The Effect of Topical Bevacizumab on Corneal Neovascularization

Sang Woo Kim, MD,[1] Byung Jin Ha, MD,[1] Eung Kweon Kim, MD, PhD,[1,2] Hungwon Tchah, MD,[3] Tae-im Kim, MD[1,2]

Purpose: To examine the effect of topical bevacizumab on corneal neovascularization (NV) over a period of 3 months.

Design: Prospective, nonrandomized, masked observational case series.

Participants: Ten eyes of 7 patients with corneal NV.

Methods: Patients received topical bevacizumab (1.25%) twice daily. Ophthalmic evaluations included visual acuity, slit-lamp examination, and tonometry.

Main Outcome Measures: Corneal NV and changes in ophthalmic evaluations.

Results: Decreased corneal NV was noted in 7 of 10 eyes, usually within 1 month of treatment. Epitheliopathy (epithelial defect, epithelial erosion) was observed in 6 of 10 eyes, 1 resulting in corneal thinning. Adverse effects generally appeared during the second month of treatment.

Conclusions: Topical application of bevacizumab was effective in reducing corneal NV within the first month. However, by the second month there was an increased risk of adverse effects. *Ophthalmology 2008;115: e33–e38 © 2008 by the American Academy of Ophthalmology.*

Recent advances in understanding the molecular mechanisms underlying angiogenesis have facilitated the development of promising new therapies for neovascular ocular diseases.[1,2] Corneal neovascularization (NV) is a challenging condition, and because corneal clarity and avascularity are critical for maintaining vision, developing treatments for corneal NV is crucial.

Corneal NV occurs as a result of disequilibrium between angiogenic and antiangiogenic stimuli.[3,4] Corneal angiogenic factors include vascular endothelial growth factor (VEGF),[2,5] with studies showing that VEGF activation can induce corneal NV, and that inhibition of VEGF can block new vessel formation in human and animal cornea model.[6,7] Bevacizumab (Avastin, Genentech, San Francisco, CA) is a full-length humanized murine monoclonal antibody directed against VEGF. Bevacizumab is a powerful anti-NV agent used for cancer therapy. There are numerous ongoing clinical studies examining the effect of bevacizumab in various solid and hematologic malignancies. A promising area of clinical research is the combination of bevacizumab with cytotoxic chemotherapy for colorectal cancer.[8] Bevacizumab was granted approval for use as first-line therapy in metastatic colorectal cancer in combination with fluorouracil-based chemotherapy. With its unique antineoplastic activity and tolerability, bevacizumab provides therapeutic benefits for cancer patients by improving overall response rates, time to progression, and survival.

Bevacizumab has also been a valuable addition to the limited treatment options available for age-related macular degeneration. Encouraging results for intravitreal bevacizumab treatment for age-related macular degeneration have led to trials involving macular edema in central vein occlusion, iris NV, and subsequently, severe proliferative diabetic retinopathy.[9–11] Such trials have encouraged investigations on the use of topical bevacizumab for corneal NV. Topically administered bevacizumab was found to inhibit corneal NV after chemical injury in an experimental rat model.[12] In humans, a short-term follow-up study showed that topical bevacizumab reduced corneal NV in patients with significant corneal NV.[13]

Systemic administration of bevacizumab is reported to have a low incidence of adverse effects such as hypertension and thrombosis,[14] and a recent report suggested that small doses delivered topically would not produce serious systemic effects.[15] Reported ocular adverse events after intravitreal bevacizumab injections include mild anterior chamber inflammation, subconjunctival hemorrhage,[16] mild vitritis,[17] corneal abrasion, and retinal pigment epithelial detachment.[1] There are few reports regarding the effect of topical bevacizumab administration on corneal wound healing.

The present study examined the effect of topical bevacizumab on corneal NV of various etiologies. In general, the treatment was found to reduce corneal NV. However, delayed side effects appeared in several patients.

Originally received: September 16, 2007.
Final revision: January 24, 2008.
Accepted: February 15, 2008.
Available online: April 23, 2008. Manuscript no. 2007-1206.

[1] Corneal Dystrophy Research Institute, Department of Ophthalmology, Yonsei University College of Medicine, Seoul, Republic of Korea.

[2] BK21 Project Team of Nanobiomaterials for Cell-based Implants, Yonsei University, Seoul, Republic of Korea.

[3] Department of Ophthalmology, Asan Medical Center, University of Ulsan, Seoul, Republic of Korea.

The authors have no conflicts of interest related to the article.

This study was supported by Korean Research Foundation grant 2006-KRF-531-E00121.

Correspondence to Tae-im Kim, MD, Severance Hospital, Yonsei University College of Medicine, Department of Ophthalmology, #250 Sungsanno, Seodaemun-gu, 120-752, Seoul, Republic of Korea. E-mail: tikim@yuhs.ac.

ISSN 0161-6420/08/$–see front matter
doi:10.1016/j.ophtha.2008.02.013

Table 1. Summary of Cases of Topical Bevacizumab Treatments

Patient	Age (yrs)/ Gender	Eye	Diagnosis	Medications Other than Bevacizumab	Comments
1	31/M	Right	Severe acid injury	Artificial tears, levofloxacin, 0.125% prednisolone acetate	Improvement of NV Epithelial defect followed by stromal thinning and descemetocele
2	64/F	Both	Stevens-Johnson syndrome	Artificial tears, levofloxacin, 0.125% prednisolone acetate	Improvement of NV on both eyes Epithelial defect on right eye
3	18/F	Both	Stevens-Johnson syndrome	Artificial tears	Improvement of NV Epithelial erosion on both eyes
4	24/M	Right	Herpetic keratitis	Artificial tears	No improvement of NV
5	39/M	Right	Penetrating keratoplasty	Artificial tears, levofloxacin, 0.125% prednisolone acetate	Improvement of NV Epithelial defect
6	24/M	Both	Stevens-Johnson syndrome	Ofloxacine, 0.1% fluorometholone	No improvement of NV on either eye
7	54/F	Right	Pterygium resection	Artificial tears, ofloxacin, 0.125% prednisolone acetate	Cessation of NV development Reduction of opacity

F = female; M = male; NV = neovascularization.

Patients and Methods

A sample of consecutive, eligible patients was identified for inclusion in the study over 1 year from 2006 to 2007. Patients with corneal NV were treated with 1.25% topical bevacizumab (12.5 mg bevacizumab with 1 mL normal saline was prepared by Severance Hospital pharmacy) twice daily for 3 months. All patients were 18–64 years of age and continued to use other topical medications (Table 1). Patients with infectious keratitis or other epithelial disease were excluded. Data collected for analysis included best-corrected visual acuity, slit-lamp photography, and tonometry. This study was approved by the Institutional Review Board of Severance Hospital. All patients were informed of the effects of topical bevacizumab and their consent was obtained.

Results

Patient 1

A 31-year-old man who had a grade IV acid injury, from the classification of Dua et al,[18] to the right eye 4 years ago underwent symblepharon lysis and conjunctival sac reconstruction 4 times, and limbal cell transplantation twice. However, the corneal NV did not respond to consecutive surgical treatments combined with topical steroid therapy. The best-corrected visual acuity in the right eye was finger counting at 30 cm. Slit-lamp examination revealed diffuse superficial and deep stromal corneal NV with significant corneal scarring and some conjunctival symblepharons (Fig 1A).

Topical bevacizumab was prescribed to improve the prognosis of subsequent limbal and/or corneal transplantation. The eye was treated with topical 1.25% bevacizumab twice daily, in addition to artificial tears and a topical steroid (0.125% prednisolone acetate, Samil Pharmaceutics, Seoul, Republic of Korea).

The patient was assessed using slit-lamp examination and tonometry. After 4 weeks of bevacizumab treatment, the superficial and deep stromal corneal NV was markedly reduced. Intraocular pressure remained at baseline levels during treatment. Three months after treatment commenced, a spontaneous epithelial defect appeared in central cornea (Fig 1B). Topical bevacizumab and steroid treatment were discontinued, a bandage contact lens was applied, and levofloxacin eye drops were administered (Fig 1C). However, healing of this epithelial defect was slow, and stromal thinning and descemetocele appeared 1 week later. One month later, a thin layer of stroma had regenerated above Descemet's membrane, but the stromal layer had not fully regained its normal thickness even 3 months after cessation of bevacizumab treatment (Fig 1D). There was a stromal defect apparent on slit-lamp examination.

Patient 2

A 64-year-old woman with Stevens-Johnson syndrome presented with ocular surface inflammation and corneal NV. Topical corticosteroid treatment for several months had little effect. Examination at this stage showed diffuse scarring, superficial and stromal corneal NV, and conjunctival injection, but corneal epithelial integrity was well maintained in both eyes (Fig 1E) and corneal conjunctivalization was not observed.

The patient was prescribed topical bevacizumab for both eyes to reduce the corneal NV. After 1 month, the superficial and deep stromal corneal NV had reduced significantly. However, a spontaneous epithelial defect subsequently developed on her right eye after 6 weeks (Fig 1F). Bevacizumab treatment was discontinued, and the epithelial defect healed 1 week later.

Patient 3

An 18 year-old-girl with Stevens-Johnson syndrome complained of decreased vision and showed corneal NV and opacity in both eyes. Topical corticosteroid therapy for several months had little effect on reducing the diffuse corneal opacity or the superficial and stromal corneal NV (Fig 2A). Topical bevacizumab was then prescribed. One month later, there was a reduction in NV, and visual acuity improved from 20/40 to 20/25 in the left eye (Fig 2B). However, diffuse corneal erosion was observed on both eyes and topical bevacizumab treatment was ceased. Two weeks later the corneal erosion had resolved.

Patient 4

A 24-year-old man with herpetic stromal keratitis had a single large conjunctival vessel emerging from the 4-o'clock position at the limbus and passing over the corneal center in the right eye (Fig 2C). The opacity resulted in a best-corrected visual acuity of 20/30. Topical bevacizumab treatment was commenced, and 2 months later the vascular caliber had slightly decreased and opacity had reduced. The best-corrected visual acuity was 20/25, and no other corneal problems were evident. Topical bevacizumab treatment was discontinued, and the improvement in visual acuity was still present at a follow-up 5 months later (Fig 2D).

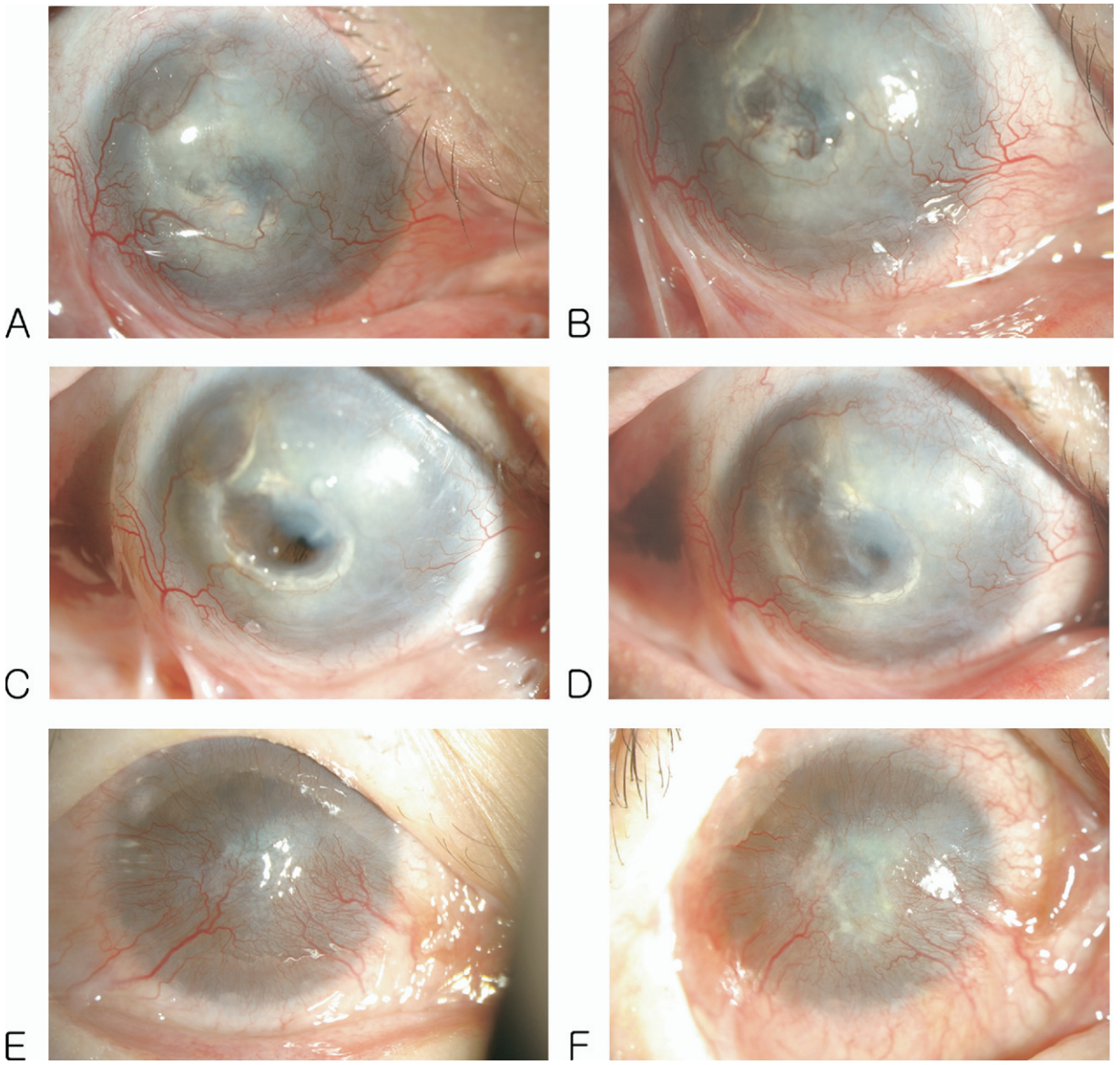

Figure 1. The effect of topical bevacizumab in cases 1 and 2. Case 1 (**A**–**D**). **A,** Before topical bevacizumab treatment. Note the corneal opacity and neovascularization (NV). **B,** Three months after topical bevacizumab treatment. Note the spontaneous epithelial defect in the central corneal area. **C,** Stromal thinning and descemetocele development. **D,** One month after cessation of treatment. Note healing of the epithelial defect and the thin stroma on top of the bare Descemet's membrane. Case 2 (**E**, **F**). **E,** Before topical bevacizumab treatment. Note the diffuse superficial and deep corneal NV from the conjunctiva passing over the limbus to the center of cornea. **F,** One month after topical bevacizumab treatment. Note the decrease in central corneal NV, and the appearance of a spontaneous epithelial defect.

Patient 5

A 39-year-old man who had undergone penetrating keratoplasty in the right eye showed slowly developing corneal NV across the graft and host junction (Fig 2E). Despite topical steroid treatment, corneal NV developed and did not regress. Twice daily application of topical bevacizumab was prescribed. One month later there was evidence of reduced corneal NV (Fig 2F). However, a spontaneous epithelial defect developed in the inferior cornea. A topical antibiotic was prescribed, topical steroid treatment was discontinued, and bevacizumab treatment was reduced to once daily. After 2 weeks the epithelial defect remained. Topical bevacizumab was then discontinued and a pressure patch was placed and changed daily. After 1 week the epithelial defect had healed completely.

Patient 6

A 24-year-old man with Stevens-Johnson syndrome complained of decreased vision and was diagnosed with corneal NV in both eyes (Fig 3A). Topical bevacizumab was prescribed. Examinations after 3 months showed the treatment had no effect on the corneal NV, nor did it adversely apparent affect the ocular surface (Fig 3B).

Patient 7

A 54-year-old woman complained of remaining vascularization 1 month after surgical pterygium removal (Fig 3C). A conjunctival vessel progressed across the limbus to the wound site. Topical steroid treatment did not prevent vascular growth. Twice daily

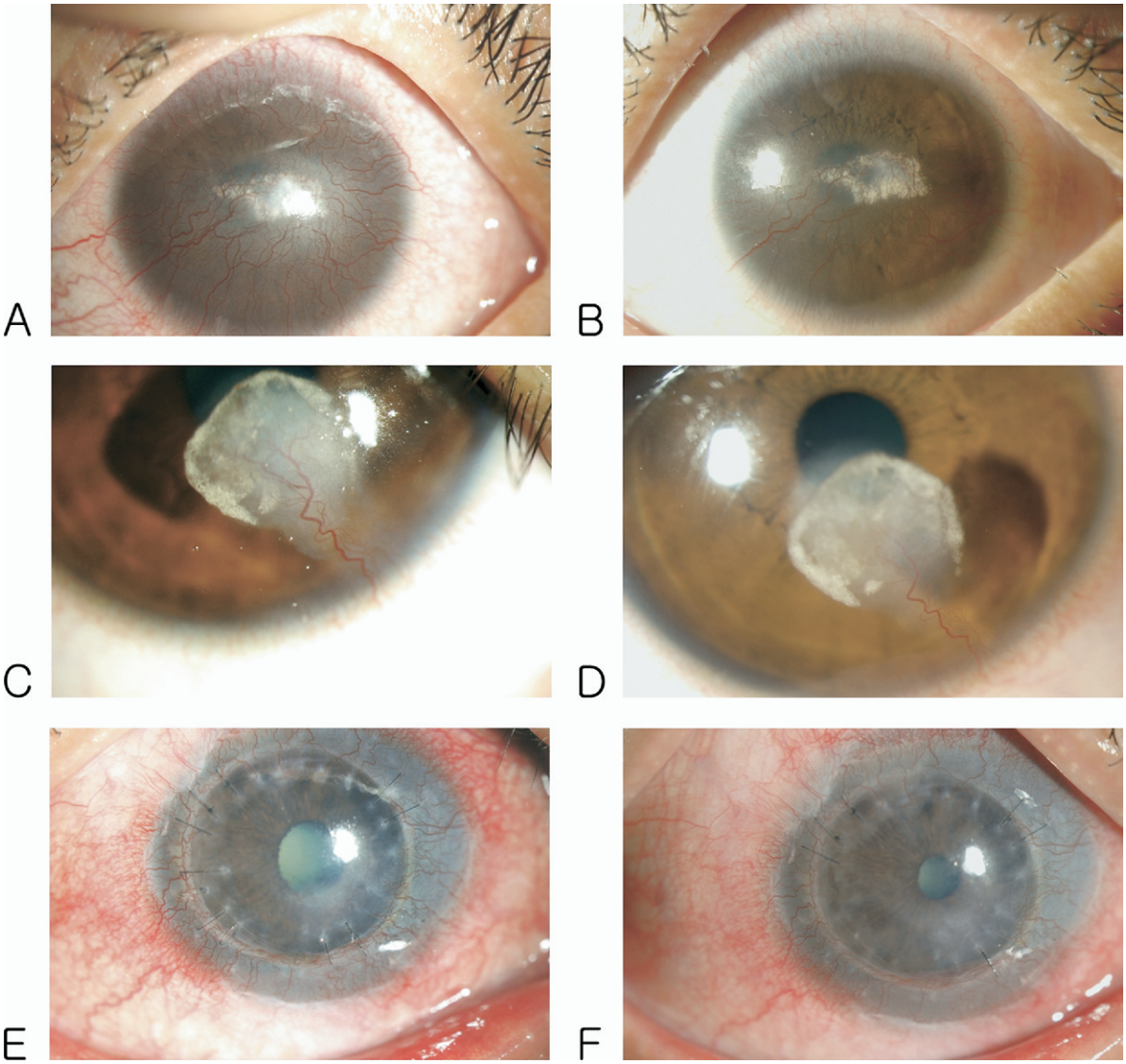

Figure 2. The effect of topical bevacizumab in cases 3–5. Case 3 (**A, B**). **A,** Before topical bevacizumab treatment. Note the severe corneal neovascularization (NV) and dense central corneal opacity. **B,** One month after topical bevacizumab treatment. Note the reduction in corneal NV and opacity. Case 4 (**C, D**). **C,** Before topical bevacizumab treatment. Note the single vessel from the limbus passing over the corneal center in the right eye. **D,** Two months after topical bevacizumab treatment. Note the slightly decreased caliber of the large vessel and reduction in vascular leakage. Case 5 (**E, F**). **E,** Before topical bevacizumab treatment. Note the corneal NV over the donor host junction in the right eye after penetrating keratoplasty. **F,** One month after topical bevacizumab treatment. Note the delayed progression of corneal NV.

topical bevacizumab was prescribed. Two weeks later, topical steroid treatment was stopped owing to an elevated intraocular pressure (up to 25 mmHg) and antiglaucoma medication was added. One month later, corneal NV progression had ceased, and the inflammatory reaction of the wound had stabilized (Fig 3D). No significant adverse effects were observed. All treatment was then ceased, and 3 months later the inflammatory reaction at the ocular surface remained under control.

Discussion

Corneal NV can lead to vision loss, is often difficult to manage, and may lead to a need for corneal transplantation. Newly or already formed corneal NV elevates the risk of subsequent graft rejection after corneal transplantation.[19] Medical and surgical therapies used to reduce corneal NV include corticosteroids, nonsteroidal anti-inflammatory agents, laser photocoagulation, and needle diathermy.[3] Many of these therapies have not only demonstrated limited success but also have associated adverse effects. Anti-VEGF therapy targeting corneal NV has recently showed successful results in many preliminary in vivo animal experiments. In rat models, topical bevacizumab (4 mg/mL) applied twice daily for 1 week reduced chemically induced corneal NV,[12] and anti-VEGF antibody implanted in neovascularized corneal stroma suppressed corneal NV.[7] However, such find-

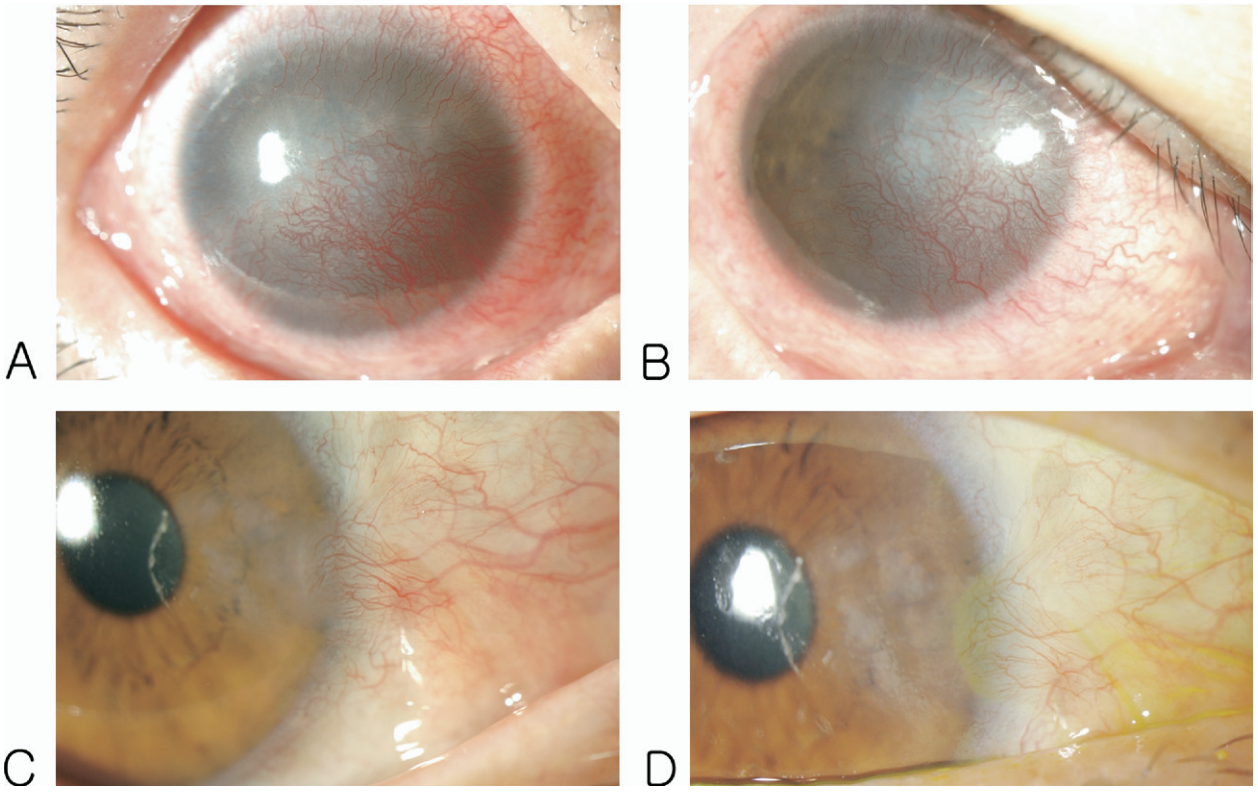

Figure 3. The effect of topical bevacizumab in cases 6 and 7. Case 6 (**A, B**). **A,** Before topical bevacizumab treatment. Note that more than half of the cornea is covered with diffuse superficial and deep corneal neovascularization (NV). **B,** At 3 months after treatment. Note no significant changes. Case 7 (**C, D**). **C,** Before topical bevacizumab treatment. Vessels from the conjunctiva crossed from the limbus towards the cornea 1 month after pterygium resection. **D,** One month after topical bevacizumab treatment. Note no significant vascular change, but reduced corneal opacification.

ings only indicate the potential of topical anti-VEGF therapy for controlling NV.

Vascular endothelial growth factor affects the extracellular matrix metabolism,[20] and may regulate structural changes associated with vascularization through intact tissue layers. It upregulates platelet activating factor activity, which enhances urokinase-type plasminogen activator gene expression in corneal epithelium.[21,22] Induced urokinase-type plasminogen activator, which is also upregulated by wounding itself,[23] has a role in cell migration, cell adhesion, and tissue remodeling, and thus plays a key role in initiating corneal wound healing and recovery.[24,25] VEGF also increases the fibrinolytic activity of endothelial cells within fibrin matrices with the involvement of VEGF receptor-2, tissue type plasminogen activator, and matrix metalloproteinases.[26] In addition, transfection of the soluble VEGF receptor (*sflt-1*) gene was shown to attenuate pulmonary fibrosis in a mouse model of bleomycin-induced pneumopathy, suggesting that an anti-VEGF approach might also offer a suitable antifibrotic therapy.[27] Thus, VEGF seems to be a critical modulator of wound healing not only via controlling angiogenesis but also via regulating wound healing and fibrosis development.

In the present study, topical application of bevacizumab resulted not only in vascular suppression but also caused spontaneous loss of epithelial integrity and progression of stromal thinning. The patients who were recruited in this study showed a stable state of corneal NV and epithelium before treatment. The corneal epithelial defects seen after use of topical bevacizumab suggest that such treatment may affect adhesion between epithelium and basement membranes, rapidly differentiate the corneal epithelium itself, or inhibit or delay normal wound healing processes. The exact mechanisms need to be elucidated through further studies. Corneal NV can be triggered by hypoxia and wound healing. But, it is difficult to know the full consequences of blocking NV. Anti-VEGF therapy seems effective in suppressing new vessel formation and vascular leakage, which can improve visual function. However, blocking VEGF function may also disrupt wound healing, causing ischemia and increasing overall tissue damage.

Anti-VEGF therapy is considered as a possible tool for controlling NV in many clinical fields. Anti-VEGF agents are becoming more broadly used for various ocular diseases, including corneal NV after chemical injury, choroidal NV, and neovascular glaucoma.[10,13,16,28] However, angiogenesis is exquisitely regulated, and while affecting the balance may provide some benefits, there may also be some adverse complications. As such, long-term studies are critical before considering clinical application. Thus, long-term clinical results and basic studies are required to provide further knowledge regarding the possible use of bevacizumab treatment for corneal diseases.

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