SUBCONJUNCTIVAL BEVACIZUMAB INJECTION IN THE SURGERY OF PRIMARY PTERYGIUM: COMPARISON WITH INTRAOPERATIVE MITOMYCIN-C

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ABSTRACT

Purpose: To assess the efficacy of subconjunctival bevacizumab injection as adjuvant therapy in the prevention of recurrence following the surgical treatment of pterygium by comparison with intraoperative mitomycin-C application.

Methods: This prospective comparative study included thirty consecutive patients with primary nasal pterygium that were surgically treated with simple excision. In addition to surgical excision, subconjunctival bevacizumab was injected in 15 eyes (group A), and, in the remaining 15 eyes, topical mitomycin-C 0.2 mg/mL was applied for 2 minutes (group B). The postoperative outcome was followed clinically for a minimum of 12 months. The main outcome criteria were recurrence rate and postoperative complications.

Results: A significantly higher recurrence rate was found in bevacizumab group (66.7%) compared to MMC group (26.7%) (P= 0.028). In group A, conjunctival wound separation was observed in 3 of 15 eyes; however, no conjunctival separation was observed in Group B and no other serious complications were observed in either group.

Conclusion: The intraoperative use of subconjunctival bevacizumab does not seem to be effective in reducing the rate of pterygium recurrence following excision when compared with intraoperative mitomycin-C application.

KEYWORDS

Pterygium, Bevacizumab, mitomycin-C, recurrence

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INTRODUCTION

Pterygium is a wing-shaped, fibrovascular tissue growing onto the cornea as an extension of the adjacent conjunctiva, generally from the nasal limbus. A conservative approach is required for silent, non-aggressive, stationary pterygia. The conditions that mainly substantiate surgical intervention are the invasion of the central cornea, excessive astigmatism, ocular discomfort, restriction of ocular motility, or unpleasant aesthetic appearance. The pathogenesis of pterygium is not well understood, but the most widely accepted etiologic factor is ultraviolet radiation.

Abnormal expression of tumor suppressor genes in pterygial epithelium suggests that mutations induced by ultraviolet radiation lead to limbal cell apoptosis and to an increase of growth factors as the earliest events in pterygium pathogenesis (1, 2, 3). Among the growth factors known to be involved in pterygium pathogenesis is VEGF, which is known to be produced in response to several stimuli, including ultraviolet radiation (2, 3).

The role for VEGF is well established in normal wound repair, because angiogenesis controlled mainly by VEGF is a key factor in the proliferative phase of wound healing by supplying oxygen and nutrients to support the process. VEGF is also required to facilitate the development of granulation tissue by means of increased vascular permeability that allows the deposition of the fibrinous substance and the migration of inflammatory cells.

The recurrence rate after primary pterygium surgery is unacceptably elevated following simple excision (4-7). Moreover, recurrent fibrovascular tissue is generally more difficult to manage than is the primary lesion. Therefore, several methods, such as postoperative β-irradiation, conjunctival autograft transplantation and intraoperative application of mitomycin-C (MMC), are used for the prevention of this fibrovascular re-growth (4-8).

Bevacizumab is a humanized antibody acting as an anti-VEGF agent that was the first U.S. Food and Drug Administration-approved anti-angiogenic drug for first- line treatment of metastatic colorectal cancer. In clinical practice, off-label use of bevacizumab has been a promising therapeutic option for treatment of ocular disorders related to neovascularization. Some studies demonstrated that bevacizumab is able to inhibit corneal neovascularization without major corneal side effects administered topically or subconjunctivally in both experimental rat model and humans but unlike MMC it has no effect on epithelial and endothelial cells (9, 10).

The purpose of this study was to investigate the effect of subconjunctival bevacizumab injection as an adjunct treatment to pterygium excision and also to evaluate its effectiveness by comparing its outcome with that of intraoperative MMC application.

MATERIALS AND METHODS

This prospective randomized study included 30 consecutive patients who underwent excision of pterygia at our ophthalmology department. Patients were eligible if they had primary pterygia crossing the limbus at least 1 mm over the cornea and not severely vascularized and congested.

A standardized classification for pterygia according to Rolando (60° Congress S.O.L. 2005) was used to define the cases. This numerical scoring system classifies the pterygia into 5 stages according to the following 5 criteria:

Stage 1 ⩽ 4 points; Stage 2: 5-8 points; Stage 3: 9-12 points;

Stage 4: 13-15 points, Stage 5: 16 points

1) Corneal invasion: Distance pterygium apex from the limbus over the cornea.
   Less than 1 mm: 1 point; 1-3 mm: 2 points;
   More than 3 mm: 3 points
   Invasion of the visual axe: 4 points

2) Limbal invasion: The width of pterygial tissue on the limbal area.
   Less than 2 mm: 1 point; 2-4mm: 2 points;
   More than 4 mm: 3 points

3) Vascularity of pterygial tissue:
   No vascularity but mild papillary reaction: 1 point; Mild vascularity: 2 points
   Moderate vascularity and congestion: 3 points;
   Severe vascularity and congestion: 4 points

Table 1: *Comparison of the two groups of patients.*

	Group A	*Group B*	*P value*
Age (years) mean ± SD (range)	51.1 ± 13.2 (31-75)	51.1 ± 10.5 (34-71)	.97(MWU)
Sex male / female	9/6	9/6	1.00 (CST)
Clinical Stage St2 / St3	6/9	7/8	.71 (CST)
Clinical Score mean ± SD (range)	8.8±1.5 (6-12)	8.9 ±1.6 (7-12)	.86 (MWU)
Follow-up time (months) mean ± SD	13.6 ± 4.1	14.1 ± 4.4	.69 (MWU)
Recurrence no./ total (%)	10/15 (66.7)	4/15 (26.7)	.028(FET)
Wound dehiscence no./ total (%)	3/15 (20)	0/15 (0)	.224(FET)

MWU: Mann-Whitney U test CST: Chi-square test FET: Fisher's exact test

4) Tissue thickness of pterygium:
   Flat pterygial tissue allowing the underlying vessels to be easily seen: 1 point
   Moderately elevated pterygium with barely discernible underlying vessels: 2 points;
   Severely raised pterygial tissue veiling completely the underlying vessels: 3 points

5) Tractional effect of pterygium:
   No traction: 0 point; Minimal traction of plica semilunare evident in lateral gaze: 1 points; Marked traction of plica semilunare evident in primary gaze position: 2 points

The patients were randomly allocated to two treatment groups. Group A comprised 15 eyes of 15 patients who underwent the procedure with subconjunctival bevacizumab injection and Group B 15 eyes of 15 patients who received intraoperative MMC application. All of the pterygia were falling into Stages 2 and 3.
Both groups were comparable regarding the preoperative criteria (Table 1).
All operations were performed by 1 author (KF). Clinical examinations were performed preoperatively and on postoperative days 1, 7, and 30 and then every 2 to 3 months at least for 12 months by a different ophthalmologist (MSK). Recurrence was defined as the postoperative growth of fibrovascular tissue over the clear cornea. Vascularization of the corneal stroma or conjunctival growth not extending over the cornea was not considered as recurrence.
The study was approved by our hospital's local ethics committee, and it followed the ethical principles of the Declaration of Helsinki. Written informed consent was taken from patients at least 24 hours prior to surgery.
A standardized surgical technique was performed for both groups. Anaesthesia was achieved by topical proxymetacaine hydrochloride drops with additional subconjunctival injection of 1% lidocaine into the pterygium body. The pterygium was dissected from the cornea with a 30° steel knife and excised with scissors. Minimal cauterization was applied only if bleeding was abundant and did not wane spontaneously. Our technique includes removal of just the pterygium's head and of the smallest area of the conjunctiva as possible, combined with excision of the subconjunctival fibrovascular tissue. The freed conjunctiva was closed with separate 8/0 polyglactin sutures to place both edges of the conjunctiva together and to cover the bare sclera but leave the limbal area uncovered.
In the first group, 1.25 mg/0.05mL bevacizumab was injected subconjunctivally on both edges (totally 0.1ml containing 2.5 mg bevacizumab), inflating minimally the sutured conjunctiva. In the second group, MMC (at a con-

centration of 0.2 mg/mL) was applied topically with a small sponge on the exposed sclera and on the underside of the conjunctiva for 2 minutes, followed by copiously irrigating the bare sclera with balanced salt solution.
Postoperative topical therapy included steroid drops (fluorometholone 0.1%) 4 times/day and antibiotic ointment (tobramycin 0.3%) 2 times/day for the first 2 weeks.
Non-steroidal anti-inflammatory drops (diclofenac 0.1%) and preservative-free artificial tear drops (hydroxypropyl methylcellulose 0.3%) were used for the following 4 weeks.
Statistical analysis was made by using the Fisher exact/Chi-square tests and Mann- Whitney tests to compare both groups. The statistical significance level was considered as $P < 0.05$.

RESULTS

In the bevacizumab group (mean follow-up time 13.6 ± 4.1 months, range 12-24 months), 10 of the 15 eyes (66.7%) had recurrence of the pterygium, and 3 of 15 eyes encountered wound dehiscence during the follow-up period probably caused by delayed healing (Table1). In 2 of these eyes with wider exposition of the sclera, a second surgical procedure was needed to reclose the conjunctival edges.
In the MMC group (mean follow-up time 14.1 ± 4.4 months, range 12-24 months), recurrence was observed in 4 of 15 eyes (26.7%) during the follow-up period. Punctate epithelial keratitis was observed in 2 of 15 eyes, but no wound dehiscence or severe complications such as scleral melting were encountered.
The rate of postoperative recurrence was significantly higher in the bevacizumab group than in the MMC group ($P = 0.028$). Even though there was a numerical difference in conjunctival dehiscence rates between both groups, it was not statistically significant ($P = 0.224$). (Table I)

All of the recurrences were observed between postoperative months 3 and 12. On the other hand, the average age of 14 patients with recurrence was 44.6±8.1 years whereas that of 16 patients without recurrence was 56.8±11.6. The statistical comparison between those average ages ($P=0.008$) showed a statistical significance indicating that the younger patients experience more recurrences than do the older patients.

DISCUSSION

VEGF has been detected in vascularized tissues of the normal eye 11, and it exists in healthy conjunctivas as well.(12) Increased levels of VEGF strongly suggest the involvement of VEGF in the pathogenesis of pterygia (12-16). Pterygium was assumed to derive from limbal tissue rather than conjunctiva, according to the increased VEGF levels in the limbus comparable to those in pterygial tissue, as opposed to normal VEGF levels in neighbouring conjunctiva (12).
Recurrence of primary pterygium is the most common and undesirable complication following simple excision. If the pterygium recurs, it is more likely within 12 months after its removal (17,18). In the present study, all recurrences were observed between postoperative months 3 and 12.
The simple excision technique has a high recurrence rate; therefore, some additional modalities and new techniques such as the use of MMC are sometimes preferred to reduce the rate of re-growth (5, 6). MMC inhibits the development of postoperative fibrosis and of new vessel growth on the wound.

Sight-threatening complications such as corneal decompensation, secondary glaucoma, scleral and corneal perforations as well as minor complications such as superficial punctate keratitis and conjunctival wound healing problems are possible with MMC use (4). The bare sclera technique with MMC application is widely used in the treatment of pterygia. Leaving the sclera uncovered at the end of surgery following intraoperative MMC application has been found to increase the risk of scleral melting, whereas the closure of the conjunctiva and the avoidance of high concentrations and long application time of MMC may prevent this complication. (19- 20) This was our reason for preferring to leave only the limbus exposed by closing the conjunctiva and for applying MMC for only 2 minutes in 0.2 mg/mL concentrations.
Several reports show that local bevacizumab with topical or subconjunctival use causes re-

gression of ocular surface neovascularisation and that it might prevent or decrease the recurrence of pterygia (21-24). Studies demonstrated that VEGF also plays a major role in the wound repair process; therefore, bevacizumab may be used to decrease scar formation in glaucoma filtration surgery (25, 26).
The dosage, the mode and the frequency of bevacizumab application in corneal vascularization are controversial. Bevacizumab of 1.25 mg is the dosage used for intravitreal injection that may be short-acting after subconjunctival injection because of the possible absorption by conjunctival and episcleral vessels that may also lead to systemic side-effects. Our bevacizumab dosage was 2.5 mg for each eye in Group A.

Razeghinejad et al. (27) used bevacizumab subconjunctivally as adjunctive to pterygium excision with a rotational flap and found that bevacizumab had no effect on recurrence rate or on postoperative complications compared with balanced salt solution. Their recurrence rate was much lower than that in our study, but, unlike our simple excision technique, a rotational flap technique was used that alone can decrease the recurrence rate even without adjuvant therapy use. Moreover the injection of bevacizumab was made to the inferior fornix. For this reason, their results are not comparable with ours. Furthermore, we compared bevacizumab with another adjuvant (MMC) that is widely used in pterygium treatment.
The limited number of our patients and single bevacizumab injection constitutes the weak points in our study. We suggest that postoperative continuation of bevacizumab in his topical form might be more effective in preventing the recurrence of pterygium than a single injection.

It has been reported that bevacizumab and anti-angiogenic agents may cause complications such as wound dehiscence because they may adversely affect the wound healing process (28, 29). In our study, in group A patients, who had intraoperative bevacizumab injection, conjunctival wound dehiscence was observed in 3 eyes, and the pterygium recurrence rate of 66.7% was significantly elevated compared with the recurrence rate (26.7%) of group B patients, who had intraoperative MMC application. The reason of this high recurrence rate that we observed with intraoperative bevacizumab was probably the lack of anti-mitotic effect on the fibroblastic and epithelial cells in contrast to MMC.

In conclusion, bevacizumab, an anti-VEGF monoclonal antibody, seems to have no adjunctive effect in reducing the rate of pterygium recurrence if injected subconjunctivally at the end of pterygium excision, compared with the intraoperative application of MMC.

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