Inhibition of Vascular Endothelial Growth Factor Reduces Scar Formation after Glaucoma Filtration Surgery

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PURPOSE. Filtration failure due to excessive postoperative scarring remains a major problem after glaucoma surgery. The authors have investigated whether glaucoma and filtration surgery are associated with increased levels of vascular endothelial growth factor (VEGF), and whether a humanized monoclonal antibody against VEGF, bevacizumab, can reduce postoperative scar formation and improve surgical outcome.

METHODS. The levels of VEGF in samples of aqueous humor were measured by ELISA. The expression of the VEGF receptors Flt-1 and KDR was analyzed in cultured Tenon fibroblasts by real-time RT-PCR and Western blotting. The effect of VEGF and bevacizumab on Tenon fibroblasts in vitro was determined using a proliferation assay. The in vivo effect of the antibody was investigated in a rabbit model of trabeculectomy by measuring the intraocular pressure (IOP) and bleb area, and by immunohistological analysis of angiogenesis, inflammation, and fibrosis.

RESULTS. VEGF levels were increased significantly in the aqueous humor of glaucoma patients and rabbits that had undergone surgery. Both VEGF receptors were expressed on Tenon fibroblasts. Fibroblast proliferation in vitro was stimulated by delivery of VEGF, and was inhibited by administration of bevacizumab. The antibody also reduced angiogenesis and collagen deposition significantly, and improved the outcome of glaucoma surgery in rabbits.

CONCLUSIONS. VEGF was upregulated in the aqueous humor of glaucoma patients and in the rabbit model, and it stimulated fibroblast proliferation in vitro. This suggests that it is involved in the scarring process after filtration surgery. Bevacizumab reduced the proliferation of fibroblasts in vitro and improved surgical outcome. (Invest Ophthalmol Vis Sci. 2009;50: 5217–5225) DOI:10.1167/iovs.08-2662

Filtration surgery remains the most effective therapy to reduce intraocular pressure (IOP) in patients with glaucoma.1,2 However, excessive postoperative scarring of the conjunctiva and Tenon at the sclerostomy site is associated with filtration failure, poor postoperative control of IOP, and the consequent progression of glaucomatous disc cupping and visual field loss.3,4 The key players in ocular wound healing are the fibroblasts in the Tenon’s capsule.5,6 Although anti-mitotic agents such as mitomycin-C (MMC) and 5-fluorouracil (5-FU) help to prevent post-surgical scarring and improve the surgical outcome, they do so by causing widespread non-selective cell death and apoptosis. Therefore, the use of these agents is associated with severe complications that can potentially cause blinding, such as hypotony, blebitis, endophthalmitis, and wound leakage.5 Recent studies showed that a monoclonal antibody (CAT-152) against transforming growth factor (TGF)-β could reduce scarring in animal models of glaucoma.7,8 However, the clinical study was terminated prematurely because of lack of efficacy with the dose used.9 Therefore, there remains a need for specific and effective anti-scarring treatments.

Vascular endothelial growth factor (VEGF) is best known as an endothelial growth and permeability factor.10 It plays a major role in physiological vasculogenesis and angiogenesis in the embryo,11 and is involved in the formation of pathologic blood vessels, as well as in tumor growth and ocular diseases.12 The concentration of VEGF is increased in all ocular diseases that involve neovascularization and/or inflammation, such as proliferative diabetic retinopathy,13 neovascular glaucoma,14,15 uveitis,16 and age-related macular degeneration.17 In addition, VEGF is associated with fibrosis, and with inflammatory diseases such as rheumatoid arthritis18 and Crohn’s disease.19 A recent study showed an association between VEGF and the healing of cutaneous wounds. Increased levels of VEGF induce scar formation in skin wounds by increasing vascularity and the deposition of collagen, while neutralization of VEGF reduces angiogenesis and cutaneous fibrosis.20 Two high-affinity VEGF tyrosine kinase receptors have been identified: fms-like tyrosine kinase (Flt)-1 and kinase domain receptor (KDR). Both receptors are expressed predominantly in endothelial cells,21 but recently they have also been found in selected non-vascular cells.22–24 VEGF stimulates inflammation by modulating Flt-1 signaling.18 The expression of VEGF can be restored not only by hypoxia25 but also by TGF-β.26 Increased levels of TGF-β have been found in patients with a failing filtration system.27 Bevacizumab (Avastin; Genentech, San Francisco, CA) is a humanized, non-selective monoclonal antibody against VEGF. It has been approved by the U.S. Food and Drug Administration as a treatment for widespread metastatic colorectal cancer,28 as

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well as breast cancer. Intra-vitreal injection of the antibody has a good safety profile, in both humans and rabbits. Although previous studies have suggested a role for VEGF in scar formation after glaucoma surgery, this has not been studied in detail. Here, we provide data to support the hypothesis that VEGF plays an important role in scar formation after glaucoma filtration surgery and that it may be a target for improvement of the outcome of this surgery.

**MATERIALS AND METHODS**

All animal procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. For human experiments, the tenets of the Declaration of Helsinki were upheld, approval was granted by the institutional human ethics committee, and informed consent was obtained.

**Rabbit Model of Filtration Surgery**

Thirty-four female New Zealand rabbits, which were aged 12 to 14 weeks and weighed 2 to 3 kg, were used in this study. Before surgery, the IOP was measured in both eyes with a tonometer (Tono-Pen; Medtronic Solan, Jacksonville, FL) under 4 mg/mL topical anesthesia (Unicain; Thea Pharma, Schaffhausen, Switzerland). General anesthesia was induced by intramuscular injection of 50 mg/mL ketamine (Ketalar; Pfizer, Ann Arbor, MI) and 2% sedative (Rompun; Bayer Health Care, Pittsburgh, PA). Surgery was performed on both eyes, using a technique that has been described previously and that results in a filtering bleb. Immediately after surgery, the left eye was injected

### Table 1. Probe and Primer Sequences (5’-3’)

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**Figure 1.** Levels of huVEGF protein in samples of aqueous humor. (A) Levels of huVEGF, as analyzed by ELISA, were significantly higher in patients with glaucoma (n = 20) than in those with cataracts (n = 20) (who served as controls) (*P < 0.005). (B) Levels of huVEGF in samples of aqueous humor from rabbits (n = 6) were increased at all time points after surgery, both in treated and non-treated eyes, compared with the level of VEGF one day before surgery. On day 4, the concentration of VEGF was significantly lower in the treated eyes than in the control eyes (*) (P < 0.05). Beyond day 6, the VEGF levels in both eyes were comparable.
with 25 mg/mL bevacizumab (Genentech); the right eye was used as a control and received an injection of 0.9% NaCl (B. Braun Medical, Bethlehem, PA). For each eye, 200 μL were injected into the anterior chamber and 100 μL were injected subconjunctivally into the filtration bleb.

ELISA for VEGF
Samples of human aqueous humor were collected from patients who were undergoing trabeculectomy for primary open angle glaucoma (POAG) or phacoemulsification for senile cataract without glaucoma (the control group) at the University Hospitals Leuven. The surgeon collected samples of aqueous humor (100 –200 μL) immediately after limbal paracentesis to avoid the influence of intra-ocular trauma/surgery. Samples (200 μL) of aqueous humor were obtained from both eyes of the rabbits the day before, and on days 1, 4, 6, 8, 14, and 30 after filtration surgery. All samples were stored immediately at −80°C until analyzed. The levels of VEGF protein in the samples of aqueous humor were analyzed with a double-antibody “sandwich” ELISA (R&D Systems Inc., Minneapolis, MN) with a detection limit of 15.6 pg/mL.

Cells and Culture Conditions
Tissue samples of human Tenon’s capsule were obtained during strabismus surgery; rabbit Tenon tissue explants were harvested during filtration surgery. Primary human and rabbit Tenon fibroblasts were propagated in Dulbecco’s modified Eagle’s medium (DMEM), supplemented with 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Rochester, NY), 2 mM L-glutamate, 100 U/ml penicillin, 100 μg/ml streptomycin, 0.01 mM non-essential amino acids, 20 mM Hepes (all from Invitrogen, Carlsbad, CA) and 11.2 μg/ml β-mercapto-ethanol (Sigma-Aldrich, St. Louis, MO), as described previously.8 The purity of the cultured Tenon fibroblasts was verified by immunostaining for vimentin. The cells were incubated overnight with a murine antibody against human vimentin (1/100; V5255; Sigma-Aldrich), and binding was visualized by fluorescent staining using a direct fluorescence detection system (TSA Cyanine 3 System; Perkin Elmer, Wellesley, MA).

Real Time RT-PCR
To investigate the expression of VEGF receptors at the mRNA level, RNA from Tenon fibroblasts was isolated using an extraction kit (RNeasy Minikit; Qiagen, Valencia, CA), as described previously.44 Expression was normalized to that of the housekeeping gene β-actin. Primers and probes (Table 1) were designed using commercial software (Primer Express 10; Applied Biosystems, Foster City, CA).

Western Blot Analysis
Cells were rinsed with ice-cold phosphate-buffered saline (PBS), extracts of total cell protein were prepared and Western blotting was performed as described previously.44 Protein concentrations were measured using a bicinchoninic acid (BCA) assay (Pierce Biotechnology, Rockford, IL).
Fibroblast Proliferation Assay

To investigate the effect of VEGF and bevacizumab on fibroblast proliferation, human and rabbit Tenon fibroblasts were cultured in DMEM supplemented with 10% FBS. In one series of experiments, the medium was removed and the cells were washed three times with PBS before the addition of DMEM supplemented with 0.1% FBS. Twenty-four hours later, medium that contained 0.1% FBS and different concentrations of VEGF (10 or 30 ng/mL) was added. In a second series, medium that contained 10% FBS and had been pre-incubated with different concentrations of bevacizumab (25, 20, 10, 5, 2.5, or 1 mg/mL; 500 μg/mL; 300 ng/mL) was added immediately after the cells were washed.

All in vitro experiments were performed in triplicate in 96-well tissue culture plates (Falcon; Becton Dickinson, Franklin Lakes, NJ) at an initial density of 5.0 × 10^3 cells per well. Medium that contained 10% FBS was used as a positive control and medium with 0.1% FBS was used as a negative control. Forty-eight hours after administration, cell proliferation was assessed using a proliferation assay (CellTiter 96 Aqueous One Solution Cell Proliferation Assay; Promega, Madison, WI).

Clinical Examination and Immunohistochemistry

All rabbits that had received surgery were examined and graded according to the Moorfield bleb grading system on day 1 after surgery and then every 2 days until the animals were killed. The IOP and bleb area (width and length) were analyzed under topical anesthesia. Bleb failure was taken as the end-point of the study, and was defined as the appearance of a scarred, flat, vascularized bleb.

On days 3, 8, 14, and 30 after surgery, rabbits were killed using a lethal intravenous injection of sedative (Rompun; Bayer Health Care). Both eyes were enucleated, fixed overnight in 4% paraformaldehyde (PFA), dehydrated, and embedded in paraffin. Serial sections (7 μm) were stained with different immunohistochemical reagents. Inflammation was analyzed by CD45 staining, and CD31 staining was performed to check the density of blood vessels. The samples were incubated overnight with a murine antibody against rabbit CD45 (1/100; MCA808; AbD Serotec, Oxford, UK) or with a murine antibody against human CD31 (1/200; M0823; DakoCytomation A/S, Copenhagen, Denmark), respectively. The following day, the bound antibodies were visualized using an amplification kit (Renaissance TSA Indirect Kit; Perkin Elmer) and diaminobenzidine (32750; DAB; Sigma) as the chromogen. Deposition of collagen was analyzed by staining with Masson’s trichrome and Sirius red. Images were obtained using a microscope (Imager Z1; Zeiss, Oberkochen, Germany) with a digital camera (AxioCam MrC5; Zeiss) at a magnification of ×20 and a resolution of 2584 × 1936 pixels. Morphometric analyses were performed using commercial software (KS300; Zeiss). The density of blood vessels was determined by calculating the CD31-positive area as a proportion of the total area. The density of leukocytes was quantified by counting the CD45-positive cells, expressed in number/mm² of the total area. Deposition of collagen was determined by measuring the percentage of collagen fibers in the total area. Sirius red staining was analyzed under polarized light.
Statistical Analysis

The data were analyzed (Statistica 6.1 Software; StatSoft, Tulsa, OK). All data were analyzed using the student’s t-test for independent samples. Data at individual time points were analyzed using one-way analysis of variance. P values smaller than 0.05 were considered to be statistically significant. Data are presented as the mean ± SEM.

RESULTS

Expression of VEGF in the Aqueous Humor

The level of VEGF in aqueous humor samples was analyzed by ELISA, and was found to be significantly lower in the eyes of control subjects (n = 20) than in glaucomatous eyes (n = 20; P = 0.001; Fig. 1A).

The concentration of VEGF in the rabbit samples was determined using the same human ELISA kit. Previous studies have shown that the antibody against human VEGF cross-reacts with rabbit VEGF, and comparison of the nucleotide sequences of rabbit and human VEGF revealed a homology of 91% (not shown). After surgery, the concentration of VEGF increased in both control and bevacizumab-treated eyes. On day 4, the level of VEGF in the treated eyes was significantly lower than in the control eyes (n = 6; P = 0.04). Beyond day 6, the VEGF levels in both control and treated eyes were comparable (Fig. 1B). Thus, the level of VEGF protein was upregulated in the aqueous humor of glaucoma patients, as well as in rabbits, after filtration surgery, and, in rabbits, this postoperative upregulation could be blocked temporarily by the application of bevacizumab at the time of surgery.

Expression of the VEGF Receptor in Tenon Fibroblasts

In cultures of both human and rabbit fibroblasts, all cells were positive for vimentin, which confirmed the purity of the fibroblast culture (data not shown). Quantitative real-time RT-PCR on human and rabbit Tenon fibroblasts showed that the different VEGF isoforms (VEGF121, VEGF165, and VEGF189) and the VEGF receptors (Flt-1 and KDR) were expressed (Figs. 2A–B). Western blotting confirmed the expression of the two VEGF receptors at the protein level (data not shown).

Effect of Administration of VEGF and Bevacizumab on Growth of Tenon Fibroblasts

The addition of 10 or 30 ng/mL VEGF induced a significant increase in cell proliferation, compared with 0.1% FBS, in human (P = 0.04 for both concentrations) and rabbit Tenon fibroblasts (P = 0.02 and P = 0.04 for 10 and 30 ng/mL VEGF, respectively; Fig. 3A). To evaluate whether inhibition of VEGF inhibits the proliferation of fibroblasts, increasing concentrations of bevacizumab were added to cells that were growing in medium containing 10% FBS. Bevacizumab reduced the number of both human and rabbit fibroblasts in a dose-dependent manner.
**FIGURE 5.** Blood vessel density, inflammation, and collagen deposition in rabbit eyes. (A) The images show representative pictures of treated eyes (top) compared with non-treated eyes (bottom) at different time points. The left panels show immunohistochemical staining for CD31. These pictures reveal the blood vessels to be less numerous and less dilated on day 3 in the eyes that were treated with bevacizumab, compared with control eyes. Morphometric analysis of the CD45 immunostaining showed increased infiltration of CD45+ leukocytes until day 8 after surgery (middle panels). Analysis of the microscopy pictures of the trichrome-stained samples showed a decrease in the deposition of collagen on day 30 after surgery in the treated eyes compared with the control eyes (right panels). (B) No blood vessels were visible on day 0, but a significant number was observed at day 3; the density then decreases. Treatment with bevacizumab decreased the formation of blood vessels and the appearance of dilated blood vessels at the filtration site on day 3 compared with control eyes (*P < 0.05). (C) Inflammation increased until day 8 and had decreased again gradually at day 14 and day 30. No leukocytes were visible on day 0. There were no significant differences between treated and untreated eyes. (D) Deposition of collagen at the filtration site increased significantly over time after surgery (*P < 0.005 at day 30 versus day 0). The increase on days 14 and 30 was effectively prevented by the administration of bevacizumab (*P < 0.01, #P < 0.005 versus control).
manner (Fig. 3B). Thus, administration of VEGF increased fibroblast proliferation, whereas bevacizumab inhibited the proliferation of human and rabbit fibroblasts in vitro.

Effects of Bevacizumab in a Rabbit Trabeculectomy Model

To determine whether administration of bevacizumab correlated with more successful filtration surgery, we analyzed the IOP and bleb area (as a measure of filtration efficacy) of rabbit eyes after surgery. Figure 4A shows the typical appearance of the blebs on days 1 and 7 after surgery. Injection of bevacizumab was associated with an elevated, diffuse, and non-vascularized bleb (top panels), compared with a flat, scarred, and vascularized bleb in the control group (bottom panels). This difference became less obvious from postoperative day 20.

The IOP decreased slightly until day 13 after surgery but then returned to normal. The IOP was similar in the treated and control eyes at each time point (n = 54; P = 0.17; Fig. 4B). However, the bleb area was significantly larger at each time point in the treated eyes compared with that in untreated eyes (n = 54; P < 0.05; Fig. 4C).

To evaluate microscopically whether bevacizumab affected inflammation, angiogenesis, and deposition of collagen, histologic and immunohistochemical staining were performed at different time points after surgery. Figure 5A shows the density of blood vessels on day 3, inflammation on day 8, and collagen deposition on day 30. Bevacizumab treatment resulted in fewer dilated CD31-positive blood vessels and in a twofold reduction in the density of blood vessels at the filtration site, compared with untreated eyes, on day 3 (n = 6; P = 0.02; Fig. 5B). No difference in the number of CD45-positive inflammatory cells could be detected between the treated and control eyes at the filtration site (n = 6; P = 0.17; Fig. 5C). In the treated eyes, compared with control eyes, the total area of collagen at the filtration site, as revealed by trichrome staining, was reduced after bevacizumab treatment by 11% on day 14 post surgery (as revealed by trichrome staining, was reduced after bevacizumab treatment by 11% on day 14 post surgery (as revealed by trichrome staining, was reduced after bevacizumab treatment by 11% on day 14 post surgery (as revealed by trichrome staining, was reduced after bevacizumab treatment by 11% on day 14 post surgery (as revealed by trichrome staining, was reduced after bevacizumab treatment by 11% on day 14 post surgery (as revealed by trichrome staining, was reduced after bevacizumab treatment by 11% on day 14 post surgery (as revealed by trichrome staining, was reduced after bevacizumab treatment by 11% on day 14 post surgery (as revealed by trichrome staining, was reduced after bevacizumab treatment by 11% on day 14 post surgery (as revealed by trichrome staining, was reduced after bevacizumab treatment by 11% on day 14 post surgery (as revealed by trichrome staining, was reduced after bevacizumab treatment by 11% on day 14 post surgery (as revealed by trichrome staining, was reduced after bevacizumab treatment by 11% on day 14 post surgery (as revealed by trichrome staining, was reduced after bevacizumab treatment by 11% on day 14 post surgery (as revealed by trichrome staining, was reduced after bevacizumab treatment by 11% on day 14 post surgery (as revealed by trichrome staining, was reduced after bevacizumab treatment by 11% on day 14 post surgery (as revealed by trichrome staining, was reduced after bevacizumab treatment by 11% on day 14 post surgery (as revealed by trichrome staining, was reduced after bevacizumab treatment by 11% on day 14 post surgery (as revealed by trichrome staining, was reduced after bevacizumab treatment by 11% on day 14 post surgery (as revealed by trichrome staining, was reduced after bevacizumab treatment by 11% on day 14 post surgery (as revealed by trichrome staining, was reduced after bevacizumab treatment by 11% on day 14 post surgery (as revealed by trichrome staining, was reduced after bevacizumab treatment by 11% on day 14 post surgery (as revealed by trichrome staining, was reduced after bevacizumab treatment by 11% on day 14 post surgery (as revealed by trichrome staining, was reduced after bevacizumab treatment by 11% on day 14 post surgery (as revealed by trichrome staining, was reduced after bevacizumab treatment by 11% on day 14 post surgery (as revealed by trichrome staining, was reduced after bevacizumab treatment by 11% on day 14 post surgery (as revealed by trichrome staining, was reduced after bevacizumab treatment by 11% on day 14 post surgery (as revealed by trichrome staining, was reduced after bevacizumab treatment by 11% on day 14 post surgery (as revealed by trichrome staining, was reduced after bevacizumab treatment by 11% on day 14 post surgery (as revealed by trichrome staining, was reduced after bevacizumab treatment by 11% on day 14 post surgery (as revealed).

Additional analysis of Sirius red staining confirmed these findings (data not shown). Thus, bevacizumab improved glaucoma filtration surgery outcome by inhibiting angiogenesis during the initial phase of healing and reducing fibrosis at later stages in a rabbit model of aggressive post-surgical scarring in glaucoma.

DISCUSSION

The failure of glaucoma filtration surgery due to excessive scarring remains a major barrier to the control of IOP and arrest of disease progression. Current anti-scarring agents, such as 5-FU and MMC, reduce the post-surgical scar formation and may help to improve the outcome of glaucoma surgery. However, the use of these agents is associated with severe side-effects and complications.

VEGF may play a role in scar formation after filtration surgery, and that blocking VEGF may improve the outcome of surgery.

Role of VEGF after Glaucoma Filtration Surgery

In this study, we demonstrated that VEGF is increased in the aqueous humor of patients with glaucoma. These data are consistent with previous studies. Tripathi et al. reported that VEGF was detected in only 4 out of 20 samples of aqueous humor from eyes with cataract, and in 27 out of 40 samples from glaucomatous eyes. We found that VEGF was detectable in all aqueous humor samples, both from patients with cataract and from those with glaucoma. These differences may be due to the sensitivities of the ELISAs that were used: a single-antibody competitive-binding assay (with a sensitivity of 195 pg/mL) was used in the previous study, whereas we used a double-antibody “sandwich” ELISA (with a sensitivity of 15.6 pg/mL). We also found that there was no significant difference in the plasma concentration of VEGF between patients who underwent trabeculectomy and control subjects (data not shown). A significant increase in VEGF in the aqueous humor, but not in the plasma, suggested that this growth factor was produced locally. Indeed, VEGF is released by numerous types of ocular cells, which include pigment epithelial cells, pericytes, endothelial cells, Müller cells, and astrocytes.

Vascular insufficiency is implicated in the pathogenesis of glaucoma, and ischemia is a potent trigger for the production of VEGF. The glaucoma patients received topical medications (prostaglandin analogues, β-blockers, carbonic anhydrase inhibitors, and α2-adrenergic receptor agonists), which may have influenced the concentration of VEGF in the aqueous humor. However, a study by Hu et al. showed no correlation between the concentration of VEGF in the aqueous humor and the use of topical medications, which suggested that the two-fold increase in VEGF in glaucomatous eyes that we observed was unlikely to have been caused solely by treatment to reduce the IOP. The documented increase in VEGF in the aqueous humor of glaucoma patients may contribute to postoperative inflammation and fibrosis, and lead to an increased risk of trabeculectomy failure.

The fibroblasts of Tenon’s capsule, which produce collagen and elastin, are the most important mediators of ocular scar formation after filtration surgery. Here, for the first time, we demonstrated that VEGF receptors were expressed in human and rabbit Tenon fibroblasts, at both the mRNA and protein level. We were able to show that VEGF stimulated the proliferation of Tenon fibroblasts in vitro, and that treatment with different concentrations of bevacizumab inhibited the proliferation of both human and rabbit fibroblasts. These data not only show that Tenon fibroblasts are susceptible to VEGF; they also confirm that bevacizumab, which is a humanized antibody against VEGF, cross-reacts with rabbit VEGF.

We were also able to show that VEGF is upregulated after filtration surgery in the rabbit trabeculectomy model. The level of VEGF rose after surgery and remained high until day 30. After application of bevacizumab, the concentration of VEGF on day 4 was significantly lower than that in control eyes. However, at later time points, the levels of VEGF were comparable in the treated and untreated eyes. The half-life of 1.25 mg bevacizumab in the aqueous humor of rabbit eyes that have not undergone operation is 4.88 days. The concentration of bevacizumab in the aqueous humor will reach a peak of 37.7 μg/mL three days after intra-vitreal administration. These findings could explain why the concentration of VEGF increased after day 4. Our data suggest, therefore, that repeated injections of bevacizumab may be necessary to attain maximum inhibition.

However, our study revealed that one injection of a high concentration of bevacizumab (25 mg/mL) was effective in improving the outcome of glaucoma surgery, and in reducing angiogenesis and fibrosis in a rabbit model of glaucoma filtration surgery. During each clinical investigation, the

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rabbits were examined for adverse effects such as conjunctival necrosis, scleral necrosis, bleb effusion, and hypotony. None of these complications was observed. In future studies, it may be beneficial to reduce the dose and evaluate the dose-response dynamics. Importantly, we demonstrated that bevacizumab was only effective in increasing the bleb area and did not affect the IOP. The latter could be explained by the fact that, in this model, rabbits do not really develop glaucoma and do not have raised IOP. On the other hand, compared with the human eye, this model of glaucoma filtration surgery is characterized by very aggressive scar formation and very early bleb failure, which occurs generally within 10 to 14 days. Therefore, an agent that can reduce scar formation in the rabbit is likely to be effective in humans, who generally show less scarring.

Finally, we studied the effect of inhibition of VEGF on the cellular processes in the filtering bleb using immunohistochemistry. Inflammation and fibrosis at the site of trabeculectomy are associated with an increased risk of bleb failure. The process of neovascularization is required to heal wounds and to restore blood flow to tissues after injury. Here we revealed that inhibition of VEGF can reduce postoperative scarring, and that this effect is associated with reduced angiogenesis on day 3 and reduced deposition of collagen on days 14 to 30, with no significant decrease in inflammation. Comparative observations have been described in the healing of cutaneous wounds and in tumor models.

CONCLUSIONS

In summary, our data suggest that VEGF is important during scar formation after filtration surgery. VEGF was expressed in samples of aqueous humor from glaucoma patients and rabbits that had undergone surgery, and stimulated the proliferation of Tenon fibroblasts in vitro. The humanized monoclonal antibody against VEGF, bevacizumab, was able to inhibit the proliferation of fibroblasts in vitro, and also improved the outcome of glaucoma filtration surgery in an animal model of aggressive scarring by reducing angiogenesis and collagen deposition. These findings reveal new possibilities for safer adjunctive therapy to improve the efficacy of filtration surgery, and thereby the prognosis of glaucoma patients.

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