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The Effect of Duration of Wound Skin Tissue on MDA Plasma Level and Micro Vessel Density (CD34 Expression) in White Rats (*Rattus novergicus)*

Danar Widyatmoko[1]*, Amru Sungkar[2], Kristanto Yuli Yarso[3] and Brian Wasita[4]

[1]Department of Surgery, Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia.
[2]Department of Surgery, Plastic Surgery and Aesthetic Reconstruction Division, Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia.
[3]Department of Surgery, Oncology Division, Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia.
[4]Department of Pathology Anatomy, Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia.

Authors' contributions

This work was carried out in collaboration among all authors. Author DW designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AS and KYY managed the analyses of the study. Author BW managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To determine the effect of wound duration on plasma MDA levels and Micro Vessel Density (CD34 Expression) in white rats (*Rattus novergicus*).
Study Design: Experimental post-test only control group design.
Place and Duration of Study: Animal care tried PAU UGM Yogyakarta, while immunohistochemical observations were conducted by experts in that field at the Anatomical Pathology Faculty of Medicine, University of Sebelas Maret Surakarta, Indonesia, between March and June 2020.
Methodology: We included male white rats (Rattus Norvegicus) which consists of 18 samples divided into 3 groups, namely the group with 5 days of wound duration (K1), the group with 10 days

**Corresponding author: E-mail: doktergrafer@gmail.com;*

of injury (K2), and the group with a wound length of 15 days (K3). The wound is made a square incision with a length of 2x2 cm. The parameters assessed were plasma MDA levels and Micro Vessel Density (CD34 Expression). Statistical analysis of plasma MDA levels and Micro Vessel Density (CD34 Expression) using OneWay ANOVA followed by Tukey HSD.

Results: The OneWay ANOVA test results showed significant values in plasma MDA levels (P=0.000) and CD34 Expression (P=0.001). The Tukey HSD test results showed a significant value (P <0.05) in the K1 with K2, K1 and K3 groups and K2 with K3 groups on the parameters of MDA plasma. The Tukey HSD test results showed a significant value (P <0.05) in the K1 with K2 and K1 with K3groups but not significant (P=0.572) in the K2 with K3 groups on the parameters of CD34 expressions.

Conclusion: Longest duration of the wound has an effect on decreasing levels of plasma MDA levels and Micro Vessel Density (CD34 Expression) in the wound healing process.

Keywords: MDA plasma; CD34; rattus novergicus; wound skin.

1. INTRODUCTION

Wounds are the result of physical trauma resulting in severe skin discontinuity. Distinguished into 2 types namely acute and chronic wounds. The optimal wound healing process is necessary for the anatomical and functional restoration of skin tissue [1]. Sorg et al (2017), states that the physiological process of wound healing is so complex, and depends on various cells and interconnected mediators [2]. The wound healing process is associated with oxidative stress that produces Reactive oxygen species (ROS). ROS function in wound healing process through complex phases includes migration phase, adhesion, proliferation, neovascularization, remodeling, as well as apoptosis [3]. ROS in the molecular aspect produce pro-inflammatory cytokine secretions and directly or indirectly become a source of damage to the function of skin fibroblasts and keratinocytes, these interacts with lipid molecules can trigger the occurrence of lipid peroxidation process and produce lipid hydroperoxides (LOOH) and malondialdehyde (MDA) [4].

Research conducted by Gonzales (2016), the necessary response occurs is vascular inflammation related to increased ROS and angiovascular (microvessel density) [5]. Therefore, we observed a longstanding relationship of wound care with MDA (malondialdehyde) and the quality of angiogenesis through CD34 expression in granulation tissue.

2. MATERIALS AND METHODS

2.1 Patient Population

Research subjects were male white rats (Rattus Norvegicus) aged 3 - 4 months, weight 150-300 grams, obtained from the Faculty of Veterinary Medicine Gajah Mada University. Male white rat food (*Rattus norvegicus*) used standard mouse feed BR I. The inclusion criteria in the subject are healthy male white rats (*Rattus norvegicus*), male white rats, feathers are not dull, active and good appetite, age 3-4 months with a weight of 150 - 300 grams. While the exclusion criteria in this study were mice that died during the research process.

2.2 Sample Collection and Processing

The animal subjects in this study were male white rat strains of wistar. Rat is acclimatized for 5 days before the wound is made, then the animal back was shaved until smooth and cleaned with 70% alcohol, after that made a square incision of wound with a length of 2x2 cm and treated, group 1 (K1) with wounds untreated for 5 days and group 2 (K2) with untreated wounds for 10 days and group 3 (K3) with the wound not treated for 15 days.

Retroorbital blood was taken on day 5 (group 1), day 10 (group 2) and day 15 (group 3) for examination of MDA levels. Plasma MDA levels in mice were obtained from blood sampling. Blood is taken from retroorbital veins that have been inhalated. MDA plasma parameters from the blood measured by the TBARS (ThioBarbituric Acid Reactive Substances) method.

Micro vessel density is assessed with CD34 expression. CD34 will expressed in tissue endothelial cells by immunohistochemical staining of IHC using CD34 monoclonal antibodies. MVD expression observed by the lowest power (10x magnification) to determine the most intense coloring area. The calculation of blood vessels carried out under the enlargement

of × 40. The area with highest number of blood vessels is referred then three high-power fields (HPF) are selected. The calculating process are carefully scanned from left to right of each slide to avoid recalculating the same area. The average of the three values is calculated and expressed as the averagen on ± SD.

2.3 Statistical Analysis

Distributions of data were analyzed using Shapiro-Wilk test. Data of this study were normally distributed. Therefore, Statistical analysis was performed using the One Way ANOVA test, and the statistical significance was accepted with $p < 0.05$. Analyses were performed using Statistical Product and Service Solution Software for Windows version 24.

3. RESULTS AND DISCUSSION

3.1 MDA Plasma

The average MDA plasma levels of each study group (K1, K2 and k3) showed different levels. The average plasma MDA data of each study group can be viewed according to the Table 1).

Table 1. The average of MDA plasma in rats

Groups	N	Mean MDA Plasma ± SD
I (K1)	6	8,99 ± 0,27
II (K2)	6	8,46 ± 0,35
III (K3)	6	6,44 ± 0,32

The data above is good with a bar chart according to Fig. 1:

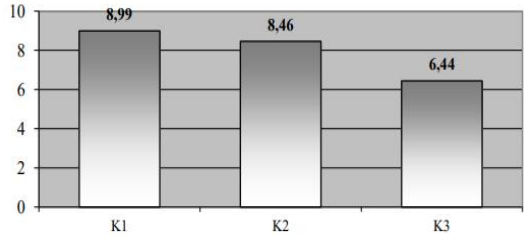

Fig. 1. Bar diagram of mean MDA plasma levels in each group of skin tissue injury duration

From the data, it was obtained that group 1 had the highest plasma MDA level value (8.99 nmol/ml) compared to group 2 (8.46 nmol/ml) and group 3 (6.44 nmol/ml).

Table 2. Shapiro-wilk test results data on the duration of skin tissue wounds against MDA levels of white rat plasma

Groups	N	P value
K1	6	0,961
K2	6	0, 948
K3	6	0, 475

The results of the Saphiro-Wilk test showed that the data distribution was normal because the p value was more than the predetermined degree of significance ($p > 0.05$).

One-Way ANOVA test results provide a value of $p=0.000$ ($p<0.05$), which means there is a difference in the effect of long-term skin tissue wounds on the significant levels of white rat plasma MDA (Rattus Norvegicus) between the three groups. Furthermore, to find out the location of meaningful differences among the sample group, the Tukey HSD test was conducted. This is in accordance with Table 3 below.

Table 3. Tukey test results duration of skin tissue injury to levels of MDA plasma white mice

Groups	Sig.	
K1 with K2	0.024	Significant
K1 with K3	0.000	Significant
K2 with K3	0.000	Significant
One-Way ANOVA	0.000	Significant

Posthoc test analysis showed a significantly difference from effect of duration skin tissue wounds on the MDA levels of white rat plasma (Rattus Norvegicus) ($p=0.024$) between the group with the duration of the wound 5 days and 10 days (K1 and K2). The group with the duration of the wound 5 days and 15 days (K1 and K3) also showed a significant difference ($p = 0.000$). In group with the duration of the wound 10 days and 15 days (K2 and K3) showed significant results ($p = 0.000$).

3.2 Expression CD 34

The average CD34 expression data of each study group (K1, K2 and k3) showed different levels. The average CD34 expression data of each study group can be viewed according to the Table 4.

Table 4. The average of CD34 expression in rats

Groups	N	Mean Expression of CD34 ± SD
I (K1)	6	151,48 ± 71.18
II (K2)	6	63,88 ± 21.31
III (K3)	6	38,08 ± 12.66

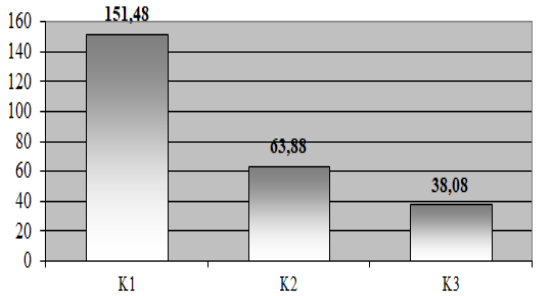

Fig. 2. Mean bar diagram of CD34 expression in each group of granulation tissue injury duration

From the data, it was obtained that group 1 had the highest average expression CD34 (151.48) compared to group 2 (63.88) and group 3 (38.08).

Table 5. Shapiro-Wilk test results data of wound duration on MVD expression of white rat granulation tissue

Groups	N	P value
K1	6	0,557
K2	6	0, 348
K3	6	0, 223

Table 6. Tukey's test result of duration of wounds on the expression of CD34 tissue granulation of white mice

Groups	Sig.	
K1 with K2	0.009	Significant
K1 with K3	0.001	Significant
K2 with K3	0.572	Significant
One-Way ANOVA	0.001	Significant

The results of the Saphiro-Wilk test showed that the data distribution was normal because the p value was more than the predetermined degree of significance (p> 0.05).

One-Way Anova test value show p=0.001 (p<0.05), which means there is a statistically significant difference in the effect of long-standing skin tissue wounds on the expression of CD34 in white rat granulation tissue (*Rattus norvegicus*) between three groups. Furthermore, to determine statistically differences among the sample of groups, a Tukey HSD test was conducted at Table 6.

Posthoc test analysis showed a significantly difference from effect of duration skin tissue wounds on the expression of CD34 white rat granulation tissue (p=0.009) between the group with the duration of the wound 5 days and 10 days (k1 and k2). Group k1 and k3 also showed a significantly difference (p = 0.001) in the group with the duration of the wound left 10 days with the group with the length of the wound left 15 days (k2 and k3) showed no meaningful results (p= 0.572) so there was no significant difference in the two groups.

3.3 Discussion

In this study, wistar strain male white rats (Rattus Novergicus) were used, which were made full thickness wound with the base of the muscle wound. Blood samples were taken for plasma MDA levels, namely group 1 on day 5, group 2 on day 10, and group 3 on day 15.

Plasma MDA levels obtained from the retroorbital vein of mice using the TBARS method gave several differences in the results of each group. The principle of this method is use the ability of the formation of a pink complex between MDA and Thibarbituric Acid (TBA).

Uncomplicated wound healing is essential for restoring skin integrity, preventing infection and dehydration. The healing cascade occurs shortly after the appearance of the wound, where there is contact between platelets and collagen from the tissue exposed to blood, causing the release of clotting factors and fibrin deposition. Platelets release clotting factors and various chemical mediators such as cytokines and growth factors, especially PDGF and TGF-β [3].

According to Gonzales (2016), the earliest necessary response of wound healing was vascular inflammation [5]. The inflammatory phase in wound healing involves inflammatory mediators in it [6]. This response is characterized by the presence of neutrophil infiltration in the wound area causing signs of inflammation. Usually the cell response is formed within the first 24 hours to two days after the wound process. Neutrophils secrete pro-inflammatory

cytokines such as Reactive Oxygen Species (ROS) and induce oxidative stress in the body's tissues or cells resulting in secondary products such as malondialdehyde (MDA) [5]. The role of ROS in the hemostasis and inflammation phase. ROS functions to cause vasoconstriction, blood coagulation, chemotaxis and fight pathogens [7]. In the proliferation and remodeling phases, ROS plays a role in cell migration, cell proliferation, fibrosis and angiogenesis. Angiogenesis occurs as a regular cascade of molecular and cellular processes at the wound bed [8]. After running for more than three days, the inflammatory response has been reduced and there is a proliferation phase that begins in the microenvironment of the lesion within 72 hours until it reaches the 14th day [5].

This is in accordance with this study where it was proven that the highest plasma MDA levels were measured in group 1 with a wound length of 5 days and decreased in group 2 (wound length of 10 days) and group 3 (wound length of 15 days). In the group with a wound duration of 10 days and 15 days there was a decrease in plasma MDA, because at this time the woud healing process had entered the proliferation phase so that the inflammatory mediator began to decrease [5].

The average plasma MDA levels in group 1 (8.99 nmol/ml) and group 2 (8.46 nmol/ml) had a difference value that was not too far compared to the group 3 (6.44 nmol/ml), but they had differences significant. This results were supported by Thiruvoth (2015) which stated that on the 3rd to 14th day of residual inflammatory response, while the15th to one year (remodeling phase) had significantly decrease in residual inflammatory cell apoptosis causing a decrease in ROS levels [9].

.Histopathology of granulated tissue in the rat group with duration of wound at 5 days and 10 days showed an increase in CD34 expression higher than in the group with duration wound of 15 days. The difference in CD34 expression in all three groups was due to differences in the duration of healing determining the type of wound healing phase. This is explained by a review of Sanchez (2018) which states that the process of proliferation in wound healing is related to the presence of MVD expressed on CD34 in the process of angiogenesis. The purpose of the proliferation stage is to reduce the area of lesions with contractions and fibroplasia, forming epithelium to activate keratinocytes. This response is responsible for the closure of the lesion itself which includes fibroplasia, angiogenesis and reepitelization. These processes begin in the micro-environment of the lesion within the first 48 hours and can reach up to the 14th day after the onset of lesions [10].

CD34 expression show as Micro Vessel Density (MVD) especially in small blood vessels (microvascular). CD34 expression show as the MVD average. MVD expression is demonstrated through immunohistochemical coloring techniques using CD34 monoclonal antibodies. Therefore, MVD expression play an important role as an indicator to assess the quality of angiogenesis in the wound healing process [11]. The strongest expression of CD34 can be found in the endothelial cells of capillaries, followed by arteries, veins, arterioles, and venules [12].

In this study expression of CD34 in each group obtained significant different values, but not in group 2 with group 3 (p=0.572). Insignificant results are possible because in group 2 (wound duration 10 days) and group 3 (wound duration 15 days) have the same phase of proliferation that begins to appear on the 3rd to 14th day. In this phase there is a response due to injury that causes cell hypoxia thus stimulating the synthesis of hypoxia inducible factor-1 (HIF1) in macrophages, fibroblasts, vascular endothelial cells and keratinocytes. Release of proangiogenic factors such as VEGF, VEGF-A, FGF2, PDGF, TGF-beta 1 may cause endothelial cells to initiate the occurrence of neovascularization [13]. The duration of each wound healing phase overlapping with the angiogenesis process led to no significant dissientation in group 2 and group 3 [14].

4. CONCLUSION

This study showed significant results of wound healing duration and that effect to plasma MDA levels and Micro Vessel Density (CD34 expression).The longest duration of wound healing associated with the lower of the plasma MDA levels in white rats and the longer duration of wound healing associated with the lower of CD34 expression in granulated tissue of white rats (*Rattus novergicus*).

CONSENT

It’s not applicable.

ETHICAL APPROVAL

Animal ethic committee approval has been taken to carry out th
is study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Barchitta M, et al. Nutrition and wound healing: An overview focusing on the beneficial effects of curcumin. International Journal of Molecular Sciences. 2019; 20(5).
2. Sorg H, et al. Skin wound healing: An update on the current knowledge and concepts. Eur Surg Res. 2017;5858:81–9481.
3. Cañedo-Dorante L. dan Cañedo-Ayala M. Skin acute wound healing: A comprehensive review. International Journal of Inflammation; 2019.
4. Huang YJ dan Nan GX. Oxidative stress-induced angiogenesis. El Sevier: Journal of Clinical Neuroscience; 2019.
5. Gonzalez, et al. Wound healing-A literature review. An Bras Dermatol. 2018;91(5):614–620.
6. Theoret C. dan Schumacher, J. Physiology of wound healing. John Wiley and Sons, Inc. 2017;1–18.
7. Zhu G, et al. Hydrogen peroxide: A Potential wound therapeutic target? Medical Principles and Practice. 2017; 26(4):301–308.
8. Huang YJ dan Nan GX.. Oxidative stress-induced angiogenesis. El Sevier: Journal of Clinical Neuroscience; 2019.
9. Thiruvoth F, et al. Current concepts in the physiology of adult wound healing. Plastic and Aesthetic Research. 2015;2(5): 250.
10. Sanchez MC, et al. Targeting oxidative stress and mitochondrial dysfunction in the treatment of impaired wound healing: A systematic review. Antioxidants. 2018;7(8):1–14.
11. Kiya K dan Kubo T. Neurovascular interactions in skin wound healing: A short review. Neurochemistry International; 2019.
12. Sarmadi S, et al. Association of neo angiogenesis by CD34 expression and clinicopathologic features in squamous cell carcinoma of cervix. Acta Medica Iranica. 2017;55(5):282–289.
13. Singh S, Young A. dan McNaught CE. The physiology of wound healing. Surgery (United Kingdom). Elsevier Ltd. 2017;35(9):473–477.
14. DiPietro LA. Angiogenesis and wound repair: When enough is enough. Journal of Leukocyte Biology. 2016;100(5):979–984.

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