Subconjunctival bevacizumab induces regression of corneal neovascularisation: a pilot randomised placebo-controlled double-masked trial

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ABSTRACT

Objective To evaluate the off-label use of subconjunctival bevacizumab for corneal neovascularisation (CoNV).

Methods 30 patients with recent-onset CoNV from various causes were randomly assigned into a double-masked, placebo-controlled trial. Each received three 0.1 ml injections containing either 2.5 mg bevacizumab or 0.9% saline at monthly intervals. Dexamethasone 0.1% drops were used four times a day for the first month, when the dose was modified if clinically indicated. The primary outcome was change in area of corneal involvement by CoNV from baseline to 3 months measured using specialised imaging technology.

Results The mean area of CoNV reduced by −36% (range −92% to +40%) in the 15 eyes that received bevacizumab compared with an increase of 90% (range −58% to +1394%) in eyes that received saline placebo (analysis of covariance (ANCOVA); p=0.007). One outlier in the placebo arm developed corneal graft rejection with aggressive neovascularisation (+1384%), but even when this patient was excluded the mean reduction in CoNV in the placebo group (−3%, range −58% to +40%) was still significantly different from the treatment arm (ANCOVA; p=0.016). Changes in best-corrected visual acuity, central corneal thickness, intraocular pressure and endothelial cell counts were similar between groups. The intervention was well tolerated with no major safety concerns.

Conclusions Three subconjunctival injections of 2.5 mg bevacizumab are more effective than placebo at inducing the regression of recent-onset CoNV. Further studies are needed to confirm this effect and our data suggest that a sample size of 40 patients per treatment group is required.

INTRODUCTION

Corneal neovascularisation (CoNV) is the invasion of blood and lymphatic vessels from pre-existing vascular structures at the limbus in response to hypoxia, inflammation, infection or corneal degeneration.[1] As well as reducing vision CoNV is a major risk factor for immune allograft rejection after corneal transplantation.[2] Current therapeutic strategies to prevent or reverse CoNV include corticosteroid, immunosuppression, or fine needle diathermy, but there are few evidence-based studies evaluating their effectiveness.[3 4]

Vascular endothelial growth factor (VEGF) is the principal mediator of CoNV in animals[5] and humans.[6] In the avascular mammalian cornea a soluble VEGF receptor (VEGF receptor-1 or sFlt-1) blocks the VEGF pathway, and disruption of this natural VEGF antagonist is thought to be the mechanism for CoNV.[7 8] Bevacizumab is a recombinant humanised monoclonal antibody that binds human VEGF-A isoforms and prevents activation of VEGF receptors. There have been a number of case series that report a beneficial effect of either topical[9 10] or subconjunctival bevacizumab[11–14] for the treatment of CoNV. However, because there is a natural tendency for CoNV to regress as the stimulus subsides, the magnitude of the therapeutic effect of bevacizumab is unclear. We therefore conducted a pilot randomised placebo-controlled, double-masked clinical trial to assess the effectiveness of subconjunctival bevacizumab in patients with progressive CoNV to gather data for a definitive study.

PATIENTS AND METHODS

Participants

Participants were recruited at Moorfields Eye Hospital between April 2009 and August 2010. Inclusion criteria were the presence of progressive CoNV, no epithelial defect, age over 18 years, and an ability to give informed consent. Progressive CoNV was defined as a minimum radial ingrowth of vessels 2 mm from the limbus in the interval of 2 months to 2 weeks before inclusion, which was confirmed by digital image analysis. Patients were excluded if CoNV had been present for more than 6 months, if there was a history of cardiovascular or cerebrovascular disease, uncontrolled systemic hypertension, or concurrent corneal conditions that could potentially be exacerbated by bevacizumab (eg, active infective keratitis, corneal melting). Only one eye per patient was included.

Injection protocol

Participants were randomly assigned to receive three 0.1 ml subconjunctival injections of either bevacizumab 2.5 mg or 0.9% saline as placebo. Random assignment was performed by pre-allocated permuted block without stratification. All syringes were prepared within 8 weeks of use and appeared identical. Topical amethocaine 1% (Minims) and povidone-iodine 5% (Moorfields Pharmaceuticals Pty Ltd.) was placed into the inferior conjunctival sac before injection. The injections were given in the subconjunctival space at the same site at least 5 mm from the limbus and adjacent to the area of most active CoNV. Injections were given at a slit lamp in the outpatient clinic. Injections were administered at enrolment, and at weeks 4 and 8 with a treatment window of ±1 week. A standard therapy of

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preservative-free dexamethasone 0.1% drops (Moorfields Pharmaceuticals Pty Ltd.) four times a day was prescribed for all patients at baseline. The assessor could modify the frequency of dexamethasone treatment at the 4, 8 and 12-week examinations if it was considered clinically necessary. The patients and the study ophthalmologists were masked to the treatment assignment.

Follow-up

One investigator (CP) examined the patients at each visit. At all visits best-corrected visual acuity was measured with an EDTRS chart, anterior segment biomicroscopy was performed, and intraocular pressure was measured with a Tono-pen-XL (Medtronic Solan Pty Ltd). At the first and final visits corneal topography (Pentacam HR; Oculus Pty Ltd) and endothelial cell density (Topcon SP-2000P; Topcon Corp.) were also performed. Any subconjunctival bleeding was recorded and each patient provided a subjective assessment of associated discomfort that ranged from zero (no pain) to 10 (worst pain in their life).

Image capture and analysis

Digital slit lamp photographs were taken at each of the four trial visits (Topcon ATE-600 slit lamp (Topcon Corp.) and Nikon D1x digital camera (Nikon Corp.)) at 10× optical zoom with standardised diffuse illumination and flash intensity. Luminance levels were not calibrated for each session. Images of the central cornea, conjunctival injection site and all four conjunctival quadrants were matched with previous images to ensure equivalent framing (figure 1). The area of CoNV was measured using computerised morphometric analysis (Image-Pro Plus V.4.5.1.22; Media Cybernetics Inc.). A detailed description of the method for flatmount corneas has been published[15] and used in a similar clinical trial.[16] The primary outcome was change in area of CoNV between baseline and the 12-week visit. Two independent masked observers analysed all of the central corneal images. To identify disagreement the results were compared, and when the difference was greater than 10% a third independent masked analysis was performed and the average of the closest two values was recorded. Reproducibility and reliability analysis was not performed. Specific protocol methods are provided (see supplementare data file, available online only).

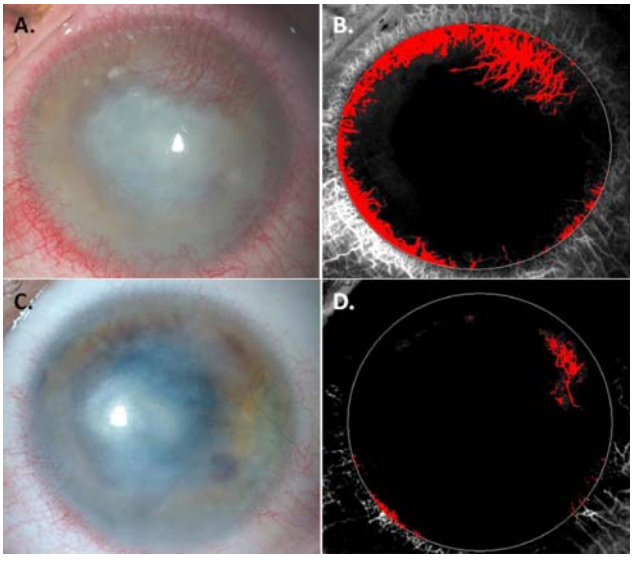

Figure 1 Image analysis series of subject 29 who received bevacizumab. (A) Digital slit lamp image taken at baseline. (B) Digital image after morphometric analysis with corneal neovascularisation (CoNV) highlighted in red and region of interest (ROI) set at the limbus. (C) Final digital slit lamp image taken at week 12 after three subconjunctival injections of bevacizumab. (D) Final morphometric analysis image with CoNV highlighted in red and total pixel count within ROI reduced by 88% compared to image B.

Statistical analysis

This was a pilot study, so a formal sample size calculation was not conducted. The aims were to assess recruitment rates, acceptability of the proposed treatment to patients, and to assess the utility of the primary endpoint to guide the design of a definitive trial. A sample size of 30 was deemed to be the smallest acceptable number to estimate efficacy. Descriptive analyses were conducted, with summary statistics (eg, mean and SD, and median and IQR) being reported. However, a large difference in the primary outcome between groups was observed and analysis of covariance (ANCOVA) was employed to assess whether this difference was statistically significant. The estimate of variability in primary outcome was then used to determine the sample size for a definitive study with 90% power and 5% level of significance. Data were independently entered twice into a trial database and all data queries and errors were resolved. All analyses were performed as intention to treat by a trial independent statistician using STATA V.11 with the level of statistical significance set at $p<0.05$.

RESULTS

Demographics

Thirty eligible patients completed the study without loss to follow-up or protocol deviations (see flow diagram in supplementare data file, available online only). All patients attended within 1 week of their final visit at 3 months. Arbitration was not required for scoring the result of any masked analysis of a digital image. The baseline characteristics are summarised in table 1.

Table 1 Baseline demographics and risk factors for CoNV

	Bevacizumab 2.5 mg (n=15)	Placebo (n=15)
Age, years (mean (SD))	43.4 (14.8)	48 (11.8)
Gender (male:female)	3:12	12:3
Study eye		
Right	8	6
Ethnic origin		
White	12	12
South Asian*	2	2
Black	1	1
Duration of CoNV, weeks (mean (SD))†	12.3 (5.8)	9.0 (4.7)
Underlying pathology‡		
Allergic eye disease	2	4
Contact lens wear	5	8
Herpes simplex/zoster keratitis	8	3
Non-viral keratitis	2	4
Corneal surgery	8	6
Other	1	4

*Indian or Pakistani.
†Before recruitment to the study.
‡Patients may have had more than one risk factor for CoNV
CoNV, corneal neovascularisation.

Table 2 Analysis of primary outcome measurements

	Bevacizumab 2.5 mg (n=15)	Placebo (n=15)	Placebo excluding subject with graft rejection (n=14)
Vascularised area at baseline*			
Mean (SD)	5.12 (5.74)	4.92 (3.25)	
Median	2.09	4.11	
Minimum, maximum	0.78, 20.12	1.20, 13.46	
Vascularised area at 3 months*			
Mean (SD)	3.19 (4.34)	6.39 (5.65)	5.43 (4.4)
Median	1.39	4.21	4.04
Minimum, maximum	0.15, 14.38	1.10, 19.87	1.10, 16.38
Change over 3 months*			
Mean (SD)	−36.2 (47)	90.1 (361.6)	−3.0 (26.6)
Median	−30.5	−5.6	−5.6
Minimum, maximum	−92.0, 39.7	−58.3, 1394	−58.3, 39.9
Statistical significance compared to treatment group (ANCOVA)		p=0.007	p=0.016
CoNV change over 12 weeks (%)			
Progression	4 (27)	5 (33)	4 (29)
Stable	1 (7)	6 (40)	6 (43)
Regression	10 (67)	4 (27)	4 (29)

*Expressed as percentage of total corneal area.
ANCOVA, analysis of covariance; CoNV, corneal neovascularisation.

The two groups were similar except for an excess of female gender and herpes simplex/herpes zoster as the cause for the CoNV in the treatment arm. The primary outcome data are summarised in table 2. Importantly, at baseline the extent of vascularisation in the two groups was similar.

Primary outcome measure

In eyes that received bevacizumab there was a significant reduction in the area of CoNV (−36.2%, SD 47.04) compared with an increase (+90.1%, SD 361.6) in the placebo group (ANCOVA; p=0.007). Expressed in terms of the percentage of corneal involvement, there was a mean reduction in CoNV of −1.93% (SD 3.87) in the treatment group compared with an increase in the placebo group of +1.47% (SD 4.96). Ten of the 15 patients who received bevacizumab showed CoNV regression, with a maximum reduction of 92%. There was progression of CoNV in four patients who received bevacizumab, but no clinical features distinguished this group from the patients who responded to treatment.

Figure 2 shows the mean area of CoNV for both groups. Patients who received bevacizumab showed the greatest reduction in CoNV in the first month but continued to have improvement with each injection. The placebo group showed little change at weeks 4 and 8 with a steep increase in CoNV at week 12. This was mainly due to one subject who developed bacterial keratitis 1 week before their 12-week exit visit, with corneal graft rejection and an aggressive increase (+1394%) in CoNV. Because this subject's primary outcome measure was 35 times higher than the next highest in the study we repeated the analysis with this subject excluded. After exclusion, the remaining placebo group (14 eyes) had a mean reduction of CoNV of −3.0% (SD 26.57) and when compared with the intervention group the statistical difference between groups remained (ANCOVA; p=0.016). When patients with herpes simplex/zoster keratitis were excluded the sample size reduced considerably, and although there was evidence of a difference between groups at 3 months this was no longer statistically significant (ANCOVA; p=0.073) although the effect estimate was similar.

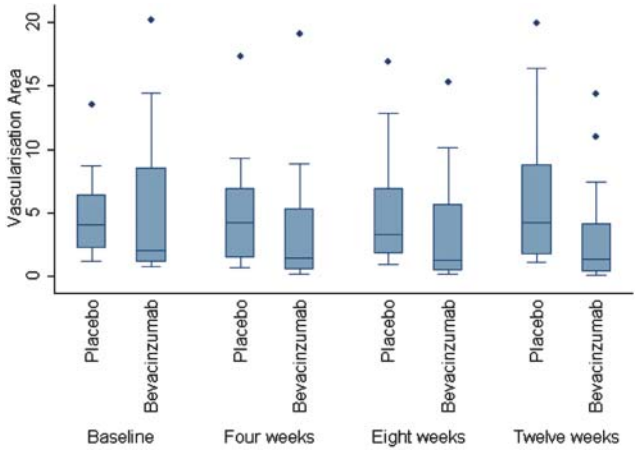

Figure 2 Box plots of the area of corneal neovascularisation of the two treatment groups at each assessment. Vascularised area in nominal units.

Additional safety data are summarised in table 3.

Table 3 Additional corneal, conjunctival and safety data

	Mean (SD)	
	Bevacizumab 2.5 mg (n=15)	Placebo (n=15)
Best corrected visual acuity (LogMar)		
Baseline	0.95 (0.83)	1.05 (0.89)
3 Months	0.77 (0.76)	1.12 (0.84)
Change	−0.18 (0.31)	0.08 (0.32)
Central corneal thickness (micrometres (SD), (subjects included with reliable data))		
Baseline	660 (194)[12]*	727 (362)[14]
3 Months	630 (213)[13]	751 (439)[14]
Change	−42 (110)[12]	22 (279)[14]
Central corneal endothelial cell count (cells/mm^2 (SD), (subjects included with reliable data))		
Baseline	1468 (738)[11]	1487 (697)[8]
3 Months	1437 (684)[11]	1403 (675)[8]
Conjunctival injection site vascularised area (% of region of interest (SD))		
Baseline	28.7% (8.3)	27.2% (10.1)
Months	25.0% (9.9)	26.4% (8.7)
Change	−3.7% (6.8)	−0.8% (4.5)
Serious reported adverse events	1 (epithelial defect)	2 (epithelial defect, corneal graft rejection)
Subconjunctival haemorrhage at injection site	3	1
Average pain score over 3 injections from 1 to 10	1.9 (1.5)	1.8 (1.1)
Proportion of patients reducing use of topical corticosteroids during trial period (n/N)	8/15	8/15

*When an investigation could not be performed reliably this is marked as incomplete data.

The LogMar best-corrected visual acuity improved in the intervention group (−0.18, SD 0.31) compared to the placebo group (+0.08, SD 0.32) but the difference was not statistically significant. Central corneal thickness and central corneal endothelial cell counts were similar in both groups. There was a small reduction in vessel area at the conjunctival injection site in both groups (bevacizumab −3.7% (SD 6.8) and placebo −0.8% (SD 4.5)).

Safety data

Six serious adverse events were reported. One patient from each arm developed a corneal epithelial defect within 3 days of their first injection, which was treated with topical non-preserved chloramphenicol 0.5% (Moorfields Pharmaceuticals plc) and a bandage contact lens (Purevision; Bausch & Lomb Corp.). A bandage lens was used prophylactically for subsequent injections and epithelial defects did not recur. The patient who received the placebo injection had a neurotrophic cornea and had a past history of corneal epithelial defects. There were four subconjuctival haemorrhages after injections, three in the bevacizumab group and one in the placebo group. The injections were well tolerated, with a pain score of one being the most commonly reported value.

The study data with ANCOVA analysis provides a relative efficiency of 1.423 with adjustment to SD of 0.838 (adjusted SD 1=4.695, adjusted SD 2=3.605). Assuming a two-armed trial with repeated measures, at the 5% probability level and power of 90%, a trial of 36 patients per arm would be required to provide evidence of effectiveness. Allowing for a 10% loss to follow-up rate, the definitive study would require 40 patients per treatment arm.

DISCUSSION

This is the first randomised placebo controlled evaluation of the off-label use of bevacizumab in patients with recent onset of CoNV. Our results show that subconjunctival bevacizumab induced a significant reduction in the area of corneal CoNV compared to placebo, with an intention to treat difference between the groups of 126%. However, there was no difference in the change in corrected vision between groups. The treatment was well tolerated with a low incidence of serious side effects.

Numerous case reports suggest that bevacizumab causes regression of CoNV. In a prospective non-randomised uncontrolled open-label study in 10 patients, a 3-week course of topical bevacizumab 10 mg/ml given two or four times a day was followed by a statistically significant reduction in vessel calibre ($p<0.001$) over 6 months, but with no effect on the area of corneal involvement ($p=0.19$).[10] This absence of vessel regression following topical treatment may be the result of poor penetration of bevacizumab (molecular weight 149) into the eye.[17 18] Although penetration into the cornea in mice is enhanced by epithelial damage, the levels in stroma are less than after subconjunctival injection.[19] A prospective uncontrolled study of 12 eyes that received a single subconjunctival injection of 2.5 mg of bevacizumab reported a maximum reduction of not of area of involvement by CoNV after 1 month, with a loss off effect over the subsequent 2 months, although the final reduction was still statistically significant ($p=0.02$).[14] The study also suggested that the treatment was less effective in cases with established CoNV.[14]

There were no human data on the pharmacokinectics of subconjunctival bevacizumab to guide the dose regimen for this study. Previous case series have used subconjunctival bevacizumab at doses of 1.25–5.0 mg given for between one and 10 injections. In rabbits[20] and rats[21] there is no increase in the effect of subconjunctival bevacizumab on CoNV regression above a dose of 5 mg. In rabbits the systemic absorption of 1.25 mg of bevacizumab is similar after a subconjunctival injection or an intravitreal injection, with a maximum plasma concentration after subconjunctival injection calculated to be 3733.1 ng/ml (SD 174.9) with a mean half-life in the plasma of 1.75 weeks.[17] For comparison the IC_{50} for VEGF inhibition of human umbilical vein endothelial cells in vitro is 22 ng/ml, while 500 ng/ml completely blocked VEGF-induced endothelial cell growth.[22] Therefore, a single subconjunctival injection of 1.25 mg in the rabbit maintains a concentration of over IC_{50} in plasma for several weeks, and it can be detected in the cornea for up to 4 weeks.[17] A similar low incidence of systemic side effects would be expected after intravitreal or subconjunctival injection.[23] Although there are significant differences between the rabbit and human eye we concluded from these data, and the absence of reported systemic side effects following subconjunctival injection of 2.5 mg,[13 24 12] that an injection of 2.5 mg of bevacizumab given every 3–5 weeks was appropriate, and that treatment limited to recent-onset CoNV would most likely identify an effect.[25] Future clinical trials are needed to confirm the optimum dose regimen.

Topical corticosteroid has also been shown to reduce CoNV in corneal grafts in a randomised study, and it is considered to be a standard of care for patients with recent-onset CoNV.[26] Therefore, we also used a standard dose of topical corticosteroid on entry to the present study. However, after exclusion of the one outlier with an exaggerated response, the placebo arm treated with topical dexamethasone 0.1% over 3 months showed only a 3% decrease in CoNV. Within this group four eyes had a marked reduction in CoNV (range −25% to −58%), but the study design did not allow us to determine if this was a subgroup particularly sensitive to topical corticosteroid. Overall, our data suggest that topical corticosteroid may stabilise CoNV but that it induces regression in relatively few patients.

Gene signal 101 has also been evaluated in a clinical trial for the treatment for CoNV. It is an antisense oligonucleotide that inhibits the expression of the scaffold protein insulin receptor substrate 1.[16 27] The interim analysis of a multicentre randomised controlled trial showed that after 3 months topical dosing of 86 mg/day twice a day produced a significant regression of CoNV of −2.04% (SD 1.57) of total corneal area compared to a placebo group that showed an increase of +0.89% (SD 2.15). These results are comparable to our data with subconjunctival bevacizumab, which resulted in a mean reduction in neovascularisation of −1.93% (SD 3.87) compared with an increase in the placebo group of +1.47% (SD 4.96). Gene signal 101 drops were also reported to be well tolerated with few side effects, although 13 of 40 patients were excluded from the interim analysis because of poor image quality, non-compliance or other adverse events. The different mechanisms of action of these two treatments may mean they are complimentary in their inhibition of CoNV, but clinical studies would be required to confirm any synergy.

In conclusion, subconjunctival bevacizumab given monthly for three injections had few side effects and appeared to cause a regression of recent-onset CoNV that was greater than topical corticosteroid alone. Our results suggest that a definitive trial of at least 40 patients per treatment group would be required to confirm an effect. The heterogenous aetiology of the CoNV in these patients might introduce study bias, and a definitive trial should be stratified according to herpes simplex/zoster keratitis status. However, we recognise that CoNV is a surrogate

measurement for patient-based outcomes such as improvement in vision or quality of life. We anticipate that a substantially larger study would be required to demonstrate a benefit for these outcomes. At this tertiary referral centre it took 16 months to enrol the necessary 30 patients, so a multicentre study would be required to complete larger definitive studies within a reasonable time period. Further controlled trials and a cost analysis are required to direct management, but this pilot study will help other researchers design future trials and the results will facilitate meta-analysis.[28 29]

Contributors SJT, CP, WX and CB contributed to the concept and design of this study. CP, SJT, KSB, JKGD and RRA contributed to the collection of data and drafting of the manuscript. KSB and WX analysed the data and CP and SJT wrote the manuscript. CP and SJT had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Competing interests None.

Patient consent Obtained.

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Data sharing statement Original data are available from the corresponding author.

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