Vascular Endothelial Growth Factors and Their Inhibitors in Ocular Neovascular Disorders

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The introduction of pharmacotherapy has been one of the most important advances in the management of retinal disorders. Age-related macular degeneration (AMD) was once considered as an irrepressible disease leading to permanent macular damage, however anti-vascular endothelial growth factor (anti-VEGF) agents are now believed to halt the progression of the condition and improve vision in a considerable proportion of patients. Anti-VEGF agents are among the most commonly used drugs in ophthalmology but questions and uncertainties still surround their indications, efficacy and complications. This paper reviews the role of VEGF under physiologic and pathologic conditions in the eye and available anti-VEGF agents in current ophthalmic practice.

Key words: Vascular Endothelial Growth Factor; Macular Degeneration

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INTRODUCTION

More than 60 years ago, Michaelson proposed the concept of stimulation of neovascular retinopathies by an unknown factor, the so-called "X factor". Nowadays the role of vascular endothelial growth factors (VEGFs) has been increasingly clarified in the pathogenesis of neovascularization processes and other mediators are expected to be recognized in future.[1] A large body of evidence suggests VEGF-A to be the main regulator of pathologic angiogenesis.[2] Therefore, targeted inhibition of this mediator seems to be biologically plausible for treatment of these conditions.

Pathologic angiogenesis has a central role in age-related macular degeneration (AMD) which is the leading causes of blindness in elderly subjects in developed countries.[1] Recently, anti-VEGF agents have received FDA approval for treatment of AMD and have greatly changed the outcomes of treatment in these patients.[3,4] Off-label administration of anti-VEGF agents is also commonly practiced for treatment of proliferative diabetic retinopathy (PDR) and diabetic macular edema (DME) and less commonly for retinal vein occlusion (RVO), other retinal vasoproliferative disorders and neovascular glaucoma (NVG).[5-10]

This review outlines current information on the mechanisms of action of VEGF under physiologic and pathologic conditions in the eye and overviews the clinical utility of available anti-VEGF agents.

VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF)

The VEGF family includes placental growth factor (PLGF), VEGF-A, VEGF-B, VEGF-C, VEGF-D and VEGF-E. These agents are the most important regulators of angiogenesis and vascular permeability; VEGF-A in particular, plays a pivotal role in pathologic ocular angiogenesis.[11] The VEGF-A gene has been localized to chromosome 6p12.3 and comprises of 8 ex-

ons and 8 intermediate introns. VEGF-A has 9 isoforms including $VEGF_{121}$, $VEGF_{145}$, $VEGF_{148}$, $VEGF_{162}$, $VEGF_{165}$, $VEGF_{165b}$, $VEGF_{183}$, $VEGF_{189}$ and $VEGF_{206}$. These isoforms differ from each other by the number of amino acids and heparin-binding affinity.[12,13] Heparin-binding affinity is nil for $VEGF_{121}$, maximum for $VEGF_{189}$ and $VEGF_{206}$, and intermediate for $VEGF_{165}$. This property determines the affinity of the molecule for binding to heparin-sulfate proteoglycans in cell membranes.[14]

VEGF appears to be essential for development and maintenance of functionally efficient retinal vasculature as well as for integrity of the retinal pigment epithelium (RPE), Bruch's membrane and choroidal endothelial cells. VEGF release mainly occurs on the basal surface of the RPE. VEGF-A production is induced by factors including hypoxia, nitric oxide, and other biologic growth factors including hypoxia-induced factor 1. The latter comprises of α and β subunits, the production of the α subunit acts as a VEGF-A gene stimulant and has been shown to be increased under hypoxic conditions.[15]

VEGF-A induces angiogenesis, increases vascular permeability, may affect female reproductive function, hair growth and wound healing in adults. It also seems to inhibit endothelial cell apoptosis and is therefore considered a survival factor.[16]

VEGF acts via two tyrosine kinase receptors including VEGF receptor-1 (VEGFR-1) and VEGFR-2 which are primarily present on vascular endothelial cell surfaces. Although the affinity of VEGFR-2 for VEGF is lower than VEGFR-1 it plays a more significant role in neovascularization processes.[17] Another receptor named neuropilin-1 has also been recognized for $VEGF_{165}$ and is present on neurons and tumor cells in addition to endothelial cells.

PLGF binds to VEGFR-1 resulting in production of VEGF-A. PLGF is a weak mitogen.[18] VEGF-B binds to both VEGFR-1 and neuropilin-1, causes destruction of extracellular matrix and has a role in cell migration. VEGF-C and VEGF-D are distinguished from other members of the family by possessing C and N terminal structures and affect endothelial cell mitosis and migration. Both are preproproteins which require plasmin for activation. VEGF-C has a major role in lymphangiogenesis in general while VEGF-D mediates lymphangiogenesis only in lymphatic tumors. VEGF-E and VEGF-F are non-human homologues of VEGF-A. The former acts via VEGFR-2 and strongly induces endothelial cell proliferation but the role of the latter remains unknown.[19-21]

RETINAL NEOVASCULARIZATION

Hypoxia is the major trigger of VEGF-associated neovascularization. Induction of hypoxia in vitro increases production of VEGF-A in different cell lines in the retina. Similarly, under hypoxic conditions in vivo, increased levels of VEGF-A were observed in the retina, vitreous and aqueous humor.[22,23] Miller et al[24] showed that VEGF-A levels in ocular tissues change proportionately with increase and decrease in the amount of new vessels.

Main sources of VEGF-A in the retina and vitreous are RPE, Muller and ganglion cells. Injection of VEGF-A in non-human primates resulted in clinical features similar to diabetic retinopathy (DR) and NVG.[25,26] In humans, VEGF-A has been implicated in the pathogenesis of retinal and choroidal neovascularization in conditions such as DR,[22,23] RVO,[23,27] iris neovascularization,[27] retinopathy of prematurity (ROP)[28] and AMD.[29-31]

VEGF-A may also play a role in intraocular inflammation through increased vasopermeability[32,33] which is probably mediated via the following mechanisms: leukocyte-mediated endothelial cell damage, destruction of tight junctions, fenestrations in vessels, and transcellular bulk flow. The concentration of VEGF and contact time for induction of vasopermeability and break-down of the blood-retinal barrier may be less than that required for angiogenesis.[26] The mechanism of action of corticosteroids in improving macular edema may be decreased leukocyte-mediated damage induced by VEGF-A.[34]

AGE-RELATED MACULAR DEGENERATION

AMD is the most prevalent disease associated with choroidal neovascularization (CNV) and

the most common subject for anti-VEGF studies in ophthalmology. Progressive macular degeneration results in drusen formation and pigmentary changes in the RPE in dry AMD leading to geographic atrophy of the macula and severe visual loss. Neovascular (wet) AMD is associated with formation of choroidal neovascular membranes. Although wet type AMD comprises only 10% of cases of AMD, it is responsible for 90% of severe visual loss due to the condition.[35] Development of CNV is associated with increased levels of VEGF.[29-31]

Oxidant agents seem to precipitate in Bruch's membrane resulting in stimulation of complement and inflammatory pathways leading to up-regulation of VEGF secretion from RPE cells eventually resulting in CNV.[36] Patients with moderate to severe types of dry type AMD profit from antioxidants.[37]

Many therapeutic modalities have been employed for neovascular AMD. Macular laser photocoagulation provides relative success in a small percentage of patients with extrafoveal CNV but may cause severe scotoma and immediate loss of vision. Photodynamic therapy (PDT) with verteporfin acts via activating a photosensitizing dye within the pathologic vessels by infrared laser leading to occlusion of choroidal new vessels with minimal damage to the retina. PDT can delay loss of vision in certain cases of CNV especially in small-sized CNVs but does not improve vision.[38]

Anti-VEGF agents have changed the paradigm of treatment for neovascular AMD with dramatic outcomes. Pegaptanib is the first FDA-approved anti-VEGF agent for intravitreal injection in the treatment of neovascular AMD.[3] The VISION study[39] showed that intravitreal injection of 0.3 mg pegaptanib every 6 weeks reduced the rate of visual loss of more than 30 letters to 10% as compared to 22% in controls; after 2 years visual acuity remained unchanged but no visual improvement was seen.

The advent of ranibizumab raised the expectations of treatment for neovascular AMD.[40] The MARINA study[40,41] evaluated the effect of ranibizumab injection in patients with minimally classic or occult CNV and the ANCHOR study[42-44] assessed ranibizumab injection in patients with predominantly classic CNV; both studies used monthly injections and reported that 90% of treated patients maintained vision or experienced only mild visual loss. After 2 years of follow-up, 40% of treated patients had visual acuity of 20/40 or better in both studies. The PIER study[45] utilized 3 injections during a 2-month period followed by repeat injections every 3-4 months, but the outcomes were not as favorable as those achieved in the MARINA and ANCHOR studies.

Intravitreal injection of bevacizumab has been shown to be effective in the management of neovascular AMD and has been widely used since 2005.[46,47] Ahmadieh et al[48] evaluated the effect of initial triple therapy using PDT combined with intravitreal bevacizumab and triamcinolone followed by additional intravitreal injections of bevacizumab when active disease persisted thereafter and concluded that this method is beneficial in terms of retinal thickness reduction and visual acuity improvement after mean follow-up of 50±15 weeks. The Comparison of AMD Treatments Trial Study compares bevacizumab with ranibizumab using monthly injections versus 3 consecutive injections followed by additional injections as needed (www. clinicaltrials.gov).

Other therapeutic methods include a single intravenous injection of VEGF-Trap, which is under phase I trial. Intravenous VEGF-Trap seems to result in adverse effects with doses higher than 1 mg/kg.[49] Intravitreal injection of VEGF-Trap has also been used.[50] Another treatment also under phase I trial is a single injection of siRNA that resulted in an average increase of 15 letters of visual acuity in 23% of treated patients.[51] More than 40 studies are currently under execution in the USA under supervision by the National Health Institute of USA evaluating different anti-VEGF therapies in AMD which are available at the clinical trials website (www.clinicaltrials.gov).

NON-AMD ASSOCIATED CNV

CNV may be seen in conditions other than AMD such as high myopia, angioid streaks, ocular histoplasmosis, and choroidal osteoma. Anti-VEGF therapy in these conditions is almost limited to off-label use of bevacizumab

and there are a few small-sized reports in the literature in this regard.[52-56]

DIABETIC RETINOPATHY

Prolonged hyperglycemia activates the β isoform of protein kinase-C (PKC-β) leading to VEGF synthesis. Elevated levels of VEGF lead to PDR, vascular leakage and fluid collection manifesting as DME. Early vascular changes in diabetic retinopathy demonstrate leukostasis (adhesion of leukocytes to the vascular bed), platelet aggregation, abnormal blood flow, degeneration of pericytes, and thickening of basement membranes.[22,23]

Retinal capillary occlusion increases synthesis of angiogenesis stimulant factors such as VEGF via regional hypoxia. These factors loosen junctions between vascular endothelial cells increasing vasopermeability.[22,23] VEGF is an important survival factor for endothelial cells, however proliferated endothelial cells degenerate and result in capillary occlusion, generation of acellular capillaries and capillary nonperfusion.[22,23]

Based on these findings, inhibition of VEGF and PKC pathways are pivotal in the treatment of diabetic retinopathy. Pegaptanib is under phase II trials for treatment of DME; it has improved visual acuity and decreased macular edema in comparison to placebo. A retrospective evaluation of these patients also revealed regression of retinal neovascularization.[5] Several studies have reported rapid regression of neovascularization in the iris (NVI), optic nerve (NVD) and retina (NVE) following intravitreal injection of bevacizumab in patients with PDR.[8-10] Some authors have used bevacizumab as an adjunct to vitrectomy in patients with vitreous hemorrhage due to PDR.[57-59]

Ruboxistaurin has been evaluated for diabetic retinopathy in 2 large scale multicenter studies as follows: in the PKC-DRS study,[60] oral ruboxistaurin (32 mg) had no effect on progression of PDR but decreased the severity of visual loss probably by decreasing macular edema. In the PKC-DRS2 study,[61] less visual loss, visual improvement and reduction in macular edema were reported.

One may conclude that in patients with PDR or DME, standard treatments such as panretinal photocoagulation (PRP), macular photocoagulation (MPC) and vitrectomy with established indications and outcomes may be advisable. However, in cases with severe and persistent macular edema unresponsive to standard therapy, anti-VEGF agents may be used as adjunctive treatment. Additionally, in patients with vitreous hemorrhage associated with advanced PDR, preoperative injection of anti-VEGF agents (usually one week before vitrectomy) seems to be advantageous.[57-59]

CENTRAL AND BRANCH RETINAL VEIN OCCLUSION

In retinal vein occlusions, VEGF levels are increased proportionate to the degree of retinal ischemia and severity of macular edema. Primary studies have reported favorable results using ranibizumab and bevacizumab.[62-64] Anti-VEGF drugs are potent agents for reduction of edema; they reduce vasopermeability but cannot eliminate the pathologic process completely. The duration of action is not clear but their effect seems to decrease after a few weeks and re-injections are required after 4 weeks.[65,66]

NEOVASCULAR GLAUCOMA (NVG)

NVG develops due to iris and angle neovascularization in response to retinal ischemia. This process may complicate diabetic retinopathy, vascular occlusions, sickle cell anemia , old retinal detachments and the ocular ischemic syndrome. Standard treatment for NVG consists of relieving retinal ischemia by using PRP along with medical or surgical control of elevated intraocular pressure (IOP). When a large area of the anterior chamber angle is involved by neovascular tissue, shunting procedures are required. Elevated levels of VEGF in the aqueous humor and vitreous have been shown in a few studies.[2] Several studies have reported rapid regression of NVI following intravitreal injection of bevacizumab.[67-69] The combination of PRP and IVB seems an appropriate treatment for NVG; IVB prevents angle closure in the time PRP needs time to take effect.[70]

RETINOPATHY OF PREMATURITY (ROP)

ROP is characterized by incomplete vascularization of the peripheral retina in a premature neonate leading to retinal neovascularization. Risk factors for ROP include premature birth, low birth weight and oxygen therapy. Experimental studies have shown that high concentrations of oxygen impair vascular formation which in turn causes hypoxia and elevates VEGF levels.[71,72] Increased levels of VEGF in the vitreous have been reported in patients with ROP.[73] It is notable that VEGF has a physiologic role in the formation of normal retinal vasculature. Another factor implicated in the pathogenesis of ROP is insulin-like growth factor-1 (IGF-1) which stimulates angiogenesis in a pathway independent of VEGF.[74] Ongoing studies[51] are evaluating the use of anti-IGF-1 and anti-VEGF agents for treatment of ROP, the results of which are not published yet.

ANTI-ANGIOGENESIS AGENTS

Pegaptanib sodium

Pegaptanib sodium (Macugen, Eyetech Pharmaceuticals, New York, USA) is a modified 28-base pegylated RNA aptamer which binds to $VEGF_{165}$ and other larger isoforms of VEGF-A.[3] It was the first VEGF inhibitor to receive FDA approval for treatment of CNV due to AMD in December 2004. In its phase III trial, three different doses of intravitreal pegaptanib (0.3, 1 and 3 mg) were used every 6 weeks for 2 years in comparison with placebo in three types of AMD (with minimally classic, classic and occult CNV). After 12 months, 30% of the 0.3 mg group and 45% of controls experienced loss of vision more than 15 letters equal to 3 lines (P=0.001). On the other hand, 6% of the 0.3 mg group and 2% of the placebo group achieved 3 lines or more visual improvement (P=0.04). After 2 years, pegaptanib significantly decelerated loss of vision but did not improve it.[39]

Ranibizumab

Ranibizumab (Lucentis, Genentech, San Francisco, USA) received FDA approval for treatment of neovascular AMD after pegaptanib in 2006. It is a humanized Fab fragment of a murine monoclonal anti-VEGF-A antibody which binds to all VEGF-A isoforms and their active cleavage by-products.[4]

The efficacy and safety of ranibizumab have been demonstrated in all types of AMD-related CNV based on the results of two phase III clinical trials.[40-44] In the MARINA study[40,41] patients with minimally classic or occult disease underwent monthly injections of 0.3 or 0.5 mg ranibizumab for 2 years. Ultimately, visual acuity increased by 7 letters in treated groups but decreased by 10.5 letters in controls (P<0.01). Another phase III clinical trial compared ranibizumab (0.3 and 0.5 mg) with PDT in the treatment of classic CNV. After 2 years, monthly injections of ranibizumab were superior to PDT.[42,43] Ranibizumab was the first anti-VEGF agent capable of increasing visual acuity in patients with neovascular AMD in addition to preventing visual loss.[40-44] The rate of ocular complications related to ranibizumab was less than 1.7% in both of the above-mentioned studies.

Bevacizumab

Bevacizumab (Avastin, Genentech, San Francisco, USA) is a full-length humanized monoclonal antibody similar to ranibizumab that binds all isoforms of VEGF-A and their active by-products. It was approved by the FDA exclusively for intravenous administration in the treatment of colorectal malignancies.[75] It has also been used as an off-label drug for ocular neovascular diseases such as AMD and has shown to be safe and effective with short-term follow-up.[76-77] The long-term safety is yet to be established; this demands large scale clinical trials with longer follow-up. Such studies have been initiated and their results are anticipated.

VEGF-Trap

VEGF-Trap (Regeneron Pharmaceuticals, New York, USA) is a protein molecule which binds to VEGFR-1 and VEGFR-2, inhibiting the effect

of all isoforms of VEGF and PLGF.[78] Intravenous administration for treatment of AMD is under phase I/II clinical trials.[49]

Sirna

Sirna (Sirna Therapeutics, Boulder, Colorado) is a short ribonucleic acid (RNA) that interferes with VEGFR-1 production at the gene level. This agent is under phase II clinical trial for treatment of AMD. Cand-5 (Acuity Pharmaceuticals, Philadelphia, USA) acts in a fashion similar to Sirna. [51]

Ruboxistaurin mesylate

Ruboxistaurin mesylate (Arxxant, Lilly, Indianapolis, USA) acts as a selective systemic inhibitor of PKC-β. It has been shown to be safe and effective in maintaining visual acuity in diabetic patients.[60,61,79]

Multireceptor Tyrosine Kinase Inhibitors

Simultaneous inhibition of several tyrosine kinase receptors including VEGFR-1, VEGFR-2 and receptors for stem cell factor, fetal liver TK_3 and PDGF is theoretically advantageous to inhibition of VEGF alone. Vatalanib (Novartis, Pharma AG, Basel, Switzerland, PTK 787/ 2K222584) is an inhibitor of tyrosine kinase receptors and currently under phase I/II clinical trials in combination with PDT for treatment of AMD.[80] The following agents from this group have also been used in animal models: AG013764/AG013711 (Pfizer Inc, New York, USA), Sutent (Sunitinib, Pfizer Inc, New York, USA), Compound A (Merck and Co., Rome, Italy), and TG 100801 (Targegen, San Diego, USA).[81-84]

Potential Hazards of Anti-VEGF Therapy

The most important adverse local effects related to anti-VEGF agents include uveitis, retinal detachment and cataracts. Based on the results of the VISION study,[39] the incidence of these complications over one year was 0.08% for retinal detachment, 0.16% for endophthalmitis, 0.7% for traumatic cataracts and 1% for moderate uveitis. For prevention of infectious complications, skin preparation and drapes, irrigation of the fornices, using a lid speculum and adherence to aseptic principles are required.

No adverse systemic effect was seen in the VISION study[39] up to 2 years after injection of pegaptanib but in the MARINA[40,41] and ANCHOR[42,43] studies, an increased incidence of MI and stroke was seen in patients who received 0.5 mg ranibizumab as compared to controls (1.3% versus 2.9%). Since then, Genentech stated that intravitreal injection of 0.5 mg ranibizumab may increase the risk of CVA in patients with a positive previous history.

Due to lack of large scale clinical trials potential side effects of bevacizumab remain vague, however this agent also seems to entail local and systemic complications similar to ranibizumab or pegaptanib.

Major concerns with anti-VEGF therapy for ocular diseases include: (1) repeat intravitreal injections; (2) risk of CVA or cardiovascular complications; (3) possible retinal and neural toxicity due to cumulative dosing; (4) interference with physiologic functions of VEGF; and (5) economic and cost-effectiveness concerns.

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