I. Studies on retinal neovascularization

Friedenwald Lecture

Arnall Patz

Evidence supporting the original concept of Michaelson and Ashton of a vasoproliferative or angiogenesis factor responsible for retinal neovascularization is discussed. The oxygen model of retinal neovascularization is described as a key example in this evidence. Newer studies on experimental retinal neovascularization and its control are summarized.

Key words: retina, neovascularization, angiogenesis, diabetic retinopathy, ischemia, oxygen, retrolental fibroplasia

Experimental retinal neovascularization or "angiogenesis" is of more than passing interest to the ophthalmologist. The clinically apparent neovascularization that occurs in the cornea, on the iris, from the retinal vessels, and from the choroidal vessels accounts for the greatest cause of severe ocular morbidity in the United States today and is a significant cause of visual loss in other countries.

It is appropriate to introduce this section with a brief discussion of certain common clinical entities associated with retinal neovascularization, not only because of the clinical magnitude but also because of what they teach us regarding mechanisms of retinal angiogenesis in general. These clinical subjects emphasize our need to understand mechanisms of angiogenesis to establish research directions toward the eventual control of neovascularization.

Retinal neovascularization is defined as the growth of retinal vessels in a configuration different from that of normal retinal vessels, with that abnormal configuration lying in a plane anterior to that of the normal retinal vessels, often extending into the vitreous cavity. In addition to a loss of the blood-retinal barrier and the consequent leakage of fluorescein dye, these blood vessels frequently bleed into the vitreous cavity, obscuring vision. Additionally, a fibrous or glial tissue often grows with the vessels to a degree that traction and distortion of the retina may occur, producing irreparable retinal damage.

The neovascularization of proliferative diabetic retinopathy is always preceded by the appearance of capillary nonperfusion and the presumably consequent retinal ischemia in those areas that are not perfused (Figs. 1 to 3). It seems that the more capillary nonperfusion is present, the greater is the likelihood for the development of neovascularization. From this observation, the hypothesis has developed that retinal ischemia leads to the production of a biochemical substance, "angiogenesis factor" or "vasoproliferative factor," that may diffuse away from the area of retinal ischemia toward adjacent retinal vessels and initiate retinal neovascularization. This hypothesis has received circumstantial support in the treatment of neovascularization by photocoagulation, a...
Fig. 1. Area of nonperfusion from retinal capillary closure. The resultant ischemia is presumed to liberate a diffusible angiogenesis substance that stimulates neovascularization in the adjacent area, at the optic disc, or on the surface of the iris.

Fig. 2. Fluorescein angiogram of young diabetic patient with preproliferative retinopathy. Note large areas of capillary nonperfusion (NP). Four months later patient developed neovascularization at the optic disc.

Technique of coagulating retinal tissue that is often followed by the regression of the retinal neovascularization. The photocoagulation treatment is not directed toward the coagulation of the abnormal retinal neovascularization itself but is directed rather toward the surrounding, presumably ischemic retinal tissue. It is presumed that photocoagulation destroys, or changes, ischemic retina that may be producing a neovascular stimulating factor in such a way that the production of the stimulating factor is reduced or eliminated.

Although photocoagulation therapy in the management of diabetic neovascularization has been demonstrated to be quite efficacious in many stages of proliferative diabetic retinopathy, it nevertheless does produce some loss of visual function and at times may be associated with ocular complications. There is therefore a continued need for the investigation of other possible methods for controlling retinal angiogenesis. Additionally, photocoagulation sometimes cannot be performed because of advanced disease or media opacities.

There are several disease entities in addition to diabetic retinopathy that support the "working hypothesis" that ischemic retina produces an angiogenesis material. These other diseases include retrolental fibroplasia and branch vein occlusion, both of which have suggested, through their clinical appearance, other features of the hypothesized angiogenesis mechanisms. It is this clinical background that has held the "working hypothesis" of an angiogenesis factor in some prominence over the past 25 years. And it is with this clinical background in mind that several laboratories have begun a biochemical and physiological search for possible retinal angiogenesis activity.

Michaelson and Ashton et al. were the first to suggest the presence of a substance or vasoproliferative factor liberated by nonperfusing or ischemic retina that stimulated the development of retinal neovascularization. Other investigators have subsequently suggested that a vasoproliferative or "angiogenesis" factor might be involved in the proliferative retinopathies.
Our studies on retinal neovascularization at the Wilmer Institute date back to the early 1950s when the proliferative stage of retrolental fibroplasia was produced in experimental animals. These laboratory studies paralleled a controlled nursery study investigating the role of oxygen in retrolental fibroplasia. The oxygen model of retinal neovascularization, which was developed in Ashton's laboratories in London and in our laboratories in Baltimore, proved to be an excellent animal model for the early proliferative stages of retrolental fibroplasia, and it is the most predictable and reproducible model now available for the experimental study of retinal neovascularization.

**Mechanism of oxygen effect on the premature retina**

The experimental counterpart of early proliferative human retrolental fibroplasia was produced in kittens by Ashton and coworkers; in mice, kittens, and puppies in our laboratory; and in mice by Gyllensten and Hellström in Stockholm. Ashton's eloquent India ink injection studies elucidated the mechanism of oxygen effect on the premature retina; the production of the lesions in three different species gave confidence to the possible application of the experimental changes to human retrolental fibroplasia.

The response to oxygen is dependent upon the stage of development of retinal vascularization. When the retina is incompletely vascularized, a positive response to oxygen was observed, whereas after full vascularization of the retina, no abnormalities developed. For example, the young kitten or puppy, during the first few days of life, has an incompletely vascularized retina and is susceptible to the oxygen-induced proliferative retinopathy. The retinal vessels reach the ora serrata temporally at approximately 21 days of age. Young kittens or puppies placed in oxygen after 21 days of age show no vascular closure and no proliferative response to the oxygen exposure. The response to oxygen was found to be inversely proportional to the degree of vascularization of the retina. The response to oxygen was also directly proportional to the concentration of oxygen administered and the duration of oxygen treatment.

When the oxygen exposure is relatively minor (e.g., 24 hr at 50% concentration), on return to room air, the neovascularization in the far periphery is seen to develop, possibly
from an angiogenesis material liberated by the peripheral ischemic tissue. As the initial oxygen exposure is increased, vascular closure occurs more posteriorly, with the resultant neovascularization similarly located more posteriorly. With prolonged exposure to oxygen (e.g., 96 hr at 85% concentration), the capillary bed near the disc is also closed, and disc neovascularization results. These experimental models of retrolental fibroplasia that can be produced in the newborn kitten and puppy mimic the human disease quite closely and probably represent the best animal models to date for the study of proliferative retinopathies.

Newer studies on experimental neovascularization

In the early 1970s, we became aware, through the publications of Folkman et al., of tumor angiogenesis factor, a substance produced by solid tumors that could induce neovascularization at a distance from the tumor itself. Folkman and colleagues had demonstrated that an avascular tumor nodule placed in a corneal pocket may induce limbal neovascularization toward the tumor nodule some millimeters away, presumably through diffusion of an angiogenesis material. Interestingly, through a similar bioassay technique, Brem and Folkman were able to demonstrate an inhibition of this tumor angiogenesis mechanism by a substance extractable from neonatal cartilage.

We became interested in tumor angiogenesis factor because of the possibility that its action on retinal vessels might provide some clues regarding mechanisms of retinal angiogenesis in general. We initiated a series of experiments in collaboration with Dr. Folkman and his colleagues (Steven Brem, Henry Brem, and Robert Langer) to determine whether tumor angiogenesis factor might produce retinal vessel neovascularization. These studies were undertaken not because we felt that tumor angiogenesis factor might be the active substance in proliferative retinopathy, but rather to begin to learn something of mechanisms of retinal neovascularization in general.

In order to test the effect of tumor angiogenesis factor on retinal vessels, we used a model system with the rabbit as the experimental animal. Although the rabbit retinal vessels may have distinct differences from those of man, the rabbit was chosen because of the availability of a homologous, transplantable rabbit tumor, the V2 carcinoma. The rabbit V2 carcinoma was used as a continuous source of tumor angiogenesis factor, with nodules of rabbit V2 carcinoma injected into the vitreous cavity of the rabbit. We succeeded in producing retinal and disc neovascularization in these studies. Retinal neovascularization occurred only after the tumor infiltrated the vascularized retina proper. Other bioassay systems that monitor tumor angiogenesis factor action, such as the corneal model, were able to show the action of tumor angiogenesis factor at a distance; therefore we concluded that a possibility might be that vitreous could interfere with the angiogenic mechanism in our test system.

A series of experiments were then conducted by Dr. Steven Brem and co-workers to investigate the possibility of an inhibitor substance being present in the normal vitreous.

The adult normal vitreous was removed, centrifuged, dialyzed, and then lyophilized. The lyophilized powder was impregnated into Elvax co-polymer sustained-release pellets. The data utilizing the corneal micropocket assay showed a significant inhibition of tumor-induced corneal neovascularization by vitreous-treated pellets compared to control pellets. The inhibition of corneal neovascularization was quite similar to that reported earlier by Brem and Folkman utilizing an extract prepared from neonatal cartilage.

Recently, Dr. Bert M. Glaser joined the research group and with his colleagues identified a substance from the retina of several species which stimulated the growth of endothelial cells in tissue culture and also stimulated the development of neovascularization on the chick chorioallantoic membrane (CAM). This active substance was obtained from the retinas of the rabbit, cow, sheep, dog, and human. Partial purification of the retinal angiogenesis substance has been ac-
accomplished by Doctors Patricia D'Amore and Alan Fenselau.

It appeared logical to test the vitreous extract material for its possible inhibitory effect on the neovascularization stimulated by the retinal extracts from these species. Lutty and his co-workers demonstrated in a highly statistically significant series of experiments that the normal adult rabbit vitreous contained a substance that inhibited the neovascularization induced by extracts prepared from rabbit retina utilizing the CAM assay.

An extension of these studies aimed at identifying vasoproliferative or angiogenic substances from human eyes with intraocular neovascularization has been done by Glaser et al. on material provided by Dr. Ronald G. Michels and Dr. Thomas A. Rice at vitrectomy surgery. Material aspirated during the vitrectomy operation for patients containing retinal, optic disc, or iris neovascularization was examined. The results were compared with the vitreous aspirate obtained from vitrectomy samples on patients who did not harbor a form of intraocular neovascularization. Although the data are still preliminary at this time, Glaser and co-workers observed a good correlation between the presence of intraocular neovascularization at surgery and the stimulation of endothelial cells in tissue culture and stimulation of neovascularization on the CAM.

Returning to the original studies on excised retina by Glaser et al., it should be pointed out that the angiogenic substances identified in these experiments may not be analogous to the postulated substances from "ischemic" retina, first suggested by Michaelson and Ashton. Although the retinas in the enucleated eyes in these experiments were obviously not perfusing, the excised retinas taken from the enucleated eye may not be precisely comparable to the "ischemic" retina that occurs in conditions such as diabetic retinopathy. Further studies are planned in our laboratories to simulate more closely the human counterpart of retinal ischemia.

The concept of the hypothetical angiogenesis factor is not the only possible explanation or model system for retinal angiogenesis. Wolbarsht and Landers have suggested that neovascularization may develop from oxygen deprivation without the intermediary of a diffusible angiogenesis factor. Shabo and Maxwell have shown that, under some circumstances, inflammation may play a role in the development of ocular neovascularization.

An excellent review of the earlier literature on retinal neovascularization can be found in Wise's American Ophthalmological Society thesis published in 1956. Wise designated the presumed vasoproliferative factor as "factor X." Although his paper appeared before the advent of fluorescein angiography, he provided keen insight into the probable role of capillary closure in the evolution of proliferative retinopathies. A comprehensive and critical contemporary review of retinal neovascularization is the Krill Memorial Lecture of Henkind published in 1978.

**Conclusion**

Neovascularization is a significant complication of several ocular disorders. Most important are the retinal conditions in which neovascularization occurs, for example, proliferative diabetic retinopathy. Common to all the proliferative retinopathies is the initial development of retinal vascular closure which presumably leads to retinal ischemia. Several experimental and clinical observations suggest that retinal ischemia is associated with liberation of an angiogenesis or vasoproliferative factor, but this concept remains to be proved.

Recent studies open new avenues to investigate the underlying pathogenesis of neovascularization and methods of controlling this important ocular complication.

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