RESEARCH LETTERS

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Intravitreous Bevacizumab as Anti–Vascular Endothelial Growth Factor Therapy for Retinopathy of Prematurity: A Morphologic Study

Overexpression of vascular endothelial growth factor (VEGF) appears important in the pathogenesis of retinopathy of prematurity (ROP). Bevacizumab (Avastin; Genentech, Inc, South San Francisco, California) is a recombinant humanized monoclonal IgG1 antibody. It binds to and inhibits the biological activity of human VEGF.[1] It has been estimated that more than 10 000 patients worldwide have been treated with intravitreous bevacizumab.[2] We report results of a study in postmortem eyes with intravitreous bevacizumab treatment for zone 1, stage 2+ ROP in an extremely low-birth-weight infant.

Report of a Case. The protocol was approved by the institutional review board for the use of intravitreous injections of bevacizumab vs conventional laser therapy for the treatment of vision-threatening ROP.

A Hispanic boy delivered at 22 weeks' gestation weighing 350 g had hypoxia at birth with development of many multisystem complications throughout his life. He developed bilateral zone 1, stage 1 ROP at postconceptual age 30.6 weeks. Zone 1, stage 2+ ROP developed 3 days later and extended 360° with multiple isolated hemorrhages. The tunica vasculosa lentis and hyaloid arteries were persistent. Laser surgery could not be used as the clinical status was poor. It was determined that intravitreous bevacizumab injections at a dose of 0.5 mg (40% of the normal adult dose) under sterile conditions would be given through the nasal pars plana of each eye at postconceptual age 31 weeks.

Within the next 6 weeks following the injections, the ROP disappeared and the vessels extended into posterior zone 2. However, zone 2, stage 3+ ROP developed at postconceptual age 41.6 weeks and intravitreous injections (0.5 mg) of bevacizumab were given again. Following these injections, the extraretinal fibrovascular proliferation gradually disappeared, leaving behind only a few traces and vessels extended into medium zone 2. No ocular complications related to the intravitreous injections were noted. Unfortunately, the patient died at postconceptual age 50.6 weeks from multiple systems failure, to our knowledge unrelated to the intraocular injections. Only the eyes were donated for study.

Histopathological analysis of both eyes showed identical changes. The corneal endothelial cells were intact.

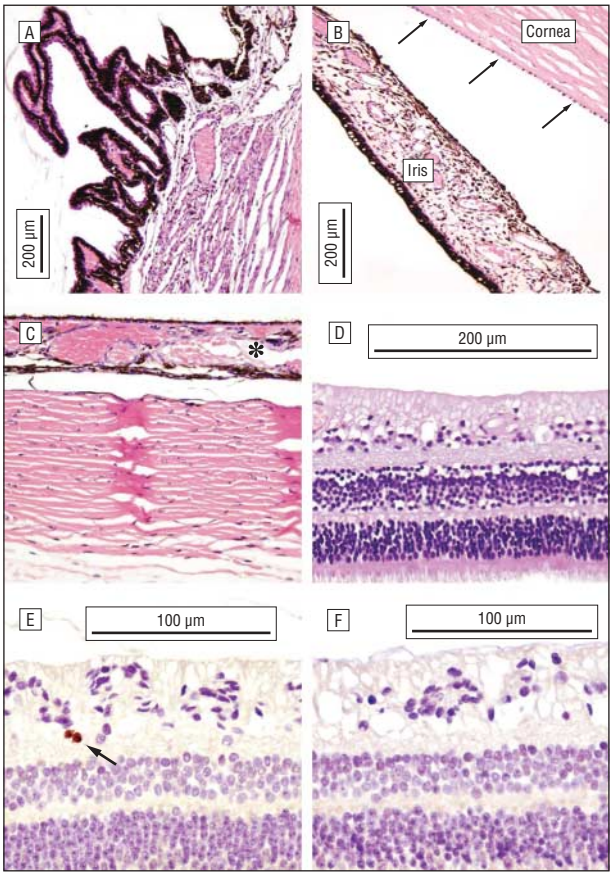

Figure 1. Histological characteristics and apoptotic activity in postmortem eyes. There was no inflammation at any level of the eye as seen by hematoxylin-eosin staining. A, Ciliary body. B, Iris, cornea, and anterior chamber showing normal endothelial cells (arrows). C, Choroid (asterisk) and sclera (original magnification ×10). D, The midperipheral retina shows normal architecture without inflammation, gliosis, or necrosis. E, Immunohistochemistry using caspase 3 for detection of cells undergoing apoptosis shows only rare cells in the right eye at the periphery in the neuronal cell layer with positive staining of the nuclei (arrow). F, Caspase 3 staining was negative in the remainder of the retina in the right and left eyes as seen in this picture from the left eye.

There was no inflammation in the anterior chamber, iris, choroid, optic nerve, or sclera (**Figure 1**), confirmed by immunohistochemistry using markers for T and B lymphocytes (CD3, CD5, CD43, and L26) and histiocytes (CD68) (**Figure 2**). All of the retinal layers were morphologically normal without inflammation, degeneration, extensive apoptosis (Figure 1), or necrosis as confirmed by caspase 3, glial fibrillary acidic protein, and vimentin immunostain. The retinal vessels extended to medium zone 2 of the retina as shown by CD31, CD34, and factor VIII antibodies (Figure 2). Few vascularization tufts through the internal limiting membrane into the vitreous were seen at the junction posterior and medium zone 2, some surrounded by histiocytes (Figure 2), but no preretinal fibrovascular membrane was seen and

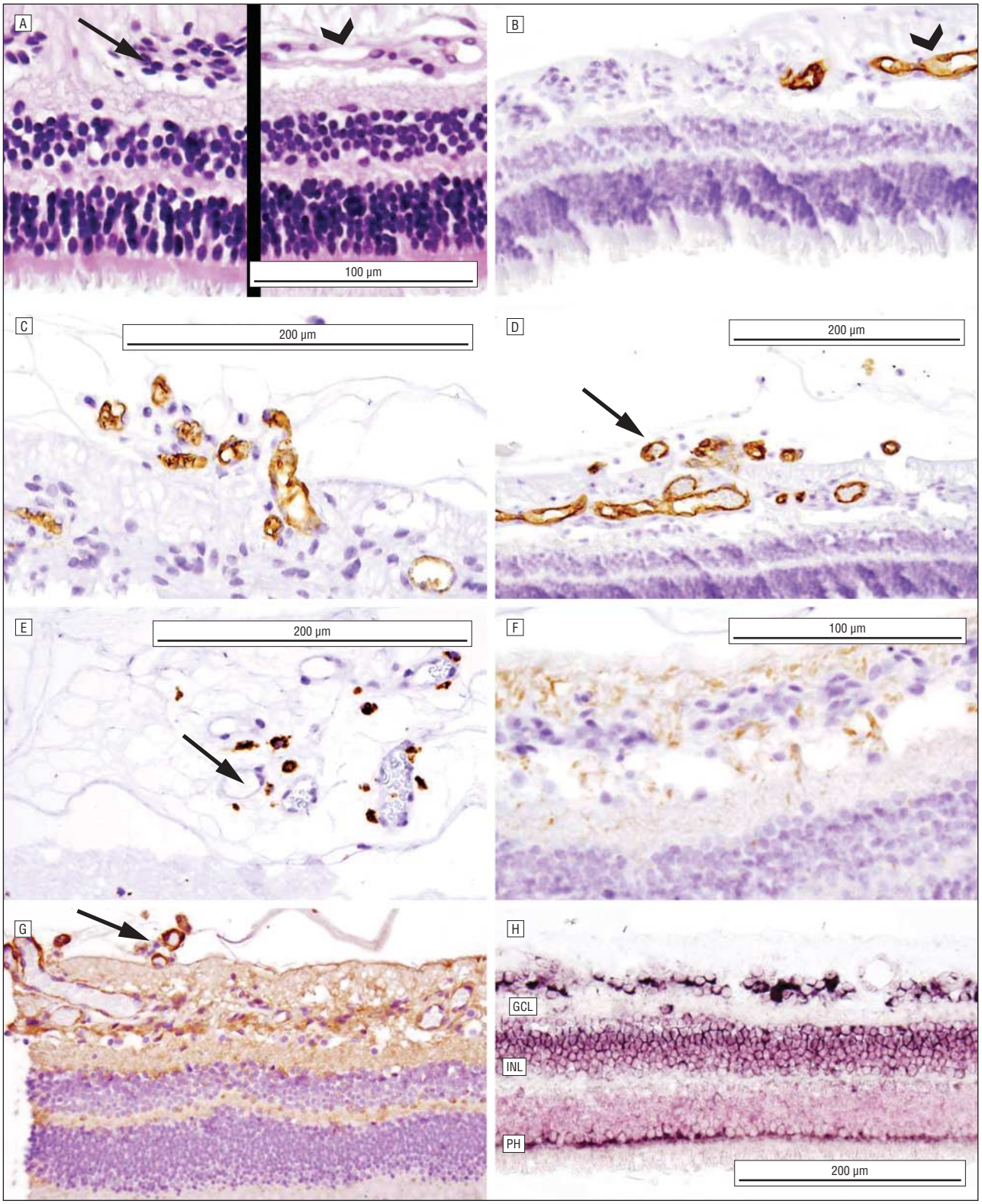

Figure 2. Vascularization and vascular endothelial growth factor expression of the eye. A, The left panel shows high magnification of an avascular peripheral retina with inner layers having precursor cells in place of vessels (arrow). The right panel shows a vascularized retina with well-formed vessels in the inner layers (arrowhead). Immunohistochemistry staining with vasculature markers was done: CD31 labels mature vessels (arrowhead) at the edge of retinal vascularization peripherally (original magnification ×20) (B); factor VIII shows staining of vessels in the inner retina along with vascular tufts breaking through the inner limiting membrane (C); CD34 labels mostly immature, newly formed vessels, both intraretinal and epiretinal (arrow) (D); CD68 labels few histiocytes surrounding the newly formed epiretinal vessels (arrow) (E); and glial fibrillary acidic protein shows staining of the inner layers of the avascular retina at the normal site of Müller cell processes and glial cells, with glial fibrillary acidic protein as the marker for glial cells (F). Vascular endothelial growth factor expression in the retina and the vascular tufts (arrow) by protein detection with immunohistochemistry (original magnification ×20) (G) and by vascular endothelial growth factor messenger RNA showing preservation of normal vascular endothelial growth factor expression in the photoreceptor (PH), inner nuclear layer (INL), and ganglion cell layer (GCL) (H).

vascularization had proceeded anteriorly. Expression of VEGF in the retina and other ocular tissues was detected at both protein and messenger RNA levels (Figure 2).

Comment. Bevacizumab in this patient was shown to be well tolerated without any signs of toxic effects; in particular, no inflammation, degeneration, or necrosis was observed. Furthermore, the results show that bevacizumab effectively controlled the neovascularization in zone 1, stage 2+ ROP. Vascular endothelial growth factor is a survival factor for retinal neurons and a critical neuroprotectant during the adaptive response to ischemic injury.[3] The retina and the proliferating abnormal vessels showed high levels of VEGF expression at both messenger RNA and protein levels. Vascular endothelial growth factor has recently been shown to influence neuronal growth, differentiation, and survival owing to its neurotrophic effects.[3-5] Therefore, the dosage of bevacizumab is critical to preserve this effect on the neuroretina for adequate development. In our case, we administered 40% of the adult dose twice. Our results show preservation of morphology and expression of VEGF in the retina.

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Descemet Membrane Rupture Accompanied by Stromal Clefting in Congenital Glaucoma

In congenital glaucoma, larger degrees of corneal distention are better tolerated by the epithelium and stroma than by the Descemet membrane. A sequential ultrasound biomicroscopical (UBM) examination of the cornea helps demonstrate both the pathophysiological mechanism for the development of breaks in the Descemet membrane as well as an accompanying clefting of stromal tissue causing acute corneal hydrops. Such examinations can provide warning of impending ruptures affecting visual prognosis.

As recently demonstrated via UBM by Nakagawa and colleagues,[1] rupture of the Descemet membrane in keratoconus is also often accompanied by clefting of the stro-

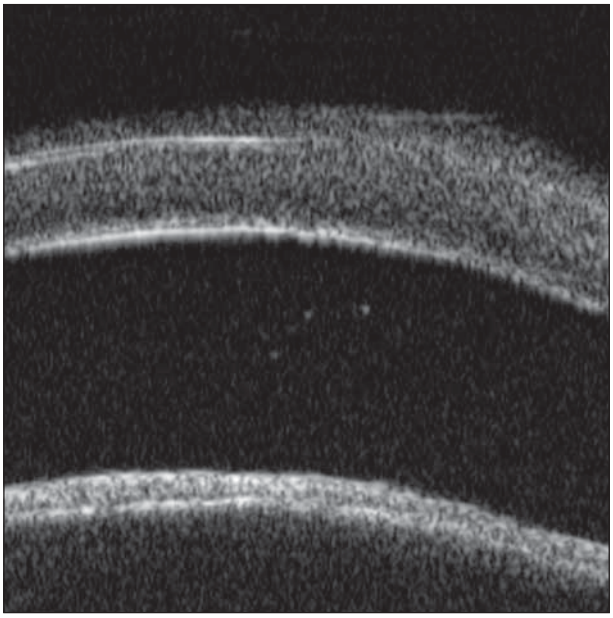

Figure 1. The corneal epithelium, Bowman membrane, stroma, Descemet membrane, and corneal endothelium are all clearly delineated. Note the significant epithelial edema at age 2 weeks without any detachment of the Descemet membrane or endothelial layer. A trabeculectomy procedure was then performed.

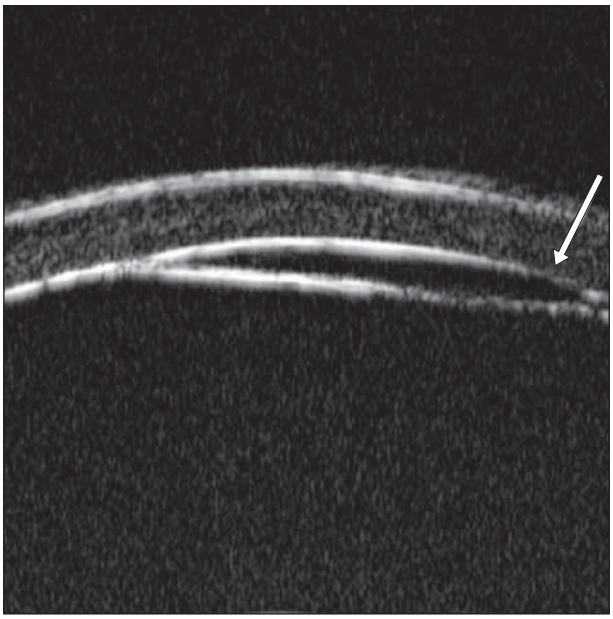

Figure 2. Visible separation of the Descemet membrane 5 weeks later during a standard follow-up examination reveals decreased extensibility of the Descemet membrane vis-à-vis the stroma and anterior corneal tissue layers. Centered beneath where the overlying epithelium edema is greatest, there appears to be a decreased reflectivity of both the posterior stromal surface (arrow) and the endothelium with the Descemet membrane. Topical pressure-lowering medications were prescribed while the contralateral eye underwent a trabeculectomy procedure.