THE EFFECT OF BEVACIZUMAB ON ANGIOGENESIS INTRABECULECTOMY AREA

(Experimental Study in Oryctolagus Cuniculus)

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ABSTRACT

Objective to examine the effect of Bevacizumab injection to the angiogenesis which the amount and density of blood vessel as the indicators after trabeculectomy procedure. Method This was a true experimental study using 16 eyes of 16 New Zealand White Rabbit eye treated by trabeculectomy procedure with eight eyes as the control group using Balanced Saline Solution (BSS) and eight eyes as the treatment group using Bevacizumab. It was injected subconjuctiva after the trabeculectomy. At the end of the study all rabbits in each group was sacrified, the eye was enucleated and the bleb area was dissected, and then processed for histological studies. The amount and density of blood vessel were evaluated using haematoxyllin eosin methode at day 14 after the eyes was done for trabeculectomy procedure. Result The mean of amount of blood vessel in control group was 22,63 ± 11,02 and treatment group was 14,75 ± 4,92 (p=0.043). The mean of density of blood vessel in control group was 19,10 ± 1,69 % and treatment group was 16,53 ± 2,90 % (p=0.029)%. The result shows there were statistically significant difference between the two groups (p<0.05). Conclusion In this study the subconjunctival Bevacizumab injection after trabeculectomy reduce the amount and density of blood vessel compared with subconjunctival BSS injection only, thus it is potential in preventing subconjunctival fibrosis after trabeculectomy.

Keyword : Bevacizumab, angiogenesis, amount of blood vessel, density of blood vessel, Trabeculectomy

INTRODUCTION

Modulation of wound healing in trabeculectomy surgery is to prevent and suppress the formation of subconjunctival fibrosis, where no fibrosis is an indicator of success. The wound healing response to tissue trauma involves a series of complex processes, including the secretion of inflammatory mediators, cellular migration (neutrophils, lymphocytes, monocytes and macrophages), release of several growth factors and cytokines, and ends with the process of tissue remodeling.

Glaucoma is an eye disorder characterized by glaucomatous optic neuropathy and typical loss of visual fields, where increased intraocular pressure (IOP) is one of the main risk factors (Cioffi et al., 2015; Stamper et al., 2009).

Trabeculectomy is the most widely used surgical procedure for uncontrolled glaucoma. This technique reduces intraocular pressure by creating an artificial channel as a place for aqueous fluid to drain from the anterior chamber into the subconjunctival space. The success of this trabeculectomy is hampered by the formation of postoperative fibrous tissue which causes bleb failure. The success rate of trabeculectomy surgery is quite low, which is due to bleb malfunction due to fibrosis, which is 24% to 74% of cases in 4 years (Mietz, 2004; Radcliffe, 2010; Sheha, 2011).

Various attempts have been made to inhibit fibrosis, ranging from modification of surgical techniques to perioperative medication, since Skuta and Parrish (1987) suggested that the main cause of failure of trabeculectomy was due to subconjunctival fibrosis. Until now, there are no effective and safe preparations to inhibit uncomplicated fibrosis. The non-specific mechanism of action of antimetabolites can lead to vision-threatening complications, including postoperative severe hypotonia, bleb leaks and endophthalmitis. These risk factors give rise to the need for other alternatives in the prevention of postoperative trabeculectomy fibrosis (Lama & Fechner, 2003; Memarzadeh et al., 2009; Sheha, 2011).

Vascular Endothelial Growth Factor (VEGF) is a growth factor that plays an important role in the wound healing process, including stimulating the process of angiogenesis or the formation of new blood vessels needed for the process of forming postoperative granulation tissue/fibrosis. The use of anti-VEGF is currently growing rapidly. Anti-VEGF in ophthalmology was first used in posterior segment disorders, such as Age-related Macular Edema (AMD), neovascular glaucoma, diabetic retinopathy and central retinal vein obstruction.

METHODS

The clinical trial in this study was a true in vivo laboratory experiment with a randomized post test only control group design using Bevacizumab as a research object to analyze the

number and density of capillary blood vessels in posttrabeculectomy tissue. The subjects in this study were New Zealand white rabbits (Oryctolagus cuniculus). The treatment is the administration of Bevacizumab via subconjunctival injection with the output being the number and density of capillaries.

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

(σ) merupakan pooled variance dari variabel struktur pembuluh darah dengan rumus sebagai berikut:

$$n = \frac{(SD1 = SD^2)}{2} = \frac{(2.07+1.17)}{2} = 1{,}62$$

μ1 and μ2 are the average values calculated from the vascular structure variables in groups I and II of 8.33 and 4.17. Based on the Z distribution table with an error rate of α = 0.05, the value of Z1-α = 1.64 is obtained, and the error rate of β = 0.10 is obtained by the value of Z1-β = 1.28.

Based on the formula above, a minimum number of repetitions of 3 is obtained. This research is a group study, so that it follows the largest researcher's repetition, namely 5.

The number and density of capillary blood vessels in the conjunctival tissue observed 14 days after treatment between groups will be analyzed by the Independent T-test with a significance level of α = 0.05.

RESULTS

During the observation period, the administration of topical drugs, both the duration and type, was the same in each eye of the rabbits where all rabbits received postoperative topical antibiotic therapy (ofloxacin) every day until the fourteenth day. The anterior chamber is deep and clear. All of the surviving rabbits appeared to be active, eating and drinking well with stable weights. The distribution of research subject groups can be seen in table 1.

Treatment Group	Amount	Percentage
Balanced Salt Salution (BSS)	8	50%
Bevacizumad	8	50%
Total	16	100%

Table 1. Distribution of Research Subject Groups

On the 14th day, rabbit termination and eye enucleation were performed in both groups. All samples were made of paraffin block preparations and stained with Haematoxylin Eosin (HE) staining which can be seen in Figure 1.

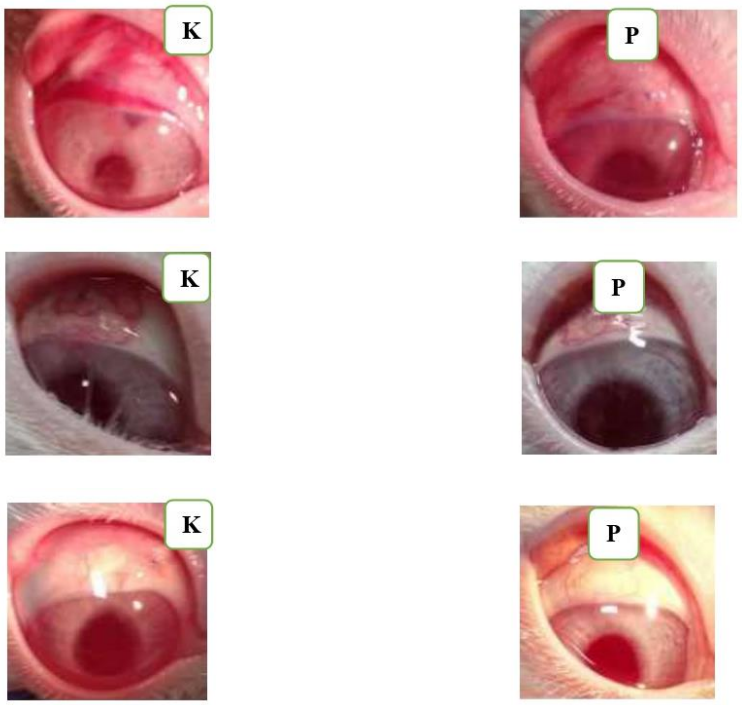

Figure 1. Bleb examination on days 1, 7 and 14

The results of examining the mean number of blood vessels in the control and treatment groups are shown in table 1. The collected data is presented in the form of descriptive data and tested using an independent t-test.

Group	Average (SD)	$P_{1\text{-tailed}}$
Balanced Salt Salution (BSS)	22,63 (11,02)	0,043*
Bevacizumad	14,74 (4,92)	

Table 2. Results of the average number of blood vessels

Description: significant at α=0.05 ($p<0.05$)

Table 2 shows that in the control group (BSS), the mean number of blood vessels was 22.63 ± 11.02. In the treatment group (Bevacizumab), the mean number of blood vessels was 14.74 ± 4.92. The statistical test to see the difference in the mean number of blood vessels between groups was tested using an independent t-test, showing that the mean number of blood vessels in the treatment group was less than the control group ($p <0.05$).

Group	Average (SD)	$P_{1\text{-tailed}}$
Balanced Salt Salution (BSS)	19,10 (1,69)	0,029*
Bevacizumad	16,53 (2,90)	

Table 3. The results of the mean blood vessel density examination

Description: significant α=0.05 (p<0.05)

The results of examining blood vessel density can be seen in the control and treatment groups shown in table 3. The data collected is presented in the form of descriptive data and tested using the Wilcoxon-Mann Whitney test.

Table 3 shows that in the control group, the mean blood vessel density was 19.10 ± 1.69%. In the treatment group, the mean blood vessel density was 16.53 ± 2.90%. The results of statistical tests using the Wilcoxon - Mann Whitney test showed that the density of blood vessels in the treatment group was lower than the control group (p<0.05).

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