International Journal of General Medicine
and Pharmacy (IJGMP)
ISSN(P): 2319-3999; ISSN(E): 2319-4006
Vol. 6, Issue 5, Aug – Sep 2017; 31-38

International Academy of Science,
Engineering and Technology
Connecting Researchers; Nurturing Innovations

CORRELATION BETWEEN MMP-1 RESPONSES AND EPITHELIALIZATION OF ACUTE STAPHYLOCOCCUS AUREUS INFECTED WOUNDS TREATED BY COFFEE POWDER, SALINE GAUZE, AND HYDROCOLLOID

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ABSTRACT

Introduction: Wound healing is a complex issue covering a variety of physical responses. It has accepted the optimal wound healing, when the wound is moist and autolytic debridement process of MMP involved here. The correlation between MMP and epithelialization would be proved.

Methods: This was a study of the wound, using Wistar rats infected by Staphylococcus aureus treated in the Groups of coffee powder (Group-1), saline gauze (Group-2 as a control group) and hydrocolloid (Group-3), each group of 9 rats. The degree of MMP-1 and epithelialization would be compared to understand the wound healing process in the three different wounds dressing. ANOVA and Kruskal-Wallis tests were used to calculate the MMP-1 concentration. Chi-square and an Exact Fisher test to analyze the epithelial existence in a wound biopsy. The correlation between the variables examined using the Spearman test.

Results: Blood serum MMP-1 was high after the exposure with Staphylococcus aureus and decreased after wound treatment. The mean of MMP-1 was 2.41±0.935 in Group-1; 5.12±1.149 in Group-2, 3.23±0.867 in Group-3. Epithelialization in Group-3 was 44.4%; in Group-1 was 66.7% and none in Group-2 (control group). The correlation between MMP-1 and epithelialization was -0.724 with p=0.001.

Conclusion: Saline-gauze could not decrease the MMP-1 level, which then influenced the delayed epithelialization. Coffee decreased the MMP-1 insignificantly better than hydrocolloid and significant to saline gauze. Coffee was the most efficient to treat an acutely infected wound. Statistically, MMP-1 has an inversely proportional effect on the appearance of epithelium growth rate.

KEYWORDS: Wound Healing, Staphylococcus Aureus, MMP-1, Coffee, Hydrocolloid

INTRODUCTION

Matrix metalloproteinase (MMP) is an important enzyme that plays a role in wound healing. The MMP enzyme is capable of destroying proteins by chopping the amino acid chain in sections. Various MMPs may work together and on some cellular extra matrix substrate. [1] MMP is a pro-inflammatory agent in which levels are found to increase in chronic wounds. [2] MMP-1 is a major component of collagenase enzymes produced by fibroblasts and serves to digest proteins in the extracellular matrix component in the inflammatory phase, digesting the basement membrane to accelerate angiogenesis in the proliferation phase and scar remodeling in the remodeling phase.[2, 3]

Since 1960, it has been agreed that optimal wound healing requires a humid environment. [4] This moist environment can help the process of autolytic debridement with the ability of enzymes that digest dead tissue. [5]

The benefits of autolytic debridement can reduce the pain caused by necrotomy (the action of cutting the tissue) but the time taken longer. [4]

In the present study, we will evaluate the rate of wound healing by measuring the levels of MMP-1 formed on Staphylococcus aureus infected wounds treated by coffee powder and compared to hydrocolloids, and saline gauze as a control group.

METHODS

Study Design

This research is an experimental study, with an entirely randomized design. The wound treated with coffee powder (Group-1), saline gauze (Group-2 as a control) and hydrocolloid (Group-3). In each group will be calculated the MMP-1 level determined in, and the epithelialization rates the presence of MMP-1 enzymes as a sign of autolytic debridement ability.

Animal

Subjects in this study used Wistar rats (Sprague-Dawley type).

Inclusion criteria

- Wistar male rats of 5 to 6 month age, weight 400-500 gram
- In good health, with reasonably current criteria with no signs of systemic infection.

Exclusion criteria

- Unhealthy mice, inactive with signs of systemic infection.
- Rat dies during treatment.

Selection of rats, according to inclusion criteria, then performed simple randomization (simple random sampling) for each treatment group. Rats are specially maintained in 1 cage containing 9 rats and placed in a room with normal room temperature with enough illumination, fed pellets and drank in moderation.

Rats anesthetized using 0.3 ml. Intramuscular injection of Ketamine hydrochloride (Ketalar, Pfizer) to its thigh.

Sample Size

The random sampling is divided into three groups and using statistical Federer formula to analyze the sample size: $(N - 1). (P - 1) \geq 15$; P = number of groups; N = number of samples each group.

$P = 3$, so $2n - 2 \geq 15$

$N \geq 8.5$

The number of rats in each group was 9, so the total rat needed to be 27.

Rats will be grouped at random according to the treatment. Food and beverages are given ad libitum and treated in animal laboratories with controlled humidity (60 mb ± 2%) with a temperature of 22˚ ± 2˚ C with air flow and an enough

illumination and dark environment that changed every 12 hours. Only laboratory personnel and prepared materials may enter the cage to prevent infection.

Skin Biopsy

A biopsy is performed by taking some of the normal tissue and wound tissue as much as 1 cm as shown below in Figure 1.

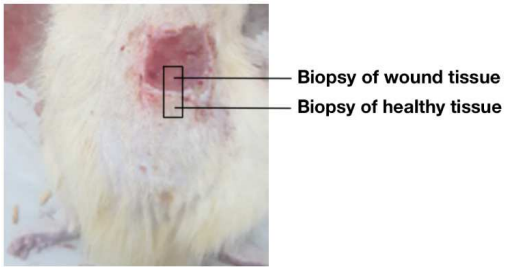

Figure 1

Figure 1. The wound and the biopsy sites: 1 cm long biopsy sample with 0.5 cm of healthy tissue and 0.5 cm wound tissue. The tissue was sent for histopathology examination.

Grouping and Operational Definition

Group-1: wound care with topical 90 mg coffee powder.

Group-2: wound care by a gauze wet through 3 ml. NaCl, 0.9% and then closed by dry sterile gauze on top and adhesive tape.

Group-3: wound care with hydrocolloids which in this study used extra thin Duoderm (manufactured by Convatec) which replaced on day-7 and day-14.

The blood samples were taken from mice with each coded for each treatment unknown to the researcher. Then sample materials were sent to the clinical pathology section for MMP-1 and the biopsy material sent to be processed using hematoxylin-eosin for epithelial examination.

Blood (1 ml.) taken from the tail of the rat for assessment of MMP-1 on day-3 and day-14.

The MMP-1 levels examined using ELISA Kit (Cloud-Clone Corp., USA, with standard value of 1.28 - 3.65 ng/ml.).

Epithelialization was inspected using histopathology examination of the biopsy tissue on day-7 and day-14.

The coffee powder utilized in this study was an Arabica coffee that was purchased from the coffee “Aroma” shop, Banceuy Street in Bandung. The coffee powder was sown as much as 80 mg. Coffee powder for (2.5x2.5) cm^2 wide and 0.2 cm thick of the wound and wrapped with a sterile gauze and adhesive tape.

Staphylococcus aureus ATCC 25923 isolates from Department of Microbiology, Faculty of Medicine, Universitas Padjadjaran, and Bandung, Indonesia. About 1 ml. Of Staphylococcus aureus standard suspension (McFarland 0.5) containing 1.0 x 10^8 bacteria were spread on the wounds.

The healing wound characterized by a dry wound, without the sign of infection or hypergranulation. The lesion assessed histopathologically whether the epithelial cell was formed or not (value 0 means no epithelial formation, and value 1 means epithelial structure exist) from a biopsy examined by a histopathologist (Figure 1).

Statistical Calculation

The analysis should be of the type of research problem and the numerical data is assessed by the Sapiro-Wilk test because the sample is less than 50. The significance test for comparing group characteristics of the MMP-1 content was used unpaired t-test if the data were normally distributed and Mann Whitney's test as an alternative if the data were not normally distributed epithelial examination using a statistical test of Chi-Square. The correlation between data used Spearman Test or the Pearson correlation test is used when abnormal data. Correlation strength (r) based on Guilford's criterion (1956) is: 0, 0 - <0, 2 = very weak; 0.2 - <0.4 = weak; 0.4 - <0.7 = medium; 0.7 - <0.9 = strong; 0.9 -1.0 = very strong.

Data analysis was performed using SPSS program version 24.0 for Windows on a confidence level of 95% with regard significant value $p \leq 0.05$.

Research Ethics

This study follows the research ethics of experimental animal of Institutional animal care and use committee (IACUC), and the research has been approved by the institutional ethics committee.

RESULTS

Before Wound Dressing Treatment

MMP-1 levels are very high at pre-treatment, but epithelialization has not been appeared yet. Table-1 shows MMP-1 levels inversely proportional to epithelialization activity.

Table 1: MMP-1 and Epithelialization: Before-Treatment and After-Treatment

Variable	
MMP-1 (Before) Mean±STD	6.41±1.455
MMP-1 (After) Mean±STD	3.59±1.497
Epithelialization (Before) Exist	0%
Epithelialization (After) Exist	37%

STD=standard deviation

Table 2: Blood Concentration of MMP-1 and Epithelialization Before-Treatment

Variable	Group-1 Group-2 Group-3		
	Coffee Powder	Saline-Gauze	Hydrocolloid
	N= 9	N= 9	N= 9
Mmp-1 Mean±STD Epithelialization	6.91±1.477	6.69±1.618	5.63±1.011
Not Exist	9 (100%)	9 (100%)	9 (100%)
Exist	0 (0.0%)	0 (0.0%)	0 (0.0%)

After-Treatment Using Wound Dressing on Day-14

Group-1 (coffee powder): the wound hyperemia was not seen; exudate readily absorbed by coffee powder, the wound width shrunk about 2 mm.

Group-2 (saline gauze): the wound showed hyperemic, complicated with exudate and many necrotic tissues. Pus was not found and the width of the wounds has not shrunk.

Group-3 (hydrocolloid): the wound showed little exudate, but no pus, the hyperemia reduced with minimal necrotic tissue and the wound width did not shrink.

No rat died.

Table 3 shows changes in MMP-1 levels and the number of epithelium formed after wound care of Group-1 and Group-3, but not seen in Group-2. MMP-1 concentration showed decreased levels and began to find epithelium in a group with hydrocolloids and coffee powder. In wound care with saline gauze, there was no new epithelial growth with MMP-1 levels that were still above standard. MMP-1 decreased in after-treatment, but epithelialization increased significantly.

Table 3: Blood Concentration of MMP-1 and Wound Epithelialization on Day-14 (After Treatment)

Variable	Group-1 Coffee Powder N= 9	Group-2 Saline Gauze N= 9	Group-3 Hydrocolloid N= 9	P
Mmp-1 Mean±Std	2.41±0.935	5.12±1.149	3.23±0.867	0.001
Epithelialization				0.012
Not Exist Exist	3(33.3%) 6 (66.7%)	9(100%) 0(0%)	5(55.6%) 4(44.4%)	

Table 4: Blood Concentration of MMP-1 and Epithelialization in Group-2 and Group-3

Variable	Group-2 Saline-gauze N= 9	Group-3Hydrocolloid N= 9	p
MMP-1 Mean±STD	5.12±1.149	3.23±0.867	0.001
Epithelialization			0.023
Not exist	9(100.0%)	5(55.6%)	
Exist	0(0.0%)	4(44.4%)	

Table 5: Blood Concentration of MMP-1 and Epithelialization in Group 3 and Group-1

Variable	Group-3 Hydrocolloid N= 9	Group-1 Coffee powder N= 9	p
MMP-1: Mean±STD	3.23±0.867	2.41±0.935	0.072
Epithelialization			0.343
Not exist Exist	5(55.6%) 4(44.4%)	3(33.3%) 6(66.7%)	

Table 6: MMP-1 Concentration and Epithelialization in Group-2 and Group-1

Variable	Group-2 Saline-gauze N= 9	Group-1 Coffee powder N= 9	p
MMP-1: Mean±STD	5.12±1.149	2.41±0.935	0.001
Epithelialization			0.003
Not exist Exist	9(100.0%) 0(0.0%)	3(33.3%) 6(66.7%)	

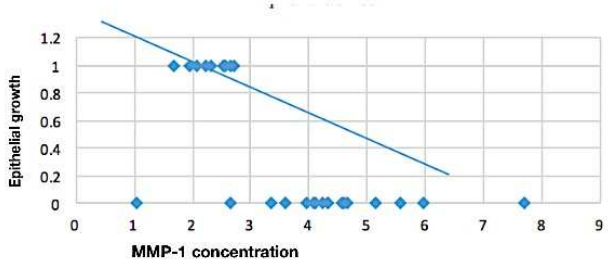

Figure 2: The Correlation Existence between MMP-1 Levels and Epithelialization

Table 7: **A Strong Correlation MMP-1 and Epithelialization Using Spearman Test**

Variable between MMP-1 and Epithelialization	r	p
Correlation	-0.724	0.001

r = correlation coefficient

A strong negative correlation between MMP-1 and epithelialization showed in Figure - 2 and Table-7. Statistically concluded that MMP-1 has an inversely proportional effect on the appearance of epithelialization.

DISCUSSIONS

On examination of MMP-1 levels on the second day after the wound exposure to Staphylococcus aureus, the MMP-1 levels increased from average values, but for five days later decreasing (Figure-2). The average number of MMP-1 levels was 6.41 ± 1.455, with the smallest being 4.36 and the largest being 9.29 (the MMP-1 standard value of 1.28 to 3.65 ng/ml). The results are consistent with the study by Kanangat (2006) that indicating Staphylococcus aureus will lead to degradation of connective tissue and increase the expression of the MMP-1 enzyme in human skin and synovial tissue. [7] The studies also stated that the MMP enzymes formed in the inflammatory process would degrade the extracellular matrix associated with wound healing. The same result as the study by Stevens (2012) conducted on Drosophila species, and by Guttierez (2007), the high MMP levels in mice can negatively interfere with wound healing process. [8, 9]

Armstrong (2002) mentioned in normal wound healing, through the epithelium migration, MMP levels will decrease as the need for collagen type III formation in the early wound healing process. [10] The presence of infectious process extends the inflammatory phase of wound healing process, and the MMP levels remain high. [1] In the presented study, the MMP-1 levels decreasing throughout the wound treatment in three groups. The infection extended the inflammatory phase inhibited by all three forms of wound treatment.

In Table 3, the Group-2 (saline-gauze) decreased the MMP-1 levels, although it did not achieve the standard levels and did not find a new epithelial growth. The epithelium was not found in the group of saline-gauze because the adherent saline-gauze at the day-7 and day-14 of dressing change, at that time the epithelial cells adhered to the gauze (Yuwono HS. 2017). That's why biopsy of the wound after saline gauze dressing removal resulted in no new epithelial layer formed. The possibility of non-selective debridement in wound dressing using saline-gauze, as described by Fleck, 2009, causes the epithelium not to be found in the biopsy tissue examination. [12]

In the group of hydrocolloids, the MMP-1 levels decreasing to the standard range and the epithelial growth were positively determined, although less than 10 epithelium/high power field.

Statistically, the Group of coffee and Group of hydrocolloid was significantly lower MMP-1 levels and increase the epithelial growth.

CONCLUSIONS

Treatment using coffee powder and hydrocolloid wound dressing was better at reducing MMP-1 levels and accelerating epithelialization compared to saline gauze dressing. Treatment using coffee powder was insignificantly better than hydrocolloid. There was a high inversely proportional correlation between MMP-1 concentration and epithelialization in wound healing.

Competing Interests

The authors declare no competing interest.

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