THE EFFECT OF BEVACIZUMAB ON α-SMOOTH MUSCLE ACTIN EXPRESSION AND FIBROBLAST COUNT IN TRABECULECTOMY AREA TOWARDS PREVENTION OF FIBROSIS

(Experimental Study on Oryctolagus cuniculus)

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ABSTRACT

Objective to analyze the effect of bevacizumab on α-smooth muscle actin expression and fibroblast count in trabeculectomy area of rabbit models in order to find a safer modulator of wound healing to improve surgical outcome. Material and methods 16 New Zealand white rabbits aged 4-6 months and weigh between 2,5-3,5 kg were performed trabeculectomy in their right eyes with postoperative subconjunctival injection of BSS and Bevacizumab. Rabbits were put into control and bevacizumab group using simple random sampling. Examination were done postoperative day 1, 7, and 14 and subjects were terminated and performed enucleation on postoperative day 14. The samples were histologically stained with Haematoxyline-Eosin to count the fibroblast and immunohistochemistry using α-smooth muscle actin antibody to differ the myofibroblast from fibroblast and the expression of α-SMA were collected using immunoreactive score. Result Mann Whitney u test and independent t-test were used to analyze the data, and we found both less expression of α-smooth muscle actin and fibroblast count on bevacizumab group compared to control group which indicates less myofibroblast, fibroblast, and less scarring potential in trabeculectomy area. Conclusion bevacizumab inhibits fibroblast proliferation and its differentiation to myofibroblast that lead to less collagen production and fibrosis.

Keyword: trabeculectomy, wound healing modulation, bevacizumab, myofibroblast, fibroblast

INTRODUCTION

Glaucoma is one of the most common causes of blindness throughout the world including Asia (Hong Kong, Japan, and India). The number of glaucoma patients is estimated to be 79,6 millions in 2020, and 50% of this number is Asian. Trabeculectomy is the gold standard if medical therapy or laser surgery is incapable of decreasing IOP. The wound healing process of episclera and Tenon's capsule is the most common cause of surgical failure that causes the failure of controlling the IOP and progressive optic nerve damage. Inhibition of fibrosis is important to improve surgical outcome and the popular agents is antimetabolites like mitomycin-C (MMC) and 5-fluorouracyl (5-FU), but the side effects are unfavorable. Antimetabolites can cause nonselective cell death through apoptosis and necrosis, which lead to complications like bleb leak, hypotony, blebitis, and endophthalmitis. Topical corticosteroids can decrease postoperative IOP but the long-term complications like cataract, secondary infection, herpes activation, and IOP increase is unfavorable for some surgeons. This situation made researchers innovate new agents that are more specific and effective in controlling fibrogenesis and have acceptable side effects, one of them is anti vascular endothelial growth factor (anti-VEGF).

Bevacizumab is monoclonal antibody of nonselective VEGF that inhibits the proliferation of fibroblast mediated by VEGF in vitro. A study by (O'Neil et al., 2010) proved that bevacizumab decreases fibroblast proliferation of Tenon's capsule and induced cell death. (Park et al., 2013) stated that VEGF induced myofibroblast transformation posttrabeculectomy through transforming growth factor β1 (TGF-β1) in rabbit model given VEGF and anti-VEGF bevacizumab. In this experimental study in rabbits, subconjunctival bevacizumab was injected postoperatively to determine the effect of bevacizumab in inhibition of fibrosis through inhibition of fibroblast proliferation and its differentiation to myofibroblast that is expressed with intraselular α-smooth muscle actin (α-SMA). Inhibiting subconjunctival fibrosis is expected to increase surgical outcome in postoperative period.

This study is a true experimental study with randomized post test only control group design to evaluate α-smooth muscle actin expression and fibroblast count in New Zealand white rabbit (Oryctolagus cuniculus) given subconjunctival injection of bevacizumab post trabeculectomy. We use 16 rabbits and we performed trabeculectomy with subconjunctival BSS and bevacizumab. The doseage of bevacizumab is 1,25 mg in 0,05 mL balanced salt solution (BSS) and injected 3-4 mm superior to the bleb area. Subconjunctival BSS in injected 0,05 mL in the same area in control group.

Postoperatively, we examined the IOP and anterior segment of the subjects and the data was recorded at day 1, 7, and 14. We terminated the subjects on day 14 postoperative and performed enucleation. Myofibroblast was examined using immunohistochemistry using α-smooth muscle

actin antibody and scored with immunoreactive score (IRS). Fibroblast was examined and counted using Hematoxyline-Eosin staining in 5 high power fields. Statistically using Mann Whitney U test, we found a significant decrease in expression of α-SMA in bevacizumab group compared to control group and with independent t-test we found less fibroblast in bevacizumab group significantly.

In conclusion, subconjunctival bevacizumab in postoperative period significantly inhibits fibrosis through decreasing the number of fibroblast and myofibroblast transformation in trabeculectomy site that play a great role in improving surgical outcome.

METHODS

This study was a true experimental study with a randomized post test only control group design to evaluate the expression of α-smooth muscle actin and the number of fibroblast cells in adult New Zealand white rabbits (Oryctolagus cuniculus) that underwent trabeculectomy and subconjunctival bevacizumab injection in the laboratory.

The experimental unit in this study was the New Zealand white rabbit (Oryctolagus cuniculus). The number of replications is estimated by the following calculation formula:

$$n = \frac{2(Z1-\propto + Z1-\beta)2.\sigma 2}{(\mu 1-\mu 2)2}$$

Information:

n : The number of replications

σ : The standard deviation of the degree of fibrosis = 0.475 (Memarzadeh et al., 2009)

μ1- μ2 : The difference in the treatment and control groups = 1.28 (Memarzadeh et al., 2009)

$Z_{1-\alpha}$ for α=0,05 is 1,64

$Z_{1-\beta}$ for β= 10% is 1,28

Based on the results of the calculation above, 2,348 ~ 3 replications were obtained. The probability of dropping out due to the death of the rabbits was 20%, so the replication for each group was 4.

RESULTS

Postoperative clinical evaluation was carried out using a handheld slit lamp on day 1, day 7, and day 14 with the results obtained a diffuse bleb and clinically there was no difference in the characteristics of the bleb between the treatment and control groups. All samples were made into paraffin block preparations and stained with H&E staining and immunohistochemical examination with α-SMA antibody (figures 1 and 2).

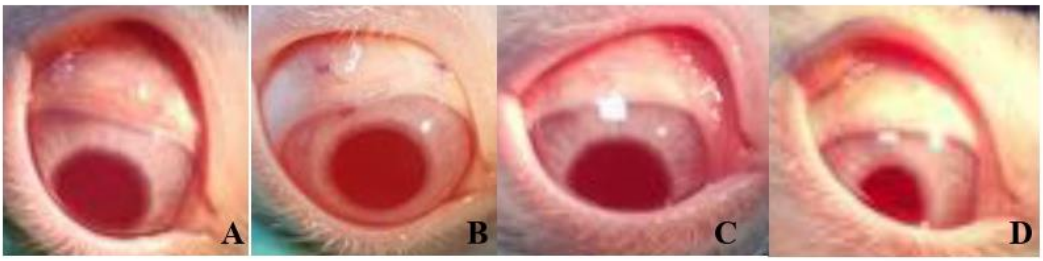

Figure 1. Bleb examination on the 7th day in: A. Control group; B. Treatment group; and the 14th day examination on: C. Control group; D. Treatment group. Blebs appeared diffuse in both groups, and were more avascular in the treated group.

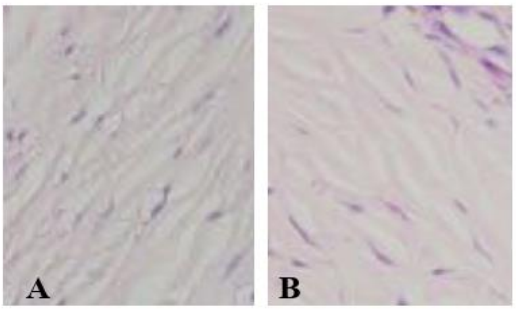

Figure 2. Fibroblasts stained with H&E at 400x magnification: A. Control group; B. Treatment group

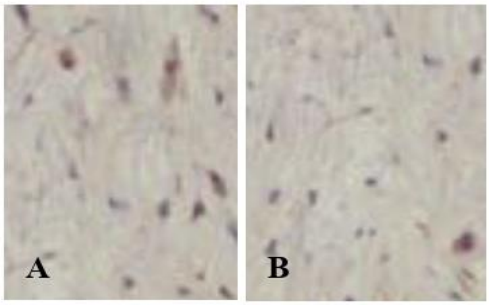

Figure 3. Expression of α-SMA on immunohistochemistry at 400x magnification: A. Control group; B. Treatment group

The results of immunohistochemical examination and counting of the number of fibroblasts can be seen in the appendix. The collected immunohistochemical examination data were analyzed and tested using the Mann Whitney U-Test, and for the number of fibroblasts using an independent t-test with $\alpha = 0.05$.

Wilcoxon statistical test - Mann Whitney test was used to see differences in α-SMA expression between groups and it was found that α-SMA expression in the treatment group was significantly lower than the control group ($p<0.05$).

Kelompok	n	Ekspresi α-SMA (IRS)				Wilcoxon - Mann Whitney test ($p_{1\text{-tailed}}$)
		Median	IQD	Minimum	Maksimum	
Kontrol	8	5,0	1,5	2	6	0,0195*
Perlakuan	8	2,5	1,0	1	6	

Table 1. Expression of α-SMA in the treatment and control groups

The results of the analysis using the independent t-test showed that the number of fibroblasts in the treatment group was significantly less than the control group ($p<0.05$). Table 5.3 Number of Fibroblasts in the treatment and control groups.

Kelompok	n	Jumlah Fibroblas (sel/LP)				Independent t-test ($p_{1\text{-tailed}}$)
		$\bar{x}$	SD	Minimum	Maksimum	
Kontrol	8	40,48	2,66	36,20	45,00	0,0005*
Perlakuan	8	34,78	2,81	31,80	39,20	

Table 2. Number of Fibroblasts in the treatment and control groups

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