Level of Vascular Endothelial Growth Factor in Tenon Tissue and Results of Glaucoma Surgery

Hae-Young Lopilly Park, MD; Jie Hyun Kim, PhD; Myung Douk Ahn, MD, PhD; Chan Kee Park, MD, PhD

Objective: To determine the levels of vascular endothelial growth factor (VEGF) in both the aqueous humor and Tenon tissue in patients with primary open-angle glaucoma (POAG) and the associations between the VEGF and outcomes of glaucoma surgery.

Methods: The study involved 19 patients with POAG who were scheduled to undergo glaucoma surgery owing to uncontrolled intraocular pressure (IOP) and 17 control subjects who were scheduled to undergo cataract surgery. At the time of surgery, about 0.1 mL of aqueous humor was collected through an anterior chamber paracentesis and a 4 × 4-mm Tenon tissue sample was cut from the eye. Concentrations of VEGF were analyzed by enzyme-linked immunosorbent assay. Spearman correlation and regression analysis were used to assess the relationship with VEGF level to the clinical characteristics and postoperative IOP.

Results: The VEGF in Tenon tissue was significantly elevated in patients with POAG compared with control subjects (P = .001). When patients with POAG were divided into success or failure groups 1 year following surgery, the VEGF levels were significantly higher in the Tenon tissue of the failure group compared with the success group (P = .014). The preoperative IOP was significantly related to the VEGF level in Tenon tissue in both the univariate (P = .001) and multivariate (P = .012) regressions.

Conclusions: The VEGF level in Tenon tissue at the time of surgery was significantly related to 1-year surgical outcomes of glaucoma surgery, and it was significantly associated with the final IOP in patients with POAG.


Vascular endothelial growth factor (VEGF) stimulates the growth of vascular endothelial cells and increases vascular permeability. It plays a major role in physiological vasculogenesis and angiogenesis in the embryo, and it is involved in the formation of pathologic blood vessels, tumor growth, and ocular diseases. Vascular endothelial growth factor also may play a role in the wound healing process other than the effect of angiogenesis, and the association between VEGF and the healing of cutaneous wounds has been reported. Increased levels of VEGF induce scar formation in skin wounds via the deposition of collagen. Related to wound healing of the eye, VEGF stimulates the proliferation of Tenon fibroblasts in vitro.

The level of VEGF in the aqueous humor is related to the success of Ahmed glaucoma valve implantation in patients with neovascular glaucoma. Blocking VEGF is a wound-modulating method that is of potential use as an adjunct in glaucoma surgery. Several animal experiments and clinical studies have compared the scar formation after glaucoma surgery with the inhibition of VEGF. Anti-VEGF agents such as bevacizumab have shown antifibrotic effects via the inhibition of fibroblast proliferation and induction of fibroblast cell death in vitro. Vascular endothelial growth factor is elevated in neovascular glaucomas as well as in other forms of glaucoma such as primary open-angle glaucoma (POAG). Tenon fibroblasts are the main effector cells in the initiation and mediation of wound healing and fibrotic scar formation after glaucoma surgery. Thus, the actual level of VEGF in the subconjunctival space or Tenon tissue may be more important in the wound-modulating process. The level of VEGF in the vitreous or anterior chamber could pass through the pores of the trabecular meshwork and openings of the Schlemm’s canal (0.5-75 µm) and reach the subconjunctival spaces because VEGF is much smaller (7.56 nm). Therefore, this study determined the levels of VEGF in both the aqueous humor and Tenon tissue in patients with POAG at the time of glaucoma surgery. The associations between the levels of aqueous VEGF or Tenon VEGF and surgical outcomes after 1 year were analyzed.
METHODS

PARTICIPANTS

Following the approval by the institutional review board at The Catholic University of Korea, 19 patients with POAG who were scheduled to undergo glaucoma surgery (14 for trabeculectomy and 5 for Ahmed glaucoma valve implantation) owing to uncontrolled intraocular pressure (IOP) were enrolled between May and July 2010. To be included, patients had to have an IOP of more than 21 mm Hg, visual field or optic disc changes characteristic of glaucoma, and be taking the maximum tolerated dose of medication. In addition, 17 control subjects scheduled to undergo cataract surgery were enrolled during the same period. Written informed consent was obtained from each patient, and all procedures were performed in accordance with the Declaration of Helsinki.

The study excluded those who were younger than 18 years of age and those diagnosed with secondary glaucoma, angle-closure glaucoma, or angle-closure suspects. Further exclusions included those with ocular trauma; previous laser therapy or intravitreal injection; ocular surgery other than cataract surgery; active or past inflammatory disease of the cornea, conjunctiva, episclera, or sclera; and active, chronic, or recurrent uveitis.

AQUEOUS HUMOR AND TENON TISSUE SAMPLING AND VEGF QUANTIFICATION

Before surgery, about 0.1 mL of aqueous humor was collected through an anterior chamber paracentesis. After a conjunctival flap incision, the conjunctiva and Tenon tissue were separated by blunt dissection. A 4 × 4-mm piece of Tenon tissue was cut from the eye at the margin of the conjunctival flap incision from the areas with no apparent vessels. Minimal manipulation was done carefully during the dissection and cutting. No significant bleeding occurred during the procedure. Each sample was stored immediately at −70°C.

The VEGF concentrations in the aqueous humor and Tenon tissue samples were determined using an enzyme-linked immunosorbent assay (Quantikine ELISA Kit; R&D Systems) according to the manufacturer’s instructions. For Tenon tissue, proteins were extracted before the enzyme-linked immunosorbent assay. The tissues were homogenized in radioimmunoprecipitation assay buffer (1% Triton X100, 5% sodium deoxycholate, 5% deoxycholic acid, 0.5M Tris hydrochloride [pH 7.5], 10% glycerol, 1mM EDTA, 1mM phenylmethylsulfonyl fluoride, 5 µg/ml aprotinin, 1 µg/ml leupeptin, 1 µg/ml pepstatin, 200mM sodium orthovanadate, and 200mM sodium fluoride). The tissue extracts were incubated for 10 minutes on ice and clarified by centrifugation at 10,000g for 25 minutes at 4°C. Total protein from the Tenon tissue was determined using a standard bicinchoninic acid assay (Pierce) and Tenon tissue extracts (30 µg total protein) were used for the enzyme-linked immunosorbent assay. After color development was stopped with stop solution, the optical density was measured with a spectrophotometer (DU-530; Beckman Instruments Inc) at 540 to 570 nm.

CLINICAL DATA

The preoperative IOP (3 recordings on separate days) by Goldmann applanation tonometry, the number of IOP-reducing medications taken before the glaucoma surgery, and mean deviation of the last field before surgery using the 24-2 Swedish Interactive Threshold Algorithm standard program (Humphrey Visual Field Analyzer; Carl Zeiss Meditec) were recorded. At each postoperative visit, IOP by Goldmann applanation tonometry, the number of IOP-reducing medications taken 12 months postoperatively, the number of IOP-reducing medications taken 12 months postoperatively was also recorded. The mean of 2 recordings of the IOP at 12 and 15 months postoperatively was regarded as the final IOP 1 year postoperatively. The patients were divided into success or failure groups based on the final IOP. Failure was defined as a final IOP of 21 mm Hg or greater, with or without IOP-reducing medications.

STATISTICAL ANALYSES

Statistical analyses were performed using SPSS software version 11.1 (SPSS Inc.). One-way analysis of variance and Scheffe multiple comparison were used to compare the continuous data among the 3 groups. Independent t tests were applied to compare the data between 2 groups. The χ² test was used to compare categorical data. The Spearman correlation test was used to assess the relationship between IOP and the VEGF level in
the aqueous humor and Tenon tissue. Univariate and multivariate linear regressions were used to assess the relation between the clinical data and VEGF level in the aqueous humor and Tenon tissue. For all analyses, the level of statistical significance was set at $P<.05$.

**RESULTS**

We found no significant differences in age ($P=.52$), sex ($P=.25$), and history of diabetes mellitus ($P=.56$) between the control subjects and patients with POAG. The pretreatment IOP ($P<.001$) and mean deviation of the perimetry ($P<.001$) differed significantly between the control subjects and patients with POAG. Of the POAG eyes that underwent glaucoma surgery, 13 eyes were classified as the success group and 6 eyes as the failure group 1 year after surgery. No significant differences existed in age ($P=.62$), sex ($P=.63$), and history of diabetes mellitus ($P=.30$) between the success and failure groups. The pretreatment IOP ($P=.70$), number of preoperative medications ($P=.67$), and mean deviation ($P=.24$) were similar between the success and failure groups. However, the final IOP differed significantly between the success and failure groups (mean [SD], 14.85 [3.38] mm Hg vs 23.20 [2.38] mm Hg, respectively; $P<.001$) (Table 1).

The VEGF level in the aqueous humor and Tenon tissue of the patients with POAG compared with the control subjects (Figure 1). When the patients with POAG were subdivided into the success and failure groups, the VEGF level of the failure group (mean [SD], 146.82 [24.66] pg/mL) was only statistically different from the control subjects (mean [SD], 95.62 [15.54] pg/mL; $P<.001$) and the success group (mean [SD], 107.11 [19.65] pg/mL; $P=.014$) in Tenon tissue. The VEGF level in the aqueous humor was a mean [SD] of 33.26 [11.54] pg/mL in the control subjects and was elevated to 56.96 [31.22] pg/mL in the success group and 63.99 [25.02] pg/mL in the failure group. Nevertheless, the VEGF in the aqueous humor did not differ statistically among the groups.

To identify factors related to the VEGF level in the aqueous humor and Tenon tissue, univariate and multivariate linear regression analyses were performed (Table 2). Among the factors analyzed, the preoperative IOP was significantly related to the VEGF level in Tenon tissue in both the univariate ($P=.001$) and multivariate regressions ($P=.01$).

The VEGF level in the aqueous humor and Tenon tissue did not show a significant relationship with Spearman correlation analysis ($r=0.029; P=.003$) (Figure 2). The VEGF level in the aqueous humor and final IOP did not show a significant correlation ($r=0.004; P=.99$). However, the VEGF level in Tenon tissue and the final IOP did show a significant correlation ($r=0.677; P=.003$) (Figure 2).

The VEGF level in Tenon tissue at the time of surgery was significantly related to the final IOP and 1-year surgical outcome of glaucoma surgery in patients with POAG. However, the level of VEGF in Tenon tissue was not correlated with that in the aqueous humor, and the VEGF level in the aqueous humor had no relationship with the final IOP. These findings suggest that the enhanced wound-modulating process by VEGF is owing to the VEGF in Tenon tissue where the wound healing occurs rather than the VEGF in the aqueous humor.

The fibroblasts of Tenon tissue, which produce collagen and elastin, are the most important mediators of ocular scar formation after glaucoma surgery. Human Tenon fibroblasts express VEGF receptors and VEGF has been shown to stimulate the proliferation of Tenon fibroblasts in vitro. Vascular endothelial growth factor is important during the proliferative phase of wound healing as an angiogenic factor. In addition, recent stud-
ies have shown that VEGF may act as an initial activator of fibrosis.\textsuperscript{4,10} Several studies have shown that the inhibition of VEGF resulted in reduced scar formation of the trabeculectomy bleb, improving the success of the surgery.\textsuperscript{5,7} Although wound modulation is a lifelong process, the early surgical results are associated with the long-term outcome.\textsuperscript{18} This is why we were interested in the VEGF at the site where glaucoma surgery is conducted. Vascular endothelial growth factor in Tenon tissue may be more important in terms of wound healing, and the VEGF level in the Tenon tissue of patients with POAG was elevated in our study. In particular, Tenon VEGF was significantly elevated in patients with failed glaucoma surgery. Our results show that VEGF in the Tenon tissue at the time of surgery is important in the long-term surgical outcome.

The aqueous VEGF concentration is elevated in various types of glaucoma such as neovascular glaucoma, pseudoexfoliation glaucoma, angle-closure glaucoma, and POAG.\textsuperscript{12,19,20} The source of VEGF production in glaucomatous eyes is not clear. However, because the VEGF level was elevated in the aqueous humor when compared with the plasma VEGF concentration in patients with glaucoma and no significant correlation was observed between VEGF in the aqueous humor and plasma, VEGF is presumed to be produced locally by ocular cells in glaucoma.\textsuperscript{19,20} Vascular endothelial growth factor is expressed and produced by the corneal endothelium, iris pigment epithelium, retinal pigment epithelium, retinal ganglion cells, astrocytes, Muller cells, uveal melanocytes, and choroidal fibroblasts.\textsuperscript{19} Although little is known of the expression and production of VEGF in conjunctival epithelial cells or Tenon fibroblasts, VEGF was elevated in the Tenon tissue of the patients with POAG in our study. The source of the VEGF in Tenon tissue may have been VEGF in the aqueous humor or vitreous cavity by diffusion or from local cells and blood vessels in the subconjunctival space. Because the VEGF in Tenon tissue was not correlated with the VEGF in the aqueous humor, diffusion may not have been the only source of the Tenon VEGF. The turnover time of the aqueous humor is about 100 minutes. This means that after 100 minutes, the anterior chamber is filled with newly produced aqueous humor and the VEGF in the aqueous humor may have passed through the trabecular meshwork pores and reached the Tenon tissue. Consequently, the VEGF in Tenon tissue may not correlate to the VEGF in the aqueous humor, although the VEGF comes from the aqueous humor via diffusion. This rapid turnover rate of VEGF in the aqueous humor may also explain why no relationship was detected between the surgical outcome of glaucoma surgery and the VEGF in the aqueous humor.

An experiment with rat models of ocular hypertension showed that VEGF was elevated in the retinal ganglion cell and inner retinal layers.\textsuperscript{11} This study showed that elevated IOP itself could trigger VEGF production. Hypoxia, ischemia, protein kinase C activator, and reactive oxygen species may also contribute to VEGF production in glaucoma.\textsuperscript{21-23} Our study demonstrated that the VEGF level in Tenon tissue was significantly associated with the preoperative IOP in both univariate and multivariate regression analyses. This is supporting evidence to show that elevated IOP triggers VEGF production. Both ischemia and axotomy induce VEGF production by the retinal ganglion cells.\textsuperscript{24,25} Elevated IOP can result in ischemia or axoplasmic flow disruption at the level of the optic nerve head. Further investigations are needed to confirm these findings, but elevated IOP may induce VEGF elevation. This finding may have clinical implication, ie, the preoperative IOP should be reduced as low as possible because a higher IOP.
IOP results in VEGF elevation, which may in turn affect the surgical outcomes of glaucoma surgery.

In summary, we evaluated the VEGF level in the aqueous humor and Tenon tissue in patients with POAG. The VEGF level in Tenon tissue was significantly correlated with the final IOP and outcome of glaucoma surgery in patients with POAG but showed no relationship with the VEGF level in the aqueous humor. Considering the wound-healing process, we should keep in mind the importance of the VEGF in Tenon tissue, where the wound healing process occurs.

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Correspondence: Chan Kee Park, MD, PhD, Department of Ophthalmology and Visual Science, Seoul St Mary’s Hospital, College of Medicine, The Catholic University of Korea, 505 Banpo-dong, Seocho-ku, Seoul 137-701, Korea (ckpark@catholic.ac.kr).

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REFERENCES