Deep Intrastromal Bevacizumab Injection for Management of Corneal Stromal Vascularization After Deep Anterior Lamellar Keratoplasty, A Novel Technique

Mohammad Naser Hashemian, MD, Mohammad Ali Zare, MD, Firoozeh Rahimi, MD, and Mehrdad Mohammadpour, MD

Purpose: To report the effect of intrastromal injection of bevacizumab in an eye with extensive corneal stromal vascularization after deep anterior lamellar keratoplasty.

Methods: Bevacizumab (2.5 mg/1 mL) was injected into the deep corneal stroma of an eye with severe and extensive stromal vascularization.

Results: Corneal vascularization regressed dramatically after deep stromal injection of bevacizumab with no recurrence after 6 months. Visual acuity was improved, and the patient's complaints subsided.

Conclusions: Corneal intrastromal injection of bevacizumab can be considered for management of intrastromal vascularization after deep anterior lamellar keratoplasty.

Key Words: bevacizumab, Avastin, anti-VEGF, cornea, vascularization, deep anterior lamellar keratoplasty

(*Cornea* 2011;30:215–218)

Corneal neovascularization (NV) is a common cause of visual impairment and a major risk factor for graft rejection and failure.[1] Corneal NV may result from inflammatory, infective, and traumatic episodes or after loose sutures after deep lamellar keratoplasty. Factors leading to corneal stromal vascularization may be especially notable in eyes with a history of vernal keratoconjunctivitis (VKC) as this case report demonstrates.

A balance normally exists in the cornea between proangiogenic factors, such as fibroblast growth factor and vascular endothelial growth factor (VEGF) and antiangiogenic factors, such as angiostatin, endostatin, and pigment epithelium–derived factor.[2] In certain diseases, however, this balance is altered in favor of the proangiogenic factors, presumably by either their upregulation or the downregulation of the antiangiogenic factors, with evidence suggesting that this is influenced by matrix metalloproteinase. Animal models have shown that argon laser photocoagulation, photodynamic therapy, nonsteroidal antiinflammatory agents, and steroids may all produce a transient inhibition of corneal NV.[3]

Upregulation of VEGF has been identified in inflamed and vascularized human corneas. Furthermore, VEGF promotes the angiogenesis stages of endothelial cell proliferation, migration, and capillary lumen formation. Several reports describe the topical,[4] subconjunctival,[5] intracameral,[6] and shield soaked application[7] of anti-VEGF therapies to inhibit corneal NV. A recent report showed no adverse effects of topical bevacizumab in human eyes when applied in drop form 4 times daily for 3 months.[8] Furthermore, an encouraging report has described an effect after subconjunctival injection of bevacizumab in reducing both the number and the caliber of corneal vessels.[5] Herein, we report the effectiveness and the safety of deep intrastromal bevacizumab (Avastin) injection for the management of extensive corneal vascularization after deep anterior lamellar keratoplasty.

CASE REPORT

A 22-year-old man complaining of decreased vision and photophobia was referred to us in February 2008. History included deep anterior lamellar keratoplasty in May 2007 in the context of advanced keratoconus and VKC, and an episode of stromal rejection in the context of loose sutures and poor compliance of the patient for topical steroids, leading to central stromal haziness. All suture removal was performed for the patient 6 months after surgery. The uncorrected visual acuity was 0.3 and did not increase with spectacle correction; slit-lamp examination showed extensive 4-quadrant deep intrastromal vascularization and a central corneal haziness for 2 months not responsive to topical steroids (Fig. 1).

Because the site of corneal stromal vascularization was too deep to respond to topical or subconjunctival injection of bevacizumab (Avastin; Roche, Basel, Switzerland), we elected injection into the corneal stroma as a more effective means to reduce the vascularization. Bevacizumab (Avastin) is commercially available as a clear solution (each 4 mL vial contains 100 mg of the active drug). Deep intrastromal bevacizumab was injected in a 2.5 mg/0.1 mL concentration in February 2008. The method of injection first used a 27-gauge needle to make a pass into the deep corneal stroma; then,

Received for publication May 18, 2009; revision received February 8, 2010; accepted February 17, 2010.

From the Cornea Department, Eye Research Center, Farabi Eye Center, Tehran Medical University, Qazvin Square, Tehran, Iran.

There is no financial interest for the authors in any methods or materials mentioned in this article.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.corneajrnl.com).

Reprints: Mehrdad Mohammadpour, Cornea Department, Eye Research Center, Farabi Eye Hospital, Tehran University of Medical Sciences, Tehran 55400010, Iran (e-mail: mahammadpour@yahoo.com).

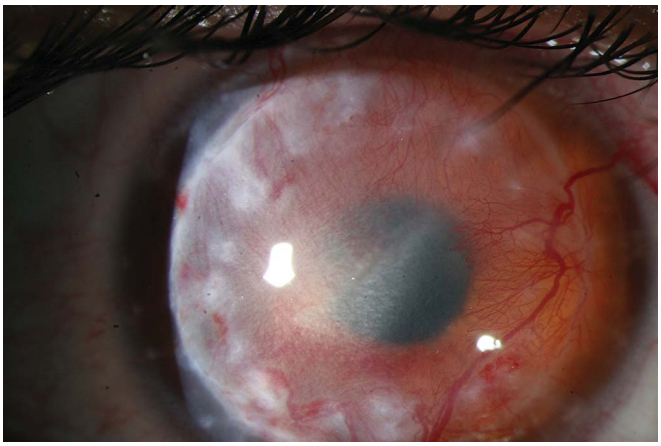

FIGURE 1. Slit-lamp examination showing massive 4-quadrant deep intrastromal vascularization and a central corneal haziness.

using an irrigation needle, the Avastin was injected into the deep stroma so that stromal whitening was seen in the paracentral area of 2 adjacent quadrants of the corneal stroma. Figure 2 shows an immediate postinjection photograph of the cornea to have an idea of how much stromal edema was observed after injection (See **Video, Supplemental Digital Content 1**, http://links.lww.com/ICO/A12).

Betamethasone 0.1% eyedrop was prescribed every 4 hours postoperatively and tapered in 1 month. Three weeks after injection of Avastin, the patient's complaints had subsided, the uncorrected visual acuity was increased to 0.4, and the corneal intrastromal vascularization regressed significantly (Fig. 3). At 50 days after injection of bevacizumab (Avastin), the eye was completely calm and the corneal intrastromal vascularization had faded except for some peripheral trunks. However, the central corneal haziness remained, which limited the visual acuity to 0.4 (Fig. 4). At the next follow-up examination, 3 months after injection of Avastin, there was no recurrence of stromal vascularization and the eye was quiescent with stable visual acuity. However, the central corneal haziness persisted (Fig. 5). Long-term follow-up of the patient, 1 year after initial treatment, revealed no recurrence of the corneal vascularization. No side effect was seen over the observation period.

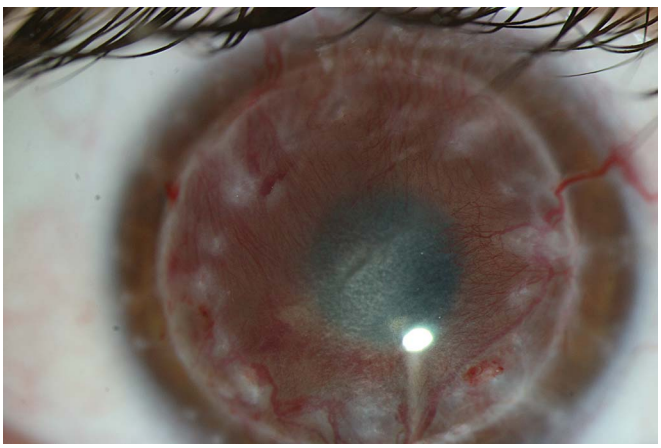

FIGURE 2. An immediate postinjection photograph of the cornea to have an idea of how much stromal edema was observed after injection.

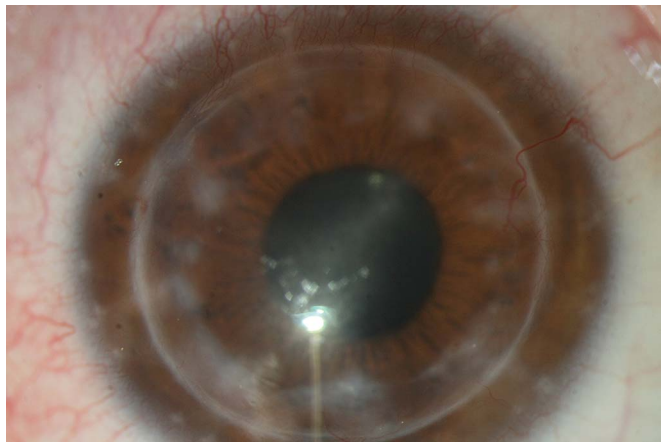

FIGURE 3. Three weeks after injection of Avastin, the corneal intrastromal vascularization regressed significantly.

DISCUSSION

Corneal NV may not only reduce visual acuity but also result in the loss of immune privilege of the cornea, thereby worsening the prognosis after corneal transplant and creating a high-risk graft.[9] Although the success rate of corneal transplantation in low-risk avascular beds surpasses 90%, graft survival rates are much lower in high-risk neovascularized beds where corneal grafts are associated with rejection rates far worse than kidney or heart allografts.[10]

Current treatments for corneal NV, including medications (such as steroids or nonsteroidal antiinflammatory agents), laser photocoagulation, fine needle diathermy, photodynamic therapy, or restoration of the ocular surface with the use of conjunctival, limbal, or amniotic membrane transplantation, have demonstrated variable and largely limited clinical success.[11,12] However, none of these treatments specifically target the molecular mediators of angiogenesis.

VEGF is thought to be a key mediator in the process of NV. It has also been shown that inhibition of angiogenesis by

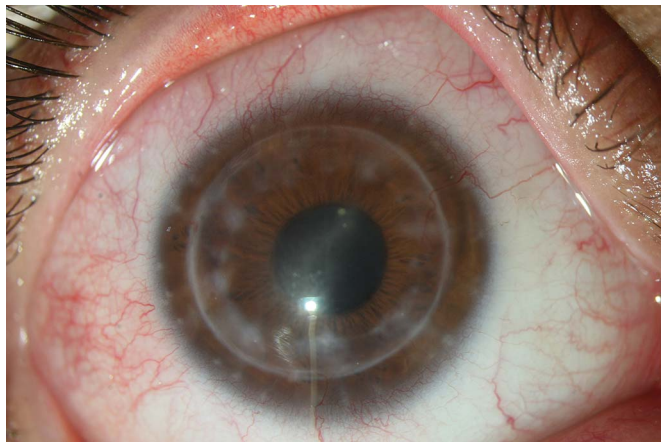

FIGURE 4. Fifty days after Avastin injection, the eye was completely calm and the corneal intrastromal vascularization was almost faded, except for some peripheral trunks. However, the central corneal haziness remained.

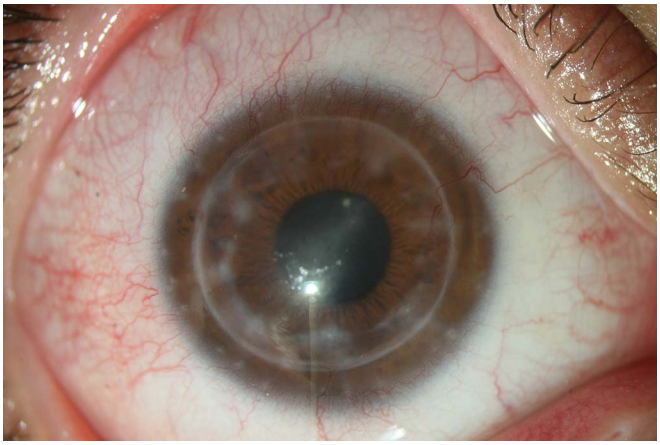

FIGURE 5. Three months after Avastin injection, there was no recurrence of stromal vascularization, the eye was quiescent, and the visual acuity was stable.

neutralization of VEGF can promote corneal graft survival in animal models. The use of bevacizumab has now been widely adopted and is arguably part of the standard of care for the treatment of neovascular age-related macular degeneration in many patients.[11] Recently, off-label use of topical and subconjunctival bevacizumab has also been considered as a new treatment modality for corneal NV.[12–15]

Topical bevacizumab was shown to inhibit corneal NV after chemical injury in an experimental rat model. In humans, a small number of studies have shown that topical bevacizumab can reduce corneal NV in a few patients with significant corneal NV.[16,17] However, many aspects of topically administered bevacizumab for the treatment of corneal NV—including long-term safety and efficacy against actively growing and established corneal NV, optimal dosing for modulating the neovascular process, and long-term stability of treatment results—have not been well developed.[18–21]

The rationale for our method of bevacizumab administration was that deep intrastromal injection of bevacizumab was expected to increase the concentration and exposure time of the drug in the deep stromal layers, perhaps providing a more effective exposure than topical or subconjunctival application. In addition, our method of application avoided the logistical difficulty of administering bevacizumab drops to the patient, and it promised to offer more effectiveness than other routes of application in this specific case. An additional basis for the deep intrastromal injection of Avastin was that the NV had been present for several weeks before the Avastin treatment and the new vessels may have been too well established to respond to other forms of Avastin application. There is no presumed side effect of this technique except inadvertent penetration of the needle into the anterior chamber.

In a recent report, the subconjunctival injection of Avastin was shown to have no effect on established corneal NV, in contrast with its effect on active NV.[22] Perhaps, in our patient, the balance had been tipped so much in favor of the proangiogenic factors that a higher concentration of this medication was required. Because the corneal NV in our case was too deep in the stroma for the Avastin to penetrate through the cornea, we decided to apply deep intrastromal injection of Avastin. Long-term follow-up of the patient 1 year after initial treatment revealed no recurrence of the corneal vascularization. No side effect was seen over the observation period. Because the presumed cause for corneal vascularization in this patient was loose sutures in the context of VKC, the blood vessels did not recur after all suture removal and control of VKC. Hence, the source of VEGF was eliminated, and there was no need for additional injections.

Recently, the combination of argon laser coagulation and bevacizumab injections has been introduced as a promising tool for the treatment of NV in association with corneal transplant surgery.[23] However, this route of administration does not seem prudent in cases of intrastromal NV because of the high risk of intrastromal hemorrhage and staining after argon laser coagulation, leading to severe and perhaps permanent visual loss. Repeated subconjunctival injection or local injection into the stroma may deliver a higher concentration and seems to offer an alternative approach. In conclusion, deep intrastromal injection of bevacizumab may be considered as a modality for management of extensive and established intrastromal corneal vascularization after deep anterior lamellar keratoplasty.

REFERENCES

1. Inoue K, Amano S, Oshika T, et al. Risk factors for corneal graft failure and rejection in penetrating keratoplasty. *Acta Ophthalmol Scand*. 2001; 79:251–255.
2. Chang JH, Gabison EE, Kato T, et al. Corneal neovascularization. *Curr Opin Ophthalmol*. 2001;12:242–249.
3. Barros LF, Belfort R Jr. The effects of the subconjunctival injection of bevacizumab (Avastin) on angiogenesis in the rat cornea. *An Acad Bras Cienc*. 2007;79:389–394.
4. Dastjerdi MH, Al-Arfaj KM, Nallasammy N, et al. Topical bevacizumab in the treatment of corneal neovascularization. *Arch Ophthalmol*. 2009; 127:381–389.
5. Awadein A. Subconjunctival bevacizumab for vascularized rejected corneal grafts. *J Cataract Refract Surg*. 2007;33:1991–1993.
6. Park HY, Kim SJ, Lee HB, et al. Effect of intracameral bevacizumab injection on corneal endothelium in rabbits. *Cornea*. 2008;27:1151–1155.
7. Mackenzie, SE, Tucker WR, Poole ERG. Bevacizumab (Avastin) for corneal neovascularization—corneal light shield soaked application. *Cornea*. 2009;28:246–247.
8. Uy HS, Chan PS, Ang RE. Topical bevacizumab and ocular surface neovascularization in patients with Stevens-Johnson syndrome. *Cornea*. 2008;27:70–73.
9. Conn H, Berman M, Kenyon K, et al. Stromal vascularization prevents corneal ulceration. *Invest Ophthalmol Vis Sci*. 1980;19:362–370.
10. Epstein RJ, Stulting RD, Hendricks RL, et al. Corneal neovascularization: pathogenesis and inhibition. *Cornea*. 1987;6:250–257.
11. Lee P, Wang CC, Adamis AP. Ocular neovascularization: an epidemiologic review. *Surv Ophthalmol*. 1998;43:245–269.
12. Cursiefen C, Kuchle M, Naumann GO. Angiogenesis in corneal diseases: histopathologic evaluation of 254 human corneal buttons with neovascularization. *Cornea*. 1998;17:611–613.
13. Liesegang TJ. Epidemiology of ocular herpes simplex: natural history in Rochester, Minn, 1950 through 1982. *Arch Ophthalmol*. 1989;107: 1160–1165.
14. Resnikoff S, Pascolini D, Etya'ale D, et al. Global data on visual impairment in the year 2002. *Bull World Health Organ*. 2004;82:844–851.
15. Dana MR, Streilein JW. Loss and restoration of immune privilege in eyes with corneal neovascularization. *Invest Ophthalmol Vis Sci*. 1996;37: 2485–2494.

16. Maguire MG, Stark WJ, Gottsch JD, et al. Collaborative Corneal Transplantation Studies Research Group. Risk factors for corneal graft failure and rejection in the collaborative corneal transplantation studies. *Ophthalmology.* 1994;101:1536–1547.
17. Williams KA, Roder D, Esterman A, et al. Factors predictive of corneal graft survival: report from the Australian Corneal Graft Registry. *Ophthalmology.* 1992;99:403–414.
18. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat Med*. 1995;1:27–31.
19. Amano S, Rohan R, Kuroki M, et al. Requirement for vascular endothelial growth factor in wound- and inflammation-related corneal neovascularization. *Invest Ophthalmol Vis Sci*. 1998;39:18–22.
20. Philipp W, Speicher L, Humpel C. Expression of vascular endothelial growth factor and its receptors in inflamed and vascularized human corneas. *Invest Ophthalmol Vis Sci*. 2000;41:2514–2522.
21. Yoeruek E, Spitzer MS, Wallenfels-Thilo B, et al. *Safety Profile of Bevacizumab on Cultured Human Corneal Cells*. Lauderdale, FL: ARVO; 2007.
22. Youn Oh J, Kim MK, Shin MS. The anti-inflammatory effect of subconjunctival bevacizumab on chemically burned rat corneas. *Curr Eye Res*. 2009;34:85–91.
23. Gerten G. Bevacizumab (Avastin) and argon laser to treat neovascularization in corneal transplant surgery. *Cornea*. 2008;27: 1195–1199.