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# COMPARISON OF TOPICALLY ADMINISTERED BEVACIZUMAB AND PROPRANOLOL IN TREATMENT OF CORNEAL NEOVASCULARIZATION IN RABBITS

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**Abstract:**

***Objective:*** *To compare the effect of topical bevacizumab with topical propranolol 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 30 rabbits (30 corneas). Two weeks after neovascularization all sutures were removed then rabbits were randomly divided into 3 groups: Group 1 received topical propranolol (10 mg/mL), group 2 received topical bevacizumab (10 mg/mL), and group 3 received only topical normal saline drops as the control group, in the right eye three times a day for two weeks. Photographs of CNV were obtained before drug administration and at 1 and 2 weeks after intervention. The images were analyzed using the NIH ImageJ software (version 1.37c).*

***Results:*** *The mean percentage of CNV area was considered as 100 % before the treatment. At the 1-week and, 2 week intervals post treatment the mean percentage of CNV area in Propranolol, Bevacizumab and saline groups were 78.01± 4.16, 75.64± 4.32 and 93.33± 4.57 and 65.72± 4.15, 61.45± 6.18 and 84.96± 5.21, respectively. After one and two weeks treatment, the neovascularization area in Propranolol and Bevacizumab groups was regressed more than saline group significantly (P<0.0001). But there was no significant difference between Propranolol and Bevacizumab groups (P=0.315, P= 0.123)*

***Conclusion:*** *Topical administration of propranolol reduces corneal neovascularization in the short term, as topical bevacizumab does but the efficacy of long term treatment needs more investigations.*

**Key Words:** *Corneal Neovascularization, Propranolol, Bevacizumab, Topical*

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## INTRODUCTION:

Corneal neovascularization (CNV) is a common consequence of several 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. VEGF-A belongs to the VEGF family and plays important role in the hemangiogenesis process and has received the most attention as the mediator of pathologic NV(2-5). VEGF and its tyrosine kinase receptors, VEGF Receptor1 and VEGF Receptor(2), promote many aspects of the angiogenic process(4-9).

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, 11). Propranolol is a nonselective beta-adrenergic receptor blocker drug. Several studies have demonstrated that beta-adrenergic system is one of the major triggering factors that increases VEGF production. Therefore, beta-blockers can reduce VEGF production and subsequently regression of NV(12, 13). Propranolol has no effect on normal level of VEGF that its mechanism remained largely unknown(12). Recent studies have shown propranolol can reduce VEGF production in oxygen induced retinopathy (12, 14, 15). Now, there is no evidence of propranolol effect on CNV except only one study in 2014 that reported non-significant effect(13).

Considering the aforementioned findings and the existed scientific gap in this regard, the present study was aimed to comparatively investigate the anti-angiogenic effects of topical administration of propranolol and bevacizumab in experimentally induced CNV model in rabbits.

## MATERIALS AND METHODS:

In this study, 30 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 School. 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 intramuscular (IM) injections of Tiletamine (dosage of 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 silk sutures (size: 7-0) were placed radially, at mid-stromal depth, at the 10-, 12-, and 2-o'clock positions on the corneas of the right eye, 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, 30 rabbits were randomly divided into 3 groups, with 10 rabbits in each group. In the groups 1, 2, and 3, the right eye received topical applications of Propranolol (10 mg/mL), 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 and Propranolol were chosen from previous studies(13, 14).

All treated and control eyes were photographed using a charge-coupled device (CCD) camera attached to a slit-lamp bio-microscope at ×32 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 J 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 measurement approach was adopted from the method described in previous studies (16-23).

Statistical analyses were performed using

statistical package SPSS (IBM, version 21.0 windows). The Mann–Whitney U test was used for

comparisons between administrations of two drugs. Differences were considered statistically significant when P values were less than 0.05.

**RESULTS:**

Biomicroscopic assessments of the rabbits' eyes at one and two weeks after the initiation of treatment revealed that corneal neovascularization in the eyes receiving Bevacizumab and Propranolol had regressed more than those animals received saline (Figure 1).

Bevacizumab – treated eyes showed the greatest degree of regression (Table 1 and 2). The mean percentage of corneal neovascularization area estimated as 100 % before treatment. After 1 week post-treatment, the mean percentage of neovascularization area in Propranolol, Bevacizumab, and Saline group were 78.01 ± 4.16, 75.64 ± 4.32, and 93.33 ± 4.57, respectively. Moreover, following 2 weeks after treatment the figures were respectively 65.72 ± 4.15, 61.45 ± 6.18 and 84.96 ± 5.21 respectively (Table 1).

After 1 week treatment, the neovascularization area in Propranolol and Bevacizumab groups was regressed more than saline group significantly (P<0.0001). However, there was no significant difference between Propranolol and Bevacizumab groups (P=0.315) (Figure 2).

After 2 week treatment, the neovascularization area in Propranolol and Bevacizumab groups was regressed more than saline group significantly (P<0.0001). However, there was no significant difference between the Propranolol and Bevacizumab groups (P=0.123) (Figure 2).

Figure 3 shows the changes of corneal neovascularization area after two weeks treatment.

The mean percentage of changes of corneal neovascularization area in Propranolol, Bevacizumab and Saline groups were 34.28 ± 4.15, 38.55 ± 6.18 and 15.04 ± 5.21 respectively (Table 2).

The mean percentage of regression of corneal neovascularization area in Propranolol, Bevacizumab groups were different to saline group significantly (P<0.0001). However, there was no difference between the Propranolol and Bevacizumab groups (P=0.384) (Figure 4).

**Table 1. Comparison between three groups**

| group | After 1 week | After 2 weeks |
|---|---|---|
| Propranolol | 78.01 ± 4.16 | 65.72 ± 4.15 |
| Bevacizumab | 75.64 ± 4.32 | 61.45 ± 6.18 |
| Normal Salin | 93.33 ± 4.57 | 84.96 ± 5.21 |

**Table 2. Comparison between three groups**

| group | Mean changes |
|---|---|
| Propranolol | 34.28 ± 4.15 |
| Bevacizumab | 38.55 ± 6.18 |
| Normal Salin | 15.04 ± 5.21 |

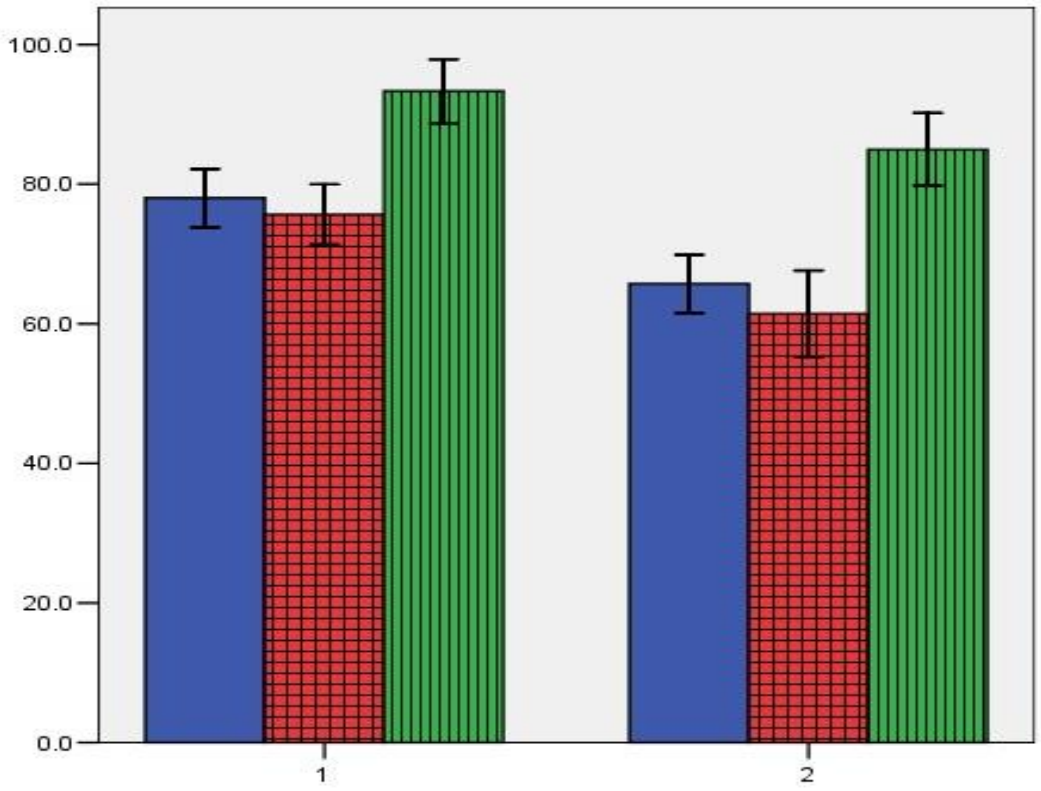


**Fig 1. Comparison of the average corneal neovascularization (CNV) area between three groups after 1 & 2 weeks. Values are presented as mean ± SD**

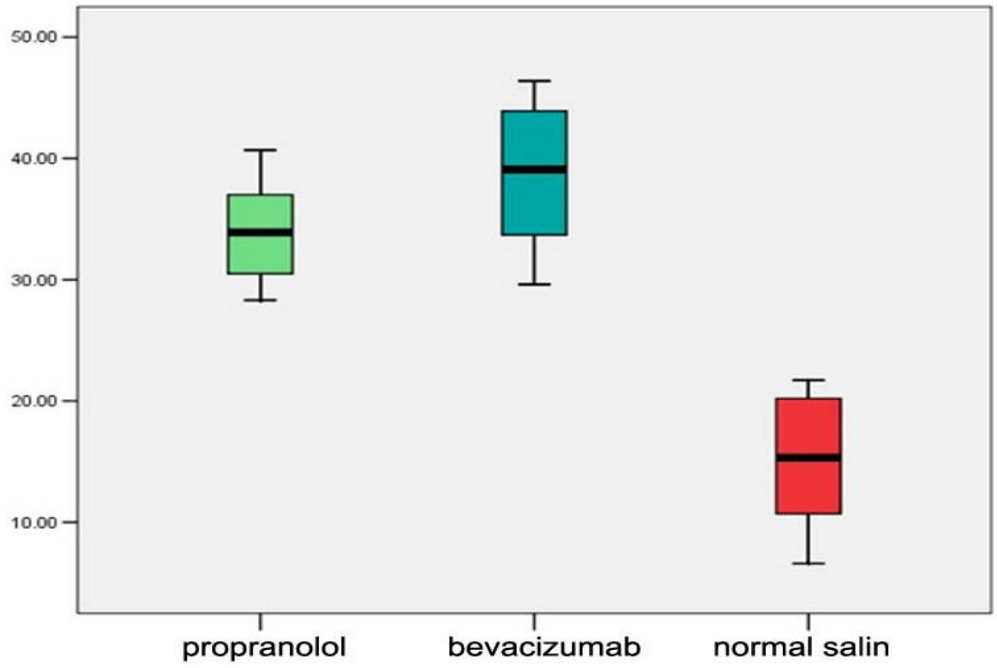


**Fig 2. Comparison of the average corneal neovascularization (CNV) area between three groups after 2 weeks. Values are presented as mean ± SD.**

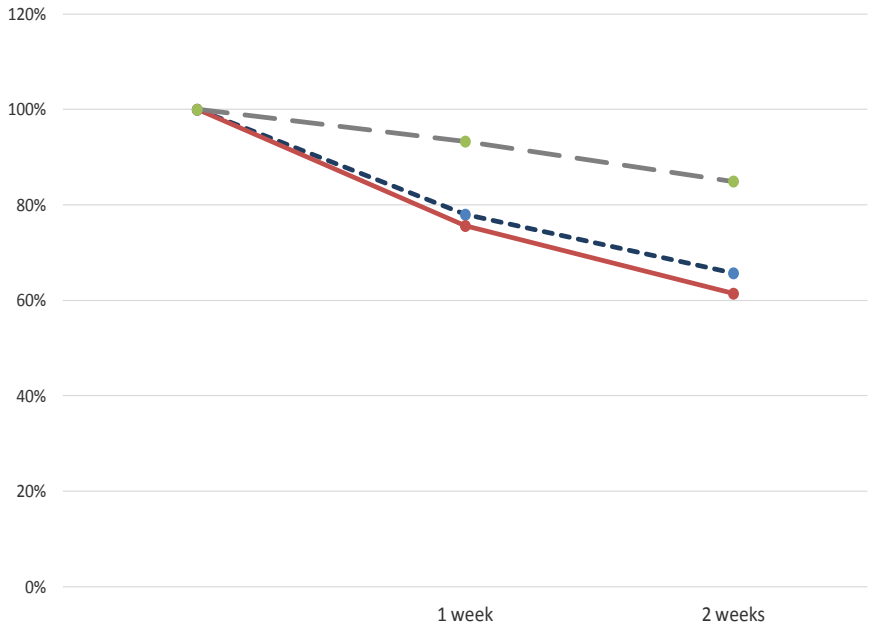


**Fig 3. Comparison of the average CNV regression between the three groups. The values are presented as Mean± SD.**

## 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(9). 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 et al. (2008) have reported subconjunctival injection of bevacizumab can inhibit experimental corneal neovascularization significantly(20). Hashemian et al in 2011 and Oner et al (2012) have reported no statistically significant difference between topical and subconjunctival bevacizumab for corneal neovascularization in an experimental rat model but both of them were effective(17, 22). In our study topical bevacizumab has been evaluated and it was effective but subconjunctival bevacizumab injection not performed. Kim et al. (2013) have shown topically administered bevacizumab had longer standing anti-angiogenic effect than subconjunctivally injected bevacizumab in rat corneal neovascularization. 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 difference can be attributed to different treatment period and follow up between the two studies as the Kim et study lasted for only 2 weeks, which may be a too short period to allow for the development of epitheliopathy (19). Simavli et al (2014) have shown topical propranolol has no significant inhibitory effect on corneal neovascularization statistically(13). In contrast, our study showed that topical propranolol can inhibit corneal neovascularization statistically.

Padrini et al. (2014) studied Pharmacokinetics and local safety profile of propranolol eye drops in rabbits and reported retinal concentration of propranolol is similar in topically and orally administration(24). Ristori et al (2011) studied the role of the adrenergic system in a mouse model of oxygen-induced retinopathy and antiangiogenic effects of beta-adrenoreceptor blockade. They reported beta receptor blockade is protective against retinal angiogenesis by reducing VEGF but no effect on normal level of VEGF.

The results of our experiments demonstrated that regression of corneal neovascularization area in topical Propranolol and Bevacizumab groups were significantly different compared with the saline group, 2.28-fold and 2.56-fold, respectively after 2 weeks. However, there was no significant difference between Propranolol and Bevacizumab groups statistically.

Several studies have shown that Propranolol can reduce VEGF and VEGF R1 and VEGFR2 production and also shown inhibition by propranolol of VEGF-induced tyrosine phosphorylation of VEGF receptor-2 lead to

inhibition of downstream signaling such as the activation of the extracellular signal-regulated kinase-1/2 and the secretion of the extracellular matrix degrading enzyme MMP-2. In conclusion, these results demonstrate that propranolol interferes with several essential steps of neovascularization and opens up novel therapeutic opportunities for the use of β-blockers in the treatment of angiogenesis-dependent human diseases. However, its mechanism of action is as yet unknown totally.

The limitations of our study include the short follow-up period and the lack of information about the biocompatibility of topical propranolol. Further trials with longer periods of follow-up will be necessary. Further studies of the optimal dosage, treatment interval, and duration are also recommended.

## CONCLUSION:

Our findings support the hypothesis that β-AR blockade can efficiently counteract neovascularization. In addition, our findings showed that topical propranolol can reverse corneal neovascularization in short term period indicating that propranolol can act as an alternative drug for bevacizumab because it is available and cost-benefit.

Our findings also suggest that topical eye application of propranolol can represent an alternative delivery route to systemic administration 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 propranolol and the efficacy and side effects of long term treatment for corneal neovascularization needs more investigation.

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