• Basic Research •

Comparison of the effects of intravitreal bevacizumab and dexamethasone in experimental posterior penetrating eye injury

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Abstract

• AIM: To compare the effects of intravitreal anti-vascular endothelial growth factor (VEGF) and dexamethasone in an experimental rabbit model of posterior penetrating ocular injury.

• METHODS: Thirty white New Zealand rabbits were included in the study. A posterior penetrating ocular injury was performed at the superotemporal quadrant. They were randomly divided into three groups. The rabbits in group 1 received intravitreal dexamethasone, in group 2 they received intravitreal bevacizumab and those in group 3 received intravitreal physiological saline solution in both eyes. All eyes were examined ophthalmologically on the 1st, 3rd, 7th, 14th and 28th days following the injury and the clinical findings were scored. On the day 28, the eyes were enucleated, evaluated and scored macroscopically, histopathologically and scanning electron microscopically.

• RESULTS: The median clinical score on the 14th and 28th days and the median macroscopic score of the dexamethasone group was significantly better than that of control (*P*=0.004, 0.018). Dexamethasone group was also better than that of bevacizumab group but the differences did not reach statistical significance. Retinal detachment rate was 8.3%, 16.6% and 12.5% in the dexamethasone group, bevacizumab group and control group, respectively (*P*=0.476). More extensive fibrocelluler proliferations were observed in controls compared with dexamethasone and bevacizumab groups. But these differences did not reach the statistical significance (*P*=0.538). In scanning electron microscopy all groups showed fibreous stalk and dense collagen fibrils in vitreous.

• CONCLUSION: This study shows that intravitreal injection of both dexamethasone and bevacizumab may reduce the intraocular fibrous proliferation after an experimental posterior penetrating ocular injury in rabbits.

• KEYWORDS: posterior penetrating ocular injury; experimental; dexamethasone; bevacizumab; proliferative vitreoretinopathy
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INTRODUCTION

Ocular injury is an important reason of visual disability and it may cause economic problems, as it occurs most commonly in young and productive population. Despite advances in medical and surgical treatment, penetrating eye injury continues to be a complicated and challenging situation[1].

Penetrating eye injuries involving posterior segment structures usually have poor prognosis because of intraocular proliferative fibrous tissue and tractional retinal detachment (RD) that may end with the phthisis of the eye. The development of fibrous tissue proliferation and tractional RD after a penetrating trauma has been linked clinically with the existence of vitreous haemorrhage (VH) and intraocular tissue incarceration to the wound site. The release of blood, serum and lens material into the vitreous causes accumulation and proliferation of retinal pigment epithelial cells, leukocytes, and fibroblasts, which lead to cyclitic membrane formation, proliferative vitreoretinopathy (PVR) followed by tractional and rhegmatogenous RD[1-5].

PVR and some various conditions associated with long standing inflammation are common after penetrating eye trauma. Although reattachment of retinas can be achieved in most PVR cases surgically, visual results remain unsatisfying. Therefore, prevention of PVR with early recognition of risk factors and signs and appropriate modification of standard surgical treatment methods remain important. There are theoretical reasons to favor strategi es drawing attention to the inhibition of cellular proliferation, inhibition of growth factors and cytokines secretion or inhibition of intracellular signaling pathways[1-5].

Medical treatment to inhibit tractional RD after posterior penetrating ocular injury is desirable to replace or enhance pars plana vitrectomy[6-7]. Corticosteroids, penicillamine, cyclosporin, synthetic peptides, colchicine, sodium hyaluronate, 5-fluorouracil, daunomycin, cryotherapy, and radiation have been used experimentally to control intraocular proliferation[8-15]. Corticosteroids are well known for their inhibitory effect on fibroblast growth[8-10]. However, recent studies have shown that anti-vascular endothelial growth factor (anti-VEGF) drugs (bevacizumab) are also a potent antifibrotic agent to limit ocular scar tissue formation[11-12]. Therefore, dexamethasone and bevacizumab could be used as a medical therapy for the prevention of posttraumatic intraocular fibrous tissue proliferation which can cause PVR and tractional RD. In this study, we aimed to evaluate the effect of intravitreal bevacizumab and dexamethasone in an experimental rabbit model of penetrating posterior ocular injury.

MATERIALS AND METHODS

This study was conducted at the Experimental Research and Application Center of the Erciyes University. It was approved by the Local Ethics Committee of Animal Experiments of Erciyes University Medical School (DA 07/14). Thirty white New Zealand rabbits weighing 2.5-4 kg were included in the study. The operations were done under general anesthesia induced by intramuscular injection of ketamine hydrochloride (35 mg/kg) and xylazine hydrochloride (5 mg/kg). The pupils were dilated with topical phenylephrine hydrochloride (2.5%) and tropicamide (1%).

Surgical Procedure and Clinical Examination The operations were done with the magnification of an operating microscope. Clean instruments were used during the surgery. A posterior penetrating ocular injury was performed with a 5-mm circumferential incision placed 8 mm behind the limbus at the superotemporal area. Full-thickness penetrating incisions were made with an 11# blade, with avoidance of the lens, similar to a method described elsewhere[12]. The prolapsed vitreous was cut with scissors, then conjunctival and scleral wound were closed with 6-0 vicryl suture. Autologus blood was injected through a 25 gauge needle inserted through the wound into the mid-vitreous in all eyes. They were seperated into three groups at random. In group 1 (n=13), 1 mg (0.1 mL) of dexamethasone was done intravitreally, in group 2 (n=13), 1.25 mg (0.05 mL) of bevacizumab (Altuzan; Genentech, Inc. South San Francisco, CA) was injected intravitreally to the right eye of the rabbits, and in group 3 (control group, n=4) the rabbits received 0.05 mL of intravitreal physiological saline solution (0.9%) to the both eyes. Rabbits were examined with indirect ophthalmoscopy on the 1st, 3rd, 7th, 14th and 28th days following the surgery and the clinical findings were scored, according to the factors described by Mehdizadeh *et al*[12].

Gross and Light Microscopy Examination of the Specimen On the day 28, all rabbits were sacrificed and the eyes of all animals were enucleated. After macroscopic evaluation of all enucleated eyes, one half of the globes were fixed in 10% buffered formaldehyde for 96h and later soaked in 50% ethanol and prepared for the histopathological evaluation. After the globes had been embedded in parafin, sections of 5 µm thickness were mounted on slides and stained with hematoxylin-eosin for light microscopy. A nominal scoring system was used to evaluate the extent of fibrosis, according to the factors similar to a method described by Mehdizadeh *et al*[12]. Limited fibrocellular proliferation at the wound site indicates mild fibrosis; small fibrocelluler proliferations containing blood vessels and a few extensions into the vitreous cavity indicates moderate fibrosis and large fibrovascular proliferations and large extensions into the vitreous cavity indicates severe fibrosis[12].

Scanning Electron Microscopy Analysis The other half of the globes were fixed with 2.5% glutaraldehyde in 0.1 mol/L phosphate. Specimens were kept in the same fixative for 2-3d. After rinsing several times in a buffer, they were postfixed with buffered 1% Osmium tetroxide for 1.5h, and then dehydrated in an ascending series of acetone. The specimens were dried by the critical point method using CO_2, sputter-coated with gold, and observed with LEO 440 scanning electron microscopy (SEM). The presence of fibrous proliferation and extensions into the vitreous cavity at the wound site, vitreous collagen were also evaluated. The examining clinician and pathologist were masked to the injected drug to the eyes of the animals.

Statistical Analysis Statistical analysis was performed using the SPSS Version 22.00 (IBM Inc., Chicago, IL, USA) program. Statistical calculations were made by nonparametric Kruskal-Wallis test, Mann-Whitney U test and Chi-square exact test. A P value <0.05 was considered as statistically significant.

RESULTS

Thirty white New Zealand rabbits were operated in the study. The animals were seperated into 3 groups including: group 1 (dexamethasone group) 13 rabbits, group 2 (bevacizumab group) 13 rabbits and group 3 (control group) 4 rabbits. One animal from dexamethasone group died because of general anesthesia complications. In bevacizumab group, endophthalmitis occured in one eye and one rabbit died because of systemic infection. These eyes were excluded from the study. Traumatic cataract developed in another eye in bevacizumab group and this eye was not included for clinical scoring. Fundus details were not visible in all groups because of blood in the vitreous at the first day of the surgery and clinical scores were not evaluated.

When we evaluate the median clinical score, the difference was not significantly among groups on the 3th and 7th days.

The median clinical scores on the 14th and 28th days and the median macroscopic score of the dexamethasone group were significantly better than that of control group (P=0.004, 0.018). Dexamethasone group was also better than that of bevacizumab group but the differences did not reach statistical significance (Table 1). The mean macroscopic scores in groups 1, 2 and 3 were 1, 2 and 4 respectively (P=0.033).

RD rate was 8.3% (n=1, tractional), 16.6% (n=2, 1 tractional and 1 rhegmatogenous) and 12.5% (n=1, tractional) in the dexamethasone group, bevacizumab group and control group, respectively (P=0.476). There was not a statistically significant difference between three groups in RD rate (P=0.476). Figure 1 showed an eye from dexamethasone group with RD.

In histopathologic examination, more extensive fibrocelluler proliferations were found in controls (mean histopathologic score: 3) compared with dexamethasone (mean histopathologic score: 1) and bevacizumab (mean histopathologic score: 2) groups (P=0.538). But these differences were not reach statistically significant. Figure 2 showed examples for histopathologic examinations.

In SEM analysis, all groups showed fibreous stalk and dense collagen fibrils in the vitreous (Figure 3).

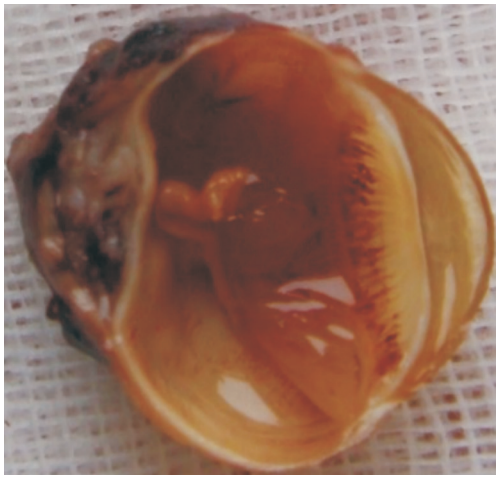

Figure 1 Dexamethasone group: an eye with RD.

Table 1 The mean clinical scores of the groups on days 3, 7, 14 and 28 mean (range)

Clinical scores	Group 1 (dexamethasone)	Group 2 (bevacizumab)	Group 3 (control)	P
Day 3	3.5 (3-4)	3 (3-5)	4 (3-5)	0.088
Day 7	3 (3-4)	3 (3-4)	4 (3-4)	0.055
Day 14	1 (1-4)	2 (1-5)	4 (2-4)	0.004
Day 28	1 (1-5)	2 (1-5)	4 (2-5)	0.018
P	0.083	0.141	0.157	-

DISCUSSION

Penetrating eye injuries of the posterior segment often cause serious problems such as tractional RD which may end with poor visual prognosis. Although these problems may in part be solved with modern vitreoretinal surgical techniques, still a safe treatment protocol which could be administered right after the initial injury to prevent the development of fibrovascular membranes would be a major determinant of the following prognosis of the eye[1].

An inflammatory response occurs in the injured eye, the extent of which is influenced by multiple factors. Clinical and histopathological observations suggest that fibrovascular proliferation from the scleral wound, vitreous haemorrhage, lens injury, and the ciliary body injurymay all contribute to a fibroblastic response within the vitreous, which often induces the formation of membranes and a condition known as PVR which is followed by RD[4,16]. The treatment of PVR is principally surgical, including vitrectomy and removal of membranes to allow reattachment of the retina[9]. Although anatomical success may be obtained, visual prognosis may be disappointing. Furthermore the manipulations during vitrectomy may stimulate the development of new membranes. Therefore, proper management and prevention of PVR and tractional RD play an important role to achieve better visual outcome. Attempts have been made to prevent membrane formation with the use of different therapies after posterior penetrating eye injury[2-6]. Radiotherapy had been used for this purpose by many investigators[13-14]. Some researchers used intravitreal cyclosporin as an adjunctive agent to radiotherapy to prevent intraocular proliferation after penetrating posterior segment injury in an experimental study[14]. However, side effects related to radiation still remains as a major disadvantage for human. Antimitotic agents, which may inhibit cellular proliferation, particularly 5-fluorouracil, have been used recently in patients with PVR. This drug is harmful for the retina and capable of deteriorating the electroretinogram at doses very close to the therapeutic range and besides there is no proven efficacy of the intraoperative use of this agent as an antiproliferative regiment for the prevention of PVR or improvement of final visual acuity[15].

Corticosteroids are known to limit the invasion and lysis of the vitreous collagen by inflammatory cells and to have an inhibitory effect on the growth of fibroblasts[10]. This inhibitory effect is attributed to the antimitotic activity on the cells[8-10]. Steroids are also known to be associated with reduction of neovascularization. Folkman and Ingber[17] were the first to suggest that the antiangiogenic effects of steroids. In an eye injury model in rabbits, intravitreal injection of 1.2 mg dexamethasone decreases the incidence of retinal detachment from 46% to 27%[18]. Other experimental works have substantiated the theory that corticosteroids can reduce Müller cell proliferation and reduce the severity of PVR[9-10].

There are some recent studies to investigate a new sustained-release formulation of dexamethasone (Ozurdex®) for inhibiting PVR and its effect on the expression of retinal glial

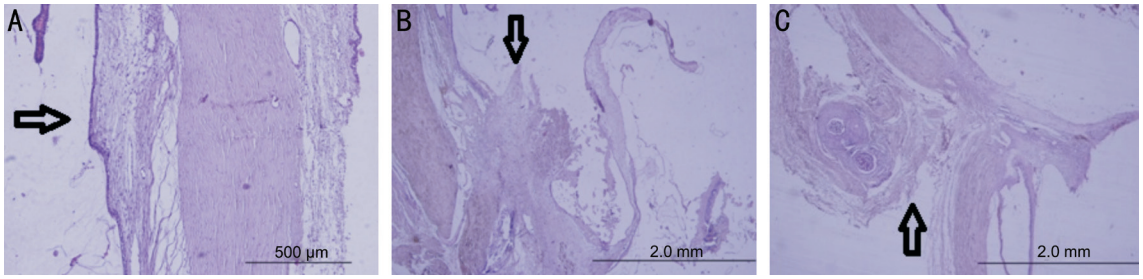

Figure 2 The histopathologic scoring according to the extent of fibrosis (black arrows) A: Mild fibrosis; B: Moderate fibrosis; C: Severe fibrosis.

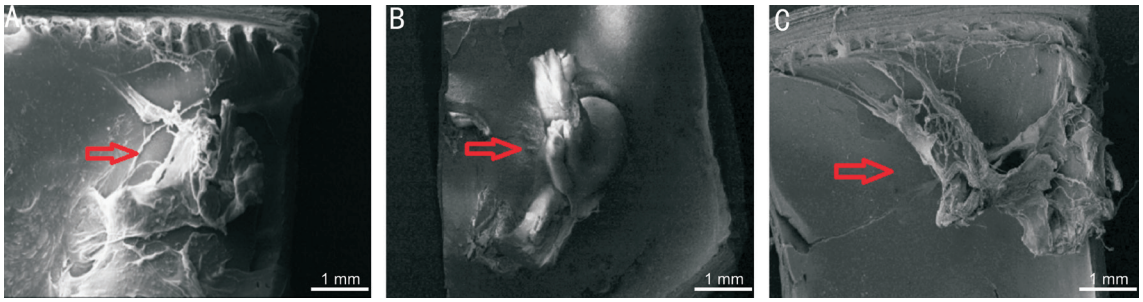

Figure 3 Examples for fibrous tissue in SEM analysis (red arrows) A: Dexamethasone group; B: Bevacizumab group; C: Control group.

reaction and inflammation in experimental PVR eyes. In one of these studies on rabbits the intravitreal injection of Ozurdex® suppressed the expression of inflammatory markers[19]. The results of a clinical trial which investigates the use of Ozurdex implant in patients undergoing vitrectomy surgery for RD with PVR showed that the implant did not improve the primary anatomic success rate and visual outcomes. On the other hand, they found that there was a greater reduction in cystoid macular edema at 6mo in vitrectomized eyes treated with Ozurdex[20].

We know that neovascularization is a key step in fibrovascular proliferation, and higher levels of vascular endothelial growth factor have been related to PVR[16]. Cellular proliferation after penetrating eye injury is triggered mostly by VH. Intravitreal blood stimulates the formation of cellular proliferation and the contraction of newly formed membranes. Thus, in theory, bevacizumab may be useful in preventing tractional fibrovascular and/or fibrocellular membranes. Besides, it reduces the vascular permeability and intraocular inflammation[11-12].

An experimental study showed that intravitreal bevacizumab reduced both the clearance time of VH and the degree of vascular proliferations. This study demonstrated that early intravitreal administration of bevacizumab at the time of primary injury repair reduces the amount of intraocular fibrocellular proliferation and the succeeding tractional powers affecting the retina. These effects are thought to be associated with the decrease in vascular permeability and secretion of cytokines and growth factors which ended in the reduction of intraocular inflammation and acceleration of the clearence of VH[12].

In our study we found that all the scores including clinical evaluation, macroscopic and histopathologic examination were better in the dexamethasone and bevacizumab-treated groups compared with the control group.

In conclusion, our study demonstrates that early intravitreal injection of dexamethasone or bevacizumab at the time of primary injury repair reduces the amount of fibrocellular proliferation and the succeeding tractional powers affecting the retina. We estimate that these effects associated with the decrease in vascular permeability and secretion of cytokines and growth factors ended in the reduction of intraocular inflammation. These drugs also help the modification of wound healing and the clearence of VH. Therefore, they reduce the development of tractional RD and enhance the final visual results. The administration of drugs is so easy, and they can be used as an adjunctive therapy to improve the results of other medical or surgical treatments.

The limitation of our study was the follow-up period. Some of the rabbits could be followed up more than 28d to observe the long-term results of the study. We believe that more research must be done to evaluate the role of intravitreal dexamethasone and bevacizumab injection in posterior penetrating ocular trauma in human eyes.

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Conflicts of Interest: Oner A, None; **Kahraman N,** None; **Ozdamar S,** None; **Balcioglu E,** None.

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