TRIAMCINOLONE ACETONIDE EFFECT ON INFLAMMATORY RESPONSE AND EXPRESSION OF COLLAGEN TYPE I AFTER STRABISMUS SURGERY

(Experimental Study in Oryctolagus Cuniculus)

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ABSTRACT

Objective to determine the effect of triamcinolone acetonide as an antifibrotic agent on inflammatory response and collagen type I after strabismus surgery in rabbit Material and methods thirty six eyes of male New Zealand white rabbits divided by two groups, 18 rabbits eyes in control group and 18 rabbits eyes in treatment group. Control group underwent recess muskulus rektus superior and irrigation of balanced salt solution in reattachment site. Treatment group underwent strabismus surgery and irrigation Triamcinolone Acetonide (TCA) (Flamicort ® Dexa-medica)(40 mg/ml) 0,15 ml (6 mg). They were terminated on 15 postoperative days. Staining Hematoxylin & Eosin were performed to evaluate inflammatory response and immunohistochemistry using a monoclonal antibody Collagen I Alpha 2 Antibody (LS-C352030, LifeSpan BioSciences, Inc) was performed to evaluate collagen type I expression. Results this study showed of inflammatory response decreased and statistically significant in the treatment group ($p=0.002$, $p=<0.05$). Expression of type I collagen obtained a decrease in the treatment group compared to BSS group ($p=0.004$, $p<0.05$). Conclusion triamcinolone Acetonide (TCA) 40 mg/ml is one of therapeutic approaches that aims to reduce fibrosis after strabismus surgery by inhibiting the accumulation of inflammatory cells activation and suppressing of type I collagen deposition.

Keywords : Triamcinolone Acetonide, strabismus surgery, fibrosis, inflammatory response, type I collagen

INTRODUCTION

Strabismus surgery aims to improve the position of the eyeball in the hope of improving vision, binocular vision and cosmetic function. The principle of this operation is to resect muscles that are too weak or recession of muscles that are too strong. As with other eye operations, complications during surgery and after surgery can occur in strabismus surgery such as the formation of fibrous tissue. (Kraft, 2016; Luederet al., 2016; (Olitsky & Coats, 2015)

Scar tissue after strabismus surgery has not been widely reported, but Ludwig et al. (2000) reported that 134 patients with scar remodeling after strabismus surgery had a 10% contribution to strabismus re-surgery and 50% overcorrection. The scar remodeling procedure involved 73 procedures for 1 muscle, 59 procedures for 2 muscles, 1 procedure involved 3 muscles, and 1 procedure involved 4 muscles. In Wan & Hunter's study (2016) explained that local complications in strabismus surgery obtained 90% of conjunctival scars with strabismus surgery that exceeds 1 muscle resulting in tissue fibrosis which allows post-strabismus surgery to occur muscle restriction. (Ludwig & Chow, 2000; Wan & Hunter, 2014)

Several surgical and medical approaches have been used to reduce postoperative strabismus inflammation and adhesions to inhibit fibrosis, namely antimetabolite drugs such as Mitomycin-C (MMC) and 5-Fluorouracil (5-FU). The non-specific mechanism of action of antimetabolites can lead to vision-threatening complications.

The inflammatory process in the wound healing process plays an important role. A severe inflammatory process will lead to a proliferative process of fibroblasts which can develop into scar tissue. Excessive accumulation of collagen will cause fibrosis. Giving Triamcinolone Acetonide (TCA) is one way to suppress collagen deposition so as to prevent fibrosis in post strabismus surgery. Triamcinolone Acetonide (TCA) has fewer side effects compared to anti-metabolites. (Carroll et al., 2002; Ganiswarna et al., 2004; Minguini et al., 2000; (Okada et al., n.d.)

Studies that have been carried out by experts show that Triamcinolone Acetonide (TCA) can play a role in inhibiting the inflammatory process, inhibiting fibroblast proliferation and collagen synthesis so that it can prevent fibrosis which causes scar tissue.

METHODS

This research is a true experimental laboratory study on post test only control group design animals to evaluate the effect of Triamcinolone Acetonide (TCA) on the inflammatory response and expression of type I collagen after 15th day of strabismus surgery. The experimental unit of this study was the eyeball of the New Zealand white rabbit.

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

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

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 results of the calculation above, 15 replications were obtained. The probability of dropping out due to the death of the rabbit was 10%, so the replication for each group was 18. (Carvalho et al., 2007)

RESULTS

This research is an experimental study where the results of the research were obtained by means of two histopathological examinations, namely examination of Hematoxylin & Eosin staining and immunohistochemistry of monoclonal antibodies against collagen type I. The collected data were subjected to statistical analysis to determine the inflammatory response and expression of collagen type I in the control and treatment groups. .

The control group was the group that underwent strabismus surgery and was given BSS irrigation in the muscle reattachment area. The treatment group was the group that underwent strabismus surgery and was given Triamcinolone Acetonide 40 mg/ml irrigation in the muscle reattachment area at a dose of 0.15 ml (6 mg).

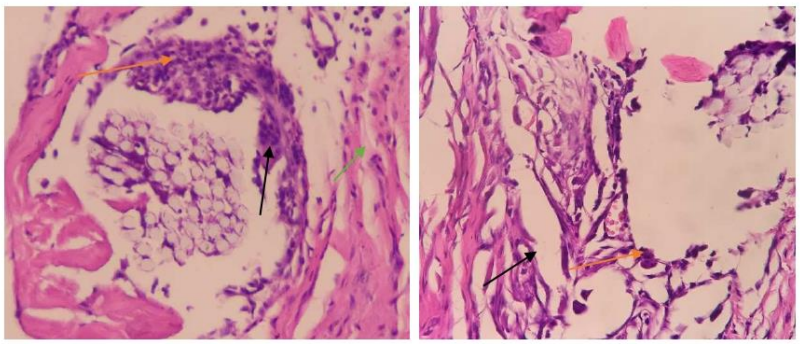
Examination of the anterior segment of the oculi before conducting the study using a handheld slit lamp and found conditions within normal limits. During the observation period, each rabbit received the same treatment. Postoperative evaluation using a handheld slit lamp to determine the anterior segment. There was conjunctival hyperemia in the superior area and clear cornea in all control and treatment groups. There was no change in eyeball position in all control and treatment groups.

Post surgery	Control Group	Treatment Group
1st day		
7th day		

Picture 1. Examination of the conjunctiva on day 1 and 7 of the control group, the 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.

Tissue samples were fixed with 10% buffered formalin and paraffin block preparations were made. The tissue samples were then stained with Hematoxylin & Eosin (HE). The histological examination of Hematoxylin & Eosin examined the inflammatory response which can be seen in picture 2.



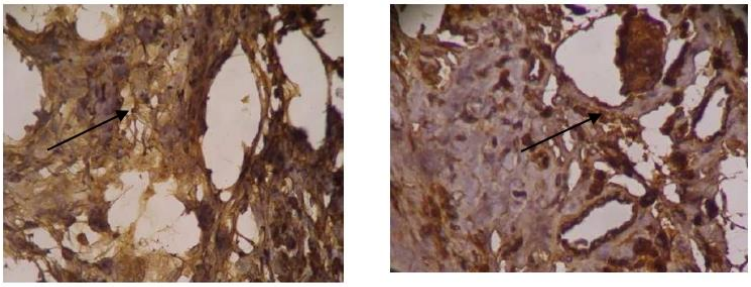
Picture 2. Inflammatory response with Hematoxylin & Eosin (HE) staining seen at 400× magnification

Grade of inflammatory response	Group				P
		Control		Treatment	
	n	Percentage (%)	n	Percentage (%)	
Grade 0	0	0	0	0	0,002*
Grade 1	0	0	0	0	
Grade 2	7	38,90%	16	88,90%	
Grade 3	11	61,10%	2	11,10%	
Total	18	100%	18	100%	

Table 1. Distribution of the inflammatory response in the superior rectus muscle of rabbits after strabismus surgery

Table 1 shows that in the control group the inflammatory response was grade 0 in 0 subjects (0%), grade 1 in 0 subjects (0%), grade 2 in 7 subjects (38.9%) and grade 3 in 11 subjects (61, 1%). In the treatment group, the inflammatory response was grade 0 in 0 subjects (0%), grade 1 in 0 subjects (0%), grade 2 in 16 subjects (88.9%) and grade 3 in 2 subjects (11.1%). The Wilcoxon-Mann Whitney U test statistic showed a significant difference in the inflammatory response where p=0.002 (p.0.05) between the control group and the treatment group. This shows that the inflammatory response in the treatment group was lower than the control group.

Expression of type I collagen was carried out by immunohistochemistry using the monoclonal antibody Collagen I Alpha 2 Antibody (LS-C352030, LifeSpan BioSciences, Inc), against type I collagen in each group. Histological picture of type I collagen expression (COL-1) can be seen in Figure 3.



a b

Picture 3. Expression of type 1 collagen by immunohistochemical staining viewed at 400x magnification, a. control group, b. treatment group.

Degree of expression of type I collagen	Group				P
		Control		Treatment	
	n	Percentage (%)	n	Percentage (%)	
There is no	0	0%	0	0%	0,004*
Weak	0	0%	0	0%	
Currently	0	0%	8	44,4%	
Strong	18	100%	10	55,5%	
Total	18	100%	18	100%	

Table 2. Distribution of type I collagen expression in the superior rectus muscle of rabbits after strabismus surgery

Description: significant at $\alpha=0.05$ ($p<0.05$)

Table 2 shows that in the control group, 0 subjects (0%) on the description of the degree of expression were absent, weak and moderate, 18 subjects (100%) had strong expression. In the treatment group, 0 subjects (0%) had no and weak degrees of expression, 8 subjects (44.4%) had moderate degrees of expression and 10 subjects (55.5%) had strong degrees of expression. The results of the Wilcoxon-Mann Whitney test statistic showed a significant difference in the expression of type I collagen where $p = 0.004$ ($p < 0.05$) between the treatment and control groups. This shows a significant decrease in the expression of type 1 collagen in the treatment group compared to the control group.

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