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THE EFFECT OF TOPICAL BEVACIZUMAB ON CORNEAL NEOVASULARIZATION IN A RABBIT MODEL

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Abstract:

Objectives: To evaluate the effect of topical bevacizumab for treatment of the corneal neovascularization (CNV) in a rabbit model of corneal injury.

Methods: Corneal neovascularization was induced by 3 sutures of the cornea in 20 rabbits (20 corneas). Two weeks later all sutures have been removed then rabbits were divided randomly into 2 groups: group 1 received topical bevacizumab at 10 mg/mL and group 2 received only topical normal salin drops as control group, in the right eyes three times a day for two weeks. Photographs of (CNV) were obtained before drug administration and at 1 and 2 weeks after therapy. The images were analyzed using NIH Image J 1.49c software.

Results: The mean percentage of CNV area estimated as 100 % before treatment. At the 1 week after treatment, the mean percentage of neovascularization area in Bevacizumab and Salin group were 75.64±4.32 and 93.33±4.57 respectively. Also at the 2 week after treatment were 61.45±6.18 and 84.96±5.21 respectively. After one and two weeks treatment, the neovascularization area in Bevacizumab group was regressed more than salin group significantly (P<0.0001).

Conclusions: Topical administration of Bevacizumab reduces corneal neovascularization in the short term,so it can be used for treatment of corneal neovascularization.

Key words: *Corneal Neovascularization, Bevacizumab, Topical.*

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INTRODUCTION:

Corneal neovascularization (CNV) is a common consequence of various inflammatory, infectious, and traumatic corneal disorders [1]. Neovascularization (NV) induces tissue scarring, lipid deposition, stromal hemorrhage, and corneal edema, all of which severely alter visual acuity [2]. In addition, vascularity reduces the immune privilege of the cornea and the likelihood of graft survival in patients who subsequently elect to undergo penetrating keratoplasty [3].

Angiogenesis is mediated by several different factors, including vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). VEGF is a homodimeric glycoprotein,heparin-binding growth factor specific to vascular endothelial cells, commonly considered the most prominent angiogenic factor. Within the VEGF family, VEGF-A is considered to be the major factor involved in hemangiogenesis and has received the most attention as the mediator of pathologic NV [2–5]. VEGF and its tyrosine kinase receptors, VEGF Receptor1 and VEGF Receptor2, promote many aspects of the angiogenic process[4-8, 22].

Bevacizumab is a recombinant humanized monoclonal immunoglobulin G1 antibody directed against all isoforms of VEGF-A. It has been used in the off-label treatment of exudative age-related macular degeneration, proliferative diabetic retinopathy, and iris rubeosis.[2,10]. Topical and subconjunctival routes of bevacizumab administration have been
investigated in experimental models and in human clinical cases examining the treatment of CNV.The majority of experimental and clinical studies have shown a statistically significant, but incomplete,reduction in the parameters reflecting NV [4-8, 35].
So the purpose of the present study was to investigate the anti-angiogenic effects of topical administration bevacizumab in experimentally induced CNV in a rabbit model.

MATERIALS AND METHODS:

In this study, 20 male wild brown rabbits, weighing 1500 to 1900 g, were used. The protocol for this experimental study was approved by the Institutional Animal Care and Use Committee of Ahvaz Jundishapur University of Medical Sciences. Animal maintenance and all in vivo experiments were performed in accordance with the institutional guidelines and the Association for Research in Vision and Ophthalmology (ARVO) Statement.
The animals were anesthetized by IM injections of tile amine (2.5 mg/kg body weight), zolazepam (2.5 mg/kg), and xylazine (3.45 mg/kg) if needed. After the application of topical tetracain, Three 7-0 silk sutures were placed radially, at midstromal depth, at the 10-, 12-, and 2-o'clock positions on the corneas of the right eyes, avoiding corneal perforation. Topical ciprofloxacin was instilled twice a day to minimize the risk of infection after surgery. Corneal sutures were removed 2 weeks after suture placement. After suture removal, the 20 rabbits were divided into 2 groups, with 10 rabbits in each group. In groups 1 and 2, the right eyes received topical applications of bevacizumab (10 mg/mL), and saline respectively. The solutions were administered three times a day for 2 weeks, starting immediately after suture removal. The concentrations of topical bevacizumab were chosen from previous studies [14,16].

All treated and control eyes were photographed using a charge-coupled device (CCD) camera attached to a slit-lamp bio microscope at ×40 magnification. Photographs were obtained before drug administration and at 1 and 2 weeks after therapy.

The images were analyzed using NIH Image J 1.49 software. The resolution of each image was 640 · 480 pixels. All images were converted to tagged information file format (TIFF) files. The quantification of NV throughout the entire cornea was performed in a blinded fashion to minimize sampling bias. The area of corneal vasculature was outlined with the computer mouse and calculated using the image software. To control for individual variation in the area of NV induced by the suture, the area before anti-neovascular treatment was set at 100%, and post treatment area values were presented as the percentage of the remaining NV. This approach to measurement is consistent with that described previously [38-45].

Statistical analyses were performed using SPSS software version 21.0 for Windows.

The Mann–Whitney U test was used to compare between administrations of two drugs. Differences were considered statistically significant when P values were less than 0.05.

RESULTS:

Bio microscopic examination of rabbit´s eyes at one and two weeks after the initiation of treatment revealed that corneal neovascularization in eyes that received bevacizumab had regressed more than those received saline (Figure 1).

The mean percentage of corneal neovascularization area estimated as 100 % before treatment. At the 1 week after treatment, the mean percentage of

neovascularization area in bevacizumab and saline group were 75.64±4.32 and 93.33±4.57 respectively. Also at the 2 week after treatment were 61.45 ± 6.18 and 84.96 ± 5.21 respectively (Table-1). After 1 week treatment, the neovascularization area in bevacizumab group was regressed more than saline group significantly (P<0.0001) (Figure-2). After 2 week treatment, the neovascularization area in bevacizumab groups was regressed more than salin group significantly (P<0.0001) (Figure-2). Figure-3 showed the changes of corneal neovascularization area in two weeks treatment. The mean percentage of changes of corneal neovascularization area in bevacizumab and saline groups were 38.55±6.18 and 15.04±5.21 respectively (Table-2).

The mean percentage of regression of corneal neovascularization area in bevacizumab group were different to saline group significantly (P<0.0001) (Figure-3).

Table 1: Comparison between two groups

Group	After 1 week	After 2 weeks
Bevacizumab	75.64±4.32	61.45±6.18
Normal Saline	93.33±4.57	84.96±5.21

Table 2: Comparison between two groups

Group	Mean changes
Bevacizumab	38.55±6.18
Normal Saline	15.04±5.21

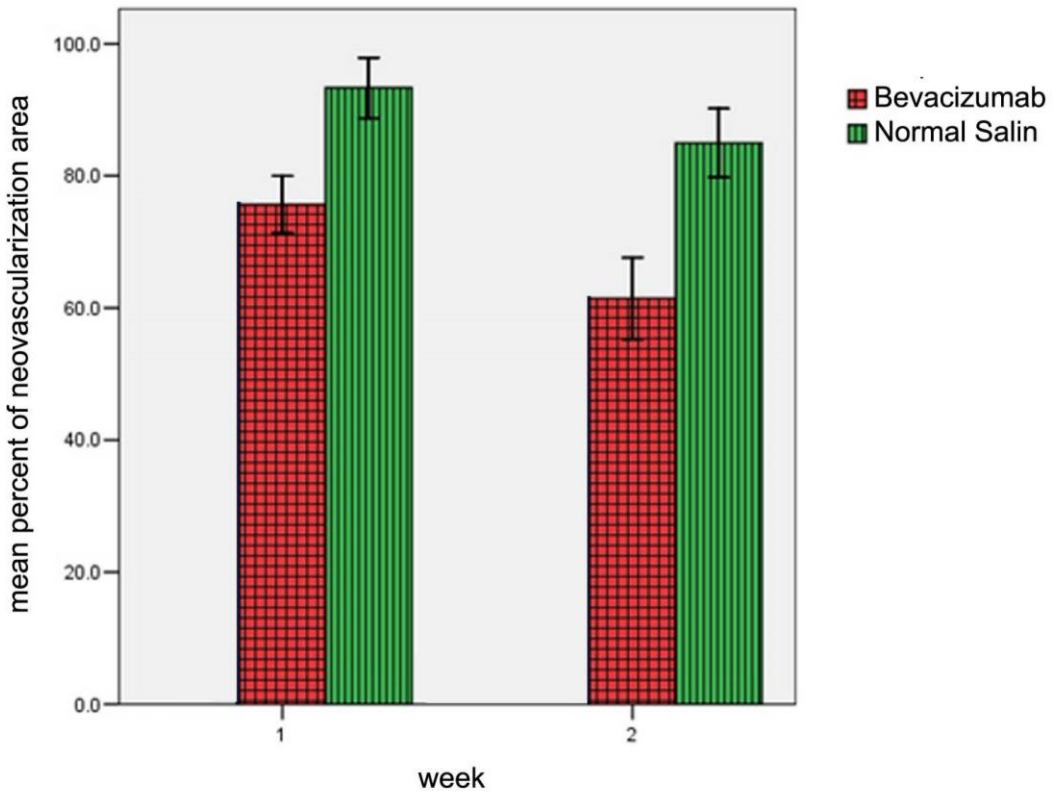

Fig 1: Comparison the mean±SD between two groups after 1 & 2 weeks

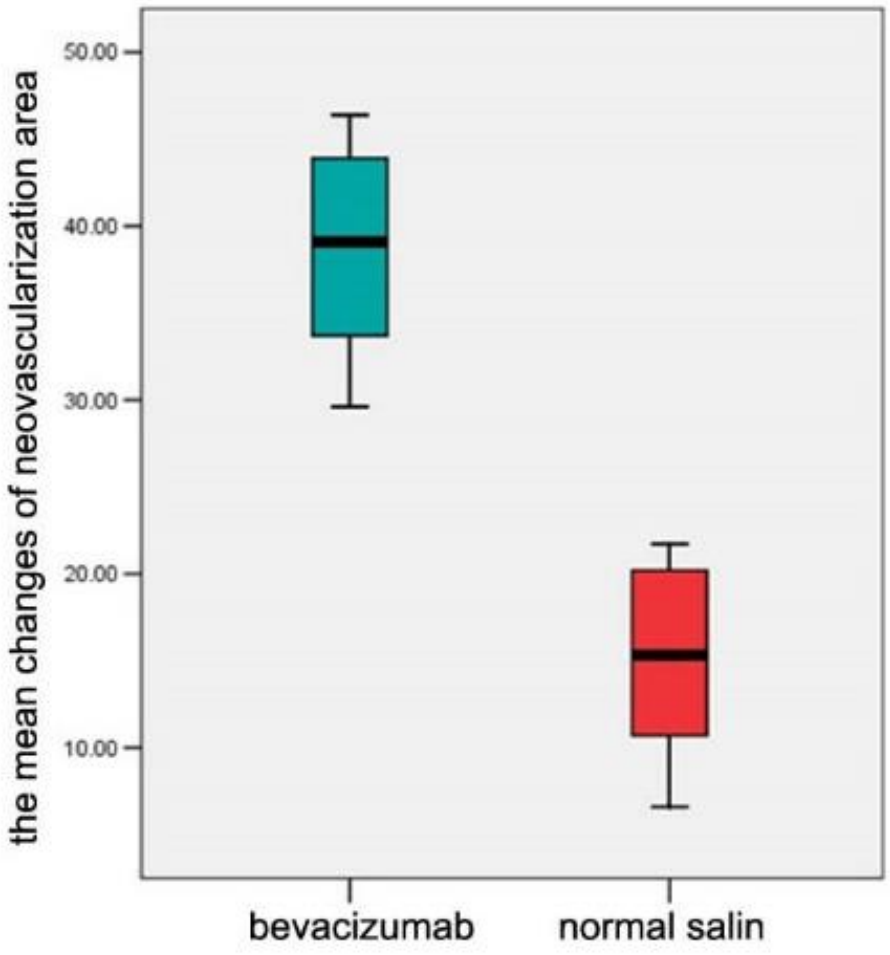

Fig 2: Comparison the mean±SD between two groups after 2 weeks

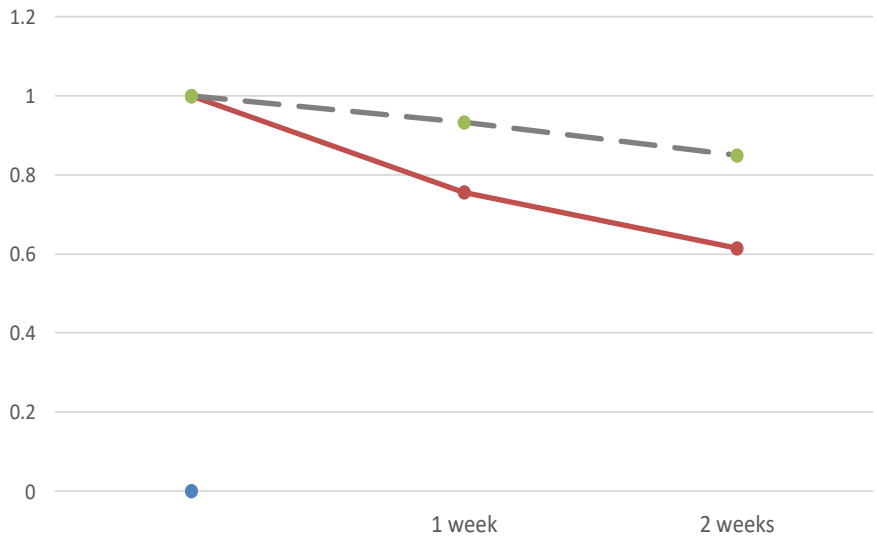

Fig 3: Comparison the regression of CN between two groups

DISCUSSION:

The treatment of CNV can be challenging and problematic.[4-8]. Various antiangiogenic therapy strategies have been used to interfere with the VEGF system. At the present time, the clinical focus in the treatment of CNV involves the use of antibodies to VEGF.22 several studies have demonstrated the effect of topical bevacizumab in the inhibition of CNV, with figures ranging 20% to79.7% in reports describing animal experiments [4-8].

Kim TI et al in 2008 have reported subconjunctival injection of bevacizumab can inhibit experimental corneal neovascularization significantly [44]. Hashemian et al in 2011 and Oner V et al in 2012 have reported nonsignificant difference statistically between topical and subconjunctival bevacizumab for corneal neovascularization in an experimental rat model but both of them were effective [(39,40]. In our study topical bevacizumab has been evaluated and it was effective but subconjunctival bevacizumab injection not performed. Kim J et al in 2013 have shown topically administered bevacizumab had longer standing anti-angiogenic effect than subconjunctivally injected bevacizumab in rat corneal neovacularization. They reported observations of epitheliopathy and corneal thinning after topical bevacizumab. These adverse effects generally appeared during the second month of treatment. On the contrary, in the current study, no instance of epitheliopathy or corneal thinning was observed. This may be because the study lasted for only 2 weeks, which may be too short a period to allow for the development of epitheliopathy.[41].

The results of our experiments demonstrated that regression of corneal neovascularization area in topical bevacizumab group was different to saline group significantly, 2.56-fold after 2 weeks.

CONCLUSIONS:

Our findings strengthen the hypothesis that anti-VEGF can efficiently counteract neovascularization and show topical bevacizumab can regress corneal neovascularization in short term period. Our study suggest that topical eye application of bevacizumab can represent an alternative delivery route to subconjunctival injection thus avoiding the risk of associated complications and side effects that could make this drug unsafe in long term treatment.. However, the evaluation of multiple doses of topical bevacizumab and the efficacy and side effects of long term treatment for corneal neovascularization needs more investigations. The limitations of our study include the short follow-up period and the lack of information about the biocompatibility of topical bevacizumab. Further trials with longer periods of follow-up will be necessary. Further studies of the optimal dosage, treatment interval, and duration are also recommended.

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