Subconjunctival Bevacizumab Injection for Corneal Neovascularization

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Purpose: To report on the clinical use of subconjunctival bevacizumab in patients with corneal neovascularization.

Methods: The charts of 10 consecutive patients with corneal neovascularization who received subconjunctival injections of bevacizumab (2.5 mg/0.1 mL) were reviewed. Digital photographs of the cornea were graded by 2 masked observers for density, extent, and centricity of corneal vascularization. Image analysis was used to determine the area of cornea covered by neovascularization as a percentage of the total corneal area.

Results: No significant ocular or systemic adverse events were observed during 3.5 ± 1.1 months of follow-up. Seven patients showed partial regression of vessels. The extent decreased from 6.0 ± 1.2 (SD) clock hours before the injection to 4.6 ± 1.0 clock hours after bevacizumab injection ($P = 0.008$). Density decreased from 2.7 ± 0.2 to 1.9 ± 0.3, respectively. ($P = 0.007$). No change was noticed in the centricity of corneal vessels. Corneal neovascularization covered, on average, 14.8% ± 2.5% (SD) of the corneal surface before the injections, compared with 10.5% ± 2.8% ($P = 0.36$, t test) after bevacizumab injection. Therefore, bevacizumab decreased corneal neovascularization by 29%.

Conclusions: Short-term results suggest that subconjunctival bevacizumab is well tolerated and associated with a partial regression of corneal neovascularization.

Key Words: corneal neovascularization, bevacizumab, VEGF

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Neovascularization is a severe complication of ischemic retinal diseases such as diabetic retinopathy, branch and central retinal vein occlusion, and retinopathy of prematurity. However, in various inflammatory corneal diseases, corneal neovascularization may also occur, particularly in the chronic course of the disease. The consequences of corneal neovascularization may not only be a severe reduction of visual acuity but also a worse prognosis for corneal transplantation because of loss of the immunologic privilege of the avascular cornea.[1,2] However, the pathogenesis of corneal angiogenesis has not yet been fully defined, and the identity and significance of different angiogenic growth factors are debatable.

Several studies have shown that vascular endothelial growth factor (VEGF), which was identified about 1 decade ago, plays a major role in vasculogenesis and in pathologic neovascularization.[3–13] This protein stimulates angiogenesis in a noninflammatory model of neovascularization in the mouse cornea[4] and was recently identified as a functional endogenous corneal angiogenic factor required for inflammatory neovascularization in a rat model.[6] Philipp et al[14] studied whether and by which cells VEGF and its receptors are expressed in vascularized and inflamed human corneas. They concluded that VEGF, Flt-1, and Flk-1 are strongly expressed in inflamed and vascularized human corneas and thus may play an important role in corneal neovascularization.

Bevacizumab (Avastin; Genentech, South San Francisco, CA) is a humanized monoclonal antibody to VEGF designed for intravenous administration and approved for the treatment of colorectal cancer.[15]

Rosenfeld et al[16–18] have reported on the use of bevacizumab systemically and intravitreally in the treatment of macular degeneration. Systemic bevacizumab has also been reported by Nguyen et al[19] to suppress choroidal neovascularization caused by pathologic myopia. Bevacizumab is currently injected into the vitreous for the treatment of proliferative and nonproliferative diabetic retinopathy, age-related macular degeneration, and neovascular glaucoma, with successful outcomes and rapid regression of the pathologic blood vessels.[20–25]

Recently, Manzano et al[26] showed that topically administered bevacizumab limits corneal neovascularization after chemical injury in a rat model, and Erdurmus et al[27] reported on the efficacy of subconjunctival bevacizumab injection in 2 patients with corneal neovascularization.

We report here on our experience with subconjunctival injections of bevacizumab for corneal neovascularization in human subjects.

MATERIALS AND METHODS

The study group consisted of 10 adults (4 men and 6 women) 32–89 years of age (mean, 57.9 ± 6.8 years) with vascularized cornea (10 eyes) secondary to herpetic eye disease (n = 3), graft-versus-host disease (n = 1), postinfectious keratitis (n = 1), chemical burn (n = 1), failed graft (n = 3), and interstitial keratitis (n = 1).

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All had extensive superficial and deep vascularization of the cornea and had a failure of steroid drops trial (4 times daily) for the treatment of these pathologic vessels. The study was approved by the Institutional Research Ethics Committee at the University Health Network, and informed consent was obtained before the procedure.

Eyes were anesthetized with topical proparacaine hydrochloride drops. Subconjunctival injection of 2.5 mg/0.1 mL bevacizumab was performed at the limbus, adjacent to the pathologic blood vessel growth/sprouting into the cornea. The injection was performed at the slit lamp after application of a topical anesthetic drop and by using an eyelid speculum. Postoperatively, patients were treated with topical tobradex (tobramycin and dexamethasone; Alcon Laboratories, Fort Worth, TX) eyedrops 4 times daily for 1 week.

As per our protocol, all eyes had at least 2 bevacizumab injections, except for 1 patient, who refused further injections. If a partial effect of bevacizumab was noted, we continued to inject the drug at 1-month intervals, unless the patient refused or stabilization was achieved.

All eyes were biomicroscopically examined preoperatively, on postoperative day 7 and at 1 and 3 months. At each visit, 2 digital corneal photographs were taken with $\times 16$ and $\times 25$ magnification by using a Nikon digital camera attached to the slit-lamp microscope.

The photographs were graded by 2 masked observers for extent, centricity, and density of corneal vascularization as follows. Extent was defined according to the number of clock hours affected by neovascularization (score 1–12). Centricity was defined as the distance the new vessels extended from the limbus toward the visual axis: 1 = vessel extended a maximum of 2 mm from limbus, 2 = extended 2–4 mm from limbus, and 3 = vessels extended to involve visual axis/central 3 mm of cornea. Density was graded 1–4 according to the density of neovascularization: 1 = very low (1 vessel), 2 = low, 3 = moderate, 4 = high (compared with standard photographs presented to the observers).

The scores given for each picture by the 2 masked observers were averaged and unmasked by a third investigator to determine the change in neovascularization extent, centricity, and density after bevacizumab injection.

Furthermore, the amount of vascularization was measured on the photographs as the percentage of the total area of the cornea by using an image processing and analysis software program (Image J 1.37v; Wayne Rasband at the Research Services Branch, National Institute of Mental Health, Bethesda, MD). The area of neovascularization was measured in terms of pixels, and its ratio to the entire corneal area was determined as the percentage of corneal neovascularization.

The Wilcoxon test was used for comparisons of ranked variables, and the Student *t* test was used for continuous variables (SPSS version 12; SPSS, Chicago, IL). Probabilities of $<5\%$ were considered statistically significant.

RESULTS

The mean duration of follow-up was 3.5 ± 1.1 months. The average number of subconjunctival bevacizumab injections per eye was 2.1 ± 0.8 (SD).

Table 1 summarizes the patients' baseline characteristics and outcomes after bevacizumab injections. Preoperative visual acuity ranged from 20/30 to hand movements.

There were no intraoperative complications. No side effects were reported by the patients, nor was pain or discomfort induced by the drug injection reported throughout 3.5 months of follow-up. Visual acuity did not change significantly in any patient in this study. Seven patients showed partial regression of vessels, whereas 3 patients did not react to the injection (2 failed grafts and 1 herpetic eye disease).

Table 2 shows the average scores before and after bevacizumab injection for each patient. During 3.5 months of follow-up, 6 (60%) patients had at least a 1–clock hour decrease in the extent of blood vessels, and 4 (40%) had at least a 2–clock hour decrease in the extent of vessels: These included 2 failed grafts, 1 graft-versus-host disease, 1 herpetic eye disease, 1 corneal ulcer, and 1 chemical burn. Seven (70%) patients had a decrease of 1 level in density (2 failed grafts, 1 graft-versus-host disease, 2 herpetic eye disease, 1 interstitial keratitis, and 1 chemical burn), but none showed any decrease in centricity of blood vessels in the cornea.

Figures 1 and 2 show preoperative and postoperative anterior-segment photographs of 2 patients. As seen in these

TABLE 1. Baseline Characteristics and Outcomes of Subconjunctival Bevacizumab Injections for Corneal Neovascularization

Patient	Age (y)/Eye	Cause of Corneal Vascularization	Visual Acuity Preoperatively	No. Injections	Side Effects	Regression of Vessels	Visual Acuity Postoperatively
1	42/OS	Herpetic eye disease, failed graft	20/100	2	—	+Partial	20/100
2	38/OD	Interstitial keratitis	20/30	2	—	+Partial	20/30
3	89/OS	Failed graft (PBK)	CF	2	—	+Partial	CF
4	62/OD	GVH	HM	3	—	+Partial	CF
5	38/OD	Herpetic eye disease	20/400	1	—	—	20/400
6	45/OD	Thyroid ophthalmopathy, failed graft	20/400	2	—	—	20/400
7	80/OS	Herpetic eye disease	20/70	4	—	+Partial	20/60
8	67/OD	Corneal ulcer	CF	2	—	+Partial	CF
9	86/OD	Failed graft	CF	2	—	—	HM
10	32/OD	Chemical burn	20/40	2	—	+Partial	20/40

PBK, pseudophakic bullous keratopathy; GVH, graft-versus-host disease; CF, counting fingers; HM, hand movement.

TABLE 2. Average Scores Before and After Bevacizumab Injection for All Patients

Patient No.	Preoperative				No. Injections	After Bevacizumab Injection			
	Extent	Centricity	Density	%		Extent	Centricity	Density	%
1	4.25	2.75	3	24.25	First	4.5	2.5	2	12
					Second	4	2.5	2	8.5
2	2.5	2	2.75		First	2	1.5	1	
3	3.5	2.5	2.5	9.76	First	3	2.5	2	10.56
					Second	3.5	2.5	2	11.5
4	12	2	3	16.07	First	12	2.5	2.75	20.67
					Second	9.5	2	2.5	14.85
					Third	9	2	2	8.69
5	1.5	3	3	7.16	First	1.5	3	3	7.87
6	4	1.5	3	17.48	First	4	1.5	3	18.72
					Second	3	2	2	15.82
7	5	3	2.5	12.61	First	5.5	2.75	2.5	13.21
					Second	5.5	3	2.5	12.43
					Third	2.5	3	1.75	10.3
					Fourth	2	1.75	1	5.5
8	12	3	3.5	30.91	First	12	3	3	26.69
					Second	10	3	3.5	32.49
9	6	2	2	15.98	First	5	2	1.5	14.5
					Second	3	2	1	9.03
10	9	1	2	6.38	First	8	1	1	4.25
					Second	7	1	1	4.5

%, proportion of vascularized cornea (computerized).

figures and in Table 3, the bevacizumab-treated eyes showed partial regression in corneal neovascularization. Indeed, a statistically significant decrease in extent and density of blood vessels is presented in Table 3. The extent decreased from 6.0 ± 1.2 (SE; median, 4.625) clock hours before the injection to 4.6 ± 1.0 (median, 3.25) clock hours after bevacizumab injection (P = 0.008). Density decreased from 2.7 ± 0.2 (median, 2.875) to 1.9 ± 0.3 (median, 2; P = 0.007).

No significant change was noticed in the centricity of vessels. Corneal neovascularization covered, on average, 14.8% ± 2.5% (SE; median, 16%) of the corneal surface before the injections compared with 10.5% ± 2.8% (median, 8.7%; P = 0.36, t test) after subconjunctival bevacizumab injection. Therefore, bevacizumab decreased corneal neovascularization by 29%.

DISCUSSION

Different substances have been identified in the past as potential vessel inhibitors, including steroids,[18,26–30] nonsteroid anti-inflammatory drugs,[31–33] heparin,[26,34] cyclosporin A,[35] methotrexate,[36] and thalidomide.[37] Although steroids have been the mainstay of therapy for corneal neovascularization and corneal graft rejection in clinical practice, they are not always effective, and chronic use may cause prominent side effects.

VEGF's role in the pathophysiology of corneal neovascularization has been shown in experimental models of corneal neovascularization,[4–8] in experimental herpes simplex keratitis,[38] and in studies from human corneal buttons.[9,10] Also, VEGF inhibition has been shown to reduce corneal neovascularization and improve corneal graft survival in experimental animals.[5,11,12,39,40]

Bevacizumab also inhibits corneal neovascularization in humans. Although our results were statistically significant, regression of corneal neovascularization was only partial. There are several possible explanations for this finding. First, the dose administered in this study was insufficient to effectively antagonize corneal VEGF (the dose we chose to inject is the dose administered to the vitreous for various retinal diseases at our institute). Second, the frequency of repeat injections should possibly be different then the one that we used. Third, cytokines other than VEGF (such as transforming growth factor-α and -β1 and fibroblast growth factor) can induce corneal neovascularization,[3,9] and these are not antagonized by bevacizumab injection. Fourth, bevacizumab inhibits new, fresh blood vessels, rather than old, well-established vessels. This fact could also contribute to a smaller effect of bevacizumab injection in 3 of our patients.

Seven of 10 patients in this study did not react to the first bevacizumab injection but did react to a repeat injection. This cumulative effect was also shown with the application of this drug to the vitreous for retinal diseases.[18–20,22–25]

The number of injections per eye to reach its maximal effect and the ideal time for a repeat injection need to be further studied.

In our study, we used both a human grading system and image processing by an analysis software program. The software objectively assessed the change in the area of pathologic vessels within the cornea, whereas our grading system evaluated which of the 3 components (extent, centricity, and

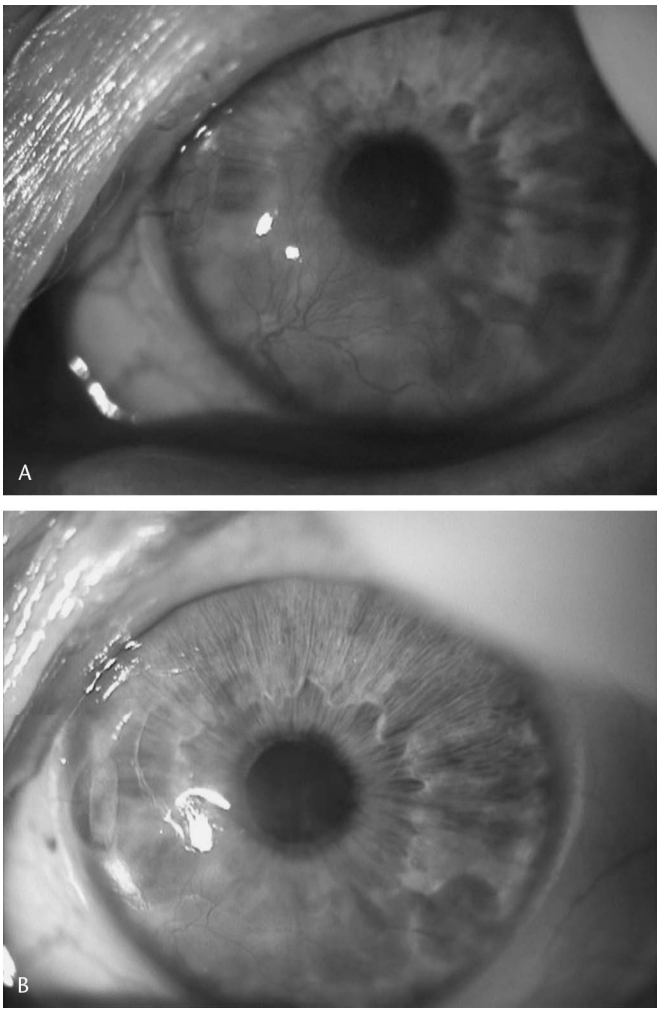

FIGURE 1. Anterior-segment photograph of patient 7 (herpetic eye disease) (A) before bevacizumab injection (extent 5, centricity 3, density 2.5, proportion of vascularized cornea 12.61%) and (B) 1 week after the fourth injection (extent 2.5, centricity 1.75, density 1, proportion of vascularized cornea 5.5%). Note the significant, although not complete, regression of blood vessels.

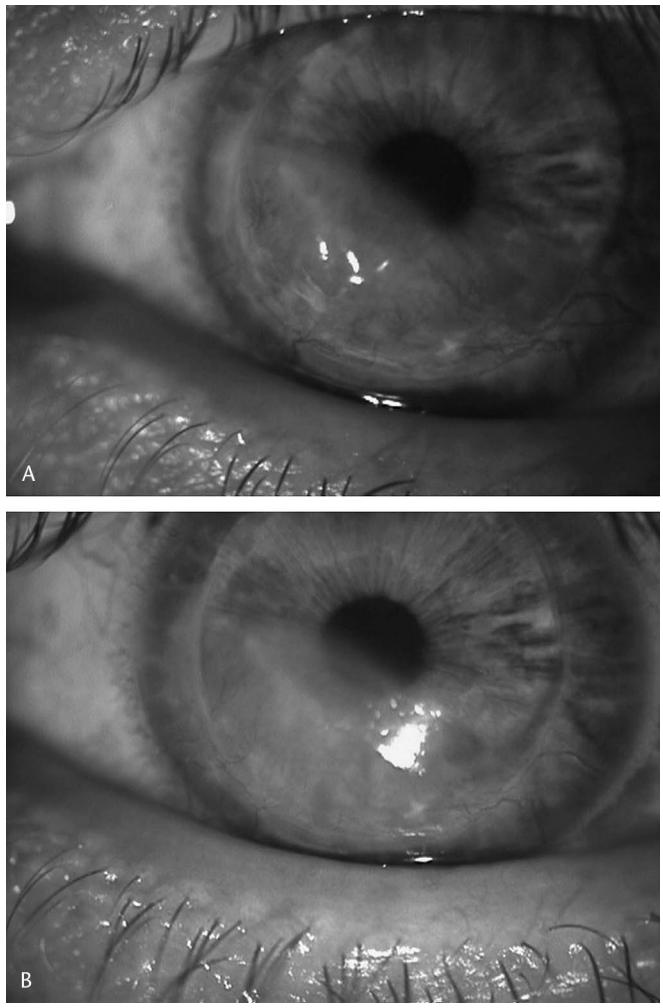

FIGURE 2. Anterior-segment photograph of patient 1 (failed graft, herpetic eye disease) (A) before bevacizumab injection (extent, 4.25, centricity 2.75, density 3, proportion of vascularized cornea 24.25%) and (B) 1 week after the second injection (extent 4, centricity 2.5, density 2, proportion of vascularized cornea 12.2%). Note the significant, although not complete, regression of blood vessels.

density) responded most to the bevacizumab injection. Indeed, a significant change in extent and density was shown, whereas centricity of vessels did not react to the subconjunctival injection. Possibly, the perilimbal application of the drug did not reach the more central part of the vessel in the deep cornea. It is possible that these central vessels would respond to a stromal injection rather than a perilimbal subconjunctival injection.

The change in extent and density of vessels that were graded by our masked observers reached statistical significance, whereas the proportion of hyperemia, which was analyzed by the software, did not. The human classification system was more diverse because it included 3 parameters, whereas the computerized system had only 1 parameter. Therefore, the human system could pick up small differences that the computer could have missed.

Bevacizumab has been used systemically for patients with colorectal cancer and has a low incidence of significant adverse effects.[41–43] It is considered unlikely that the small doses delivered by periocular injection would produce such adverse effects. Indeed, no patient in this series experienced any side effects, although such safety data are preliminary.[44] Bevacizumab has been injected intravitreally in humans for the treatment of choroidal and retinal neovascularization without significant side effects.[18–20,22–25,45]

Recently, Manzano et al[26] reported on the effect of topically administered bevacizumab on experimental corneal neovascularization in rats. They showed that topically administered bevacizumab at a concentration of 4 mg/mL limits corneal neovascularization and decreased it by 40% after chemical injury. They used a model of recent, fresh, neovascular vessels that are more likely to react to bevacizumab

TABLE 3. Corneal Neovascularization Before and After the Last Subconjunctival Injection of Bevacizumab

Vascularization Measure	Before Treatment [Mean ± SE (Median)]	1 Week After Last Treatment [Mean ± SE (Median)]	*P* (Wilcoxon Test)
Extent	6.0 ± 1.2 (4.625)	4.6 ± 1.0 (3.25)	0.008
Centricity	2.3 ± 0.2 (2.25)	2.1 ± 0.2 (2)	0.38
Density	2.7 ± 0.2 (2.875)	1.9 ± 0.3 (2)	0.007
Proportion of vascularized cornea (computerized)	14.8 ± 2.5 (16.0)	10.5 ± 2.8 (8.7)	0.36 (*t* test)

injection, whereas our series is composed of different etiologies for neovascularization and both recent and old blood vessels. Nevertheless, we showed similar results, despite the different mode of application of the drug (subconjunctival in our series vs. topical in the study of Manzano et al) and the different subjects used in these 2 studies (humans vs. rats).

Erdurmus et al[27] reported on the efficacy of subconjunctival bevacizumab injection (2.5 mg/0.1 mL) in 2 patients with corneal neovascularization. One patient had dry eye, whose vessels dramatically regressed 1 week after the injection, and the other patient had a failed graft, who had only a minor vessels' regression. These results compare well to our findings.

In conclusion, subconjunctival bevacizumab injection is efficacious in limiting corneal neovascularization in human subjects. Whether this partial regression of blood vessels is of clinical value needs to be studied in a randomized clinical trial with a larger sample size and longer follow-up. Bevacizumab may also be used in the future, as an adjunct to other drugs, for the treatment of corneal neovascularization.

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