Anti-VEGF therapy for glaucoma

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Purpose of review

The role of antivascular endothelial growth factor (anti-VEGF) agents in treating various ophthalmic diseases is currently being investigated. There have been many advances in the understanding of how anti-VEGF agents work and speculation on when to implement them clinically for neovascular glaucoma. Recent studies exploring the utility of anti-VEGF agents for wound modulation after trabeculectomy reveal promising results.

Recent findings

Anti-VEGF agents have been shown to be beneficial in treating neovascular glaucoma. Their use leads to regression of both iris and angle neovascularization, intraocular pressure control when the angle remains open and, in many cases, prompts symptomatic improvement. In addition, research on the wound modulatory properties of anti-VEGF agents has revealed a dose-dependent inhibition of fibroblast proliferation. Studies exploring the use of anti-VEGF agents at time of trabeculectomy or in bleb revision procedures suggest a beneficial effect on bleb survival and subsequent improvement in intraocular pressure control. Prospective randomized clinical trials are still needed.

Summary

The recent use of anti-VEGF agents for neovascular glaucoma as well as wound modulation after trabeculectomy has shown great promise. Through future research, the antiangiogenic and antifibroblastic properties of anti-VEGF agents may prove to be beneficial in patients being treated for various forms of glaucoma.

Keywords

antivascular endothelial growth factor, bevacizumab, neovascular glaucoma, ranibizumab, trabeculectomy, wound modulation

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Introduction

The idea of developing angiogenic inhibitors to treat cancer was first proposed by Folkman in 1971 [1]. In an attempt to further classify the properties of tumor blood vessels, a protein that induced vascular leakage was partially purified in 1983 [2]. Later, advances in molecular science allowed this protein to be isolated. It was subsequently named vascular endothelial growth factor or VEGF [3]. In-vitro studies indicated this protein to be an important regulator in endothelial cell growth and the inflammatory response [4]. Many VEGF isoforms exist, varying in size as well as spatial distribution, from cell bound to diffusible. Early research revealed that VEGF mRNA is highly expressed by glioblastoma multiforme, an aggressive central nervous system (CNS) tumor [5]. Through this and other studies, it became apparent that blood vessel recruitment to ischemic tissues was derived, in large part, through hypoxia-induced VEGF expression.

Initially proposed by Michaelson [6], the development of pathologic intraocular angiogenesis was due to a diffusible angiogenic factor, or factor X, that was released by an ischemic retina.

The VEGF cytokine, both diffusible and induced under hypoxic conditions, became a possible candidate for Michaelson's factor X. Intraocular concentrations of VEGF were then found to be increased in patients with active proliferative diabetic retinopathy, central retinal vein occlusion and those with retinopathy of prematurity [7–9]. More recently, VEGF has been implicated in choroidal neovascularization seen in age-related macular degeneration (AMD). These findings led to research focused on developing VEGF inhibitors to block the angiogenic drive of these sight-threatening diseases.

What is an antivascular endothelial growth factor agent?

Angiogenesis inhibitors such as hydrocortisone 21-phosphate and AGM-1470, a fumagillin analogue, were initially found to have significant effects on Tenon's fibroblast proliferation and migration. This discovery

led researchers to believe that angiogenesis inhibitors may play a role in the wound modulation seen after glaucoma filtration surgery [10].

Bevacizumab (Avastin; Genentech Inc., San Francisco, California, USA) was approved by the US Food and Drug Administration (FDA) for metastatic colon cancer in 2004 [11]. Bevacizumab is a humanized anti-VEGF-A monoclonal antibody. By binding to two receptor kinases [VEGFR-1 (Flt-1) and VEGFR-2 (KDR, Flk-1)], bevacizumab is able to downregulate the mitogenic, angiogenic and permeability-enhancing effects of VEGF-A [12,13]. Recently, ranibizumab (Lucentis; Genentech Inc.), a Fab fragment of a recombinant humanized IgG1 kappa isotype murine monoclonal antibody, was FDA approved for treating choroidal neovascular membranes related to AMD. The use of anti-VEGF agents has continued to expand. It has been shown to be effective for patients with diabetic macular edema as well as central retinal vein occlusions [14,15].

Bevacizumab has been utilized extensively as an off-label treatment for ocular neovascular disease, including neovascular glaucoma (NVG). Due to a direct effect on vascular and fibroblast proliferation, as well as their indirect effect of decreasing the influx of pro-inflammatory cytokines into the bleb by way of surrounding vessels, anti-VEGF agents may also have utility in glaucoma surgery through wound modulation. This article will discuss the use of anti-VEGF agents in NVG as well as its use in wound modulation after trabeculectomy.

Neovascular glaucoma and antivascular endothelial growth factor agents

NVG is a serious ocular disease associated with poor visual outcomes [16,17]. It is seen in conditions that lead to retinal ischemia, such as proliferative diabetic retinopathy (PDR), central retinal vein occlusions (CRVOs) and chronic retinal detachments. In the later stages of NVG, iris and angle neovascularization can lead to the development of peripheral anterior synechiae that occludes the angle and results in elevated intraocular pressure (IOP) that does not respond to traditional topical glaucoma therapy.

Historically, treatment has consisted of multiple panretinal photocoagulation (PRP) treatments to the posterior pole. These treatments have been shown to downregulate the release of VEGF into the vitreous cavity [18]. Often extensive and multiple laser treatment sessions are needed. In addition, there are some patients in whom media opacities such as a mature cataract or vitreous hemorrhage preclude the use of laser. Finally, PRP does not lower the IOP and does not improve the ocular discomfort that these patients experience in the acute phase. Therefore, cyclophotocoagulation or invasive surgery is often required.

VEGF, a potent mitogen specific for vascular endothelial cells, is upregulated under conditions of retinal ischemia and NVG [19]. Therefore, a downregulation in its production through the use of VEGF inhibitors should stifle the neovascular properties of the aforementioned ocular diseases, which lead to retinal ischemia. This finding was established with a recent report that described a decrease in aqueous VEGF concentrations in NVG in patients who received intracameral bevacizumab [20]. There have also been multiple case series, which highlight regression of neovascularization of both the iris and angle with the use of VEGF inhibitors [18–26].

Initially, there were few case reports of the benefits of intravitreal bevacizumab (IVB) in patients with NVG. Kahook *et al.* [21] reported a patient who was treated with bevacizumab, 1 mg in 0.04 ml, after having failed IOP control with transscleral cyclophotocoagulation and PRP. This patient showed an immediate decrease in IOP and was symptomatically improved as well. Avery [22] reported a single case of rapid resolution of iris and retinal neovascularization with the administration of IVB. Davidorf *et al.* [23] reported similar results in a single patient with regressed neovascularization of the iris (NVI) after a single injection of bevacizumab.

Iliev *et al.* [24] described the use of IVB, 1.25 mg/0.05 ml, for six consecutive patients with NVI and refractory NVG. This treatment resulted in marked regression of anterior segment neovascularization and relief of symptoms in the first 2 days. In three patients, IOP was significantly reduced and the other three were controlled after the addition of cyclophotocoagulation and PRP. Oshima *et al.* [25] reported a case series of seven eyes with NVI due to PDR. NVI regressed with IVB in all eyes at 1 week. Two eyes had recurrences at 2 months and were stabilized with repeated IVB injections. Also, IOP was stabilized without the use of topical antiglaucoma medications in four eyes.

With the use of iris fluorescein angiography, Grisanti *et al.* [26] studied the effects of IVB on NVI. They presented a case series of six eyes in three patients who had NVG and NVI due to CRVO or PDR and received 1.0 mg of IVB. They noted a decrease in iris fluorescein angiography leakage as early as 1 day after injection. Of the six eyes, there was no regression at 1-month follow-up.

Bevacizumab's role and length of therapeutic benefit was explored by Gheith *et al.* [27]. They presented a case series of six patients with an average of 9.7 months

of follow-up. Each patient received 1.25 mg/0.05 ml of bevacizumab followed by PRP 1 week later. All patients had a complete regression of iris and angle neovascularization. However, two patients had recurrence of NVI after 3 and 5 months, respectively. They received another injection of bevacizumab that eradicated the recurrent neovascularization. They noted that topical medications did not control IOP in the patients who already had developed peripheral anterior synechiae. They also highlighted the need for continued monitoring of NVI due to the long-term regression seen in two of their patients. The pharmacokinetics of bevacizumab was further elucidated by Bakri *et al.* [28]. They found the vitreous half-life of 1.25 mg IVB is 4.32 days in a rabbit eye. Interestingly, they also found small amounts of bevacizumab in the serum and in the fellow uninjected eye.

Wakabayashi *et al.* [29] described a case series of 30 patients, 41 eyes, with NVI or NVG secondary to ischemic retinal disorders. With a mean of 13.3 months of follow-up, they reported no side effects from the use of IVB treatment. After dividing their patients into three groups – those with NVI alone, patients with NVG with open angles and those with neovascularization with closed angles – they concluded that IVB effectively stabilized NVI activity and controlled IOP in patients with NVI alone. But, in patients with advanced NVG and closed angles, IVB did not control IOP. It did, however, show promise as an adjunct to improve subsequent surgeries. Cornish *et al.* [30•] reported two cases of young diabetic patients with NVG managed with bevacizumab and mitomycin-C-augmented trabeculectomy. Both were treated with PRP and maximal medical topical therapy for their glaucoma and had persistently elevated IOP. With 6-month follow-up, both had controlled IOP. The authors postulated that in addition to bevacizumab's antineovascular effect, it modulates wound healing, thereby allowing the trabeculectomy to potentially be more successful in this often difficult to manage subgroup.

Through the antiangiogenic properties of anti-VEGF agents, their use in NVG has been promising in retarding and reversing the growth of vessels in the angle. When promptly evaluated and the angle has not been occluded with peripheral anterior synechiae (PAS), this pharmacological therapy has profound effects on IOP control. In addition to rapid IOP lowering, patients experience less ocular discomfort soon after its use. In conjunction with PRP, the use of anti-VEGF agents holds great promise in improving the otherwise dismal long-term visual prognosis for patients with NVG. Due to anti-VEGF's effect on wound healing, it has been studied as an adjunct in trabeculectomy surgery as well.

Antivascular endothelial growth factor agents and wound modulation in trabeculectomy surgery

Creation of a fistula between the anterior chamber and the sub-Tenon's space, as is done at the time of trabeculectomy surgery, leads to a decrease in IOP. A major impediment to the development of a successful bleb posttrabeculectomy is the body's innate healing response. Through fibroblast proliferation, migration and contraction, scarring can develop, which may limit the outflow of aqueous. With the advent of wound modulators such as mitomycin C (MMC) and 5-fluorouracil (5-FU), there was an improvement in surgical outcomes [31–33]. But, with this enhanced IOP control came an increase in complications such as bleb leaks and endophthalmitis [31,34–36]. Alternative antifibrotic agents have been explored, but with little success in supplanting MMC or 5-FU.

The use of anti-VEGF agents as an adjunct to trabeculectomy has recently been proposed [37,38••]. The wound healing process is potentiated through both fibroblast activity and angiogenesis. Therefore, an anti-VEGF agent should decrease new vascular growth and potentially lead to a healthier bleb with less scarring and better long-term IOP control.

To further elucidate the direct effect of anti-VEGF agents on fibroblasts, Guerriero *et al.* [39] illustrated in-vitro effects of bevacizumab on human corneal and conjunctival fibroblast cell lines. Their research concluded that when corneal stromal fibroblasts are exposed to bevacizumab, loss of cell-to-cell adhesions and morphological changes are seen. They further stated that these changes are dose-dependent. However, Yoeruek *et al.* [40] reported no change in human corneal fibroblast cells with high-dose bevacizumab. But, Yu *et al.* [41] later found that conjunctival stromal fibroblasts exhibited similar changes when exposed to anti-VEGF agents. Other reports have concluded that a dose-dependent alteration is evident in human Tenon fibroblast morphology and proliferation when exposed to bevacizumab and ranibizumab *in vitro* at doses similar to or higher than those used for intravitreal injection [42,43].

Welsandt *et al.* [44] have shown that the inhibition of the neovascular cascade by anti-VEGF agents decreases fibroblast proliferation through cytokines like fibroblast growth factor (FGF), thereby inhibiting the synergy between VEGF-A and FGF-2. Therefore, anti-VEGF agents modulate wound healing through both fibroblast proliferation and angiogenesis.

A recent report by Memarzadeh *et al.* [45•] investigated the utility of subconjunctival injections of bevacizumab

on bleb morphology in rabbits after trabeculectomy. They noted that bevacizumab prolonged bleb survival and led to more favorable bleb morphology compared with 5-FU and control (balanced salt solution) groups. Bleb survival was 16.0 days for the bevacizumab group versus 6.9 and 7.4 days for the 5-FU and control groups, respectively.

In the first report describing the use of bevacizumab to modulate wound healing in humans, Kahook *et al.* [37] noted a significant and lasting decrease in IOP after a bleb needling procedure after failed trabeculectomy. They injected 1 mg of bevacizumab adjacent to the bleb at the end of the needling procedure. Many subsequent reports have illustrated the utility of both bevacizumab and ranibizumab as sub-Tenon's injections after filtration surgery or at time of bleb needle revision [43–44,45•,46].

Kapetansky *et al.* [46] studied the utility of subconjunctival bevacizumab injections administered proximal to blebs after trabeculectomy at the earliest sign of vascularization. They noted that nearly two thirds of the blebs had an observable reduction in vascularity while decreasing IOP from a mean of 17.8 to 14 mmHg 1 month after injection. Improved results were noted when the injections were given earlier in the postoperative phase. Purcell *et al.* [47] noted decreased IOP and bleb vascularization after bleb needle revision using ranibizumab. But, this effect was short-lived, as increased vascularization was noted after 1 month of follow-up. Coote *et al.* [48] presented a case of subconjunctival injection of bevacizumab that resulted in a dramatic reduction of bleb vascularity for 6 weeks. In their case, even 6 months after injection, a healthy bleb with minimal scar tissue was seen.

Grewal *et al.* [38••] recently reported results from their nonrandomized, open-label, prospective, interventional case series of 12 patients receiving subconjunctival injection of bevacizumab (1.25 mg/0.05 ml) adjacent to the bleb at time of trabeculectomy. The mean preoperative IOP was 24.4 mmHg and decreased to a mean of 11.6 mmHg at 6 months. They concluded that subconjunctival bevacizumab is a potential adjunctive treatment for reducing the incidence of bleb failure after trabeculectomy. An interesting observation in their study was the increasing bleb vascularity that occurred at month 3, which they stated might decrease the incidence of thin cystic blebs that frequently develop after MMC-augmented trabeculectomy surgery. They also added that further studies are needed to better understand the dose and route of injection as well as the side effect profile of bevacizumab on the corneal endothelium and trabecular meshwork. Jue [49] postulates that anti-VEGF agents may have a synergistic effect with MMC and 5-FU in those patients whose trabeculectomies may fail with the use of MMC or 5-FU alone.

A case series by Kitnarong *et al.* [50] highlights the benefits of IVB preoperatively. They studied six patients with NVG who underwent trabeculectomy with MMC. Preoperatively, PRP was performed and IVB (1.25 mg/0.05 ml) was administered. They noted that short-term NVI regressed within 1 week of IVB treatment in four patients. In addition, there were no intraoperative complications such as hemorrhage. Finally, they reported five patients had an IOP less than 21 mmHg without medication after a mean of 24.7 weeks of follow-up.

Research focused on the most appropriate dosage of intracameral bevacizumab was studied by Gupta *et al.* [51]. In their study, patients received either 1.25 or 2.5 mg of intracameral bevacizumab prior to trabeculectomy with MMC for NVG. Although there was no difference in recurrence of neovascularization or IOP control after 3 months, this study highlights an area of interesting research.

Further studies are needed to better understand how anti-VEGF agents might benefit patients undergoing glaucoma filtration surgery. There are ongoing safety studies to better analyze the importance of route of administration – intracameral, sub-Tenon and intravitreal – and to determine whether unknown side effects co-exist. It is important to delineate duration of action when anti-VEGF agents are injected in the intra or sub-Tenon's space and how this might influence efficacy. Protein aggregation preinjection and postinjection are poorly understood and require further study. These new compounds may be unstable under various temperatures and denatured proteins might lead to downstream effects on the outflow system of the eye. It is also important to further study the pharmacokinetics and pharmacodynamics of anti-VEGF agents when they come in contact with the sclera, as this might prolong their retention and lead to improved long-term efficacy if applied directly to the surgical bed as is the case with MMC [52]. Further studies are also needed to investigate the utility of combination therapy (i.e. 5-FU + ranibizumab) for wound modulation after trabeculectomy. There is no reason to believe that any single agent might provide improved outcomes compared to approaching the wound healing process from multiple angles.

Conclusion

The antiangiogenic and antifibroblastic properties of the recently introduced anti-VEGF agents have led to their early adoption in treating NVG and influencing wound modulation posttrabeculectomy. Prospective multicenter studies are still lacking for these pharmacotherapies and

studies will be needed to better outline proper treatment regimens, most appropriate route of delivery, optimum dose for each agent, as well as potential patient populations that might be more susceptible to currently unknown side effects. In addition, the potential for anti-VEGF delivery systems used in conjunction with glaucoma drainage devices may be explored. Future research investigating the role of anti-VEGF agents in NVG, trabeculectomy surgery as well as other areas of glaucoma may lead to an improvement in IOP lowering without compromising patient safety.

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