of the retina (Fig. 5). Further contraction of the membrane drew the retinal vessels into the middle and anterior portions of the vitreous cavity (Figs. 6 and 7). Two to three days later, the myelin sheaths degenerated further and appeared to peel off from the vessels, leaving the vessels surrounded only by a diaphanous membrane (Fig. 8).

Of the 24 eyes injected with 20 mg. of NH₄Cl, six eyes showed complete obliteration of retinal vessels following intravitreal injection of 30 mg. of NH₄Cl.
Fig. 5. Separation of vascularized portion of rabbit retina with myelin sheaths (arrows) from the rest of the retina following intravitreal injection of 15 mg of NH₄Cl.

Fig. 6. External photograph showing intravitreally displaced retinal vessels and myelin sheaths following injection of 15 mg of NH₄Cl.

Fig. 7. Schematic diagram of intravitreally displaced retinal vessels attached to posterior hyaloid membrane after injection of 15 mg of NH₄Cl.

Histologic examination of the retina immediately after ammonium chloride injection demonstrated complete necrosis of the sensory retina in affected areas (Fig. 11). Later, the retina completely disappeared and the choroid was scarred (Fig. 12). The intravitreally displaced vessels were patent (Fig. 13) and surrounded by a collagenous cellular membrane.

Discussion

The described changes may have been caused by the additive effects of the following mechanisms. First, the presence of a hypertonic solution in the vitreous tends to draw water into the vitreous to establish osmotic equilibrium. The water drag from
the choroid draws the retina into the vitreous cavity and effectively produces an exudative or, more appropriately, a transudative retinal detachment. Second, the ammonium ion presumably enters the retinal cells in sufficient concentration to swell and burst them in a manner analogous to ammonium chloride's effects in causing osmotic hemolysis in red blood cells.10

Apparently, blood vessels are spared this gross disruption, possibly because of the fast-moving circulation within them, which can either wash out the ammonium chloride solution through the bloodstream or at least mitigate its effects to allow the
endothelial cells and pericytes to survive. If the vessels are exposed to excessive local concentrations of ammonium chloride, the vessel wall is also damaged, and bleeding, segmentation, and eventual obliteration occurs. The part of the rabbit retina that is not vascularized has no way of diluting this solution, and thus will be destroyed. Interestingly, since the damage is caused by a readily diffusible hypertonic salt solution, the process of diffusion will dissipate the noxious agent and limit further destruction. During our preliminary studies, we have not found these changes with all hypertonic solutions. Injection of hypertonic sodium chloride (30 mg.), while bringing about similar changes on the retina, caused complete obliteration of the retinal vessels. Lower concentrations fail to produce progressive retinal detachment. The third possible mechanism to explain the observed changes is that intravitreal injection of ammonium chloride causes liquefaction of the vitreous gel and contraction of the posterior hyaloid membrane. The vessels of the rabbit retina are mostly on the surface of the retina and in close contact with the posterior hyaloid membrane. The contraction of the posterior hyaloid membrane draws the retinal vessels with the myelin sheath into the vitreous cavity. The myelin sheath then degenerates further, peels off the vascularized area, and disappears, leaving the retinal vessels surrounded only by an almost transparent membrane.

Our experiments show that it is possible to produce relatively undamaged intravitreal vessels suitable for experimentation in practically all injected rabbit eyes on at least one side of the optic disk. We did, however, find a slight attenuation of these blood vessels two to three months after injection. This attenuation might be explained by the fact that when retinal tissue is destroyed, there is no need for nutrition, and the vessels might thus have a tendency to regress.

Although this model does not produce a pathologic process resulting in true intravitreal neovascularization, it is useful for a number of reasons. It allows com-
parison of treatment modalities such as xenon-arc photocoagulation, argon laser photocoagulation, and intraocular diathermy, which rely on the production of heat energy to achieve closure; the fact that the retinal vessels have been displaced into the vitreous cavity and are free of the energy-absorbing effects of the retina, choriocapillaris, and pigment epithelium permits a realistic comparison. Such a comparison allows appraisal of newer treatment modalities prior to use in humans and permits an evaluation not possible on human subjects.

The patency and anterior displacement of these vessels provides a means of testing various new instruments designed to treat intravitreal vessels surgically.

In conclusion, we have produced a new, inexpensive, easily reproduced model for simulating intravitreal neovascularization.

The authors wish to thank Nirmal Seth for preparing histologic sections and Norbert Jednock and Bruce Busse for their assistance in taking the photomicrographs.

REFERENCES