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Comparison of the efficiency of subconjunctival aflibercept ranibizumab and bevacizumab in corneal neovascularization in a rat model

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ABSTRACT

BACKGROUND: Corneal neovascularization (CN) might be a sight-threatening condition via reducing the corneal transparency. One of the most significant proangiogenic factors is vascular endothelial growth factor (VEGF) that is shown to be upregulated in CN. Accordingly, we aimed to evaluate and compare the effectiveness of aflibercept, ranibizumab, and bevacizumab in CN in a rat model.

MATERIAL AND METHODS: Twenty-eight male Wistar-Albino rats were administered general anesthesia. 2-mm-diameter central burns were formed on the corneas. Rats were divided into four groups randomly, and one group was administered subconjunctival saline solution as the control group. Subconjunctival aflibercept, bevacizumab, and ranibizumab were applied to the other three groups, respectively. Three rats from each group were randomly selected, and digital photographs of the corneas were taken under general anesthesia. Then, rats were euthanized, and eyes were enucleated one week and one month after the corneal injury. Three enucleated eyes from each group were prepared for histological evaluation with hematoxylin and eosin.

RESULTS: According to the first week results, there was no central CN in all groups except the control group. There was no statistically significant difference in total CN among the groups according to the Kruskal-Wallis test ($p = 0.09$). The control group had a higher inflammation score than the other groups in the central cornea. On the other hand, the control group had less intense fibrosis than the other groups in the central cornea. In the first month, there was a spontaneous regression in central CN in the control group. There was again no statistical difference among the groups regarding the CN ($p = 0.46$). There was no inflammation in the central cornea in any groups.

CONCLUSION: Single-dose subconjunctival injection of aflibercept, ranibizumab, and bevacizumab prevents the central CN and reduces the inflammation.

KEY WORDS: aflibercept; bevacizumab; corneal neovascularization; ranibizumab

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INTRODUCTION

Corneal neovascularization (CN) might be a sight-threatening condition via reducing the corneal transparency and/or causing refractive errors. The cornea has an equilibrium between the proangiogenic and antiangiogenic factors, which can be disrupted by trauma, infection, inflammation, and hypoxia [1]. One of the most significant proangiogenic factors is Vascular Endothelial Growth Factor (VEGF) that was shown to be upregulated in CN [2]. Therefore, anti-VEGF molecules have been considered as potential candidates for CN treatment [3].

Bevacizumab is a humanized monoclonal antibody that selectively antagonizes all isoforms of VEGF-A and has been extensively used off-label in ophthalmology [4]. Ranibizumab is a similar molecule to bevacizumab that is generated for ophthalmic use, including only Fab without Fc domain and having a smaller molecular weight. Aflibercept is a VEGF trap that binds all VEGF-A isoforms, VEGF-B, and placental growth factors 1 and 2. In age-related macular degeneration treatment, aflibercept is considered the highest affinity VEGF blocker and more potent than ranibizumab and bevacizumab [5]. To date, various studies investigating and comparing the efficiency of these anti-VEGFs have been performed in animal models with conflicting results [6–8]. However, there is no study including all three together in an animal model yet. Accordingly, we aimed to evaluate and compare the effectiveness of aflibercept, ranibizumab, and bevacizumab in CN in a rat model.

MATERIAL AND METHODS

This study was approved by the Gazi University Animal Experiments Local Ethics Committee (No: G.Ü.ET-15.055). All the protocols of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research were followed. Twenty-eight male Wistar-Albino rats weighing between 250–350 grams with healthy clear corneas were administered general anesthesia via intramuscular 50 mg/kg body weight ketamine hydrochloride and 5 mg/kg xylazine. Topical 0.5% proparacaine hydrochloride eye drops were applied before the chemical burns were formed on the right eyes of each rat. The corneas were exposed to a stick covered with 75% silver nitrate and 25% potassium nitrate for 10 seconds to create a 2mm diameter central burn (Fig. 1). Then, eyes were irrigated with

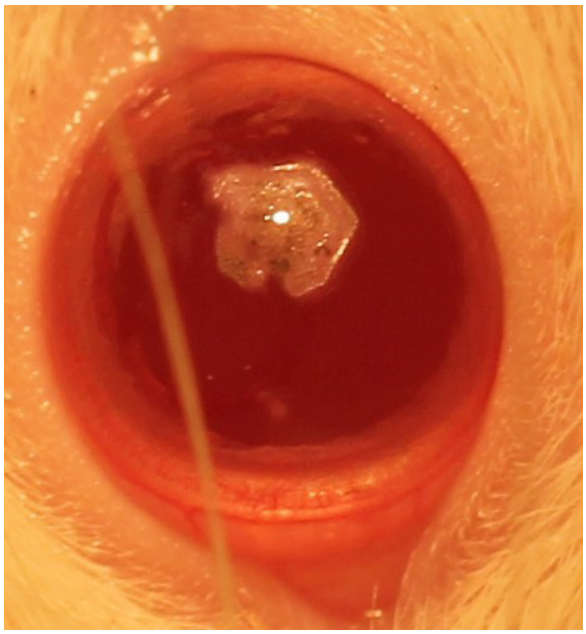

FIGURE 1. Central corneal burn

5 ml balanced salt solution for 1 minute. Rats were divided into four groups randomly, and one group was administered subconjunctival saline solution (0.05 mL) as the control group. Subconjunctival aflibercept 2 mg (0.05 mL), bevacizumab 1.25 mg (0.05 mL) and ranibizumab 0.5 mg (0.05 mL) were applied to other three groups respectively.

Three rats from each group were randomly selected, and digital photographs of the corneas were taken under general anesthesia. Then, rats were euthanized, and eyes were enucleated. This procedure was carried out one week and one month after the corneal injury.

After three enucleated eyes from each group were fixated with 10% formaldehyde for 24 hours, limbus to limbus corneal sections, including the most dense neovascularized corneal areas, were excised. These sections were dehydrated and embedded in paraffin blocks. From the sections, 5 μm thickness sagittal cuts were made with microtomes and prepared for hematoxylin and eosin staining. The stained vessels were counted under the light microscope with ×400 magnification and then compared using the Kruskal-Wallis test. Also, sections were evaluated regarding the inflammation based on the inflammation scale described before (1: focal fibroblast activity and focal a few mixed type inflammatory cells, 3: diffuse and intense fibroblast activity and lots of mixed types inflammatory cells, 2: between 1 and 3) [9]. The results were presented with column bars.

RESULTS

According to the first week results, there was no central CN in all the groups except the control group (Fig. 2, 3). There was no statistically significant difference in total CN among the groups (p = 0.09) (Tab. 1). The control group had a higher inflammation score than the other groups in the central cornea. On the other hand, the control group had less intense fibrosis than the other groups in the central cornea (Fig. 4).

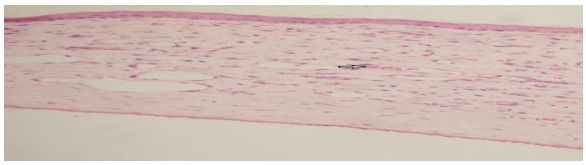

FIGURE 2. Control group staining with hematoxylin and eosin. Arrow showing vessel lumen involving erythrocytes in central cornea

After the first month, there was a spontaneous regression in central CN in the control group. Again, there was no statistical difference among the groups regarding the CN (p = 0.46) (Tab. 2). There was no inflammation in the central cornea in any groups. There was no fibrosis in the aflibercept group in the central cornea at the first month. Other groups had grade 1 and more fibrosis scores.

DISCUSSION

Our study indicated that early anti-VEGF treatment is effective in preventing central CN after corneal insult. There was no central CN in all treatment groups at one week and one month after the corneal injury. The CN presented in the control group at the first week was regressed one month after the injury. This possible spontaneous regression was demonstrated in rat corneas [10]. Although there was no statistically significant

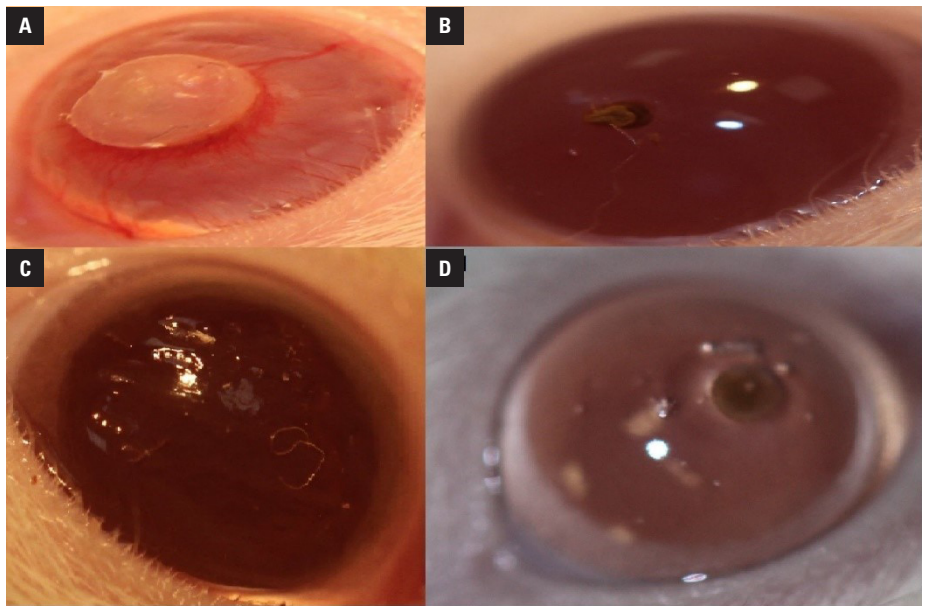

FIGURE 3. Cornea photographs of the control group (**A**) bevacizumab group (**B**) aflibercept group (**C**) ranibizumab group (**D**) at the first week

Table 1. Vessel counts in histological specimens between the groups at the first week (Kruskal-Wallis test)

Groups	Number	Mean	Standard deviation	Median	Minimum	Maximum	p
Control	3	16.3333	6.02771	17.0000	10.00	22.00	0.090
Ranibizumab	3	12.3333	3.78594	14.0000	8.00	15.00	
Aflibercept	3	6.3333	1.52753	6.0000	5.00	8.00	
Bevacizumab	3	10.6667	4.04145	10.0000	7.00	15.00	

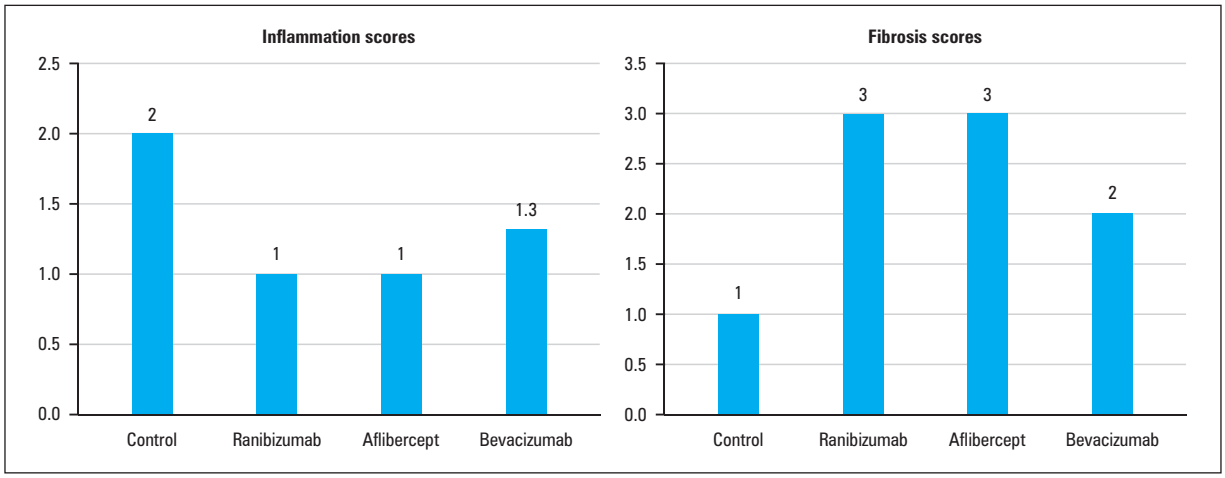

FIGURE 4. Inflammation and fibrosis scores of the groups at the first week

Table 2. Vessel counts in histological specimens between the groups at the first month (Kruskal-Wallis test)

Groups	Number	Mean	Standard deviation	Median	Minimum	Maximum	p
Control	3	12.00	3.61	13.0000	8.00	15.00	0.464
Ranibizumab	3	15.33	4.04	13.0000	13.00	20.00	
Aflibercept	3	15.00	5.00	15.0000	10.00	20.00	
Bevacizumab	3	10.67	2.89	9.0000	9.00	14.00	

difference in vessel counts among the groups in histological evaluation at the first week, we think that this result was due to small number of the groups, and the difference between the groups was clinically significant. However, this statistical insignificancy was higher at the first month. This result might have arisen from the single-dose treatment and probable short-term therapeutical effect of anti-VEGFs. It is possible that low-dose anti-VEGF treatment can inhibit only early vessel formation and may be insufficient to prevent the late-term CN. This theory was supported by earlier studies indicating that bevacizumab was ineffective in preventing late-formed CN in experimental animal models [11, 12]. Also, it was demonstrated that repeated doses of subconjunctival or topical treatments had better outcomes in reducing early and late formed CN [13, 14].

One of the purposes of this study was to compare the efficacy of aflibercept, ranibizumab, and bevacizumab in CN treatment. Despite the fact that we couldn't apply the Bonferroni test to analyze the differences between the two groups as the Kruskal-Wallis test did not demonstrate a statistically significant difference among the groups, we could observe that the aflibercept group had the lesser amount of vessel count in specimens one week after the treatment. However, there was no difference between any groups after one month. In a comparative rat study comprising the subconjunctival bevacizumab and ranibizumab, it was found that the bevacizumab group had less CN intensity than the ranibizumab group on the 8th day regarding the histological evaluation. Moreover, there was not a statistically significant difference between the ranibizumab and control group [9]. On the other hand, in another experimental animal model, there was no difference between subconjunctival bevacizumab and ranibizumab seven days after the treatment regarding their inhibitory effect in CN [15]. Gal-Or et al. conducted a study comparing the inhibitory effects of subconjunctival aflibercept and bevacizumab in an experimental rat CN model. They showed that aflibercept prevented central CN formation contrary to bevacizumab. They stated that this result might be due to insufficiency of single-dose bevacizumab injection or the impact of other proangiogenic factors in CN other than VEGF-A, like placental growth factor 1 and 2 which beva-

cizumab couldn't inhibit contrary to aflibercept. They indicated that a possible stronger interaction between the aflibercept and rodent VEGF-A than bevacizumab and rodent VEGF-A might cause this outcome [16]. Moreover, similar outcomes were reached when topical aflibercept and bevacizumab were compared again in an experimental rat CN model [17].

At the first week, there was less inflammation involving the central cornea in treatment groups compared to the control group in our study. On the contrary, the control group had less fibrosis involving the central cornea than the treatment groups. The former result is expected as VEGF increases vascular permeability and helps the transportation of inflammatory cells. Also, certain previous studies confirmed this result [18, 19]. The unexpected latter result can be explained by the theory that even though VEGF has a role in preventing fibrosis, it also has a role in fibrosis resolution [20]. Therefore, we think that anti-VEGF treatment may decrease the fibrosis resolution which is required for an already formed corneal insult. At the first month, there was no inflammation in the central cornea in any groups indicating that the activity of the inflammatory factors had already been finished and the fibrosis had already been established before the first month after the corneal insult.

CONCLUSION

Single-dose subconjunctival injection of aflibercept, ranibizumab, and bevacizumab prevents the central CN. Aflibercept seems to be superior to bevacizumab and ranibizumab in inhibiting the CN. However, there is no difference between ranibizumab and bevacizumab. Repeated dose anti-VEGF injections could be more effective in preventing the total CN.

Acknowledgements

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Conflict of interest

There is no conflict of interest.

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