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Original Research Article

The Effect of Duration of Administration and Discontinuation of Fluorometholone Eye Drops on The Trabecular Meshwork

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Abstract

Background: The extracellular matrix (ECM) in the trabecular meshwork (TM) may play a role in the steroid-induced aqueous humor outflow resistance pathophysiology. Topical steroids such as fluorometholone can cause elevated intraocular pressure. Increased synthesis and accumulation of TM ECM proteins due to steroids result in TM dysfunction and elevated intraocular pressure.

Purpose: This study aims to analyze the effect of duration of administration and discontinuation of fluorometholone eye drops on the thickness of the TM ECM of Wistar rats**.**

Methods: This study was an experimental study with a posttest-only control group design. The total sample of 25 rats was divided into 5 groups: Treatment 1 was given topical fluorometholone for 4 weeks and stopped for 4 weeks. Treatment 2 was given fluorometholone for 6 weeks and stopped for 4 weeks. Control 1 was given topical fluorometholone for 4 weeks, Control 2 was given topical fluorometholone for 6 weeks, and the negative control was used as a baseline. TM ECM examination was assessed using histopathological score grading. This study used the Mann-Whitney test and the Kruskal-Wallis comparative hypothesis test.

Results: The difference in ECM thickness was not statistically significant either between treatment group 1 and treatment group 2 (p=1.000) or control group 1 (p=0,1000). There was a significant change in the thickness of the TM ECM in treatment group 1, treatment 2, control 1, control 2 compared to the negative control (p=0.003, p=0.003, p=0.003, p=0.004) and The thickness was statistically significant betweent treatment 2 and control group 2 (p = 0,014).

Conclusion: TM ECM thickness did not return to normal thickness after administration of fluorometholone eye drops one drop 4 times a day for 4 weeks and 6 weeks followed by discontinuation of the drug for 4 weeks. The thickness of the TM ECM was thicker in experimental rats with a longer duration of administration of fluorometholone eye drops.

Keywords: *Extracellular matrix; Fluorometholone;, Trabecular meshwork.*
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INTRODUCTION

Glaucoma is a condition characterized by optic neuropathy which is associated with changes in the connective tissue structure of the optic disc and neural tissue thickness associated with decreased vision and visual fields.1

The exact pathophysiology of corticosteroid-induced glaucoma is still not fully understood. Steroid eye drops are known to induce physiological changes in the ocular tissue after more than 2 weeks of use. The extracellular matrix of the trabecular meshwork plays an important role in the pathophysiology of corticosteroid-induced glaucoma.

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Table 1. A comparative test of extracellular matrix thickening of the trabecular meshwork group between NC and T1, T2, C1, C2.

Groups	Thickening			Compared Groups	Thickening			P value
	None	Mild	Severe		None	Mild	Severe	
NC	5(100%)	0 (0%)	0(0%)	T1	0(0%)	5(100%)	0(0%)	P= 0,003*
				T2	0(0%)	5(100%)	0(0%)	P= 0,003*
				C1	0(0%)	5(100%)	0(0%)	P= 0,003*
				C2	0(0%)	1(20%)	4(80%)	p= 0,004*

Description: *significant if *p value* < 0,05

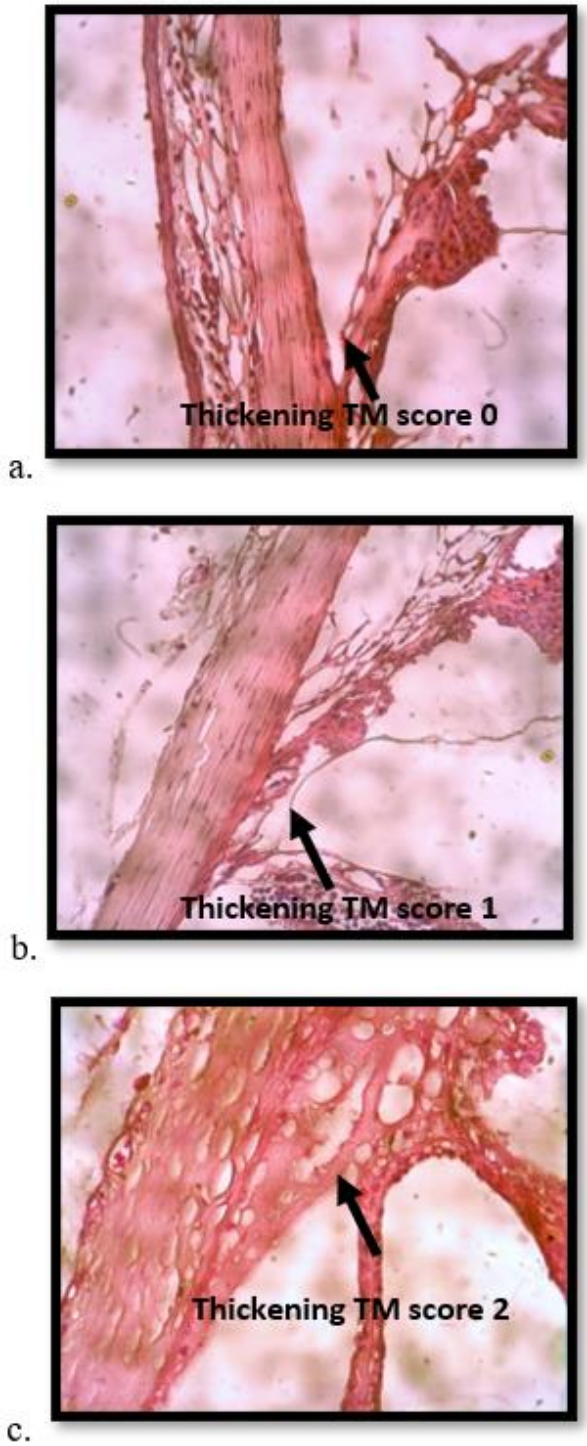

Figure 1. Histopathological examples of research preparations (H.E. coloring, 400x magnification). a. sample picture of preparations with normal thickness from the NC group (score 0). b. An example of a mild thickened preparation from group T1 (score 1). c. Example of obvious/severe thickening of the preparations from group C2 (score 2).

Existing theory suggests that corticosteroids increase the resistance to outflow of aqueous humor by the extracellular matrix of the trabecular meshwork. Phulke (2017) study showed that the changes that occur were highly dependent on the duration of exposure to steroids. Disruption of the extracellular matrix may be the result of an accumulation of glycosaminoglycans or increased protein production in the extracellular matrix trabecular meshwork induced by the glucocorticoid response, leading to the obstruction of the outflow of aqueous humor[1]

The study of Mandelkom (2001) and wordinger (1999) demonstrated that corticosteroid-induced cytoskeletal changes could inhibit pinocytosis of the aqueous humor or inhibit glycosaminoglycan elimination. Furthermore, protease inhibition and suppression of the phagocytic function of trabecular meshwork endothelial cells caused the accumulation of cellular debris further obstructing the flow of aqueous humor.[2–4]

The extracellular matrix of the trabecular meshwork is suggested to play a role in the corticosteroid-induced pathophysiology of thickness. Corticosteroids are thought to increase the resistance to aqueous humor outflow due to changes in the extracellular matrix of the trabecular meshwork. The resistance might be due to the accumulation of glycosaminoglycans or increased protein production in the extracellular matrix of the trabecular meshwork induced by the glucocorticoid response, therefore, obstructing aqueous humor outflow and inhibiting glycosaminoglycan degradation.[2–5]

Currently, the use of fluorometholone is increasing along with the increase in autoimmune diseases. Although it is said to be a safe steroid, in some cases, the use of fluorometholone results in an increase in IOP even after cessastion of fluorometholone. Therefore, this study aimed to determine the effect of the duration of administration and discontinuation of fluorometholone eye drops on the trabecular meshwork. The effect of elevated IOP due to fluorometholone has been well documented in patients who responded to steroid (steroid responders) and other steroid preparations such as prednisolone, dexamethasone, betamethasone, or other steroid preparations.

Table 2. A comparative test of extracellular matrix thickening of the trabecular meshwork group between T1 and T2

Groups	Thickening			Compared Groups	Thickening			P value
	None	Mild	Severe		None	Mild	Severe	
T1	0(0%)	5(100%)	0(0%)	T2	0(0%)	5(100%)	0(0%)	P = 1,000

Description: *significant if *p value* < 0,05

Tabel 3. A comparative test of extracellular matrix thickening of the trabecular meshwork group between C1 and T1, C2.

Groups	Thickening			Compared Groups	Thickening			P value
	None	Mild	Severe		None	Mild	Severe	
C1	0(0%)	5(100%)	0(0%)	T1	0(0%)	5(100%)	0(0%)	P = 1,000
				C2	0(0%)	1(20%)	4(80%)	P = 0,014*

Description: *significant if *p value* < 0,05

In steroid-responsive patients, intraocular pressure was elevated in the first week of steroid use, but this varied. It could occur within hours or even years after routine using the steroid. After the steroid is discontinued, the intraocular pressure will return to normal after 1-4 weeks. The duration of steroid exposure is believed to affect the reversibility of the increase in intraocular pressure.[6] Lately, there are no studies on the exposure time and discontinuation time of topical steroids that explain the correlation between the use of topical fluorometholone preparations on the thickness of the extracellular matrix in the trabecular meshwork. This study is expected to determine the description of the thickness of the trabecular meshwork on the administration time and discontinuation time of topical fluorometholone corticosteroids with particular durations.

MATERIALS AND METHODS

This was an experimental study with posttest only control group design conducted for six months at Lembaga Pengembangan Penelitian Terpadu (LPPT) Universitas Diponegoro Semarang for the maintenance, treatment, and termination of experimental animals. Preparation of research samples from the trabecular meshwork of Wistar rats was conducted at the Central Laboratory of the Diponegoro National Hospital. The study population was wistar male rats, age 2-3 months, weight 200-300 grams, active motion, with no anatomical abnormalities in the eyes of the rats. Corticosteroids used in this study were fluorometholone eye drops (posop ® 1%) with doses 4x1 per day (1 drop = 0.05 ml). The total sample of 25 rats was divided into 5 groups: Treatment 1 (T1) was given fluorometholone eye drops one drop 4 times a day for 4 weeks and stopped for 4 weeks. Treatment 2 (T2) was given fluorometholone eye drops one drop 4 times a day for 6 weeks and stopped for 4 weeks. Control 1 (C1) was given fluorometholone eye drops one drop 4 times a day for 4 weeks, control 2 (C2) was given fluorometholone eye drops one drop 4 times a day for 6 weeks, and negative control (NC) was used as a baseline. The rats were acclimatized for 1 week and then randomized to be divided into 3 groups: treatment group (T1, T2), negative control (NC), and control group (C1, C2). Then, intervension on the sample were carried out at the beginning of the same time according to the division of the group. After the intervention, (according to the group) the rats in the group were terminated and enucleated. The Rats were immediately prepared for specimens the next day.TM ECM anatomical pathology examination was assessed using histopathological score grading. This study uses the Mann-Whitney test and the Kruskal-Wallis comparative hypothesis test.

RESULTS

Based on Table 1, in the NC group, there was no change in the thickness of the extracellular matrix of the trabecular meshwork in all samples, meanwhile, in T1, 5 samples (100%) experienced mild extracellular matrix thickness of the trabecular meshwork. There was a significant difference in the NC group and T1 group p =0.003 (p<0.05). Significant differences are also shown in NC group compared to T2 group p = 0.003 (p<0.05), NC group compared to C1 group p = 0.003 (p<0.05) ,and NC group compared to C2 group p = 0.004 (p <0.05).

Both T1 group and T2 group had 5 samples (100%) with mild trabecular meshwork extracellular matrix thickness and there was no significant difference (p = 1,000) (Table 2).

Based on Table 3, C1 group had mild thickening of the extracellular matrix of the trabecular meshwork in 5 samples (100%). In comparison, C2 group had mild thickening of the extracellular matrix of the trabecular meshwork in 1 sample (20%) and severe thickening in 4 samples (80%). There was a significant difference in C1 group and C2 group (p = 0.014). Meanwhile, Both T1 group and C1 group, 5 samples (100%) had mild trabecular meshwork extracellular matrix thickening and can be seen in Table 3 that there was no significant difference in T1 group and C1 group p=1,000 (p>0.05).

Table 4 explains that there was mild thickening of the extracellular matrix of the trabecular meshwork in 1 sample (20%), and severe thickening of the extracellular matrix of the trabecular meshwork in 4 samples (80%) in C2 group meanwhile, T2 group had 5 samples (100%) with mild trabecular meshwork extracellular matrix

thickness. There was a significant difference p = 0.014 (p <0.05) in the C2 group and T2 group.

Table 5 shows the results analysis of the thickness of the extracellular matrix of the trabecular meshwork using the Kruskal Wallis test. The comparison results between the NC, C1, C2, T1 and T2 groups showed a significant difference (p<0.001) in the thickness of the extracellular matrix of the trabecular meshwork. Groups T1, T2, and C1 had mild thickening of the trabecular meshwork in all specimens. Severe thickening was found in almost all C2 groups, 20% having mild thickening and 80% having severe thickening. The NC group had no thickening in the entire specimen.

meshwork ultrastructure such as denser trabecular meshwork lamellae and thickening of the trabecular meshwork extracellular matrix in Levi rats after being exposed to topical prednisolone implantation for 3 weeks. Raghunathan, et al. (2015), who examined dexamethasone exposure to human trabecular meshwork cultures, found an increase in human trabecular meshwork extracellular matrix deposits that increased 4 times thicker than the control group after being exposed to dexamethasone for 28 days (p<0.05). Dexamethasone disrupts the dynamics of cytoskeletal function and trabecular meshwork cells, resulting in decreased contractility.[7,8]

Table 4. A comparative test of extracellular matrix thickening of the trabecular meshwork group between C2 and T2

Groups	Thickening			Compared Groups	Thickening			P value
	None	Mild	Severe		None	Mild	Severe	
C2	0(0%)	1 (20%)	4 (80%)	T2	0(0%)	5(100%)	0(0%)	P= 0,014*

Description: *significant if *p value* < 0,05

Table 5. A comparative test of extracellular matrix thickening of the trabecular meshwork group T1, T2, C1, C2, dan NC.

Groups	Thickening ECM			Total	P
	Normal	Mild	Severe		
Negatif control	5 (100%)	0 (0%)	0 (0%)	5	<0,001*
Control 1	0 (0%)	5 (100%)	0 (0%)	5	
Control 2	0 (0%)	1 (20%)	4(80%)	5	
Treatment 1	0 (0%)	5 (100%)	0 (0%)	5	
Treatment 2	0 (0%)	5 (100%)	0 (0%)	5	

Description : *significant if p value < 0,05; *Kruskal Wallis*.

DISCUSSION

This study found that the duration of administration of fluorometholone eye drops one drop 4 times a day for 4 weeks and 6 weeks affected the thickness of the extracellular matrix of the trabecular meshwork. Based on Table 1, the group with the duration of administration of fluorometholone eye drops one drop 4 times a day for 4 weeks found mild thickening of the extracellular matrix of the trabecular meshwork in 5 samples (100%). Whereas, exposure to topical fluorometholone for 6 weeks caused mild thickening of the extracellular matrix of the trabecular meshwork in 1 sample (20%) and severe/obvious thickening in 4 samples (80%). It compared to the negative control, which was not found thickening of the trabecular extracellular matrix tissue. The Mann-Whitney comparative test showed a significant difference in the thickness of the extracellular matrix of the trabecular meshwork, which was significant (p>0.05) between the NC, C1, and C2 groups. It explains that the longer administration of fluorometholone eye drops, the thicker the thickness of the extracellular matrix of the trabecular meshwork.

This is related to the research results of Li et al. (2012), who investigated the increase in the thickness of the extracellular matrix of the trabecular meshwork after being given steroid exposure. Changes in the trabecular

This study found that stopping the drug for 4 weeks in groups T1 and T2 affected the thickness of the extracellular matrix of the trabecular meshwork. The extracellular matrix thickness of the T1 group was thinner than the C1 group although it was not statistically significant (Table 3, p=1,000) and the extracellular matrix thickness of the T2 group was thinner than the C2 group (table 4, p = 0.014). The differences of extracellular matrix thicknesses analysis between T1 and T2 groups were not statistically significant. However, the extracellular matrix thicknesses in the T1 and T2 groups were significantly higher than the NC group. The results of this study showed a decrease in the thickness of the extracellular matrix after discontinuation of eye drops for 4 weeks but not as much as the thickness of the extracellular matrix compared to the NC group. In table comparison of T1 and C1, T2 and C2 shows that the longer exposure to fluorometholone steroids, the thickness of the TM ECM will increase and after stopping for 2 weeks, there is a thinning of the thickness of the TM ECM but it has not returned to normal when compared to NC. This can be because of permanent damage or shorter dicontinuation of the eye drops.

The longer exposure to fluorometholone steroids, the thickness of the TM ECM will increase (C1 dan C2 tabel 3) while, discontinuation of eye drops for 2 weeks will reduce the thickness of the TM ECM, but not return to

normal (T1 T2 table 2 dan T2 C2 table 4). In this study, it was shown by the results of the analysis that the thickness of the extracellular matrix of the C1 group was thinner than the C2 group (table 3), the thickness of the extracellular matrix of the NC group was thinner than C1 group (table 1) and the thickness of the extracellular matrix of the NC group was thinner than C2 group (table 1). Corticosteroids have been shown to trigger the accumulation of extracellular matrix, proteoglycans, elastin, glycoproteins, collagen, and myocillin (MYOC). Corticosteroids will also cause cytoskeletal changes that will block pinocytosis by aqueous humor so that glycosaminoglycans will accumulate. This will trigger an increase in IOP (intraocular pressure) due to steroi-induced.[9] Elevated IOP causes mechanical stretching and distortion of both extracellular matrix and the juxtacanalicular cells. This will initiate a cascade involving MMPs. MMPs will initiate extracellular matrix turnover. Extracellular matrix remodeling is very useful for maintaining the function of the trabecular meshwork cells so that normal IOP can be maintained. Mechanical stretching of the trabecular meshwork cells or an increase in perfusion pressure in the anterior segment of the organ culture will cause an increase in the expression of MMPs. This mechanism triggers changes in the expression of extracellular matrix proteins. Elevated IOP will also affect the expression of extracellular matrix genes which can be seen from changes in mRNA. The changes of the extracellular matrix will undergo slight changes in terms of composition, organization and/or amount, to maintain normal IOP. The process can take from hours to several days to complete. This is relate to the theory of extracellular matrix remodeling to achieve IOP homeostasis. [10]

In a study conducted by Sekar (2021) on Wistar rats exposed to prednisolone eye drops, the results of the extracellular matrix of the trabecular meshwork in the administration of prednisolone eye drops for 28 days followed by discontinuation of the eye drops for 14 days and 28 days, were thinner than the administration of prednisolone eye drops without a discontinuation period of the drug.[11] However, this study has not been able to conclude how long it takes to stop the extracellular matrix of the trabecular meshwork from reaching normal limits compared to the control group. The limitation of this study is that it did not measure the TM ECM with an electron microscope due to equipment limitations. The method chosen to compare the thickness of the TM ECM was done subjectively by 2 anatomical pathologists.

CONCLUSION

TM ECM thickness did not return to normal thickness after administration of fluorometholone eye drops one drop 4 times a day for 4 weeks and 6 weeks, followed by discontinuation of the drug for 4 weeks. The thickness of the TM ECM was thicker in experimental rats with a longer duration of administration of fluorometholone eye drops.

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