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Topical Ranibizumab as a Treatment of Corneal Neovascularization

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Abstract

Purpose—To examine the effect of topical ranibizumab on clinically stable corneal neovascularization (NV).

Methods—This was a prospective, open-label, monocentric, uncontrolled, non-comparative study. Ten eyes of 9 patients with corneal NV received topical ranibizumab (1%) 4 times a day for 3 weeks with a follow-up of 16 weeks. The main corneal neovascularization outcome measures were: neovascular area (NA), the area occupied by the corneal neovessels; vessel caliber (VC), the mean diameter of the corneal neovessels; and invasion area (IA), the fraction of the total cornea area covered by the vessels. This study was conducted at the Massachusetts Eye and Ear Infirmary, Boston, MA, USA.

Results—Statistically significant decreases in NA (55.3%, P<0.001), which lasted through 16 weeks, and VC (59%, P<0.001), which continued to improve up to week 16, were observed after treatment. No significant decrease was observed in IA (12.3%, P=0.49). There was no statistically significant change in visual acuity or intraocular pressure. No adverse events ascribed to the treatment were noted.

Conclusions—Topical application of ranibizumab is effective in reducing the severity of corneal NV in the context of established corneal NV, mostly through decrease in VC rather than IA.

Keywords

corneal neovascularization; VEGF; ranibizumab

INTRODUCTION

Corneal neovascularization (NV) is a significant clinical problem[1,2]. In the United States it is estimated that approximately 1.4 million patients suffer from corneal NV. Of these, 12% are associated with a decrease in visual acuity. Moreover, corneal angiogenesis acts as a strong risk factor for penetrating keratoplasty[3]. Indeed, survival rates for corneal grafts

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Conflicts of interest
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placed into vascularized recipient beds (so called 'high-risk' keratoplasty) often decrease to below 50% even with local and systemic immune suppression[4]. In addition to its effects on corneal immunoinflammatory responses, corneal NV can lead to corneal scarring, edema, and lipid deposition and is associated with visual impairment and blindness worldwide.

Current treatments for corneal NV include pharmacological approaches such as corticosteroids and non-steroidal anti-inflammatory agents – both of which may be associated with serious side effects, such as ocular hypertension and corneal melting respectively [1,5]. Other treatments include laser photocoagulation, fine-needle diathermy and photodynamic therapy[6–11]; however, vessel recanalization and stromal injury have been associated with these approaches. Surgical approaches, aimed at restoration of the ocular surface, including amniotic membrane-based transplantation, have also been described with varying degrees of success[1].

Members of the vascular endothelial growth factor (VEGF) family mediate angiogenesis, and it has been shown that VEGF is up-regulated in inflamed and vascularized corneas in both humans and animal models[12]. Clinically, VEGF inhibitors such as pegaptanib sodium (Macugen™, OSI/Eyetech), ranibizumab (Lucentis™, Genentech) and off-label bevacizumab (Avastin™, Genentech) are currently used for the treatment of various retinal diseases such as neovascular age-related macular degeneration[13,14]. In addition, inhibition of angiogenesis by neutralization of VEGF-A has been shown to promote corneal graft survival in animal models[15]. Specifically, ranibizumab has been tested in animal models where it was found to significantly reduce corneal neovascularization[16,17].

Bevacizumab and ranibizumab have similar VEGF-binding characteristics because both drugs are related to each other. Bevacizumab is a full-length, humanized monoclonal antibody whereas ranibizumab is a humanized antibody fragment against VEGF-A[18]. Given the encouraging results using bevacizumab for the treatment of corneal NV[19] and the fact that ranibizumab has a smaller molecular weight (48kD) than bevacizumab (149kD), we tested the efficacy and safety of topical ranibizumab as a potential treatment modality for corneal NV.

MATERIALS AND METHODS

This was an open label, single center uncontrolled, study investigating the safety and efficacy of off-label topically administered ranibizumab in patients affected by corneal NV. This study was approved by the Institutional Review Board of the Massachusetts Eye and Ear Infirmary. Potential patients signed an informed consent at the time of the screening visit.

Patients

Consecutive, eligible patients were identified for inclusion in the study. Adult patients of either sex with clinically stable corneal NV were eligible for participation. Ten eyes of 9 patients (4 females and 5 males) were recruited. Stable corneal NV was defined with inclusion and exclusion criteria as follows. Patients with superficial or deep corneal neovessels extending more than 2 mm from the limbus were considered. Exclusion criteria were: (a) current or recent (≤3 months) episode of corneal and ocular surface infection (of whatever origin); (b) ocular surgery in study eye, including cataract surgery, keratoplasty, ocular surface reconstruction, limbal stem cell transplantation or amniotic membrane transplantation within 3 months prior to study entry; (c) current or recent (≤3 months) use of contact lens; (d) current or recent (≤3 months) persistent corneal epithelial defects (of at least 14 days in duration measuring more than 1 mm^2); (e) recent (≤1 month) change in dose and frequency of topical steroids and/or nonsteroidal anti-inflammatory agents; and (f)

ocular or periocular neoplasias. Other non-ocular exclusion criteria were: uncontrolled hypertension (systolic blood pressure ≥150mmHg or diastolic blood pressure ≥90mmHg), history of a thromboembolic event, coagulation abnormalities (including current anticoagulant except aspirin) and diabetes mellitus.

Patients were consented for enrollment according to an institutional Human Studies Committee-approved process and were treated with 1% (10mg/ml) topical ranibizumab 4 times a day for 3 weeks. This study was administered under an Investigational New Drug application (IND 100,859) provided by the Food and Drug Administration.

Study medication

A topical ranibizumab solution was aseptically formulated by the Massachusetts Eye and Ear Infirmary Clinical Pharmacy as follows: 2ml single-use vials were prepared using a 1% (10 mg/ml) of preservative-free, single use ranibizumab aqueous solution containing 10 mM histidine HCl, 10%, a-trehalose dihydrate, and 0.01% polysorbate 20 (pH 5.5). Each patient received 84 single use dropper containers with instructions to use one single use dropper container for each application and discard each dropper container after one use. Patients were instructed to refrigerate the study drug at 2–8°C (36–46°F). In order to reduce the chance of systemic absorption of ranibizumab, both puncta of the study eye were temporarily plugged for the duration of treatment (3 weeks).

Study protocol

To determine the safety and efficacy of topical ranibizumab for the treatment of corneal NV, follow-up visits were scheduled at weeks 1, 3, 8, and 16. Patients were instructed to strictly follow the study visit schedule. At all visits a full ocular examination was completed, including visual acuity (VA) measurements, corneal photographs, central corneal thickness measured by ultrasound pachymetry and a detailed review of the general, medical and pharmacological history. Adverse event monitoring was performed throughout the study. Systolic and diastolic blood pressure measurements were obtained at all visits.

Main outcome measures

Safety—Safety was monitored via occurrence of adverse events (AEs). Both ocular and systemic AEs were monitored during the study. Ocular adverse events were identified by eye examination, VA testing, intraocular pressure, and ocular surface fluorescein staining up to week 16. Systemic adverse events were identified by blood pressure measurements, patient reporting, and physical examinations up to week 16. This study did not include blood sampling or any pharmacokinetic measures.

Efficacy—We considered the extent of corneal NV, as measured by neovascular area, vessel caliber, and invasion area, to be the primary efficacy variable. By comparing baseline corneal photographs with follow-up photographs, as detailed below, the change in corneal NV was evaluated. Secondary efficacy variables included measuring the changes in best-corrected visual acuity and central corneal thickness from baseline to the last visit.

Three primary metrics for corneal NV (Fig. 1) were considered, as previously described[15]. The following metrics were extrapolated from the digital pictures: Neovascular Area (NA), the area of the corneal vessels themselves when projected into the plane of a photograph; Vessel Caliber (VC), the mean diameter of the corneal vessels; Invasion Area (IA), the fraction of corneal area in which vessels are present. Digital slit-lamp corneal pictures were analyzed morphometrically using graphic editing software (Photoshop® CS2; Adobe Systems Inc.) and a mathematical program (written using Matlab®; Mathworks Inc.).

After the total corneal area was outlined, the blood vessels were enhanced and traced with Photoshop tools and filters, then NV parameters were extrapolated as described elsewhere[19].

Statistical analysis

To assess the difference in the three metrics, three different time points were considered: (1) the screening visit, (2) the 3-week visit (end of treatment), and (3) the 16-week visit. Repeated–measures ANOVA with complementary post-hoc Bonferroni test was performed comparing cohort scores for each metric. In each case the hypothesis was a reduction in cohort scores for a given metric from the earlier time point to the later time point. $P \leq 0.05$ was considered statistically significant.

RESULTS

Ten eyes of 9 patients (4 females and 5 males) were included in this study. The mean age of the subjects was 57.3 (SD 14.5) and ranged from 30 to 78 years. Table 1 provides the demographics of the study population. Patient medications at time of screening are described in Table 2.

Neovascular Area

A statistically significant decrease in NA was found across the cohort ($P<0.001$). Post-hoc Bonferroni test revealed a significant reduction from the screening visit to the 3-week visit ($P<0.05$). The treated eyes also showed a significant reduction in the NA from the screening visit to the 16-week visit ($P<0.001$) (Fig. 2 and 3). The mean change in the NA from the initial to the 3-week visit was 39.8% (SD, 24.1%). The mean reduction in NA across the cohort was 55.3% (SD, 44.4%) ranging from 27% to 99.6%. No significant change was noticed from the 3-week visit to the 16-week visit ($P=0.13$) indicating sustained treatment outcome up to week 16.

Vessel Caliber

A statistically significant decrease in VC was observed across the cohort ($P<0.001$). Post-hoc Bonferroni test showed a significant reduction from the screening visit to the 16-week visit ($P<0.001$) (Fig. 2). The mean change in the VC from the initial to the 3-week visit was 25.8% (SD, 18.8%). The mean reduction in VC across the cohort was 59% (SD, 34.9%) ranging from 36.3% to 98.9%. There was a significant reduction in VC from the 3-week visit to the 16-week visit ($P<0.05$) indicating continued improvement up to week 16.

Invasion Area

No significant change in the IA was observed across the cohort ($P=0.26$). The mean change in IA was 31.6% ($P= 0.87$, SD, 67.4) and 12.3% ($P=0.49$, SD, 54.7) from the baseline visit to the 3-week and the 16-week visits, respectively..

Other End-points and Adverse Events

Visual acuity—Conversion to logMAR equivalents of Snellen visual acuity was performed for analysis of visual acuity changes. Mean corrected logMAR visual acuity (the lower the logMAR score, the higher the degree of acuity) was 0.68 (SD, 0.62) at the screening visit, 0.48 (SD, 0.43) at the 3-week visit, and 0.55 (SD, 0.37) at the last visit. Changes in visual acuity from the baseline to any of the follow-up visits were not found to be significant.

Corneal thickness—Mean values for central corneal thickness were 632um (SD, 106um) at baseline, 571um (SD, 109um) at 3 weeks, and 571um (SD, 45um) at 16 weeks; there were no statistically significant differences between these time points.

Intraocular pressure—No significant change was found in intraocular pressure from baseline to the 3-week visit and from baseline to the 16-week visit.

Systemic blood pressure—Mean arterial blood pressure (MAP) ([(2×diastolic blood pressure) + systolic blood pressure]/3) at week 1, week 3, and week 16 were compared with baseline visit measurements. The mean (±SD) of MAP for all patients was 90.6 (±12) mmHg at the baseline, 89.8 (±7.1) mmHg at 1 week (P =0.85), 86.9 (±10.1) mmHg at 3 weeks (P=0.46), and 86.7 (±9.6) mmHg at 16 weeks (P =0.47). In summary, MAP did not appear significantly affected by ranibizumab topical application.

No systemic or ocular adverse events including thromboembolic events, hemorrhage, allergic reaction, ocular surface toxicity and epitheliopathy (superficial punctate keratopathy, epithelial erosion or defect) or burning upon instillation were reported. Self-reported compliance was extremely favorable; no patients reported to have missed doses of the study drug throughout the entire treatment period.

DISCUSSION

Corneal NV represents a challenging clinical condition that may also lead to significant visual impairment. Current therapies aiming to induce the regression of corneal vessels are not uniformly effective and are variably associated with undesirable side-effects[5,9]. Several VEGF inhibitors are currently used for the treatment of neovascular age-related macular degeneration and macular edema[20,21]. Several studies have evaluated the application of topical bevacizumab, at different concentrations,[19,22–24] for treatment of corneal NV. Concerns have been raised in regard to prolonged topical application of bevacizumab, as VEGF may be a critical modulator of wound healing,[25] and has also been implicated as a nerve trophic factor [26]. Indeed, the loss of epithelial integrity has been reported with topical use of bevacizumab at 1.25% concentration when applied for prolonged periods (2 months)[22]. In light of these findings, although we did not observe the development of epithelial defects in the course of our study with ranibizumab, we suggest caution should be taken when treating patients with sub-optimal ocular surface integrity.

Ranibizumab, a Fab fragment related to bevacizumab, has been used to treat pterygia via subconjunctival injection with no reported side effects[27]; prompt regression of microvessel in the pterygium bed has been described[28]. Additionally, subconjunctival ranibizumab has been shown effective in inhibiting neoplastic NV in ocular surface neoplasias[29,30]. However, topical application of ranibizumab has not been reported to date in a clinical setting.

In the aggregate, the existing literature suggests that local delivery of ranibizumab to the anterior segment of the eye is not associated with significant side effects. Moreover, studies from intravitreal administration of ranibizumab suggest that it is well tolerated and not associated with clinically significant safety risks during and up to two years of treatment[31].

However, no reports are available describing application of topical ranibizumab in corneal NV. The current pilot study was performed to evaluate the efficacy of topical ranibizumab in the treatment of corneal NV and to make comparisons with a similar treatment regimen for topical bevacizumab (same concentration [10mg/ml], treatment frequency [4x daily] and duration [3 weeks]) reported by our group[19].

In the current study, we found that topical ranibizumab 1% is effective in the treatment of clinically stable corneal NV as evidenced by a significant reduction in two corneal NV parameters (NA and VC). The average reduction in NA from baseline was 39.8% by week 3 and 55.3% by week 16, with no statistically significant difference between these two time points indicating sustained treatment effect up to week 16. Interestingly, VC continued to decrease significantly up to week 16, suggesting not only sustained, but potentially progressive, treatment efficacy beyond treatment termination at week 3. The average decrease in VC was 25.8% by week 3 and 59.0% by week 16. This progressive effect on VC is in line with our observations with use of topical bevacizumab[19]. Given that patients enrolled into this study, and our earlier trial with bevacizumab with stable NV, we do not believe that this sustained efficacy beyond drug termination is simply a reflection of the natural history of NV regression. This is in contrast with the well-known requirement for repeat treatment in neovascular AMD and other proliferative retinopathies[13,14,32]. In our study, the significant reduction of NA and VC in the absence of a significant change in IA suggests that the main outcome of ranibizumab treatment is to induce narrowing of blood vessels more than a reduction in their length. It is important to emphasize again that in this study only patients with stable neovascularization were treated. Stable NV is less influenced by VEGF blockade as opposed to newly formed vessels; this may explain the absence of significant reduction in the NV invasion area.

Topical application of bevacizumab 1% for treatment of corneal NV has similarly been studied by our group.[19] In that study 10 patients with corneal NV were treated with 1% topical bevacizumab for 3 weeks. Since our current study used the same efficacy parameters to evaluate corneal NV, a comparison between these two studies can be made. For NA we found a reduction of 55% (SD, 44%) with ranibizumab as opposed to 47% (SD, 37%) with bevacizumab by week 16. VC was reduced by 59% (SD, 35%) with ranibizumab as opposed to 54% (SD, 28%) after bevacizumab by week 16. No significant difference of IA was found in either trial. Though not statistically significant, the decreases in NA and VC were consistently greater for patients treated with ranibizumab at the comparable time points. Thus, given the small sample sizes of these studies, we cannot derive definitive conclusions regarding the relative efficacies of these two anti-VEGF-A agents. Indeed, this was an open-label and non-comparative study and was designed as a pilot study to test the efficacy of topical ranibizumab in patients with corneal NV.

Ranibizumab, designed for intraocular injection, has a relatively low (49kD) molecular weight (MW) – approximately one third of the MW of bevacizumab. Thus, it is possible that it could better penetrate the corneal epithelial barrier than larger MW biologic agents such as bevacizumab[33] and thus reaching higher therapeutic concentrations in the stroma. Clearly, however, additional comparative efficacy studies are required before any conclusion can be drawn regarding the apparent, modestly enhanced efficacy of topical ranibizumab as compared to bevacizumab in the treatment of corneal NV.

Finally, as is increasingly true for any therapeutic strategy, the efficacy of the treatment should be balanced against its costs. Thus, it is important to emphasize that there is a significant cost difference between bevacizumab and ranibizumab. It is noteworthy that clinical adoption of topical ranibizumab for treatment of corneal NV may be prohibitive due to the current pricing of the drug.

In summary, this study provides novel evidence for the efficacy and safety of topical ranibizumab in the treatment of corneal NV across a diverse spectrum of corneal neovascular pathology. Further and larger studies are needed to better establish the efficacy profile of this drug and optimize its dosing and formulation.

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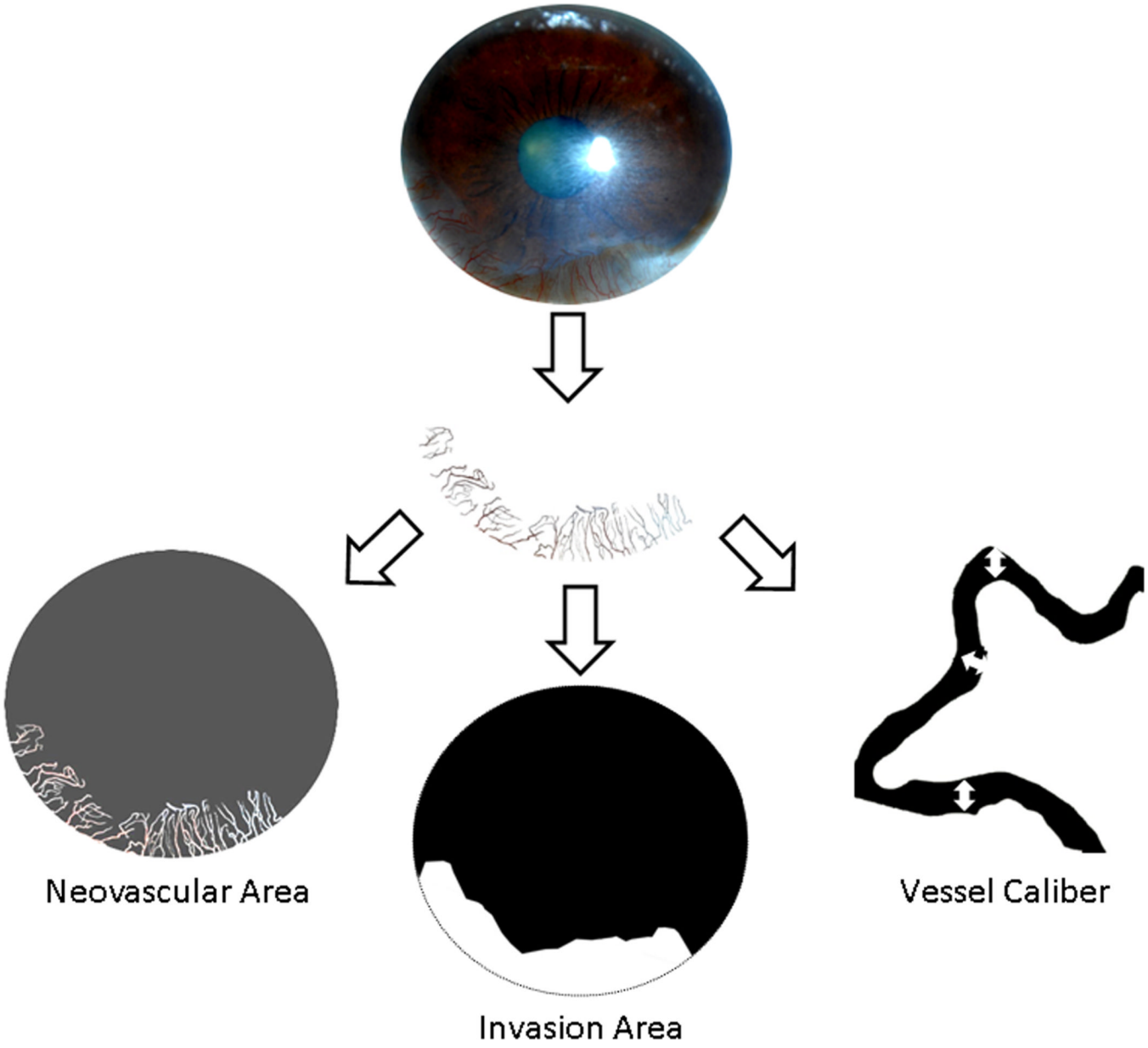

Fig. 1.
Quantification of corneal neovascularization. Digital slit lamp corneal photographs were analyzed using Photoshop CS2 and a mathematical program (Matlab, Mathworks Inc.). The total area of the cornea was isolated and blood vessels were manually extracted using Photoshop. To analyze the effect of ranibizumab three metrics were considered using a Matlab script: Neovascular Area, measuring the area of the corneal vessels themselves; Vessel Caliber, determining the mean diameter of the corneal vessels; and Invasion Area, measuring the fraction of the corneal area in which the vessels are present.

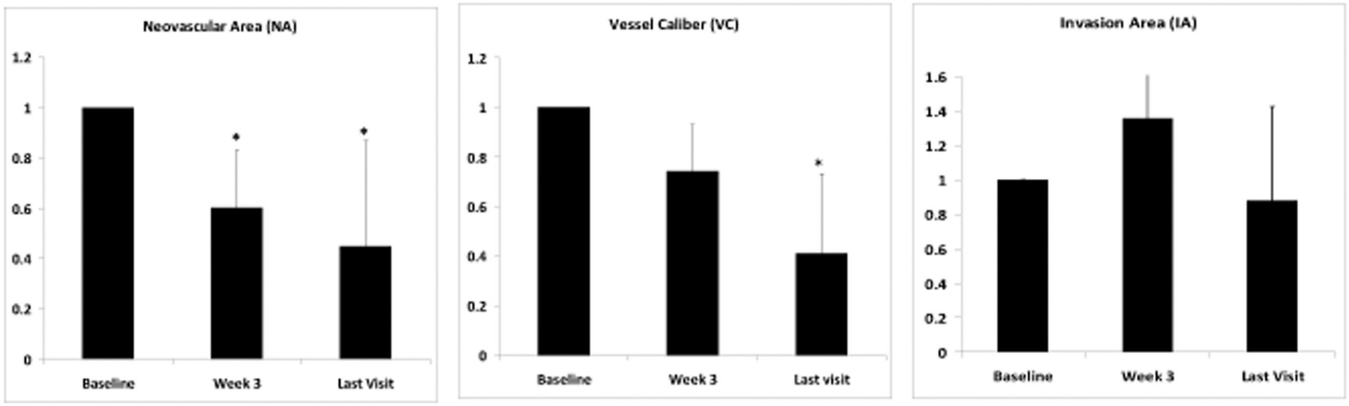

Fig. 2.

Summary of changes in response to ranibizumab topical therapy at the different time points in the neovascular area, vessel caliber, and invasion area. The reduction in the neovascular area and vessel caliber were statistically significant. There was no statistically significant change in the invasion area.

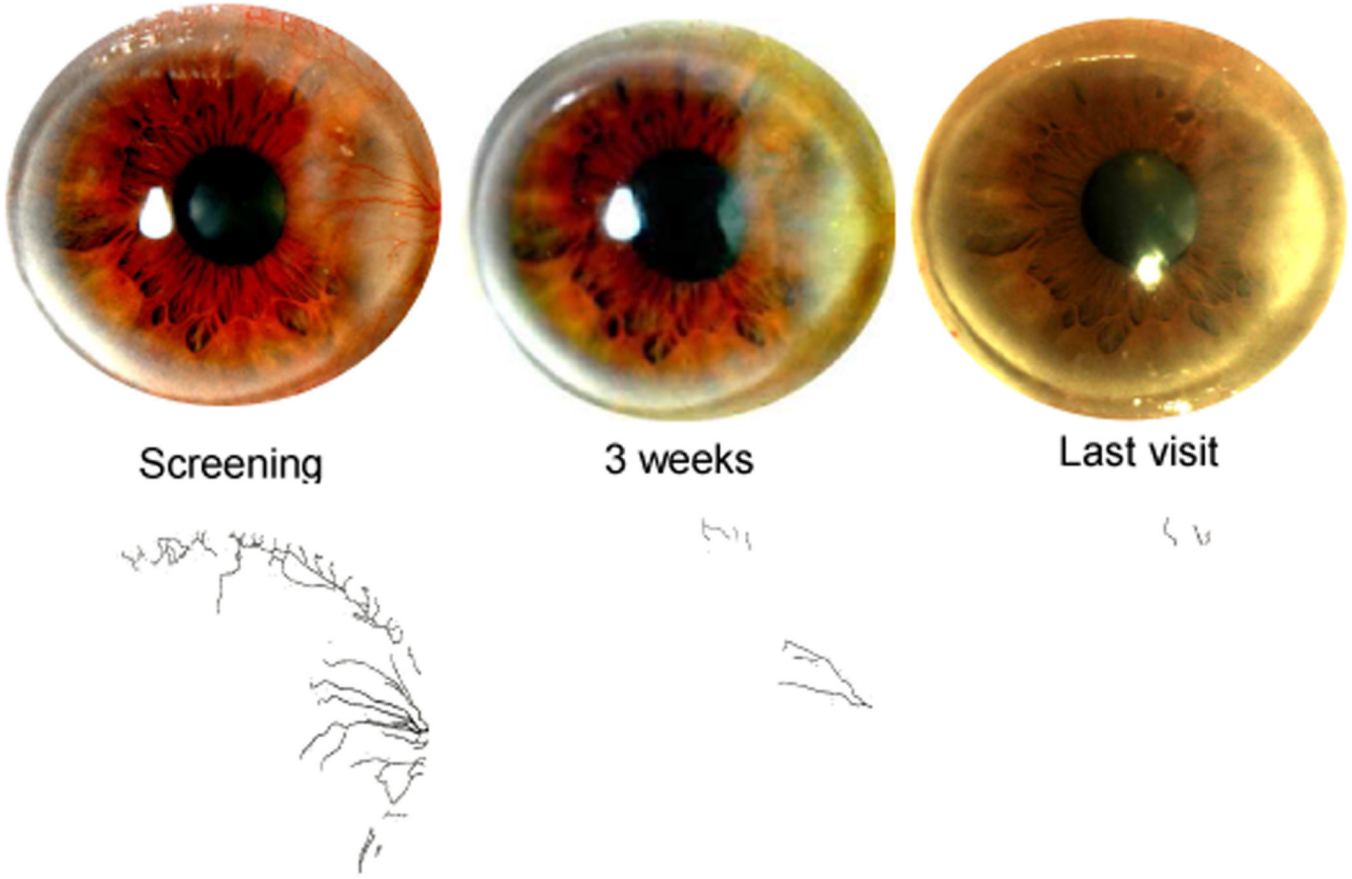

Fig. 3.

The effect of topical ranibizumab on patient number 5. Upper row: The screening picture shows neovessels branching into the cornea from the 3 o'clock position. The vessels were progressively reduced in caliber and in extension already 3 weeks after the initiation of the treatment; the effect appeared to continue up to week 16. Lower row: corneal neovessels extracted from the corresponding pictures.

TABLE 1

Study population characteristics.

Patient	Eye	Gender	Age at enrollment	Diagnosis at enrollment
1	OS	F	78	Corneal NV; s/p band keratopathy LID 11/20/07
2*	OD	M	57	Corneal NV OU; h/o perforated Pseudomonas corneal ulcer, s/p patch graft LID 12/26/07
2*	OS	M	57	Corneal NV OU; s/p lamellar excision of corneal scar, amniotic membrane transplantation LID 12/26/07
3	OS	F	52	H/o HZV keratitis; neurotrophic keratopathy, lipid keratopathy LID 04/07
4	OS	M	65	s/p chemical burn OU; limbal stem cell deficiency OS LID 06/10/08
5	OD	M	70	s/p excision of primary pterygium with conjunctival autograft and amniotic membrane LID 10/22/08
6	OD	F	69	HSV Keratitis, central corneal scar LID 1985
7	OS	F	30	HSV Keratitis, central corneal scar LID 2/18/08
8	OD	M	39	HZV Keratitis LID 01/08
9	OD	M	56	s/p LASIK; s/p PK for Pseudomonas corneal ulcer LID 03/11/09

NV: neovascularization; s/p: status post; LID: last inflammation clinically detected; HZV: herpes zoster keratitis; HSV: herpes simplex keratitis.

* Both eyes of Patient 2 were analyzed in this study.

TABLE 2

Current medications at time of enrollment. Oral medications are shown in parentheses.

Subject number	Meds at screening visit
1	Erythromycin 0.5% OD Artificial tears Prednisolone acetate 1% TID OS
2 (OD)	Prednisolone acetate 1% BID OS Medroxyprogesterone 1% QID OS Artificial tears OD Erythromycin 0.5% TID OS (Doxycycline 100mg QD)
2 (OS)	Prednisolone acetate 1% BID OS Medroxyprogesterone 1% QID OS Artificial tears OD Erythromycin 0.5% OS (Doxycycline 100mg QD)
3	Prednisolone acetate 1% QD OS
4	Bacitracin OU (Doxycycline 100mg QD)
5	Prednisolone Acetate 1% BID OD
6	Artificial tears (Acyclovir 400mg BID)
7	Artificial tears (Acyclovir 400mg BID)
8	Artificial tears
9	Prednisolone acetate 1% TID OD