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Original Research Article

The Discontinuation Effect of Topical Prednisolone on Extracellular Matrix Trabecular Meshwork in Wistar Rat

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Abstract

Background: Intra ocular pressure (IOP) elevation is one of topical steroids' side effects. Corticosteroid will initiate matrix metalloproteinase cascade that leads to extra cellular matrix (ECM) of trabecular meshwork (TM) turnover (remodeling) and its accumulation in TM.

Objective: To analyse the reversibility of ECM TM in Wistar rat after discontinuation of topical prednisolone 1% at different period (14 days and 28 days).

Methods: This was an experimental study with posttest only control group design. Total samples of 28 rats were divided into 4 groups: Treatment groups 1 was treated with topical prednisolone for 28 days, and was terminated 14 days after discontinuation of the drug. Treatment groups 2 was treated topical prednisolone for 28 days and was terminated 28 days after discontinuation of the drug. Control 1 was treated with topical prednisolone for 28 days, and Control 2 was treated with topical saline for 28 days and terminated without period of discontinuation. Histopathological grading score was used to evaluate ECM TM deposition. Mann-Whitney test and Kruskal-Wallis test were used to analyze the data.

Result: Deposition of ECM in TM was not statistically different in treatment group 1 and treatment group 2 ($p>0.05$). Deposition of ECM in TM were statistically different between treatment group 2 and control group 1 ($p<0.05$). Comparative test showed $p<0.001$, which means that there was a change in the thickness of ECM after discontinuation of instillation.

Conclusion: ECM TM was thinner in the experimental animals with a longer duration of topical prednisolone discontinuation, which demonstrate that maintenance remodeling of ECM was happen.

Keywords: *Prednisolone; extracellular matrix; trabecular meshwork*
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INTRODUCTION

Glaucoma is one of the world's major causes of blindness (14%).[1] By 2020 the number of patients with glaucoma is expected to reach 37 million in Asia and 111.8 million worldwide.[2] Global prevalence of glaucoma is 0.5%-1.5% and increase by 2.5%-4.5% in the elderly.[3] Prevalence of glaucoma in Asia is 3.54% and in Southeast Asia is 3.7%.[4] Prevalence of glaucoma in Indonesia based on Riset Kesehatan Dasar 2007 is 2.53%.[5]

Topical corticosteroids are widely used to treat inflammations and have been found as a risk for intraocular hypertension and glaucoma.[6,7]

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The effect of corticosteroids on intraocular pressure (IOP) varies depending on their chemical structures, potency, ability to penetrate ocular tissue, dose, duration, and route of administration. Topical prednisolone is one of the corticosteroids derivatives that mostly prescribed as topical treatment.[7,8]

Corticosteroids induced IOP elevation is thought to be caused by 2 cellular mechanism: (1) increased barrier function in Schlemm's canal and (2) changes in cells contractility and extracellular matrix (ECM) turnover in trabecular meshwork (TM).[7] ECM is a dynamic structure that constantly maintains the remodeling activity. Corticosteroid causes decrease in ECM activity which leads to ECM accumulation in TM and eventually increasing of IOP. High IOP will initiate matrix metalloproteinase cascade in TM that leads to ECM turnover. This reversibility is known as maintenance remodeling of ECM TM.[7,8,9]

The objective of this study was to analyze the reversibility of ECM TM in Wistar rat after 28 days instillation of topical prednisolone 1% and discontinued at different periods, which is 0 days, 14 days and 28 days. Histopathology specimen of ECM TM then evaluated using histopathological grading score.

MATERIAL AND METHODS

Research Design and Sampels

This was an experimental study with posttest only control group design. The study took place in Diponegoro University Animal Laboratory during March – May 2020, in accordance with the KEPK (Komisi Etik Penelitian Kesehatan) Universitas Diponegoro No.15/EC/H/FK-UNDIP/II/2020. Total samples of 28 healthy male rats between 2-3 months and weighted between 200-300 grams were selected based on random allocation sampling.

Drugs and Protocols

Wistar rats were acclimatized for new environment with standard food ad libitum for one week. They were divided into 4 groups: treatment group 1, treatment group 2, control group 1 and control group 2. Both topical prednisolone 1% (Cendo p-pred ED®, Bandung, Indonesia) and topical saline were given every 8 hours every day in the right eye.

Treatment group 1 was treated with topical prednisolone 1% (cendo p-pred ®, Bandung, Indonesia) for 28 days and discontinued for 14 days before terminated. Treatment group 2 was treated with topical prednisolone 1% for 28 days and discontinued for 28 days. Control group 1 was treated with topical prednisolone 1% for 28 days and control group 2 was treated with saline topical for 28 days. The control groups were terminated without discontinuation period. After termination with cervical dislocation, enucleation and hematoxylin staining procedure of eye specimen was done. Hematoxylin eosin staining was done by immersing the object glass sequentially in a solution of xylol, alcohol 100%, alcohol 96%, alcohol 70%, aquadest, mayer hematoxylin (Selec Tech, Leica Biosystem, California), eosin (Selec tech, Leica Biosystem, California), alcohol 80%, alcohol 90%, alcohol 100% and xylol. The preparations were ready to be read under a Nicon Eclipse E200 microscope connected to a Sony CMOSsensor IMX265 camera and the indomicro view measurement application to measure the extracellular matrix thickness of the trabecular meshwork. Histopathology of ECM TM was evaluated using histopathological grading score (0: no thickening, 1: mild thickening, 2: severe thickening) by two Anatomical Pathologist doctors.

Statistical Analysis

Statistical Analysis were performed using the SPSS 24.0 software package. Reliability test thickening ECM TM between two Anatomical Pathologist were analyzed using the Cronbach Alpha test. Differences between 2 groups were analyzed statistically using Mann-Whitney test and differences between all group were analyzed using Kruskal-Wallis test.

RESULT

There were 28 Wistar rats in this research divided into 4 groups. Each group contained 7 Wistar rats. There was no dead or sick animal during the research. Data were reliable with high inter-rate agreement (Cronbach Alpha test between two Anatomical Pathologists showed kappa (κ) 0.935 or 93.5%). Normality test with Saphiro-Wilk test showed that the data were not normally distributed ($p<0.05$)

Mann-Whitney test between 2 groups showed no statistically significant difference of ECM TM thickness between treatment group 1 and treatment group 2 ($p>0.05$) (Table 1). Comparative test with Mann-Whitney between treatment group 2 and control group 1 showed statistically significant difference of ECM TM thickness ($p<0.05$) (Table 2).

Comparative test between treatment group 1, treatment group 2, control group 1, and control group 2 with Kruskal-Wallis test showed statistically significant difference of ECM TM thickness ($p<0.05$) (Table 3).

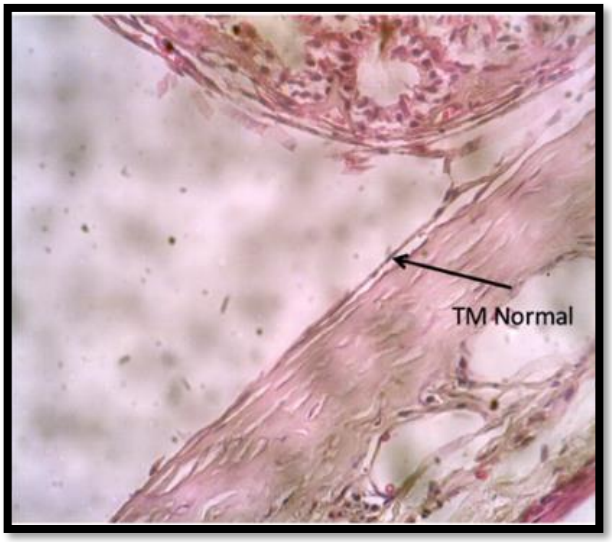


Figure.1. Trabecular tissue with normal thickening ECM of control group 2 (score 0)

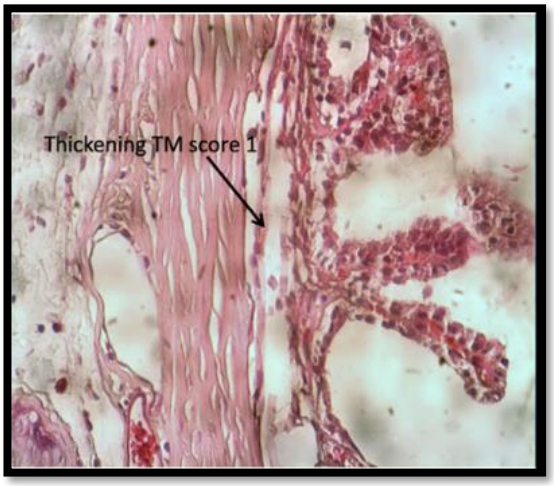


Figure 2. Trabecular tissue with mild thickening ECM of treatment group 2 (score 1)

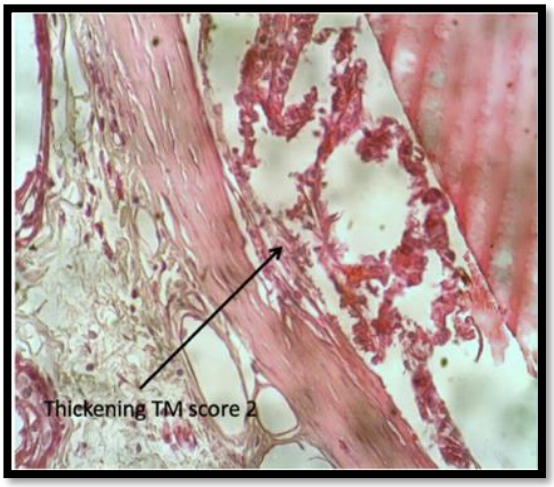
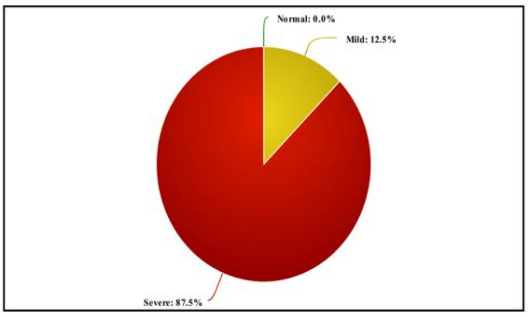
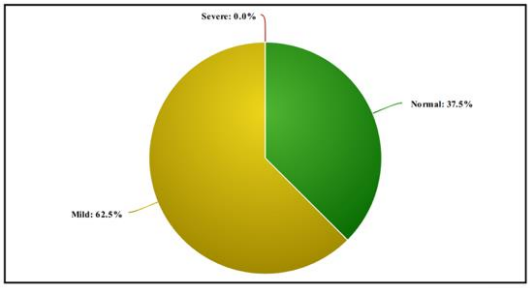


Figure 3. Trabecular tissue with severe thickening ECM of treatment group 1 (score 2)





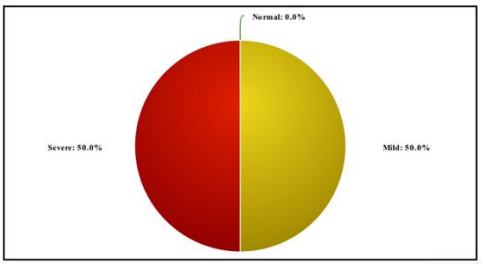


Figure 4. Pie chart of control group 1, Treatment Group 1 and Treatment Group 2

DISCUSSION

The main function of TM is to regulate aquos humor outflow. Plugging of trabecular tissue/ trabecular beam will increase resistance of aquos humor outflow and result in increased IOP.[10,12] The ECM plays an important role in resistance to aquos humor outflow. Aquos humor resistance most commonly occurs in the TM, either in conventional or uveoscleral pathways. This resistance is responsible for the elevation of IOP.[12-14]

According to this study, the thickening of ECM TM is affected by the period of topical prednisolone discontinuation. Table 1 shows the thickening of ECM TM in treatment group 1 (normal 37.5% and mild 62.5%) is thinner compared to treatment group 2 (mild 50% and severe 50%) (p>0.05). Table 2 shows the thickening ECM TM in the treatment group 2 (normal 37.5% and mild 62.5%) is thinner compared to control group 1 (mild 12.5% and severe 87.5%) (p<0.05).

There is no statistical difference in ECM TM thickness after discontinuation of topical prednisolone for 14 days (treatment group 1) compared to 0 days discontinuation (control group 1). However, after 28 days of topical prednisolone discontinuation (treatment group 2) there was statistical difference when compared to 0 days discontinuation (control group 1). This was consistent with a previous study by Guorong, et al (2017) who evaluate ECM proteins (MYOC, matrix metalloproteinase (MMPs) and fibronectin respond to dexamethasone (Dex, 100 ηm) administered for 4 weeks on human TM. Mean MYOC expression increased 17 folds and continued to increase until 2 weeks after discontinuation and decrease after more than 2 weeks. MMPs expression increase by 40% and returned to baseline 3 weeks after discontinuation of dexamethasone. Fibronectin expression increased 1.52 folds and returned to baseline after 4 weeks of discontinuation.[7]

Based on time analysis, it can be concluded that the longer time of topical prednisolone discontinuation, the thinner. The ECM TM becomes. It was shown in this study that the thickening of ECM in the treatment group 1 was thinner than the control group1 (Table 1, p>0.05), the thickening of ECM in the treatment group 2 was thinner than control group 1(Table 2, p> 0.05), and the thickening of ECM treatment group 2 is thinner than control group 1 (Table 2, p <0.05).

Table 1. Comparative thickening of ECM TM between treatment group 1 and treatment group 2

Groups	Thickening ECM TM			*p*
	Normal	Mild	Severe	
Treatment group 1	0 (0%)	4 (50%)	4 (50%)	<0.068*
Treatment group 2	3 (37.5%)	5 (62.5%)	0 (0%)	

*Significant (p<0.05); *Mann Whitney*

Table 2. Comparative thickening of ECM TM between treatment group 2 and control group 1

Groups	Thickening ECM TM			*p*
	Normal	Mild	Severe	
Treatment group 2	3 (37.5%)	5 (62.5%)	0 (0%)	<0.003*
Control group 1	0 (0%)	1 (12.%)	7 (87.5%)	

*Significant (p<0.05); *Mann Whitney*

Table 3. Comparative thickening of ECM TM between research groups

Groups	Thickening of ECM			*p*
	Normal	Mild	Severe	
Treatment group 1	0 (0%)	4 (50%)	4 (50%)	<0.001*
Treatment group 2	3 (37.5%)	5 (62.5%)	0 (0%)	
Control group 1	0 (0%)	1 (12.5%)	7 (87.5%)	
Control group 2	8 (100%)	0 (0%)	0 (0%)	

*Significant (p<0,05); *Kruskal Wallis*

Corticosteroids have been shown to trigger the accumulation of ECM, proteoglycan, elastin, glycoprotein, collagen, and myocilin (MYOC), and connective tissue growth factor (CTGF). The accumulation of ECM that consist of type IV collagen, reduced ctoplasmic glycogen, and increasing of material resembling basement membrane of TM. Corticosteroids will also cause cytoskeletal changes that will block pinocytosis by the aquos humor so that glycosaminoglycan will accumulate. All the mechanisms above will trigger an increase in IOP due to the use of steroids.[15-19]

Mechanical stretching and distortion of the ECM and juxtacanalicular cells and Schlemm's canal caused by the increase of IOP will initiate a cascade that implicates MMPs. MMPs will initiate ECM turnover and eventually ECM remodeling. ECM remodeling is very useful for maintaining the function of TM cells so that normal IOP can be maintained.[10,16, 20]

This study is the first to histopathological observe the thickening of the ECM TM after topical prednisolone was discontinued for a period of time in Wistar rat. This study shows that ECM TM thickening will decrease by time. The longer the time of discontinuation of topical prednisolone instillation, the thinner the ECM TM. Thinner ECM TM will increase aquos humor outflow and decrease IOP. So, the increase in IOP due to topical steroid should be reversible if the steroid treatment is discontinued. But we still do not know how long ECM TM will back to baseline condition. Until now there have been no reports or studies on the length of time prednisolone persist in the trabecular meshwork. A report by Guorong (2017) as mentioned above, gave assumption on whether dexamethasone clearance from human TM tissue is about 2-4 weeks. [7]

The limitation of the study is that in this study, only one type of corticosteroid and one route of administration was implemented. So, this research could not compare the effect of therapy on ECM TM based on the type and routes of administration. The time of study was also limited, so that it could not observe how long the ECM TM thickening return to normal.

This study is expected to give more information about reversibility of ECM TM thickening due to topical steroid.

CONCLUSION

The conclusion of this research is ECM TM was thinner in the experimental animals with a longer duration of discontinuation of topical prednisolone.

However, it is necessary to carry out further experimental studies to assess IOP at the time of administration and after discontinuation of prednisolone so that the relationship between IOP and ECM TM histopathological changes can be determined.

Study with longer duration is required to know how long the histological changes of ECM TM will return to the baseline condition. Also, further experimental study with different routes of prednisolone is needed to determine which route plays a greater role in the changes of histopathology of ECM TM.

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