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Original Article

The effect of intracameral bevacizumab on current hyphema

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Abstract

Aim: The aim of this study was to investigate the effect of intracameral bevacizumab on the current hyphema.

Material and Methods: The animals were assigned to the following 4 groups; Group 1: One 2.5 mg bevacizumab injection to the anterior chamber; Group 2: One 1.25 mg bevacizumab injection to the anterior chamber; Group 3: One 1cc balanced salt solution injection to the anterior chamber; and Group 4: Untreated hyphema group. Non-heparinized blood that obtained from the rabbit ear was used to fill the anterior chamber to create total hyphema. Intraocular pressures (IOP), hyphema resorption time, clot formation, peripheral synechia formation, and corneal staining were recorded.

Results: IOP results were 26±1.2, 30±2.1, 24±2.9, and 22±0.0 mm Hg for groups 1, 2, 3, and 4, respectively, and were significantly higher in group 2 than in the other groups (p= 0.001). Resorption times of hyphema were 13±2.2, 13±3.2, 9±1.7, and 9±1.6 days for groups 1, 2, 3, and 4, respectively, and were significantly longer for the groups receiving bevacizumab than for the others (p=0.018). The clot formation scores were 0.16±0.41, 0.14±0.38, 0.86±0.38, and 1.0±0.0 for groups 1, 2, 3, and 4, respectively, and were significantly lower for the groups receiving bevacizumab than in the other groups (p= 0.002). The peripheral synechia formation scores were 0.0±0.0, 0.0±0.0, 0.43±0.53, and 0.50±0.54 for groups 1, 2, 3, and 4, respectively, and were not significantly different (p= 0.213). The corneal staining scores were 0.85±0.35, 0.86±0.38, 0.14±0.38, and 0.14±0.38 for groups 1, 2, 3, and 4, respectively, and were significantly higher for the groups receiving bevacizumab (p= 0.035).

Conclusion: Intracameral bevacizumab may increase complications that related current hyphema.

Keywords: Bevacizumab; Corneal blood staining; Glaucoma; Hyphema.

INTRODUCTION

The accumulation of blood in the anterior chamber is called hyphema. This accumulation can occur due to trauma or intraocular surgery, or it can occur spontaneously (as in rubeosis iridis, juvenile xanthogranuloma, retinoblastoma, metastatic tumors, iris melanoma, myotonic dystrophy, keratouveitis, leukemia, hemophilia, thrombocytopenia, and Von Willebrand disease) (1). Hyphema may cause intraocular pressure (IOP) increases, inflammation, peripheral anterior synechia, optic atrophy, corneal staining, secondary bleeding and accommodative disorder (2). Resorption of hyphema usually occurs spontaneously, but a delay in the resorption of the blood can increase the rate of hyphema-associated complications. No consensus has yet been reached regarding the best treatment for hyphema. Many different supportive therapeutic and medicinal regimens continue to be tested in an attempt to avoid complications and promote hyphema resorption. Nevertheless, despite medical treatment, approximately 5% of patients with hyphema may require surgical treatment (3).

Bevacizumab (Avastin, Genentech, San Francisco, CA) was first used to inhibit angiogenesis in metastatic colorectal cancers (4). Recent studies, have also determined that the administration of intravitreal bevacizumab is an effective treatment for various neovascular eye diseases (5-7). Although its use in the eye is still regarded as off-label intracameral bevacizumab administration has shown promising results for the treatment of neovascular glaucoma (8). Intracameral bevacizumab reduces the aqueous humor levels of vascular endothelial growth factor (VEGF) and can prevent neovascularization of the anterior segment (9).

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Despite the frequent use of bevacizumab in neovascular conditions, its effects on the accompanying hyphema have not been investigated. The aim of the present study was to investigate the effect of injection of intracameral bevacizumab on the current hyphema and to determine the effect of this application on hyphema-related complications in these eyes.

MATERIAL and METHODS

This experimental animal study was designed according to the Animal Research: Reporting of in Vivo Experiments (ARRIVE) guidelines (10). The authors confirm adherence to the Association for Research in Vision and Ophthalmology (ARVO) statement for the Use of Animals in Ophthalmic and Vision Research. The experimental protocol in the current study was approved by the local Ethics Committee (Reference Number: 2015/A-42) and the Guidelines for Animal Research from the National Institutes of Health were followed in all experimental procedures.

Twenty-eight healthy adult New Zealand white rabbits, each weighing approximately 2.5 kg were enrolled in this study. Animals were obtained from the Inonu University Laboratory Animals Research Center and were placed in a temperature-cotrolled (21 ± 2°C) - and humidity-controlled (60 ± 5%) room, in which a 12:12 h light/dark cycle was maintained.

The right eye from each rabbit was chosen for creation of a total hyphema. The rabbits were anesthetized with an intramuscular injection of ketamine (35 mg/kg) + xylazine (5 mg/kg). Corneal anesthesia was established by administering 1–2 drops of proxymetacaine hydrochloride 0.5% (Alcaine®, Alcon, USA). After placement of a lid speculum, one drop of 5% povidone-iodine solution was dropped onto the eye, and the eye was washed two minute later. The anterior chamber was evacuated by paracentesis with a 30 gauge needle near the limbus. The non-heparinized blood obtained from the rabbit ear was immediately used to fill the anterior chamber. The procedure was terminated by leakage control and IOP control Post-procedure analgesia was done for each animal (Figure 1).

The animals were assigned to the following four groups: Group 1: One dose of 2.5 mg bevacizumab injected into the anterior chamber, Group 2: One dose of 1.25 mg bevacizumab injected into the anterior chamber, Group 3: One dose of 1 cc balanced salt solution injected into the anterior chamber, Group 4: Untreated hyphema group. The injections were performed into the anterior chambers of the treatment groups using a 30 gauge needle five minutes after the creation of the hyphema.

The IOP was measured daily with a Perkins tonometer (Haag-Streit, Essex, UK), and portable slit lamp biomicroscopic examinations were also performed daily. These daily evaluations were performed without sedation, but under topical corneal anesthesia with Alcaine®, and were conducted by the same researcher. The hyphema resorption time, clot formation, peripheral synechia formation, and corneal staining were evaluated by biomicroscopy examination. The hyphema resorption time was the number of days required for clearing of the hyphema from the anterior chamber. Clot formation, peripheral synechiae formation, and corneal staining were scored as '1' for presence and '0' for absence. No animals were lost during the procedure or during follow-up.

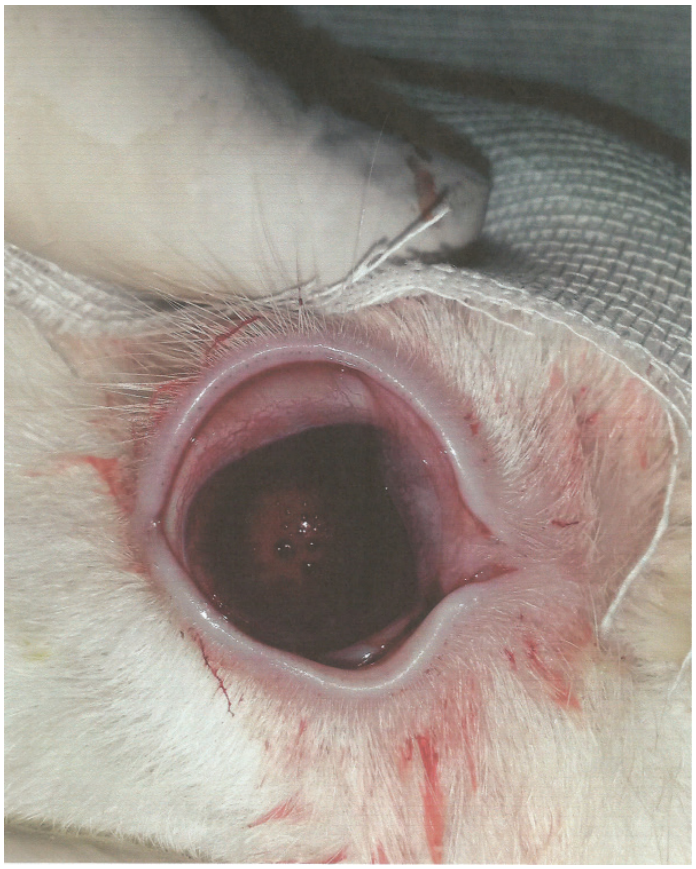

Figure 1. Experimental total hyphema in rabbit eye

After 15 days, each animal was anesthetized and then euthanized. The corneas were excised to include the scleral rim and examined histopathologically. The corneas were fixed in 10% formalin, embedded in paraffin, and cut into sections 4 µm thick. The sections were stained with hematoxylin and eosin and examined under 40× magnification. Histopathological examination revealed endothelial swelling, endothelial breakdown, stromal inflammation, and stromal vascularization. For statistical analysis, histopathological scoring was performed with numerical values ranging from 0 to 4.

Statistical analysis

The SPSS 22 package program was used for statistical analysis of the data. ANOVA and post hoc Tukey tests were used to compare the results. A P value of 0.05 was considered statistically significant.

RESULTS

The IOP results were 26 ± 1.2, 30 ± 2.1, 24 ± 2.9, and 22 ± 0.0 mm Hg for groups 1, 2, 3, and 4, respectively, and the IOP was significantly higher in group 2 than in groups 1, 3, and 4 (p = 0.001). The hyphema resorption times were

13 ± 2.2, 13 ± 3.2, 9 ± 1.7, and 9 ± 1.6 days for groups 1, 2, 3, and 4, respectively, and were significantly higher for group 1 and group 2 than for group 3 and group 4 (p = 0.018). The clot formation scores were 0.16 ± 0.41, 0.14 ± 0.38, 0.86 ± 0.38, and 1.0 ± 0.0 for groups 1, 2, 3, and 4, respectively, and were significantly lower in groups 1 and 2 than in groups 3 and 4 (p = 0.002). The peripheral synechia formation scores were 0.0 ± 0.0, 0.0 ± 0.0, 0.43 ± 0.53, and 0.50 ± 0.54 for groups 1, 2, 3, and 4, respectively, and these values showed no statistically significant differences (p = 0.213). The corneal staining scores were 0.85 ± 0.35, 0.86 ± 0.38, 0.14 ± 0.38, and 0.14 ± 0.38 for groups 1, 2, 3, and 4, respectively, and the scores were significantly higher for groups 1 and 2 than for groups 3 and 4 (p = 0.035) (Table 1).

The endothelial swelling scores determined by histopathological examination were 0.83 ± 0.98, 1.29 ± 0.49, 0.86 ± 0.90, and 1.0 ± 1.10 for groups 1, 2, 3, and 4, respectively, and these values were not significantly different (p = 0.991). The endothelial breakdown scores were 1.33 ± 1.21, 1.86 ± 0.90, 1.71 ± 0.76, and 1.0 ± 1.10 for groups 1, 2, 3, and 4, respectively, and these scores also were not significantly different (p = 0.576). The stromal inflammation scores were 1.0 ± 0.82, 1.0 ± 0.90, 0.14 ± 0.38, and 0.0 ± 0.0 for groups 1, 2, 3, and 4, respectively, and the scores were significantly higher for groups 1 and 2 than for groups 3 and 4 (p = 0.044). The stromal vascularization scores were 0.86 ± 0.90, 1.0 ± 0.90, 0.0 ± 0.0, and 0.0 ± 0.0 for groups 1, 2, 3, and 4, respectively, and the scores were significantly higher for groups 1 and 2 than for groups 3 and 4 (p = 0.044) (Table 2) (Figure 2).

Table 1. Biomicroscopic results of groups

	Group 1 (n=7)	Group 2 (n=7)	Group 3 (n=7)	Group 4 (n=7)	P
IOP (mmHg)	26±1.2	30±2.1*	24±2.9	22±0.0	0.001
Resorption times of hyphema (Days)	13±2.2*	13±3.2*	9±1.7	9±1.6	0.018
Clot formation scores	0.16±0.41	0.14±0.38	0.86±0.38*	1.0±0.0*	0.002
Peripheral synechia scores	0.0±0.0	0.0±0.0	0.43±0.53	0.50±0.54	0.213
Corneal blood staining scores	0.85±0.35*	0.86±0.38*	0.14±0.38	0.14±0.38	0.035

Clot formation, peripheral synechia formation, and corneal staining were scored as '1' for presence and '0' for absence.
IOP: Intraocular pressure, * statistically significant

Table 2. Corneal histopathological results of groups

	Group 1 (n=7)	Group 2 (n=7)	Group 3 (n=7)	Group 4 (n=7)	P
Endothelial swelling scores	0.83±0.98	1.29±0.49	0.86±0.90	1.0±1.10	0.991
Endothelial breakdown scores	1.33±1.21	1.86±0.90	1.71±0.76	1.0±1.10	0.576
Stromal inflammation scores	1.0±0.82*	1.0±0.90*	0.14±0.38	0.0±0.0	0.044
Stromal vascularization scores	0.86±0.90*	1.0±0.90*	0.0±0.0	0.0±0.0	0.044

Histopathological scoring was performed with numerical values ranging from 0 to 4. *Statistically significant

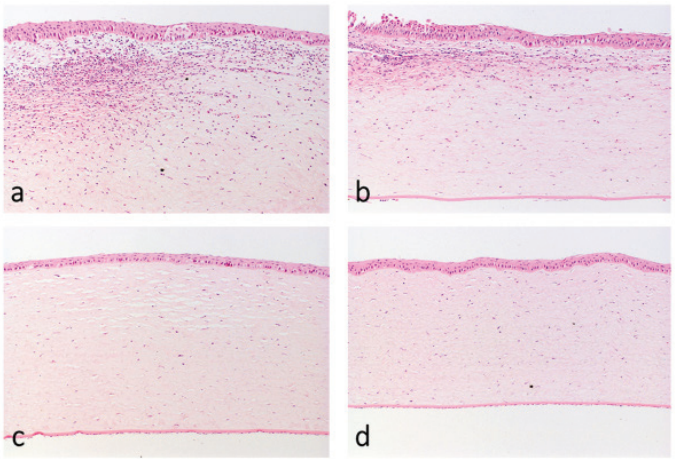

Figure 2. a) The image of a corneal histopathological specimen from group 1 shows severe stromal inflammation and stromal vascularization. b) The image of a corneal histopathological specimen from group 2 shows severe stromal inflammation and stromal vascularization. c) The image of a corneal histopathological specimen from group 3 shows slight stromal inflammation and stromal vascularization. d) The image of a corneal histopathological specimen from group 4 shows no stromal inflammation and stromal vascularization (H&E, X100)

DISCUSSION

To the best of our knowledge, this is the first study about the effect of injection of intracameral bevacizumab on the current hyphema. Hyphema is often encountered in neovascular conditions. Bevacizumab reduces new hyphema and associated complications in neovascular conditions (11). But, there are not any study about how bevacizumab effects current hyphema although that uses in neovascular conditions frequently. The primary goal of a hyphema treatment is to prevent the development of complications. The results of our study indicated that bevacizumab extended the time required for absorption of the hyphema from the anterior chamber. Bevacizumab use is known to cause wound healing complications (12); therefore, we believe that the extended duration required for hyphema resorption was due to disruption of the wound healing process. In a normal wound, the blood turns into a clot during the healing process. Under normal conditions, the blood coagulates, becomes organized into a clot, and then gradually resorbs after clot lysis (13). Hyphema-associated clots can be treated with thrombolytic agents (14). In our study, clot formation was significantly lower in

the groups that received bevacizumab. Previous studies have reported that administration of anticoagulants and antiplatelet drugs after the hyphema can increase the risk of persistence of the hyphema and of re-bleeding (15). Anti-fibrinolytic agents are used to stabilize the fibrin clot in hyphema conditions (16), and corticosteroids are administered to inhibit fibrinolysis. Clot formation is therefore an important condition that controls the fate of the hyphema.

Obstruction of the trabecular meshwork by erythrocytes, fibrin, debris, and platelets increases the IOP (1). A disruption of the wound healing process could prevent clot formation and allow blood to fill up the trabecular meshwork. We found that the IOPs were higher in the bevacizumab-treated groups, but the difference reached statistical significance only for the group treated with 1.25 mg bevacizumab and not for the group treated with 2.5 mg. This may reflect a dilution effect due to the greater volume injected to deliver 2.5 mg bevacizumab. Non-clotting blood can increase inflammation in the anterior chamber, but we found no significant difference between the groups in terms of the formation of inflammation-induced peripheral synechia. Bevacizumab was previously found to have no effect on inflammatory cytokines other than VEGF (17).

Decreased resorption of blood from the trabecular meshwork will increase the duration of contact between the blood and cornea. Elevation of IOP also increases the risk of corneal staining (18). In addition, the incidence of corneal blood staining increases in response to a number of factors, including large hyphema, secondary hemorrhage, prolonged clot retraction, sustained increases in IOP, and previous endothelial dysfunction (19-21). Non-clotting blood becomes hemolyzed and over-product occurs. These hemolysis products impair endothelial functions and also pass in increasing amounts to the cornea, resulting in a more intense corneal staining (20,22). In the present study, the bevacizumab-treated groups showed a higher degree of macroscopic staining. The histopathological examinations also indicated greater stromal damage and corneal staining in the bevacizumab groups.

The passage of blood elements into the corneal stroma can cause inflammation and neovascularization in the stroma (2). Our histopathology results revealed a significant vascularization and inflammation in the stroma in the bevacizumab-treated groups, even though bevacizumab is considered an anti-neovascular drug. This suggests that the observed stromal injury may represent a response to a stronger vascular signal than can be compensated for by this drug. Alternatively, bevacizumab administered to the anterior chamber might have had no effect on stromal vascularization. Intracameral administration of bevacizumab appears to have no toxic effects on the cornea (23-25). Our histopathological examination results did not show a statistically significant difference in endothelial damage, although a trend toward more damage was indicated in the group given 1.25 mg bevacizumab. Our opinion is that this endothelial damage is caused by the non-resorbed blood products and the high IOP in this group.

Overall, our results indicated no therapeutic effects of bevacizumab administration as a treatment for non-neovascular hyphema. In fact, the use of bevacizumab could possibly worsen the situation. Therefore, we do not recommend the use of bevacizumab as a therapy for non-neovascular hyphema. Bevacizumab is frequently used in neovascular conditions; for example, intracameral bevacizumab administered 24 hours prior to filtration surgery has been reported to effectively prevent re-bleeding (26). Similarly, intravitreal and intracameral bevacizumab have been demonstrated to induce regression of iris neovascularization (27-29). Based on our findings, we believe that a current hyphema must be surgically removed prior to injection of bevacizumab if the hyphema is present in the neovascular state. Otherwise, the hyphema can cause increased damage and complications.

Study Limitations

In the present study, we investigated only the acute period of hyphema. We did not investigate late complications, such as hyphema-related late-onset glaucoma. This can be regarded as a limitation of our study.

CONCLUSION

In conclusion, intracameral bevacizumab may worsen current hyphema-related complications. We recommend surgical removal of a current hyphema if bevacizumab is to be used to prevent recurrent bleeding in neovascular conditions. Further studies are warranted.

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Competing interests: The authors declare that they have no competing interest.
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