BEVACIZUMAB EFFECT ON EXPRESSION OF COLLAGEN TYPE I AFTER TRABECULECTOMY (EXPERIMENTAL STUDY ON ORYCTOLAGUS CUNICULUS)

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ABSTRACT

Objective to determine the effect of bevacizumab as an antifibrotic agent on collagen density, collagen thickness and collagen type I after trabeculectomy in rabbit. Materials and methods : Sixteen male New Zealand white rabbits divided by two groups, 8 rabbits in control group and 8 rabbits in treatment group. Control group underwent trabeculectomy and injection of balanced salt solution. Treatment group underwent trabeculectomy and subconjunctival injection of bevacizumab (1.25mg, 25mg/mL). They were terminated on 14 postoperative days. Masson Trichrome were performed to evaluate collagen collagen density, collagen thickness. Immunohistochemistry using a monoclonal antibody to collagen type I was performed to evaluate collagen type I expression. Results This study showed the density of collagen fibers decreased and statistically significant in the treatment group ($p = 0.075$, p 0.05). Expression of type I collagen obtained a decrease in the treatment group compared to BSS group ($p = 0.006$, $p<0.05$). Conclusion Bevacizumab reduces bleb fibrosis by inhibition of angiogenesis and accumulation of extracellular matrix. Postoperative subconjunctival injection of bevacizumab may limiting scar tissue formation at the site of trabeculectomy by blocking collagen synthesis.

Keywords : bevacizumab, trabeculectomy, fibrosis, collagen type I

INTRODUCTION

Glaucoma is an eye disease characterized by progressive optic neuropathy and can cause irreversible blindness if not treated properly. Trabeculectomy is the most commonly used surgical filtration procedure to treat IOP that is

not controlled by drugs or lasers. The failure of this bleb is the result of fibroblast proliferation and migration of inflammatory cells in the wound healing process, thus forming excessive subconjunctival scar tissue in the filtration area and inhibiting new pathways of aqueous fluids (Radcliffe, 2010; Sheha 2011; Cheng, et al. 2015; Masoumpour, Nowroozzadeh, Razeghinejad 2016).

Glaucoma is the leading cause of irreversible blindness in the world. The number of glaucoma sufferers (aged 40-80 years) globally in 2013 reached 64.3 million, both open-angle and closed-angle glaucoma. The number of open-angle glaucoma sufferers globally is more than angle-closure glaucoma. The prevalence of national blindness in Indonesia in 2014 according to IAPB (International Agency for Prevention of Blindness) data is 1.5% of the population (3 million people) and glaucoma is the second cause of blindness after cataracts (0.2%) (IAPB, 2013; Tham, et al., 2014).

The use of antimetabolites such as Mitomycin-C (MMC) and 5-fluorouracil (5-FU) to prevent episcleral fibrosis has been widely used for the past three decades. Amniotic membrane transplantation does not cause side effects like the use of antimetabolites (Hyung & Kim 2001; Sheha 2011; Liu, et al. 2014; Bergen, et al. 2015; Masoumpour, Nowroozzadeh, Razeghinejad 2016).

This study was a true experimental study with a randomized post test only control group design. The aim of study was to evaluate collagen density, collagen thickness and expression of type I collagen. Sixteen male New Zealand white rabbits (Oryctolagus cuniculus) divided in two groups, 8 rabbits in control group and 8 rabbits in treatment group. The control group carried out trabeculectomy and given subconjunctival Balanced Salt Solution (BSS) injection in the outer area of bleb whereas the second group was the treatment group where the experimental animals included in this group were trabeculectomy and given subconjunctival bevacizumab injection (1.25mg, 25mg/mL). They were terminated on 14 postoperative days. Masson Trichrome were performed to evaluate collagen density and collagen thickness. Immunohistochemistry using a monoclonal antibody to collagen type I was performed to evaluate collagen type I expression. The results showed the density of collagen fibers decreased and statistically significant in the treatment group ($p = 0.075$, p 0.05). Expression of type I collagen obtained a decrease in the treatment group compared to BSS group ($p = 0.006$, $p < 0.05$).

METHODS

This research was a true experimental laboratory study in rabbits with a randomized post test only control group design to see the effect of bevacizumab on the density and thickness of collagen fibers and expression of collagen type I after trabeculectomy day 14.

The number of replications is estimated by the following calculation formula:

$$n = \frac{2(\mathrm{Z}\alpha + \mathrm{Z}\beta)2.\sigma 2}{(\mu - \mu 2)2}$$

Information :

n	: The number of replications
σ	: The standard deviation in the control group = 291,949
μ1 - μ2	: The difference in the area of granulomatosis in the treatment and control groups =273,119
$Z_1/2\alpha$ untuk	: 0.05 is 1.64
Zβ untuk β	: 20% is 0,84

Based on the calculation results above, 5 replications were obtained. The probability of dropping out due to the death of rabbits was 20%, so replication for each group was 7. The dropout criteria in this study were rabbits that were sick, died, and/or complications of trabeculectomy surgery such as infection , scleral perforation, vitreous prolapse, and bleeding during and after trabeculectomy surgery.

RESULT AND DISCUSSION

This study is an experimental study in which the results were obtained by means of two histopathological examinations, namely Masson trichrome examination and monoclonal antibody immunohistochemistry against collagen type I. The collected data were subjected to statistical analysis to determine the density of collagen fibers, thickness of collagen fibers, and expression of collagen type I in control and treatment groups.

The control group was the group that underwent a trabeculectomy and was given BSS injections to the outside of the bleb. The treatment group was the group that underwent a trabeculectomy and was given a subconjunctival bevacizumab injection in the area outside the bleb, at a dose of 1.25 mg in 0.05 mL BSS.

Gambar 1. Segmen anterior kelinci sebelum operasi

Postoperative evaluation using a handheld slit lamp to determine the anterior segment and the condition of the bleb. Obtained bleb that is diffuse and there is no difference in the characteristics of the bleb between the control and treatment groups. Deep chambers and clear corneas. IOP within normal limits.

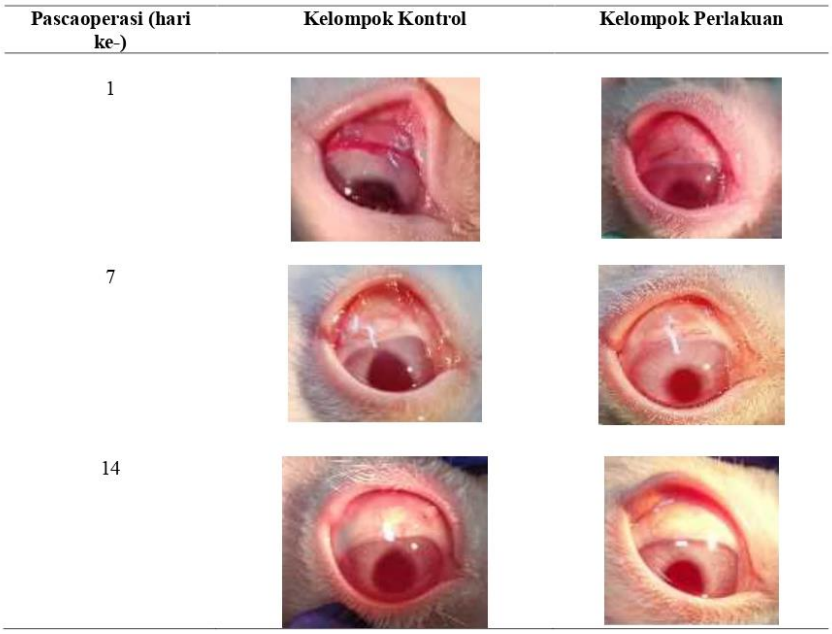


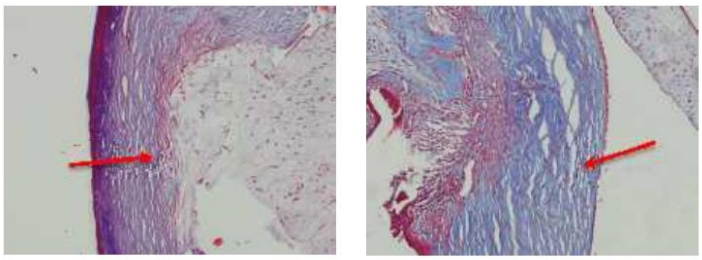
Figure 2 Bleb examination on days 1, 7 and 14, a. Control group, b. Treatment group

The condition of all rabbits during the treatment period was very good, their movements were active, and their weight was stable. Postoperative complications were not found. There were also no rabbits that died or dropped out during the study period.

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

Table 1. Distribution of research subject groups

Observation and antibiotic therapy were carried out for 14 days, then the rabbits were terminated and enucleated. Tissue samples were fixed with 10% buffered formalin and paraffin block preparations were made. The tissue samples were then stained with Masson trichrome.



a b

Figure 3 Density of conjunctival collagen fibers with Masson trichrome staining seen at 200x magnification (collagen is colored blue) indicated by arrows, a. Control group, b. Treatment group.

Kepadatan serabut kolagen	Kelompok				p
	Kontrol		Perlakuan		
	n	Persentase (%)	n	Persentase (%)	
Skor 1	0	0	0	0	0,0075*
Skor 2	2	25,0	7	87,5	
Skor 3	6	75,0	1	12,5	
Median		3		2	
IQD		0,5		0,0	

* Keterangan : Signifikan pada α=0,05 ($p<0,05$)

Table 2. Distribution of fiber density in the rabbit conjunctiva

Table 2 shows that in the control group the density of collagen fibers was obtained with a score of 1 for 0 subjects (0%), a score of 2 for 2 subjects (25.0%), and a score of 3 for 6 subjects (75.0%). The treatment group obtained the density of collagen fibers with a score of 1 for 0 subjects (0%), a score of 2 for 7 subjects (87.5%), and a score of 3 for 1 subject (12.5%). This shows that the density of collagen fibers in the treatment group is lower than the control group.

In Masson trichrome staining, an examination of the thickness of the collagen fibers is also carried out which can be seen in Figure 4.

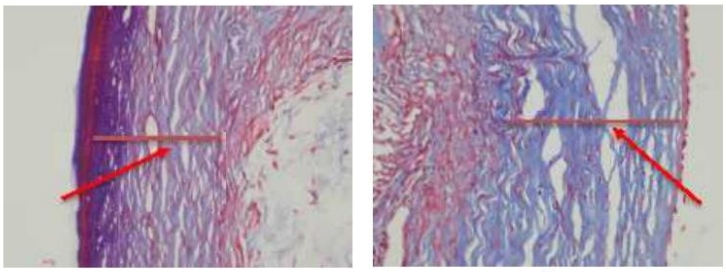


Figure 4. Thickness of n conjunctival collagen fibers with Masson trichrome staining viewed at 400x magnification. The arrows indicate the thickness of the collagen as measured using a ruler on the computer. The overall thickness measurement is calculated and reduced by the formed vacuoles, a. Control group, b. Treatment group

Group	Thickness of collagen fibers					P
	n	Average	Standard Deviation	Minimum	Maksimum	
Control	8	491,36	243,03	114,3	954,0	0,323*
Treatment	8	543,15	195,13	303,3	831,6	

Table 3. Distribution of collagen fiber thickness in rabbit conjunctiva

Table 3 shows that in the control group, the average thickness of collagen fibers was 491.36 μm ± 243.03. Whereas in the treatment group, the thickness of the collagen fibers was 543.15 μm± 195.13. The results of the independent t-test statistic showed that the thickness of the collagen fibers in the treatment group did not differ from the control group, as evidenced by the value of p = 0.323 (p > 0.05).

Type I collagen expression was carried out by immunohistochemistry using Collagen I Alpha 2 Antibody in each group. The results of examination of type I collagen expression according to the IRS score obtained are presented in table 4.

Kelompok	Skor IRS Ekspresi Kolagen Tipe I								Medium	IQD	p
	1	2	3	4	5	6	7	8			
Kontrol	9	12	8	12	4	9	9	6	5,50	0,75	0,006*
Perlakuan	2	9	4	2	3	6	4	3	2,88	1,50	

Table 4. Distribution of type I collagen expression in rabbit conjunctiva

Table 4 shows that in the control group the median IRS score for type I collagen expression was 5.50 ± 0.75. In the treatment group, the median IRS score for collagen type I expression

was 2.88 μ 1.50. The Wilcoxon-Mann Whitney test statistic showed a significant decrease in type I collagen expression in the treatment group compared to the control group. This is evidenced by the value of p = 0.006 (p <0.05).

BIBLIOGRAPHY

Radcliffe, N.M. 2010, Trabeculectomy revision as a treatment for failed trabeculectomy, Glaucoma Today, hal. 25-28

Sheha, H. 2011, Update on Modulating Wound Healing in Trabeculectomy, S.Rumelt ed. Glaucoma Basic and Clinical Consepts, Rijeka In.Tech., Kroasia, hal 401-411

Cheng, J.W., Cheng, S.W., Wei, R.L., Lu G.C. 2016, 'Anti-Vascular Endothelial Growth Factor for Control of Wound Healing in Glaucoma Surgery', Cochrane Database of Systematic Review, John Wiley & Sons, Ltd.,DOI:10.1002/14651858.CD009782.pub2, diakses 30 November 2017, http:// www.cochranelibrary.com

Masoumpour, M.P., Nowroozzadeh, M.H., Razeghinejad, M.R., 2016, 'Current and Future Techniques in Wound Healing Modulation after Glaucoma Filtering Surgeries', The Open Ophthalmology Journal volume 10, hal 68-85, DOI: 10.2174/1874364101610010068, diakses 28 Desember 2017

International Agency for Prevention of Blindness (IAPB) 2014, Report of Vision 2020 IAPB Workshop Indonesia, Jogjakarta, Indonesia, hal 6-7

Tham, Y.C., Li, X., Wong, T.Y., Quigley, H.A., Aung, T., Cheng, C. Y. 2014, 'Global Prevalence of Glaucoma and Projections of Glaucoma Burden through 2040: A Systematic Review and Meta-Analysis', Ophthalmology, volume 121(11), hal 2081-2090, doi: 10.1016/j.ophtha.2014.05.013, diakses 4 September 2017, https://www.ncbi.nlm.nih.gov/pubmed/24974815

Hyung, S.M., Kim, S.K. 2001, Mid-Term Effect of Trabeculectomy with Mitomycin C in Neovascular Glaucoma Patients, Korean Journal Ophthalmology vol. 15, hal 98-106

Liu, X., Du, L., Li, N. 2016, 'The Effects of Bevacizumab in Augmenting Trabeculectomy for Glaucoma A Systematic Review and Meta-Analysis of Randomized Controlled Trials', Medicine volume 95, Wolters Kluwer Health, Inc., DOI: 10.1097/MD.0000000000003223, diakses 12 November 2017

Bergen, T.V., Vandewalle, E., Moons, L., dan Stalmans, I. 2015, 'Complementary Effects of Bevacizumab and MMC in the Improvement of Surgical Outcome after Glaucoma Filtration Surgery', Acta Ophthalmology vol. 93, hal 667–678