

Review article

Long term effects of anti-VEGF agents: patho-physiological perspectives

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Abstract

Monoclonal antibodies directed against different targets have become a new tool for the treatment of various disorders. More than 20 monoclonal antibody-based therapies have been approved in the USA and hundreds more are in development. Some of these therapies are finding applications in ocular disorders. The role of anti-VEGF in the treatment of wet ARMD is now well known. Anti-VEGF, which were initially discovered to treat carcinomas like bevacizumab in metastatic colorectal carcinoma, have now found place in ophthalmology to treat disorders where neovascularization/angiogenesis leads to blindness.

Key-words: VEGF, ranibizumab, revacizumab

Introduction

Intra-vitreous anti-VEGF drugs are targeted against Vascular Endothelial Growth Factor (VEGF) which was first suggested by Michaelson as a diffusible "factor X" stimulating the retinal and iris neovascularization seen in diabetic retinopathy (Michaelson et al, 1948). Upregulation of VEGF occurs in many ischemic conditions of the retina, including age-related macular degeneration (ARMD) and diabetic retinopathy (Ferrara et al, 2007, Duh et al 2004). Higher intraocular VEGF levels lead to subretinal and vitreous hemorrhage, retinal detachment, and often blindness (Aiello et al, 1994). Therefore, VEGF formed an optimal therapeutic target for treatment of ocular diseases where neovascularization formed the main pathology.

Wet ARMD is a leading cause of blindness over 55 years of age. Previously treatment options were suboptimal specially for subfoveal CNV (Choroidal Neovascularization). This search for better treatment

led to the discovery of anti-VEGF for intravitreal use.

Pegatanib sodium (Macugen) was the first antiangiogenic agent that was approved by FDA in 2004 for treatment of neovascular/wet ARMD. It is an aptamer (RNA oligonucleotide that selectively binds and inhibits VEGF-165 (isoform of VEGF-A). It is injected intravitreally in a dose of 0.3mg/0.09ml after taking informed consent.

Second anti-VEGF drug that was approved by FDA in 2006 for intravitreal use in wet-ARMD was ranibizumab (Lucentis). It is a humanized monoclonal antibody fragment against VEGF inhibiting all isoforms of VEGF-A. The most convincing information regarding its efficacy and safety comes from the MARINA & ANCHOR trials using intravitreal ranibizumab. Both the trials showed stable visual acuity and morphology of the lesion with the use of ranibizumab. Infact, improvement in visual acuity was also noticed. Apart from the ocular side effects due to intravitreal procedure (like uveitis, endophthalmitis, retinal tears)

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no significant systemic adverse effects were noticed. Increased rates of serious and non-serious ocular haemorrhage were seen. Doses used are 0.3mg/0.05ml and 0.5mg/0.05ml. Ranibizumab, however, is costly at over \$2000 a dose.

Anti-VEGF drug which especially caught the attention of ophthalmologists is bevacizumab. Bevacizumab (Avastin®; Genentech, Inc., San Francisco, CA, USA) is a widely used recombinant antibody against vascular endothelial growth factor (VEGF). It is a full length humanized monoclonal antibody which also inhibits all isoforms of VEGF-A. Avastin is a low cost alternative to lucentis. It comes in a preservative free 100mg vial containing 4cc of 25mg/ml drug and since only 0.05ml is used in one patient it can be divided into multiple aliquots to be stored in sterile conditions and used later. But presently it is FDA approved only for intra venous use in metastatic carcinoma colon. In ophthalmology it is being used off label after explaining to the patient and taking informed consent in a dose of 1.25mg/0.05ml. Easy availability, low cost (\$15-50/dose) and efficacy are responsible for its increasing popularity. As a result of routine off-label clinical use, the toxic effects of Avastin® on retinal cells are subject to ongoing investigation

Anti-VEGF has also been tried in macular edema following vein occlusions, proliferative diabetic retinopathy and diabetic macular edema (CSME), iris rubeosis and neovascular glaucoma. Till date none of the trials/studies have reported significant systemic side effects or ocular toxicity with the use of intra-vitreous anti-VEGF. But, this is just a beginning. Not much long term data is available on their use. Also, anti-VEGFs are injected locally at monthly intervals for a year or longer and since VEGF is required for normal retinal functioning such chronic inhibition might prove toxic in long run.

Discussion

Vascular endothelial growth factor (VEGF), a secreted 46 kDa glycoprotein, is an endothelial cell-specific angiogenic as well as a vasopermeable factor (Ferrara et al, 1989; Keck et al, 1989).

Although initially thought to be endothelial-specific, VEGF plays a major role during the development and maturation of neural tissue, including the retina (Wang et al, 2007). During development, VEGF is expressed by astrocytes in the retinal ganglion cell layer, by cells of inner nuclear layer, Müller cells, and retinal pigment epithelial cells (Darland et al, 2003; Saint-Geniez et al, 2006). In the adult retina, VEGF is expressed in the absence of active neovascularization and is implicated in the maintenance and function of adult retina neuronal cells (Saint-Geniez et al, 2008). VEGF-A, an isoform of VEGF, reduces apoptosis in retinal neurons after ischemic injury and delays degeneration of retinal ganglion cells after axotomy (Kilic et al, 2006). VEGF is a neurotrophic and neuroprotective cytokine produced by a variety of cells in the retina, including retinal ganglion cells.

Hypothetically, repeated treatment with anti-VEGF agents should interfere with the neuroprotective action of VEGF. Recently, (Saint-Geniez et al, 2008) have shown in animal studies that small, interfering RNA (siRNA)-mediated gene silencing of VEGF led to a reduction in the thickness of retinal cell layers. They concluded that VEGF has a neuroprotective role in the survival of retinal neurons. Target therapy, though aimed against the angiogenic effect of VEGF, can reduce the potential benefit of VEGF in neuroprotection from oxidative stress. In vitro evidence shows that VEGF protection against oxidative stress is blocked by bevacizumab, in a model of differentiated retinal ganglion cells (Vikram et al, 2010). Bevacizumab might interfere with the autocrine or paracrine functions of retinal ganglion cells. In addition, it is also possible that bevacizumab, or potentially other antibody therapies, may exert non-specific effects by altering the internal milieu of the eye (e.g. by altering soluble protein content) or through Fc receptors for which several ocular cells, including iris and ciliary body cells, have receptors (Kim et al, 2008). Scientists at Schepens Eye Research Institute (Saint-Geniez et al, 2006) have found that reducing the levels of vascular endothelial growth

factor (VEGF), which is best known as a stimulator of new blood vessel growth, in adult mice causes the death of photoreceptors and Muller glia - cells of the retina that are essential to visual function. This finding, published on November 3, 2008 *PLoS ONE*, holds implications for the chronic use of promising new anti-VEGF drugs such as Lucentis, which eliminate abnormal and damaging blood vessel growth and leakage in the retina by neutralizing VEGF. Mice eyes differ from human eyes in many ways, so we cannot directly extrapolate these results to humans, but this study is an important heads-up that clinical application of anti-VEGF therapy in the eye needs to proceed with caution.

On the contrary, studies have also demonstrated that bevacizumab was non-cytotoxic to RGC-5 (Retinal Ganglion Cell) cells in doses relevant for treating intraocular conditions. The most commonly used intravitreal dose of bevacizumab is 1.25 mg/0.05 ml. Assuming an average vitreous cavity volume of 5 ml, this will translate into a concentration of 0.25 mg/ml. Experiments have examined doses up to eight times higher than this without significant cytotoxicity (Sharma et al, 2009). A commercially available bevacizumab preparation demonstrated a significant stimulatory effect on RGC-5 proliferation (Sharma et al, 2009). Bevacizumab treatment, especially in higher doses, may also add to the protein load, in which case the additional protein (bevacizumab) may act as enhanced growth medium. The addition of 0.1 mg/ml, 1 mg/ml or 2 mg/ml FBS or BSA also stimulated proliferation, which suggests that the stimulatory effect of bevacizumab may be related to non-specific changes in the growth environment. The normal concentration of soluble proteins in vitreous is about 0.88 mg/ml (1% of serum value). The majority of these are albumin and transferrin (Sjostrand et al, 1992). In pathological conditions such as proliferative vitreoretinopathy or proliferative diabetic retinopathy, the soluble protein may increase to up to three times the normal concentration (Sjostrand et al, 1992). Intra-vitreous bevacizumab

injection would increase this concentration by 0.25–0.31 mg/ml (injection of 1.25 mg in 4–5 ml of vitreous). This amounts to an almost 33% increase in the soluble protein concentration of vitreous. This increase could potentially affect cell proliferation in certain cell types, especially those outwith VEGF control (Sharma et al, 2009). The highest concentration of bevacizumab used in experiments (Sharma et al, 2009), where the stimulatory effect was most potent, was several times higher than the typical therapeutic dose. Moreover, the growth characteristics of cells in culture are likely to be very different to those of cells *in vivo*. It remains to be seen how primary cell cultures for retinal membranes will respond to bevacizumab *in vitro*.

Corneal neovascularization contributes to corneal opacification in inflammatory conditions of the cornea and severely compromises the success of corneal transplantation. Vascular endothelial growth factor (VEGF) plays an important role in stimulating and maintaining corneal neovascularization. Anti-VEGF therapy, especially the use of anti-VEGF antibody bevacizumab, has gained popularity in the management of retinal neovascularization and is being used topically for corneal neovascularization. In the cytotoxicity experiments, there was no difference in cell numbers after 24-hour exposure compared with control in corneal epithelial and fibroblast cell lines at the concentrations tested. Bevacizumab was thus shown to be nontoxic to human corneal epithelial and fibroblast cells at 3 different concentrations (Shalam et al, 2009).

One study demonstrated that the effect of anti-VEGF therapy on the in oculo pathophysiological environment extended beyond neutralization of VEGF (Grover et al, 2009). Another 24 analytes were identified that distinctly changed (1.5 fold or more) in the aqueous from the pathological samples as compared to normal values (Sharma et al, 2010). Analysis of these analytes in the same patients after the bevacizumab injection demonstrated that all analytes upregulated in pre-treatment tended to normalize after the treatment. Many of these analytes are not directly related to the function of VEGF.



These results provide strong and unequivocal evidence that the bevacizumab treatment affects the pathophysiological state of the eye beyond what could be expected by neutralization of VEGF alone (Sharma et al, 2010). These results were confirmed by a global view of the changes provided by the cluster analysis. Bevacizumab treatment had a wide-ranging impact on the aqueous profile toward normalization. It is possible that some of the changes noted may be the result of the regression of the disease process (indirect effect of the treatment) or the reestablishment of the blood-retinal barrier rather than the direct effect of the bevacizumab injection. Clinical observations suggest that the bevacizumab treatment changes the clinical course of neovascularization in a manner that cannot be entirely explained by the VEGF neutralizing effect of bevacizumab (Sharma et al, 2010). For instance, intracameral injection of bevacizumab for iris neovascularization lasts longer than bevacizumab availability in the aqueous (Sharma et al, 2010) considering aqueous is produced at a rate of 2–3 $\mu\text{l}/\text{min}$ and the volume of anterior chamber is approximately 250 μl . Similarly, the effect of intravitreal injection of bevacizumab lasts for months, longer than the bioavailability of bevacizumab (Krohne et al, 2008, Miyake et al, 2010). In addition, bevacizumab treatment not only arrests the progression of neo-vascularization, but in many cases reverses it, which again cannot be explained by the effect of VEGF on endothelial cell proliferation and migration alone (Grover et al, 2009). The trend toward the global normalization of the ocular physiologic environment by bevacizumab injection could alter the pathophysiology of the disease process and thus potentially provide an explanation for these observations.

Several mechanisms have been elaborated to explain the cytoprotective effect mediated by VEGF against oxidative stress (Madhavan et al, 2008; Siner et al, 2007). In a study evaluating the role of growth factor-modulated antioxidant expression, Madhavan et al, 2008, found elevated expression of the

antioxidant enzyme superoxide dismutase 2 (SOD2). This enzyme protected neuronal cells from oxidative stress when exposed to 3-NP, an inhibitor of mitochondrial respiratory complex, in the presence of VEGF. Similarly, in animal models of hyperoxic acute lung injury, VEGF has been shown to protect vascular endothelial cells through induction of heme oxygenase-1 (HO-1) (Siner et al, 2007). In the human eye, there is increasing evidence that pathogenic oxidative mechanisms contribute to the progression of pathological conditions such as age-related macular degeneration and glaucoma (Beatty et al, 2000; Jin GF et al, 2001). Chronic, repeated use of anti-VEGF might therefore interfere with this action of VEGF against oxidative stress, neutralizing its benefit in long run.

Regarding AMD, the optimal frequency and duration of dosage are still unknown. Speculations are still regarding the impact of exposing patients to continual VEGF blockade and whether it leads to some unintended consequences. The benefits of preventing or stopping aberrant vascular formation have undoubtedly changed the course of ARMD, VEGF also serves important homeostatic, anti-oxidative functions. Even if administered systemically for eye diseases, unforetold cardiac and/or pulmonary complications could ensue. Even local pan-VEGF blockade theoretically prevents normal microvascular function following intravitreal injection of anti-VEGF agents.

Conclusion

Anti-VEGF therapy has become a widely accepted treatment for several diseases where neovascularization and permeability plays a pivotal role, including cancer and retinal disorders. Few *in vitro* studies, which are at best approximate and cannot mimic *in vivo* conditions, do show evidence of retinal toxicity by these drugs but further research needs to be done before coming to a conclusion.

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