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EC OPHTHALMOLOGY
Research Article

Mitomycin C Isolated or Anti-Vegf Associated? Bevacizumab Use as an Adjunct to Trabeculectomy

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Received: July 13, 2021; **Published:** November 16, 2021

Abstract

Objective: To compare the therapeutic response, intraocular pressure (IOP), bleb characteristics and possible surgical complications of fistulizing anti-glaucoma surgery between eyes with or without bevacizumab subconjunctival injection (Avastin®).

Materials and Methods: A non-randomized retrospective study in which 131 eyes (from 117 patients) with primary angle-closure glaucoma and primary open-angle glaucoma were enrolled. The conventional trabeculectomy was performed and they were divided into two groups, with and without bevacizumab subconjunctival injection. There was no preference for gender, race and age.

Results: Sixty-tree females and 68 males (51.9%) were included. The mean initial IOP was 22.67 mmHg (SD ± 8.55 [12 - 50]) and 11.14 (SD ± 6.79 [0 - 42], 11.13 (SD ± 5.51 [0 - 30]) and 10.81 (SD ± 5.08 [1 - 30]) mmHg on 1st, 2ndand 3rd DPO, respectively. In addition, there was no difference in the IOP on1st, 2nd and 3rd DPO between the groups with and without bevacizumab injection. Considering the groups with and without bevacizumab injection application, there was no difference on the variables flap suture lysis, bleb needling and use of post-op hypotensive topical medication on 2nd and 3rd DPO.

Conclusion: Subconjunctival administration of per-operative bevacizumab is safe and may be an effective adjuvant on trabeculectomies, especially in refractory and secondary glaucoma, reducing local angiogenesis and modulating the fibroblast activity, reducing the healing process and increasing the survival of filtration surgery.

Keywords: *Mitomycin C; Anti-Vegf; Bevacizumab; Trabeculectomy*

Introduction

Glaucoma is a progressive, multifactorial degenerative optic neuropathy, associated with optic nerve damage and its corresponding visual field loss [1]. The main risk factor related to the development and progression of glaucoma is the elevated intraocular pressure (IOP). Among the option treatments, the fistulizing anti-glaucoma surgery (trabeculectomy) is one of the most effective and is indicated for those with uncontrolled disease under treatment with laser therapy or topical medications [2]. According to Stalmans [3], 30% of surgical failure cases are secondary to scarring and the four major processes that contribute to post-operative conjunctival wound healing are clot formation, inflammation, angiogenesis and fibrosis.

The primary stimulus for angiogenesis is a deficiency of blood supply, resulting in tissue hypoxia and triggering factors that increase the expression of pro- angiogenic factors such as vascular endothelium growth factor (VEGF) and fibroblast growth factor (FGF), besides

inhibiting the occurrence of anti-angiogenic factors, including angiostatin [4]. Clinically, the persistence of inflammatory stimulus is associated with the increased healing response and induction of fibrosis, which occurs with the chronic use of topical medication for the treatment of glaucoma [5,6].

Angiogenesis is essential in the tissue repair process. The lack of healing process in the postoperative period is one of the major limitations of fistulizing anti- glaucoma surgery [4]. Accordingly, to Roshmi M and Keith B, the development of conjunctival and episcleral fibrosis occurs as a result of progressive fibroblast migration, collagen deposition and angiogenesis at the fistula site, demonstrating that maximum subconjunctival fibroblasts proliferation occurs between the 3rd and 5th day after surgery [7].

The healing process usually starts at the episcleral layer, with scarring of the scleral flap, thickening of the conjunctiva and the consequent failure of the filter tunnel [8]. As an alternative to reduce the risk of surgical failure and improve the effectiveness of trabeculectomy (TRAB), antimitotic agents, such as mitomycin C (MMC) and 5- fluorouracil (5-FU), became part of the current surgery. Unfortunately, their use is not totally safe, with some serious complications, such as cataract, choroidal effusion, hypotonia, endophthalmitis and toxicity of the corneal epithelium, among others [9-11].

Mitomycin C increases TRAB effectiveness when applied during surgery on patients with high risk of surgical failure. It is an antineoplastic agent, isolated from *Streptomyces caespitosus*, which acts by inhibiting the proliferation of fibroblasts in the surgical site. The recommended dose of MMC is from 0.3 mg/dl to 0.5 mg/dl at the subconjunctival space, applied for a period between two to five minutes [12].

The action of anti-VEGF compounds as modulators of scar tissue is based on the fact that VEGF is an angiogenesis mediator related to the granulation tissue formation [13,14]. Bevacizumab (Avastin®; Genentech Inc., San Francisco, California, USA) is a humanized monoclonal antibody against all isoforms of VEGF. It was FDA approved in February 2004 for intravenous administration for metastatic colorectal cancer treatment [4,15].

In 2005, the use of bevacizumab in ophthalmology was initiated as an intravitreal injection for retinal and choroidal disease treatment, enabling significant inhibition of neovascularization in these tissues and decreased vascular permeability [16,17]. Its use has been extended to prevent an excessive scarring and neovascularization at the TRAB site, maintaining the aqueous humor drainage through the fistula [18-20].

Objective of the Study

The objective of this study is to compare the therapeutic response, IOP, bleb characteristics and possible surgical complications of fistulizing anti-glaucoma surgery between eyes with or without bevacizumab subconjunctival injection (Avastin®).

Materials and Methods

This is a non-randomized retrospective study in which 131 eyes (from 117 patients) with primary angle-closure glaucoma and primary open-angle glaucoma were enrolled from August 2017 to November 2018. The conventional TRAB was performed and they were divided into two groups, with and without bevacizumab subconjunctival injection. There was no preference for gender, race and age.

The data analyzed were age, gender, topical hypotensive therapy, pre-op IOP, bevacizumab subconjunctival injection at a dose of 1.25 mg/0.1 ml, immediately after surgery, postoperative interventions and surgical complications.

Univariate and bivariate statistical analysis was used to analyze the quantitative data. The univariate test was used for frequency distribution, mean, median and dispersion (standard deviation). Parametric tests were performed to evaluate the differences between the bevacizumab variable (patients who received and patients who did not) and IOP variables. For the bevacizumab variable, which had only two categories, the Student's t test for independent samples was used to compare the means between categories.

Contingency tables were used to analyze the association between bevacizumab variable and clinical variables of interest. The chi-square of Fisher's test was adopted to test the statistical significance of the association between these variables. The survey data was analyzed through the statistical program Predictive Analytics Software (PASW 18). In all statistical tests, we considered a 5% significance level. Thus, statistically significant associations are considered those whose p value was less than 0.05.

All the surgeries were performed by the same surgeon (FK) using the same surgical technique, using per-op MMC in all eyes, in a period of 3 - 5 minutes.

Surgery steps

Caruncular anesthesia with lidocaine 2% without vasoconstrictor and neocaine 0.75%, silk 8.0 corneopexia, superior conjunctival fornix base opening, 3 x 2 mm rectangular scleral flap, application of soaked sponges with MMC 0.03%, washing with 10 ml balanced salt solution, accessory paracentesis, main incision at the level of the scleral spur, TRAB with scleral punch, peripheral iridectomy, two 10.0 nylon sutures at scleral flap, two lateral conjunctival suture, one central modified Peng-Khaw suture and eye occlusion.

The eyes were examined post-operatively (DPO) as follows: 1 day, 7 - 10 days, 15 - 20 days and 30 - 45 days. At each visit, we evaluated the IOP, bleb's appearance, the need of scleral flap suture lysis, needling, introduction of hypotensive topical medications and the presence of any complications.

Results

This section is divided in two parts: Initially the results shown are related to the distribution of frequencies and descriptive measures; subsequently the clinical variables are associated to the bevacizumab subconjunctival injection.

Descriptive analysis: General data

Sixty-three females and 68 males (51.9%) were included. Seventy-eight percent were under Timolol Maleate 0,5% eyedrops, 67% under Brimonidine tartrate and 73% under Prostaglandin Analog (Table 1). There was no difference between eyes with 51.1% being right and 48.9% being left eyes. Among all included eyes, 51.1% had a bevacizumab subconjunctival injection and MMC, while 48.9% had only MMC.

Variable		N	%
Gender	Men	68	51.9
	Female	63	48.1
Timolol Maleate 0.5%	No	29	22.1
	Yes	102	77.9
Brimonidine tartrate, Brinzolamide or Dorzolamide	No	43	32.8
	Yes	88	67.2
Prostaglandin analogues	No	35	26.7
	Yes	96	73.3
Operated eye	Right	67	51.1
	Left	64	48.9
Bevacizumab injection	No	64	48.9
	Yes	67	51.1
Total		131	100.0

Table 1: *Frequency distribution of 131 patients according to clinical characteristics.*

The mean age of patients was 62.77 (SD ± 12.17 [23 - 88]) years table 2.

Variable	Descriptive measures						
	Average	DP	Minimum	Maximum	1Q	Median	3Q
Age	62.77	12.17	23.00	88.00	56.00	64.00	71.00
Initial IOP	22.67	8.55	12.00	50.00	17.00	20.00	26.25
1DPO	11.14	6.79	0.00	42.00	7.00	10.00	14.00
2DPO	11.13	5.51	0.00	30.00	7.00	10.00	14.00
3DPO	10.81	5.08	1.00	30.00	8.00	10.00	13.00
PIO - HIGH	10.39	5.17	2.00	50.00	7.00	10.00	12.00

Table 2: *Descriptive measures of the total sample patients according to age and IOP.*

The mean initial IOPA was 22.67 mmHg (SD ± 8.55 [12 - 50]) and 11.14 (SD ± 6.79 [0 - 42], 11.13 (SD ± 5.51 [0 - 30]) and 10.81 (SD ± 5.08 [1 - 30]) mmHg on 1st, 2nd and 3rd DPO, respectively.

Bivariate analysis

Comparative analysis between the two groups, with and without bevacizumab injection application indicates no difference in IOP, as the p-value found was higher than 5% in both situations. In addition, there was no difference in the IOP on 1st, 2nd and 3rd DPO between the group with and without bevacizumab injection (Table 3).

Variable	Use of Avastin	N	Average	DP	P-v
Initial IOP	No	64	22.06	7.59	0.4
	Yes	66	23.26	9.40	
1DPO	No	64	11.61	7.53	0.4
	Yes	67	10.69	6.02	
2DPO	No	64	11.02	5.66	0.8
	Yes	66	11.24	5.41	
3DPO	No	62	10.66	5.20	0.7
	Yes	65	10.95	5.01	
PIO – HIGH	No	62	10.26	3.98	0.7
	Yes	65	10.51	6.12	

Table 3: *Frequency distribution of 66 patients using bevacizumab and 64 patients who did not use on IOP measures.*
Note: The significance probabilities (p) refer to the student t test for independent samples.

There was no difference between the groups with and without bevacizumab injection application, on scleral flap suture lysis, bleb needling and use of post-op hypotensive topical medications on 2nd and 3rd DPO (Table 4).

Clinical variables		Use of Bevacizumab				p--value
		No		Yes		
	N	%		N	%	
Suture lysis (2 DPO)	No	52	81.3%	60	90.9%	0.132
	Yes	12	18.8%	6	9.1%	
Needling (2 DPO)	No	64	100.0%	63	95.5%	0.244
	Yes	0	0%	3	4.5%	
Eye drops (2 DPO)	No	64	100.0%	64	97.0%	0.496
	Yes	0	0%	2	3.0%	
Suture lysis (3 DPO)	No	57	91.9%	62	95.4%	0.485
	Yes	5	8.1%	3	4.6%	
Needling (3 DPO)	No	61	98.4%	62	95.4%	0.619
	Yes	1	1.6%	3	4.6%	
Eye drops (3 DPO)	No	59	95.2%	57	87.7%	0.207
	Yes	3	4.8%	8	12.3%	
	Total	64	100%	66	100%	

Table 4: *Frequency distribution of 66 patients using bevacizumab and 64 patients who did not use bevacizumab on clinical characteristics.*
Note: p--value: descriptive level the chi--square test.

Discussion

The TRAB surgery is still the most effective method to reduce IOP in glaucoma patients, despite the introduction of many modern minimally invasive procedures [21]. Several surgical approaches have been developed and clinical trials conducted for greater success of glaucoma filtration surgeries such as the use of steroids (topical and systemic) and MMC, reducing inflammation and fibrosis [22,23].

It is well known that elevated VEGF levels in the aqueous humor of glaucoma patients, as well as elevated levels of other cytokines, may predispose towards an increased risk of scarring of the scleral flap and conjunctiva after TRAB [24]. VEGF antagonist, bevacizumab (Avastin®) has been used clinically to reduce vascularization, improving the outcome of fistulizing surgeries and increasing the patency of the filter tunnel with the formation of greater and diffuse blebs and reduction of local conjunctival vasculature [7].

The drugs injected into the subconjunctival space have two features: direct transscleral infiltration into the intraocular tissues and absorption by conjunctiva and lymphatic pathways. Ambati., *et al*. showed that IgG antibodies have a high scleral permeability. As bevacizumab is an IgG1 monoclonal antibody, it can reach the intraocular tissues via sclera when injected subconjunctivally. Thus, it was thought that subconjunctival injected drugs have short duration, requiring repeated injections [25].

Accordingly, to Bochmann., *et al*. the use of anti-VEGF at sub-Tenon space within per-op could not cover the main peak of the scar formation that occurs 2 - 3 weeks after surgery and suggested that topical application for 4 weeks would be better to avoid the cystic formation peak. Vascularized blebs are associated with poor prognosis, requiring modulation of fibroblast activity, though the use of antiangiogenic could be indicated [2].

Antimitotic agents like MMC and 5-FU, help in prevention of post-operative scar and increase the surgery survival, although they are associated with several complications, such as hypotonia, blebites, endophthalmitis and Seidel [18]. There are reports that the conventional TREC has less surgical success in eyes with flat blebs or in cases of poor prognosis of diseases such as neovascular glaucoma or uveitis, consequent of a more aggressive healing process associated with inflammation, adhesions or angiogenesis. Thus, bevacizumab could be effective and safe adjuvant for TREC in eyes with refractory glaucoma [26]. On the other hand, according to Kahook, there is no advantage of using subconjunctival or intravitreal injection of bevacizumab as a single therapy, but the bleb morphology tends to be better when its use is adjuvant with 5-FU or MMC [27].

In relation to bevacizumab use in filtering glaucoma surgery, several studies showed more encapsulated blebs with bevacizumab as compared with MMC, suggesting that MMC is more effective than Bevacizumab in the formation of diffuse blebs [21]. In Mathew and Barton study, the pre-operative IOP was 24.4 ± 7.1 mmHg, ranging from 22 to 44 mmHg and at 6 months of follow-up, the IOP was 11.6 ± 2.2 mmHg, ranging from 8 to 14 mm Hg [7]. Our clinical results are similar to the described by Kahook, where the IOP was 9 ± 1.22 mmHg only with MMC, compared to 10.80 ± 2.17 mm Hg with MMC and anti-VEGF injection. He also described the presence of diffuse blebs and lower degree of vascularization [24]. With respect to our study, we observed a pre-operative IOP of 22.67 ± 8.55 mmHg, and 10.39 ± 5.17 at the 3rd DPO, with no significant statistical differences when comparing MMC alone with MMC and anti-VEGF injection.

Few studies compare MMC use alone vs. MMC and bevacizumab use. Mitomycin C and anti-VEGF are associated with lower IOP, but in comparison with isolated anti-VEGF agents, antimetabolites are associated with greater IOP reduction. Another study, comparing the adjunctive use of anti-VEGF and MMC vs. MMC alone, observed no statistical significance on their findings [28]. One reason for this, is that antimetabolites are not selective to cell death and apoptosis in comparison to anti-VEGF. Thus, mitomycin C not only inhibits the proliferation of fibroblasts in the sclera and conjunctiva [28].

A weak point of our study is that the bevacizumab group had more refractory glaucoma cases, including eyes with intense pre-op hyperemia or secondary neovascular glaucomas and trauma. Considering the very close surgical outcome between the groups, we would suggest the occurrence of some benefits on bevacizumab use, resulting in greater probability of success. The purpose of antimetabolites

and anti-VEGF as an adjunct to TRAB is not only to control the IOP, but also to improve the surgical outcome, increase the surgery survival, decrease the bleb healing and local neovascularization [28].

Conclusion

Subconjunctival administration of per-operative bevacizumab is safe and may be an effective adjuvant on trabeculectomies, especially in refractory and secondary glaucoma, reducing local angiogenesis and modulating the fibroblast activity, reducing the healing process and increasing the survival of filtration surgery.

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Volume 12 Issue 12 December 2021