THE EFFECT OF SUPER OXIDE DISMUTASE (SOD) ON THE HEALING OF WHITE RAT CORNEAL EPITHELIAL WOUNDS CORNEA TRAUMA MODEL

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Abstract

Keratitis and corneal erosion are part of eye trauma. Injury to the cornea will cause cell damage, triggering an inflammatory reaction that can increasethe production of ROS (Reactive Oxygen Species). High amounts of ROS can damage cells, modify DNA, destroy proteins and damage mitochondria. SOD (Superoxide Dismutase), one of the endogenous enzymatic antioxidants works by limiting the level of inflammation of the corneal damage caused by oxidation. SOD concentrations were found in the corneal epithelium and endothelium. The aim of this study was to determine the effect of superoxide dismutase concentrations of 2%, 3%, 5% and placebo on the healing of corneal epithelial wound lesions in whiterats with corneal trauma models. Experimental study pre and post-test only with control group design in vivo with 24 samples of white wistar rats that met the inclusion and exclusion criteria. The research was conducted at the Animal House Laboratory, the Biotechnology Laboratory of the UNSRI Medical Faculty and the Eureka Research Center laboratory within 6 months. In this study, 5% SOD showed a smaller reduction in wound area with the largest reduction (mean 0.67cm ± 0.007) followed by SOD 3% (mean 0.67cm ± 0.005). SOD 3% and 5% significantly correlated with weak strength at 48 hours and 72 hours. A significant reduction in the area of corneal epithelial wound lesions was only seen at 48 hours. SOD 5% most affected the healing of corneal epithelial wound lesions, followed by SOD 3%. SOD concentrations of 3% and 5% had a significant correlation on corneal epithelial wound healing at 48 and 72 hours with weak correlation strength.

Key Words: Superoksida Dismutase (SOD), Corneal Erosion, Keratitis

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1. Introduction

Ocular trauma is an ocular injury that can occur in the form of blunt or sharp trauma, chemical, thermal and radiation trauma. Trauma results in damage to the eye tissue anterior to posterior. Eye trauma is an emergency case, if it is not treated immediate it can cause decreased vision (low vision) to blindness. A decreased visual acuity if the visual acuity ranges from 6/18 to 3/60 and blind if the visual acuity is less than 3/60.[1]

Corneal erosion or abrasion is part of eye trauma. Corneal erosion or abrasion can occur in all age groups, in America 15 per 1000 employees experience corneal abrasion at work. Erosion or abrasion is part of eye trauma.[42] Epidemiological studies show that many eye injuries occur in developing countries such as America and Italy where the proportion of eye injuries caused by sharp objects ranges from 5% -25%.[3] Meanwhile, in industrialized countries such as Taiwan and China have 40% of eye injuries due to sharp objects.[4] Almira et al (2019) also found that 66.66% of eye trauma patients were caused by open trauma by sharp objects at DR. H Abdul Moeloek Lampung in 2016-2017.[5]

The process of injury to the cornea will cause damage to cells that cause Damage Associated Molecular Pattern (DAMP) and will eventually trigger an inflammatory reaction. Inflammation is a redox sensitive mechanism, oxidative stress can activate transcription factors such as NF-κB, which regulates the release of proinflammatory cytokines such as TNF-α, IL-1, IL-10, IL-12. Production of Reactive Oxygen Species (ROS), also triggers the production of enzymatic antioxidants such as catalase (CAT), Hydroperosidase (HPx), Superoxide Dismutase (SOD) As endogenous antioxidants. The amount of ROS that is formed will disrupt homeostasis or stimulation of growth, survival, and cell signaling, depending on how much ROS is produced. If the production of high ROS levels exceeds the existing capacity, it can damage cells through lipid peroxidase, DNA modification, protein destruction, and mitochondrial damage. Oxidative stress causes damage to various cell components and contributes to the pathogenesis of various diseases.[6,7]

Arima et al found that there was activation of the SOD enzyme and correlated with repair of injured tissue in mice. [8,9] A 2018 study by Ikeda et al found that SOD1 derived from Diquafosol sodium eye drop at a dose of 3% was effective in the treatment of dry eyes in rats,[10] however Kojima et al 2018 concluded that topical use of rebamipide 2% (SOD1) drops was observed to increase conjunctival epithelial differentiation and suppress keratinization in rats. [11] Yagi-yaguchi et al in 2020 found that 3% diquafosol sodium eye drop was effective as an antiglaucoma in rats. [12]

Based on the above studies, as well as the absence of research on the effect of using SOD on the healing of corneal epithelial wounds caused by mechanical trauma, this study was designed to evaluate the effect of 2%, 3%, 5% superoxide dismutase (SOD) on the healing of rat corneal epithelial wounds. white model of corneal trauma. There is hypothesis for this research;

H_0: There was no difference between administration of superoxide dismutase (SOD) with concentrations of 2%, 3%, 5% and placebo on wound healing of the corneal epithelium of white rats of the corneal trauma model.

H_a: There was difference between administration of superoxide dismutase (SOD) with concentrations of 2%, 3%, 5% and placebo on wound healing of the corneal epithelium of white rats of the corneal trauma model.

2. Research Methods

This research will be conducted at Animal House Laboratory, the Biotechnology Laboratory of the UNSRI Medical Faculty and the Eureka Research Center laboratory within 6 months. This study was experimental study pre and post-test only with control group design in vivo with 24 samples of white wistar rats that met the inclusion and exclusion criteria. There are several inclusion criteria used in this study, namely; a) healthy and male white Wistar rats, aged 6-8 weeks with body weight 150-200 grams. The exclusion criteria used were; a) unhealthy or died rats before intervention, b) eye infection on rat after intervention.

Samples were total 24 rats and divided into 4 groups: 3 intervention and 1 control group. The intervention group given SOD eye drop based on different concentration: 2%, 3%, 4% respectively on trauma eye. The rat cornea was dripped with 0.5% Fluorescein. Then, using a loop and caliper, measurements of the lesion (mm) were carried out at 6 hours, 12 hours, 24 hours and 36 hours. Data Analysis used SPSSS version 25. Normality test was performed and analyzing further with one-way Anova if data was normal distributed and if it was not, Kruskal Wallis was considered.

3. Results and Discussion

Effectiveness of Superoxide Dismutase with Concentration 2%, 3%, 5% and Placebo on Extensive Healing of Corneal Epithelial Wound Lesions of The White Rat Corneal Trauma Model

Observation		Treatment Group				p
	hours	SOD 2%	SOD 3%	SOD 5%	Placebo	
0		1±0	1±0	1±0	1±0	1,000
6		0,98±0,41	1±0	1±0	1±0	0,083
12		0,97±0,05	0,97±0,05	0,97±0,08	0,97±0,08	0,757
24		0,88±0,02	0,87±0,01	0,88±0,02	0,88±0,05	0,572
36		0,88±0,05	0,87±0,004	0,87±0,05	0,88±0,05	0,081
48		0,80±0,01	0,78±0,05	0,78±0,014	0,80±0,07	**0,046**
72		0,71±0,05	0,68±0,05	0,68±0,07	0,71±0,05	**0,000**

At 6 hours, the treatment group with 2% SOD began to show a reduction in the area of the wound lesion. However, at the 48th hour, there was a significant difference between the treatment groups, especially the 3% SOD group, which showed that the average reduction in the area of epithelial wound lesions was greater, namely 0.78 ± 0.05 and SOD 5% had an average epithelial area of 0.78 ± 0.014. At 72 hours it can be seen that there is a significant difference, especially at 5% SOD where the mean area of corneal wound lesions is 0.68 ± 0.07 and at 3% SOD is 0.68 ± 0.05

In line with this study, Kost et al conducted a study on the treatment of eye inflammation using SOD 1 at a dose of 5% in 5 rabbits and found that the results of conjunctival edema and hyperemia seemed to improve, neovascularization of the cornea in the acute phase of uveitis was observed in 20-30% of the eyes. Yagi-yaguchi et al 2020 compared SOD 3% and antiglaucoma in mice with samples of mice suffering from glaucoma, the results showed that administration of antiglaucoma and SOD 3% had equally significant results with antiglaucoma and secretagogue eye drops in treating glaucoma eyes.[12] Ikeda et al 2018 concluded that administration of 3% SOD eye drops increased the number of lipid droplets, tear stability and tear production which in turn appeared to have a beneficial effect on the ocular surface epithelium. 3% diquafosol sodium eye drops may be a potential treatment for age-related meibomian gland and dry eye disease based on the observations of the current study.[10] A review article conducted by Dougru et al in 2018 concluded that SOD 1 can cure some of the pathogenesis in eye diseases such as keratitis and ocular inflammation.[13]

Effectiveness of Superoxide Dismutase with Concentration 2%, 3%, 5% and Placebo on Extensive Healing of Corneal Epithelial Wound Lesions of The White Rat Corneal Trauma Model

Observation hours	**Wound area (mm)**	**SOD2% n(%)**	**SOD3% n(%)**	**SOD5% n(%)**	**Placebo n(%)**	**r**	**p**
0						-	-
	1	6(25%)	6(25%)	6(25%)	6(25%)		

6						0,280	0,372
	0,98	1(100%)	-	-	-		
	1	5(21,7%)	6(26,1%)	6(26,1%)	6(26,1%)		
1						0,258	0,380
2							
	0,96	-	-	2(50%)	2(50%)		
	0,97	4(40%)	2(20%)	2(20%)	2(20%)		
	0,98	2(20%)	4(40%)	2(20%)	2(20%)		
2						0,223	0,294
4							
	0,80	-	-	-	1(100%)		
	0,81	-	-	-	1(100%)		
	0,82	-	-	-	1(100%)		
	0,85	1(100%)	-	1(100%)	-		
	0,86	1(33,3%)	1(33,3%)	1(33,3%)	-		
	0,87	1(33,3%)	2(66,7%)	-	-		
	0,88	1(20%)	2(40%)	1(20%)	1(20%)		
	0,89	1(33,3%)	1(33,3%)	1(33,3%)	-		
	0,90	-	-	1(50%)	1(50%)		
	0,91	-	-	1(100%)	-		
	0,93	1(50%)	-	-	1(50%)		
3						0,190	0,051
6							
	0,87	-	-	3(100%)	-		
	0,88	2(16,7%)	5(41,7%)	3(25%)	2(16,7%)		
	0,89	4(44,4%)	1(11,1%)	-	4(44,4%)		
4						**0,350**	**0,004**
8							
	0,77	-	1(50%)	1(50%)	-		
	0,78	-	2(50%)	2(50%)	-		
	0,79	1(16,7%)	3(50%)	1(16,7%)	1(16,7%)		
	0,80	3(42,9%)	-	1(14,3%)	3(42,9%)		

In line with the results of the Anova test which showed a significant relationship at 48 and 72 hours, the results of the Pearson correlation test also showed that there was a significant correlation with weak strength at 48 hours (r=0.350, p=0.030) and 72 hours (r= 0.321, p=0.027)

A 2018 study by Ikeda et al found that SOD1 derived from Diquafosol sodium eye drop at a dose of 3% was effective in treating dry eyes in rats and concluded that there was a strong correlation between giving SOD and healing dry eyes in rats. [10]

Zhou et al in 2021 added that SOD is not only effective for injuries to the cornea but to the retina, the antioxidant enzymes from SOD enter the corneal cells or the retina can downregulate oxidative stress in the retina and cornea depending on the area focused. The efficiency of intracellular proteins such as boronic acid polymer is brought to SOD into nanoparticles by binding affinity and transporting cargo proteins to each cell line with maintained bioactivity and low cytotoxicity. Zhou et al concluded that treatment using the nano SOD formulation has high efficacy and is safe for the treatment of eye injuries In line with Zhou et al, Tokuc et al in 2021 also concluded that SOD1 can be recommended for the treatment of eye diseases.[14]

According to Zhou et al, Tokuc et al, also concluded inflammation in eye disease is associated with the production of reactive oxygen species (ROS) and causes fatigue in endogenous antioxidant systems, often causing tissue degeneration, blurred vision and even blindness. Antioxidant enzymes in SOD can function as a counterweight to excessive ROS production so that it can accelerate the inflammatory process and

trigger re-epithelialization.[15]

4. Conclusion

5% SOD has the most effect on healing the extent of corneal epithelial wound lesions, followed by 3% SOD. SOD concentrations of 3% and 5% had a significant correlation with wound healing of the corneal epithelium at 48 and 72 hours with weak correlation strength.

5. References

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