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Anti–Vascular Endothelial Growth Factor Agents in the Treatment of Retinal Disease

From Bench to Bedside

Peter A. Campochiaro, MD,[1] Lloyd Paul Aiello, MD, PhD,[2] Philip J. Rosenfeld, MD, PhD[3]

The association of retinal hypoxia with retinal neovascularization has been recognized for decades, causing Michaelson to postulate in 1948 that a factor secreted by hypoxic retina was involved. The isolation of vascular endothelial growth factor (VEGF), characterization of its angiogenic activity, and demonstration that its expression was increased in hypoxic tissue made it a prime candidate. Intraocular levels of VEGF are elevated in patients with retinal or iris neovascularization, and VEGF-specific antagonists markedly suppress retinal neovascularization in mice and primates with ischemic retinopathy. Vascular endothelial growth factor antagonists also suppress choroidal neovascularization, and transgenic expression of VEGF in the retina of mice causes subretinal neovascularization. Clinical trials using a VEGF antagonist that blocks all isoforms of VEGF-A in patients with neovascular age-related macular degeneration (nAMD) demonstrated dramatic benefit. Similar results have been obtained with 2 other VEGF antagonists. Retinal hypoxia also contributes to diabetic macular edema (DME), and because of the absence of good animal models, small clinical trials were used to test the role of VEGF. The results clearly implicated VEGF as a major contributor to DME and have been confirmed by several large multicenter trials. A similar strategy demonstrated that VEGF is a major contributor to macular edema resulting from retinal vein occlusion, also confirmed in multicenter trials. Secondary outcomes in these large clinical trials have shown that VEGF inhibition improves retinal hemorrhages, retinal vessel closure, and progression of nonproliferative diabetic retinopathy. Anti-VEGF agents also provide therapeutic benefits in proliferative diabetic retinopathy. Thus, the development of VEGF antagonists has revolutionized the treatment of nAMD, diabetic retinopathy, and other ischemic retinopathies, but in many patients, the upregulation of VEGF is prolonged. Although the molecular signaling by which hypoxia and some other insults lead to upregulation of VEGF has been elucidated, it has not yet led to a treatment that reliably reduces the production of VEGF, necessitating continued neutralization by repeated intraocular injections of VEGF antagonists in many patients. The next horizon in the evolution of anti-VEGF therapy is the development of longer-acting agents or delivery platforms that provide sustained neutralization with fewer injections. *Ophthalmology 2016;123:S78-S88 © 2016 by the American Academy of Ophthalmology.*

An association among normal retinal vascular development, retinal neovascularization, and retinal nonperfusion has been appreciated for well over a half-century. Beginning in the late 1940s, Michaelson,[1] Ashton,[2] and others postulated that there must be an angiogenic factor produced by hypoxic retina that mediated these processes. Numerous candidate molecules with angiogenic activity were evaluated, but none met all essential criteria. For example, fibroblast growth factor-2 was isolated from retina and had angiogenic activity,[3,4] but it was not secreted or regulated by hypoxia, and knockout of fibroblast growth factor-2 or its overexpression in retina had no effect on retinal or choroidal neovascularization.[5] However, with the isolation of vascular permeability factor,[6] later cloned and characterized as vascular endothelial growth factor (VEGF),[7] a single molecule was identified that was produced within the retina, was induced by hypoxia, increased retinal vascular permeability, and was angiogenic.[6–10] The demonstration that VEGF levels were increased markedly in ocular fluids of patients with ischemic retinopathies and correlated with disease activity and that VEGF was the active angiogenic factor within the human vitreous[11] clearly implied that if local inhibition of VEGF within the eye could be achieved, it might have substantial beneficial effect on a variety of ischemia-induced retinal vasculopathies.

Preclinical Research

To test potential involvement of a protein in a disease processes, it is necessary to have animal models that mimic key aspects of the disease and specific antagonists of the protein. Vascular endothelial growth factor expression is increased in primates with laser-induced occlusion of

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retinal veins, and iris neovascularization, but not retinal neovascularization, develops.[12] The mouse model of oxygen-induced ischemic retinopathy[13] is most analogous to retinopathy of prematurity, but the presence of retinal ischemia and retinal neovascularization make it relevant to the proliferative stages of all ischemic retinopathies. Vascular endothelial growth factor was implicated in the pathogenesis of this model because hyperoxia was associated with reduced retinal levels of VEGF, followed by regression of newly developed retinal vessels leaving patches of nonperfused retina, followed by a sudden increase in VEGF and retinal neovascularization as soon as oxygen levels were reduced.[14] The reduction in VEGF is not associated simply with regression of developing vessels; it is the cause. Injection of VEGF prevents regression of developing vessels,[15] and the increase in VEGF is the cause of neovascularization, as shown when neovascularization was suppressed by intraocular injection of a specific VEGF-binding protein.[16] A single intravitreous injection of VEGF in primates causes iris neovascularization,[17] and multiple injections cause dilated retinal vessels, microaneurysms, retinal hemorrhages, and capillary closure, but not retinal neovascularization.[18] Sustained delivery of VEGF in primates causes iris neovascularization and severe macular edema with large cystic spaces in the retina but no retinal neovascularization.[19] These observations suggest different responses to VEGF or sensitivity to VEGF in different vascular beds. Indeed, transgenic expression of VEGF in photoreceptors of mice results in sprouting of neovascularization from the deep capillary bed of the retina but not superficial or intermediate retinal capillaries or the choriocapillaris.[20]

In view of differences among vascular beds and uncertainty as to whether hypoxia contributes to choroidal neovascularization, it could not be assumed that VEGF plays the same critical role in choroidal neovascularization as it does in retinal neovascularization. Laser-induced rupture of Bruch's membrane causes choroidal neovascularization in primates,[21] rats,[22] and mice.[5] Vascular endothelial growth factor antagonists suppress choroidal neovascularization in the mouse and primate models, predicting possible benefits in neovascular age-related macular degeneration (nAMD) and other disease processes complicated by choroidal neovascularization.[23–25]

The upregulation of VEGF in hypoxic retina occurs because it is transcriptionally regulated by the transcription factor called hypoxia-inducible factor-1 (HIF-1).[26] Hypoxia-inducible factor-1 is a heterodimer made up of HIF-1α and HIF-1β.[27] Under normoxic conditions, HIF-1α is dehydroxylated by a prolyl hydroxylase, resulting in ubiquitination and degradation. Hypoxia inactivates the prolyl hydroxylase, causing HIF-1α to accumulate and bind to HIF-1β to form HIF-1, which translocates to the nucleus and binds to the hypoxia response element (*HRE*) located in the promoter region of *VEGF* and several other genes. Figure 1 shows many of the HIF-1–regulated gene products that have been implicated in retinal neovascularization.[28] Mice in which the *HRE* has been removed from the *Vegf* promoter have marked reduction of ischemia-induced retinal neovascularization and choroidal neovascularization at Bruch's membrane rupture sites.[29] Because HIF-1 is involved in choroidal neovascularization, many of the factors involved in retinal neovascularization also contribute to choroidal neovascularization (Fig 2).[28] Angiopoietin 2 is a hypoxia-regulated gene product that is responsible for the different sensitivity of various vascular beds to VEGF.[30] Although the role of several other hypoxia-regulated gene products is being defined in ocular neovascularization, it is clear that VEGF is a critical and major contributor.

Vascular Endothelial Growth Factor in Neovascular Age-Related Macular Degeneration and Other Disease Processes Is Complicated by Choroidal Neovascularization

The first 2 anti-VEGF drugs to enter clinical trials for nAMD were ranibizumab (Lucentis; Genentech/Roche, South San Francisco, CA), an antibody fragment that binds all isoforms of VEGF-A, and pegaptanib sodium (Macugen; OSI/Eyetech, New York, NY), an aptamer that binds $VEGF_{165}$ and larger isoforms (Fig 3).[31] Pegaptanib was approved in 2004 when phase III trials showed that it prevented severe loss of vision over 1 year.[32] In 2004, bevacizumab (Avastin; Genentech/Roche) was approved for metastatic colon cancer, and it was tested as an intravenous therapy for nAMD[33,34] and for choroidal neovascularization in other diseases.[35] This full-length anti-VEGF antibody was engineered from the same precursor anti-VEGF mouse monoclonal antibody clone as ranibizumab and, similar to ranibizumab, binds all isoforms of VEGF-A.[36,37] However, the risk of potential adverse side effects resulting from systemic bevacizumab led to off-label intravitreal injections of bevacizumab, which were found to be extremely effective in reducing subretinal and intraretinal fluid in patients with nAMD, like ranibizumab.[38] Off-label intravitreal bevacizumab rapidly became the treatment of choice in 2005 because of its perceived clinical efficacy and superiority over pegaptanib, its widespread availability, and its low cost. The phase III trials testing ranibizumab confirmed the value of blocking all isoforms of VEGF-A in nAMD. In the MARINA (Minimmaly Classic/Occult Trial of the Anti-VEGF Antibody Ranibizumab in the Treatment of Neovascular AMD) trial, monthly intravitreal injections of ranibizumab 0.3 or 0.5 mg in eyes with occult or minimally classic choroidal neovascularization increased the percentage of patients who experienced substantial improvement in vision (15 letters), from 5% in the sham injection group to 24.8% and 33.8% in the 0.3- and 0.5-mg ranibizumab groups, respectively.[39] In the ANCHOR (Anti-VEGF Antibody for the Treatment of Predominantly Classic Choroidal Neovascularization in Age-Related Macular Degeneration) trial, the percentage of patients with predominantly classic choroidal neovascularization gaining 15 letters or more was 5.6% in eyes treated with photodynamic therapy every 3 months compared with 34.7% and 40.3% in eyes given monthly injections of 0.3