Original Article

Side Effects and Effectiveness of Subconjunctival Bevacizumab Injection in Patients with Corneal Neovascularization

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Purpose: To evaluate the side effects and effectiveness of subconjunctival bevacizumab injection in reducing corneal neovascularization

Material and Methods: Patients selected according to inclusion and exclusion criteria were randomly allocated to either group 1 (patients were given subconjunctival bevacizumab injection) and group 2 (patients were given subconjunctival sham injection), its effectiveness in the form of decrease in neovascular area and side effects were compared between the two groups.

Results: 41 patients were included in each group. There was no statistical significance between the 2 groups in effectiveness of the subconjunctival injection as well as in the complications except decrease in the fibrinogen level which was observed in group 1.

Conclusion: Subconjunctival bevacizumab injection failed to show significant improvement in either corneal neovascularisation or visual acuity.

The cornea has the only one of its kind feature of being normally avascular, but under pathologic conditions, vessels march into the cornea from the limbal vascular plexus. A wide range of insults, including infection, inflammation, ischemia, degeneration, trauma, and loss of the limbal stem cell barrier, can results in corneal neovascularization (NV)[1]. Even though corneal neovascularization can occasionally serve a advantageous role in the clearing of infections, wound healing, and arresting stromal melts,[2] its disadvantages are abundant. Corneal neovascularization leads to tissue scarring, edema, lipid deposition, and persistent inflammation that may significantly alter visual acuity[3]. Corneal neovascularization accompanies the most frequent causes of corneal infectious blindness[4,5]. Corneal neovascularization is also noteworthy in extended wear of hydrogel contact lenses[6,7]. Corneal neovascularization not only reduces visual acuity but it also results in the loss of the immune privilege of the cornea, thus, increasing the chances of failure after penetrating keratoplasty[8]. Preexisting corneal stromal blood vessels have been identified as strong risk factors for immune rejection after corneal transplantation[9,10].

Existing treatments for corneal neovascularization, includes medications (such as steroids or nonsteroidal anti-inflammatory agents), laser photocoagulation, fine-needle diathermy, photodynamic therapy, or restoration of the ocular surface with the use of conjunctival, limbal, or amniotic membrane transplantation, have established variable and largely limited clinical success[1]. The uneven efficacy and innumerable adverse effects (cataract, glaucoma, and increased risk of infection) of topical and systemic corticosteroids are well recognized, but none of these treatments particularly target the molecular mediators of angiogenesis[11]. Vascular endothelial growth factor (VEGF) has found to be a key mediator in the process of neovascularization[11]. The important role of VEGF in the pathophysiology of corneal neovascularization has been confirmed in experimental models of corneal neovascularization[12]. It has been revealed that VEGF is up regulated in inflamed and vascularized corneas in humans and animal models[13].

Vascular endothelial growth factor inhibitors, such as pegaptanib sodium, ranibizumab, and bevacizumab, are at present used for the treatment of neovascular age – related macular degeneration[14]. Lately, off-label use of topical as well as subconjunctival bevacizumab has also been well thought – out to be a new treatment modality for corneal neovascularization[15-18]. Bevacizumab was demonstrated to inhibit corneal neovascularization after chemical injury in an experimental rat model[15]. In humans, a small number of studies have shown that topical bevacizumab can reduce corneal neovascularization in a few patients with significant corneal neovascularization[16,17]. However,data regarding the efficacy and side effects of sunconjunctival bevacizumab is lacking.

The purpose of this study is to report the long-term (6-month) results of the side effects and, efficacy of treatment of clinically stable corneal neovascularization in patients using subconjunctival bevacizumab in a prospective, randomized clinical study.

MATERIAL AND METHODS

This was a randomized controlled study. Side effects / efficacy of subconjuctivally administered bevacizumab in subjects with corneal neovasculari-zation was observed. This study was conducted at Isra Postgraduate Institute of Ophthalmology and approved by the Ethical committee of Isra Postgraduate Institute of Ophthalmology. Patients, who were selected in the study, signed an informed consent before any intervention.

Patients with clinically stable superficial corneal NV that extended farther than 2mmfrom the limbus were selected. Patients older than 25 years, of either sex, were included. However, stable corneal neovascularization were only considered when there had no current or recent (less than 3 months) episode of corneal and ocular surface infection (bacterial, viral, fungal, or parasitic). Patients with history of ocular surgery such as keratoplasty, amniotic membrane transplantation or ocular surface reconstruction, were excluded. Similarly patients who had history of use of contact lens were also excluded. Also excluded were the patients having persistent corneal epithelial defects. Patients having age greater than 75 years, uncontrolled hypertension (defined as systolic blood pressure of > 150 mm Hg or diastolic blood pressure of > 90 mm Hg) were also excluded. History of a thromboembolic event (including myocardial infarction or cerebral vascular accident), Diabetes mellitus and renal or liver abnormalities were also among the exclusion criterias. Patients who had history of use of corticosteroid antithrombotic drugs or aspirin were not included. History of ocular or periocular malignancy, pregnancy or lactation were also among the exclusion criteria's for the study.

A written consent was taken from all the participants of the study. A subconjunctival injection of bevacizumab (0.2 ml of 1.25 mg / 0.05 ml) solution was formulated and aseptically prepared from commercially available intravenous bevacizumab (Avastin; Genentech Inc, San Francisco, California). Patients were randomly divided into 2 groups, group A (patients will be injected with subconjunctival Bevacizumab injection) and group B (patients in this group will be injected with sham / normal saline subconjunctival injection). Injections were given at only single site, which was selected as the site of entry of main vessel into the cornea, in cases of diffuse vascularization the area of most dense neovessels was selected. Only patients with superficial neovascularization were selected. Patients included had corneal neovascularization secondary to infective, noninfective keratitis, corneal degeneration and trauma / foreign body. Causative agents in most of the cases were microbes including bacteria and fungai, it also included trauma such as chemical burn, or instrumental injury.

Follow-up visits were scheduled after 1week, after first month, after 3 months and after 6 months. On every visit, comprehensive eye examinations were done including digital corneal photographs. Blood pressure measurements were obtained in all visits. Fibrinogen level, platelet count, Prothrombin time (PT), and APTT were tested in blood at baseline (before injection) and at the 6th month follow up.

MAIN OUTCOME MEASURES

Ocular complications

Ocular side effects were monitored. All side effects (ocular and systemic) were recorded throughout the study. Ocular adverse events were identified by eye examination, visual acuity testing, intraocular pressure, biomicroscopy and corneal fluorescein staining. Systemic side effects were identified by physical examination, blood pressure recordings, and blood tests of fibrinogen level and platelet count.

Efficacy

The primary efficacy variables were the size of neovascular area. By comparing baseline corneal

photographs with follow-up photographs, the efficacy of bevacizumab in treatment of corneal NV was evaluated. Matlab software was used to exactly compare the photographs. Other efficacy variables such as the changes in best-corrected visual acuity were also recorded.

Statistical analysis was done using SPSS version 17. Paired t test was used to compare baseline and 6th month recordings.

RESULTS

82 Patients were included in the study, 41 were in the group A (Bevacizumab) while rest of the 41 were in the group B (Sham). Out of these 82 patients, 62 (76.5%) were males while 20 (23.5%) were females. They were between 45 years of age to 59 years of age with mean age of 53.23 years. (Out of 41 patients, although) patients had decrease in the neovascular area in group A (decreased area was considered when there was decrease in area in mm), compared to only 1 in group B, but this difference is not significant statistically ($p > 0.05$), (Table 1). Similarly, 2 patients had improvement in visual acuity in group A, compared to only 1 in group B, but this difference is also not significant statistically ($p > 0.05$), (Table 2). (visual improvement was considered when single line improvement was seen after the treatment in visual acuity) The only ocular complication observed was subconjunctival hemorrhage, which was almost equally observed in both groups, showing that it is injection related rather than drug related (Table 3). Among the systemic changes observed among the 2 groups, only serum fibrinogen level was decreased in group A ($p = 0.003$) after the injection. The systemic features observed among the 2 groups before and after the injection are shown in (Table 4).

Table 1: Efficacy of subconjuctival bevacizumab injection. (Comparison between baseline, before injection, and after 6 months)

	Reduction in neovas-cular area	No reduction in neovascu-lar area	Increase in neovascular area	Total
Group A (Bevacizu-mab group)	3	34	4	41
Group B (Sham group)	1	34	6	41

N = 41 (In each group)

Table 2: Best corrected visual acuity (Comparison between baseline, before injection, and after 6 months)

	Improveme nt in best corrected visual acuity	No improve-ment in best corrected visual acuity	Decrease in best corrected visual acuity	Total
Group A (Bevacizu-mab group)	2	38	1	41
Group B (Sham group)	1	39	1	41

Table 3: Ocular complications

Complication	Group A (Bevacizumab)	Group B (Sham)
Subconjunctival Hemmorhage	05	06

Table 4: Sytemic complications

		Group A (Bevacizumab)		Group B (Sham)	
Fibrinogen level	Before the injection	286.88mg /dl	P= 0.003	256.48mg/ dl	P>0.05
	After 6 months	255.01 mg/dl		255.01 mg/dl	
Systolic blood pressure	Before the injection	142.22	P>0.05	134.34	P>0.05
	After 6 months	146.00		133.20	
Diastolic blood pressure	Before the injection	88.89	P>0.05	89.32	P>0.05
	After 6 months	91.23		90.13	
Platelet count	Before the injection	234500.00	P>0.05	233450.00	P>0.05
	After 6 months	228875. 00		233875.0 0	

DISCUSSION

Newly or already formed corneal neovascularisation increases the risk of subsequent graft rejection after corneal transplantation as well.[19] Medical and surgical therapies used to diminish corneal neovascularisation include corticosteroids, nonsteroidal anti-inflammatory agents, laser photocoagulation, and needle diathermy[20]. Many of these therapies have not only confirmed limited success but multiple side effects have also been repoerted. Anti-VEGF therapy targeting corneal neovascularisation has recently

showed triumphant results in animal experiments. In rat models, topical bevacizumab (4 mg/mL) applied twice daily for 1 week reduced chemically induced corneal neovascularisation[21]. Anti-VEGF antibody entrenched in neovascularized corneal stroma suppressed corneal NV[22]. Such findings only indicate the potential of anti-VEGF in controlling neovascularisation. Vascular endothelial growth factor affects the metabolism,[23] and may result in changes related with vascularization through intact tissue layers. It upregulates platelet activating factor, increases plasminogen activator gene expression in corneal epithelium[24-26]. Plasminogen activator has a role in cell migration, cell adhesion, and tissue remodeling, it thus plays a key role wound healing and revival[27,28]. VEGF also increases fibrinolytic activity of endothelial cells in fibrin matrices with the involvement of VEGF receptor-2, tissue type plasminogen activator, and matrix metalloproteinases[28].

Many studies have shown that anti-VEGF is effective in suppressing new vessel formation and vascular leakage, which can improve visual function, but in this study we have not observed any significant improvement in either neovascularization or visual acuity. Anti-VEGF therapy is considered as a possible tool for controlling neovascularisation in many clinical fields, but our study showed no usefulness in corneal neovascularisation.

Although, fibrinogen level decreased after the injection, but no other systemic or ocular drug related complication was seen in our study, which shows that it is a safe drug.

The main limitation of our study was that it was conducted in a single centre rather than multiple centers and patients of similar racial background were included in the study.

CONCLUSION

Subconjunctival Bevacizumab in our study failed to prove its effectiveness in reducing the neovascular area in cases of corneal neovascularization when it was injected subconjunctivally.

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REFERENCE

1. **Chang JH, Gabison EE, Kato T, et al.** Corneal neovascularization.CurrOpinOphthalmol. 2001; 12: 242-9.
2. **Conn H, Berman M, Kenyon K, et al.** Stromal vascularization prevents corneal ulceration. Invest Ophthalmol Vis Sci. 1980; 19: 362-70.
3. **Epstein RJ, Stulting RD, Hendricks RL, et al.** Corneal neovascularization: pathogenesis and inhibition. Cornea. 1987; 6: 250-7.
4. **Liesegang TJ.** Epidemiology of ocular herpes simplex: natural history in Rochester, Minn, 1950 through 1982.Arch Ophthalmol. 1989; 107: 1160-5.
5. **Resnikoff S, Pascolini D, Etya'ale D, et al.** Global data on visual impairment inthe year 2002. Bull World Health Organ. 2004; 82: 844-51.
6. **Lamer L.** Extended wear contact lenses for myopes: a follow-up study of 400cases. Ophthalmology. 1983; 90: 156-61.
7. **Stark WJ, Martin NF.** Extended-wear contact lenses for myopic correction. ArchOphthalmol. 1981; 99: 1963-6.
8. **Dana MR, Streilein JW.** Loss and restoration of immune privilege in eyes with corneal neovascularization. Invest Ophthalmol Vis Sci. 1996; 37: 2485-94.
9. **Maguire MG, Stark WJ, Gottsch JD, et al.** Collaborative Corneal Transplantation Studies Research Group. Risk factors for corneal graft failure and rejection in the collaborative corneal transplantation studies. Ophthalmology 1994; 101: 1536-47.
10. **Williams KA, Roder D, Esterman A, et al.** Factors predictive of corneal graft survival: report from the Australian Corneal Graft Registry. Ophthalmology. 1992; 99: 403-14.
11. **Folkman J.** Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med. 1995; 1: 27-31.
12. **Amano S, Rohan R, Kuroki M, et al.** Requirement for vascular endothelial growth factor in wound- and inflammation-related corneal neovascularization. Invest Ophthalmol Vis Sci. 1998; 39: 18-22.
13. **Philipp W, Speicher L, Humpel C.** Expression of vascular endothelial growth factor and its receptors in inflamed and vascularized human corneas. Invest Ophthalmol Vis Sci. 2000; 41: 2514-22.
14. **Kim TI, Kim SW, Kim S, et al.** Inhibition of experimental corneal neovascularization by using subconjunctival injection of bevacizumab (Avastin). Cornea. 2008; 27: 349-52.

15. **Manzano RP, Peyman GA, Khan P, et al.** Inhibition of experimental corneal neovascularisationby bevacizumab (Avastin). Br J Ophthalmol. 2007; 91: 804-7.
16. **Kim SW, Ha BJ, Kim EK, et al.** The effect of topical bevacizumab on corneal neovascularization. Ophthalmology 2008; 115: 33-8.
17. **DeStafeno JJ, Kim T.** Topical bevacizumab therapy for corneal neovascularization. Arch Ophthalmol. 2007; 125: 834-6.
18. **Bahar I, Kaiserman I, McAllum P, et al.** Subconjunctival bevacizumabinjection for corneal neovascularization. Cornea. 2008; 27: 142-7.
19. **Volker-Dieben HJ, D'Amaro J, Kok-van Alphen CC.** Hierarchyof prognostic factors for corneal allograft survival. Aust NZ J Ophthalmol. 1987; 15: 11-8.
20. **Chang JH, Gabison EE, Kato T, et al.** Corneal neovascularization. CurrOpin Ophthalmol. 2001; 12: 242-9.
21. **Manzano RP, Peyman GA, Khan P, et al.** Inhibition of experimental corneal neovascularisation by bevacizumab (Avastin). Br J Ophthalmol. 2007; 91: 804-7.
22. **Amano S, Rohan R, Kuroki M, et al.** Requirement for vascularendothelial growth factor in wound and inflammation-relatedcorneal neovascularization. Invest Ophthalmol Vis Sci 1998; 39: 18-22.
23. **Wu Y, Zhang Q, Ann DK, et al.** Increased vascular endothelial growth factor may account for elevated level of plasminogenactivator inhibitor-1 via activating ERK1/2 in keloid fibroblasts. Am J Physiol Cell Physiol. 2004; 286: 905-12.
24. **Bernatchez PN, Allen BG, Gélinas DS, et al.** Regulation of VEGF-induced endothelial cell PAF synthesis: role of p42/44MAPK, p38 MAPK and PI3K pathways. Br J Pharmacol. 2001; 134: 1253-62.
25. **Tao Y, Bazan HE, Bazan NG.** Platelet-activating factor enhances urokinase – type plasminogen activator gene expression in corneal epithelium. Invest Ophthalmol Vis Sci. 1996; 37: 2037-46.
26. **Watanabe M, Yano W, Kondo S, et al.** Up-regulation of urokinase – type plasminogen activator in corneal epithelial cells induced by wounding. Invest Ophthalmol Vis Sci. 2003; 44: 3332-8.
27. **Irigoyen JP, Muñoz-Cánoves P, Montero L, et al.** The plasminogen activator system: biology and regulation. Cell MolLife Sci. 1999; 56: 104-32.
28. **Blasi F.** Proteolysis, cell adhesion, chemotaxis, and invasiveness are regulated by the u-PA-u-PAR-PAI-1 system. Thromb Haemost. 1999; 82: 298-304.