Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2018, 10(7): 187-197

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

+D Alethyne Potential Toward Epithelialization of Wound on Skin of Mrsa Infected Rats

Kayapan Satya Dharshan[1]

[1]PT. Dermozone Pratama, Mayapada Tower, South Jakarta, Indonesia

---

ABSTRACT

Wound healing is part of the regeneration of skin tissue from the damage. This will be hampered by the Methicillin resistant Staphylococcus aureus (MRSA) infection. This study aims to determine the effect of +dalethyne against epithelialization in wound healing of the skin MRSA-infected rats. Thirty six Wistar rats, 3 months old, are divided into 6 groups, 2 groups of negative control, 2 groups of positive control, 2 groups of treatment group (All are sacrificed on the fourth day and sixth day). Incision to the back skin of rats by a knife along the incised 2 cm and depth to subcutaneous was done. Wound of positive control groups infected by MRSA, wound in the treatment groups also infected MRSA and then applied topically +dalethyne. Each groups are sacrificed on day 4 and 6, the skin tissue is fixed, made histological preparations, stained with HE. The measurement of the epithelial length using Optilab mounted on a light microscope. The data are analyzed by comparing the mean and SD. The epithelial length in the treatment group was higher than the positive control {(0,46±0,19)vs(0,21±0,16);(0,63±0,76)vs(0,42±0,301), being compared with the negative control is not much different. Conclusion: Topical +dalethyne accelerates epithelialization in wound healing of the skin MRSA-infected rat.

Keywords: Wound healing; MRSA infection; +D Alethyne; Epithelialization

---

INTRODUCTION

Nosocomial infection is still a serious problem, especially in patients who need healthcare in hospital for long period of time. Several bacteria causing nosocomial infection are resistant to one or more antibiotics. It worsens the condition of patients being cared in hospital, even causes death and increases the cost of hospitalization for patients. This is without a doubt brings a loss to public who uses healthcare facility, such as hospital. One of the indicators used to measure hospital performance is nosocomial infection, which is an indicator of healthcare quality in the hospital. Bacterial infection inhibits wound healing process on skin of patients who receive healthcare in hospital, such as patient who has surgery, prosthetic bone implantation, urinary catheter application, infusion application for a long period, gangrene and bedsore following a chronic metabolic disease with or without complication, or immune system deficiency. Several microbes causing nosocomial infection on the skin are Staphylococcus aureus, Streptococcus pyogenes, Acinetobacter sp., Pseudomonas sp. Staphylococcus aureus is selected to be used in this research as the cause of nosocomial infection on the skin because this bacteria is often found in severe infection or in patients with immune system deficiency or combined infection with other bacteria. These bacteria can also cause severe infection on the skin[1].

Methicillin resistant Staphylococcus aureus (MRSA) is a classified as bacteria which can produce biofilm, toxin, and superantigen which can avoid immune system and protect itself from antibiotic damage by using staphylococcal cassette chromosome mec (SSCmec) transfer which forms protection from antibiotic with methicillin structure. This type of Staphylococcus aureus can cause much severe pain when infecting skin with open wound.

Wound on skin can be caused by several things, which are wound from trauma (mechanical, chemical, thermal, electric) or from blocking in blood vessel (such as in Buerger disease). Wound can cause the loss of skin structure, which affects epidermis, dermis, or even muscle. Wound affecting dermis structure or muscle will be followed by bleeding because blood vessel is also affected. Wound becomes port d'entry for microorganism to enter the body, causing wound to be unsterile. Pathogenic microorganism which penetrates through wounded skin can cause infection, which can be localized, can spread to tissue beneath the skin, and can even spread systematically to other organs depending on pathogenicity of the microorganism. Infection stimulates body immune response to eradicate the microorganism. The immune response can be non-specific (innate) and specific (acquired)[2].

When trauma happens and disrupts skin structure due various things, inflammation is triggered as the first defense to keep skin tissue and prevent wider damage. When inflammation happens, there is infiltration by inflammatory cells, such as PMN (polymorphonuclear), macrophage, and lymphocyte, to destroy pathogenic microorganism which penetrates the wound area. PMN and macrophage do phagocytosis to microorganism which penetrates through the wound and destroy the microorganism by producing free radical. On the other hand, lymphocyte is activated by dendritic cell expressed receptor which recognizes pathogen. Activated lymphocyte produces pro-inflammation cytokines (TNF-α, IL-1, IL-6, IL-8)[3].

Epithelialization is one of the basic mechanisms in wound healing. Three main tissues that play role in wound healing are connective tissue, blood vessel, and epithelial tissue. Epithelialization, a complex process involving epithelial cell, is modification of epithelial cell internal structure which involves migration, proliferation, and differentiation. In healing phases, there is often an overlapping of time, physiology, and cell type. It depends on etiology of the cause of wound, whether infection happens, whether there's medicine or intervening action.

Bacterial resistance to antibiotic increases the number of death caused by MRSA infection. The need of medicine which effectively eradicates multiple antibiotic resistant microorganisms becomes a challenge today, with hope of suppressing the number of death caused by MRSA infection.

+dalethyne is a new active compound extracted by ozonisation, consists of combination of compounds: essential oil (aldehyde), fatty acid (stearic, oleic, linoleic, palmitic), iodine, and peroxide. +dalethyne forms an antimicrobial agent that can kill bacteria and induce formation of new tissue on wounded skin.

Literature Review

Skin Structure and Function:

Skin is the largest organ in the body with total weight of 2.7 – 3.6 kg and receives a third of total blood volume in the body. Skin thickness ranges between 0.5 – 6 mm, skin consists of extracellular cells and matrix. Cell structure consists of 3 layers, epidermis which is the thin layer and the outer most layer of skin, dermis which is a thick layer and located on the inside, and subcutaneous adipose tissue beneath dermis (hypodermis). Hypodermis is a loose connective tissue beneath dermis[4].

Human skin has many important functions, especially as first defense, protecting from various elements outside the body. When there's a wound the skin, integrity of skin defense is compromise and this can be a door for various microorganism, such as bacteria and virus, to enter the body. Skin can also be important factor in maintaining mental health and social stage of human.

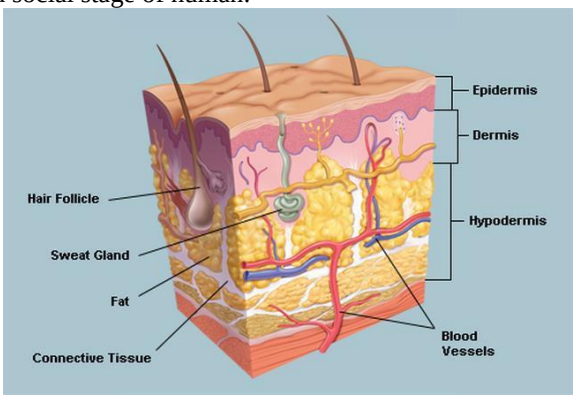

Figure 1: Skin structure

Epidermis functions as the outer most defenses against environment outside the body. Acidic nature of skin protects it against microorganism. Keratin is a hard layer protecting the body from microorganism invasion and

infection, as well as maintaining moisture. Langerhans cells form receptors that recognize microorganism, virus, and even foreign compound which then activate immune system. The ability to keep water concentration is important in maintaining skin health. The amount and distribution of melanin pigment causes variety in human skin color. Vitamin D is synthesized in epidermis with the help of ultraviolet ray; synthesis is done by keratinocyte which is located in stratum basale and stratum spinosum of epidermis[5].
Dermis is home of additional epidermis component. Dermis consists of immune cells that fight against infection in the skin. Dermis provides supply of blood, nutrition, and oxygen to itself as well as epidermis. It also functions as skin temperature regulator by using superficial blood vessel and sense of touch by using nerve receptors.
Hypodermis or subcutaneous is a layer which consists of fat and connective tissue which is rich in blood vessel and nerves. This layer is crucial in regulating body and skin temperature.

Wound and Healing Process:
Wound is a condition in which there's a disruption in normal skin structure with depth and severity vary between different wound conditions. Wound is not only a cut on skin layer, but can also reach tissue beneath the skin. There are open and close wounds. Examples of open wound are incision, laceration, abrasion, stab injury, and penetrating injury. Examples of close wound are contusion due to blunt object, bruises, and wound with damage on tissue beneath the skin but leaving both epidermis and dermis intact. The depth of wound varies; wound within the epidermis is classified as superficial and wound which affects part of the dermis is classified as partial thickness wound. Full thickness wound covers all the epidermis and dermis, and even the tissue beneath skin, such as subcutaneous tissue, fascia, and muscle. Acute wound is a wound with healing process that takes around 7 – 14 days.
There are 4 phases of wound healing process: hemostatic phase, inflammation phase, proliferation phase, and remodeling or re-formation of skin structure phase. Figure 2 shows the estimation of starting time and period of each phase in functional wound healing process (with no complication or infection).

Hemostatic Phase:
Hemostatic phase happens as soon as trauma happens. In order to stop bleeding, open blood vessel goes through vasoconstriction, then activated platelet adheres with each other and aggregates around wound area. Platelet is activated by extracellular collagen (type I). As platelet interacts with collagen, platelet releases mediator (growth factor and cyclic AMP) and glycoprotein, which signal platelet to be become stickier and accumulate. Alpha platelet granules releases glycoprotein in the form of fibrinogen, fibronectin, thrombospondin, and factor von Willebrand. When platelet aggregation occurs, blood clotting factors is released, causing fibrin to aggregate in the wound area.

Inflammation Phase:
This phase happens 24 hour after the trauma to skin, and can last up to 2 weeks, depending on whether there's an infection which prolongs the phase. Mast cell releases granules containing enzyme, histamine, and other active amines which induce inflammation symptoms, rubor (redness), calar (heat), tumor (swelling), and pain, around the wound area. Neutrophil, monocyte, and macrophage are the main cells in this phase. These cells clean up infection and debris in the wound, also release soluble mediators, such as pro-inflammation cytokine (TNF-α, IL-1, IL-6, and IL-8) and growth factor (PDGF, TGF-β, TGF-α, IGF-1 and FGF) which are involved in activation of fibroblast and epithelial cell for preparation of next phase in wound healing process.

Proliferation Phase:
Fibroblast migrates to the wound as a response to soluble mediators that are released by platelet and macrophage. Fibroblast migration to extracellular matrix depends heavily on the recognition and interaction of fibroblast with specific components in the matrix. In normal dermis condition, fibroblast in inactive and not distributed, while in matrix around wounded area and in granulating tissue, fibroblast becomes active and increases in number. Fibroblast binds matrix components, such as fibronectin, vitronectin, and fibrin, by using fibroblast's integrin receptor. Integrin attaches to specific amino acid (R- G-D or arginine-glycine-aspartate acid) or to side of matrix component. When one side of fibroblast binds to matrix component, the cell expands its cytoplasm to find other sides to bind. Once finding new side to bind, cell releases previous binding (by local protease activity) and uses cytoskeleton actin filaments to move forward. Fibroblast secretes proteolytic enzyme to facilitate fibroblast migration to matrix. Secreted enzymes are three types of MMP which are collagenase

(MMP-1), gelatinase (MMP-2 and MMP-9) that destroy gelatin compound, and stromelysin (MMP-3) which has several proteins in ECM (extracellular matrix).

Remodeling Phase:

Remodeling is the last phase of wound healing which happens after granulated tissue turns into scarred tissue and skin elasticity increases. Granulated tissue maturation involves the decrease in number of capillaries by becoming one with bigger blood vessel and decrease of glycosaminoglycan (GAG) concentration, water bound to GAG, and proteoglycan. Cell density and metabolic activity decrease in granulated tissue which goes through maturation. Medication also happens in type, amount, and arrangement of collagen, which improve elasticity. At the beginning, type III collagen is synthesized in high amount. Then, it's replaced by type I collagen, dominated by nerve collagen in skin. New epithelial elasticity is only 25% compared to normal tissue. Repaired skin tissue after wounded will never be as strong as normal tissue which is never wounded.

Epithelialization

Epithelialization is a process that involves adhesion of epithelial cell and structure modification which then migrates, proliferates, and differentiates. Mature and intact epidermis tissue has five layers as explained before; its epithelial cells are differentiating starting from keratinocyte in stratum basalis which is bordered with cuboid shape dermis. Only epithelial cells in stratum basalis can proliferate, then adhere with both surrounding cells and basalis membrane with the help of intercellular connector, desmosome (for intercellular adhesion) and hemidesmosomes (for epithelial cell and basalis membrane adhesion). Released growth factors (EGF, keratinocyte growth factor / KGF, TGF-α) bind with each growth factor's receptor on epithelial cell, and then induce migration and proliferation. Bound between growth factor and receptor triggers desmosome and hemidesmosome to be soluble, so that cells can migrate. Integrin receptor is expressed, and basalis epithelial cell with cuboid shape turns into flat shape then migrates to form thin layer on top of new granulated tissue, following the length of collagen filaments.

Basalis epithelial cell proliferation in the wound area provides new cells in epithelial cell layer on top of granulated tissue. Epithelial cells in this layer forms and secretes proteolytic enzyme (MMP) that allows cells to penetrate scar, necrosis surface, or eschar. MMP plays role in re-formation of ECM, cell migration, mitogenic factor activation. Cell Migration continues until epithelial cells reach other additional cells and forms unified layer. As soon as this happens, all epithelial layer proliferates and layered epidermis is formed and maturating to repair skin defense functionality. TGF-β is one of the growth factors that help maturation (modification and keratinization) of epidermis layer. Intercellular desmosome and hemidesmosome attach to the new basalis membrane. Epithelialization is a clinical sign of wound healing, but not the end of healing process.

Methicillin-resistant *Staphylococcus aure us* ((MRSA)

***Staphylococcus aureus* morphology**

S. aureus is a single cell organism, called prokaryotes. It has shape of coccus (round) that forms a groupd (Figure 2), its cytoplasmic structure consists of nucleoid, and its chromosome is double helix DNA, extrachromosomal and no nucleosome (plasmid), and ribosome 70s which consists of subunit 30s and 50s. Cytoplasmic membrane has lipid (cholesterol) bilayer structure. It has a capsule with polysaccharide, pili for adhesion, fimbrae and flagella for bacterial motility.

Bacterial cell wall is a complex with less elastic structure and affects cell shape. Main function of cell wall is protecting bacterial cell from higher intracellular pressure compared to extracellular that carries risk of rupture (bursting). Clinically, cell wall is important because it contributes to bacteria ability to cause disease, has part to adhere with APC (antigen presenting cell) receptor, and becomes location for antibiotic to work. S. aureus cell wall consists of macromolecule peptidoglycan, teichoic acid, and lipoteichoic acid.

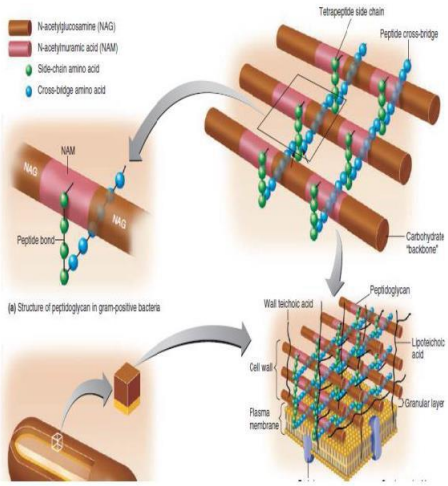

Figure 2 : *S. aureus* cell wall structure

Staphylococcus aureus Pathogenicity

Two factors suspected to be the virulence factors of *S. aureus* are gene ica which codes the formation of *poly-N-acetylglucosamine/polysaccharide* intercellular adhesion (PNAG / PIA) and insertion of gene IS256. IS256 plays role in genetic adaptation during infection by inserting itself to ica or agr locus. Insertion to ica locus increases the formation of PNAG / PIA, while insertion to agr inhibits biofilm formation regulation, therefore biofilm will grow thicker. These two virulence factors help S. aureus to colonialize both for commensal and infection purposes[5].

PNAG / PIA, PGA, and protease ScpA play role in protecting bacteria from antimicrobial protein which is produced by non-specific immune system (innate). Intercellular adhesion by PIA and biofilm protein is important factors as bacteria stress mechanism on skin environment. PGA plays role in osmotic tolerance, which is the original function of this polymer in non-infectious S. aureus. There's still no conclusive data about the difference between commensal and infectious strains in term of their surface components recognizing adhesive matrix molecules (MSCRAMMs). Due to these virulence factors, S. aureus can clinically cause skin infection depending on the bacterial contamination frequency, bacterial adhesion mechanism to skin, and bacterial ability to avoid immune system which help to colonialize. High number of bacteria population can cause infection.

S. aureus synthesizes several toxins, which are exotoxin (5 cytolytic toxin, 2 exfoliative toxin, enterotoxin, and *toxin shock syndrome toxin*- 1/TSST-1). Exfoliative A toxin, enterotoxin, and TSST-1 are classified as polypetides, called superantigen.

Methicillin-resistant S. aureus has a gene which plays role in certain antibiotic resistance, especially methicillin, which is the first antibiotic option for *Staphylococcus* infection. Gene coding for methicillin resistance in *mobile genetic elements* (MGEs) is called *staphylococcal cassette* chromosome mec (*SCCmec*), which consists of *mecA*, coding for protein that binds to penicillin / β lactam, PBP2a (penicillin-binding protein 2a). PBP2a is an enzyme in bacterial membrane that catalyzes transpeptidation reaction, which is crucial for cross binding in peptidoglycan chain. Since PBP2a has low affinity to all β-lactam antibiotics, Staphylococcus can survive after exposure to high dosage of this antibiotic group. There are 10 identified *SCCmec* structures in S. aureus; one of them is *SCCmec* type IV. Methicillin resistant S. aureus also shows resistance to other antibiotics, such as rifampicin, flouroquinolone, gentamisin, tetracycline, chloramphenicol, erythromycin, clindamycin and sulfonamide.

Skin Immune System against MRSA Infection

Non Specific Immune System:

Infection starts when pathogen penetrates anatomical barrier of host. Several non-specific immune mechanisms activated are several groups of soluble molecule that are present in extracellular fluid, blood, and secreted by epithelial cells. Soluble molecules includes antimicrobial enzyme, such as lysozyme to digest bacterial cell wall ; antimicrobial peptides, such as defensin to lyse bacterial cell membrane ; plasma protein system known as

complement system to lyse bacterial cell. Phagocytosis in non-specific immune system is done by neutrophil and macrophage. If phagocytosis fails to kill bacteria, non-specific immune cell is activated by pattern recognition receptor (PRRs) that recognizes molecules called pathogen- associated molecular patterns (PAMPs) which is an identifier of microbe. Activated cells in non-specific immune system involve a lot of effector mechanisms to eliminate infections. If infection can defeat non-specific immune system, specific immune system, which destroys pathogen specifically and forms long term memory cells, is involved.

Specific Immune System:

APC (antigen presenting cell), induced by S. aureus phagocytosis and TLR stimulation by S. aureus cell wall components, expresses MHCL class II. MHC class II binds with TCR (T cell receptor) CD4+. T helper cell (Th cell) CD4+ differentiates to Th17 which produces cytokine IL- 17 and IL-22. IL-17 stimulates chemokines, other cytokines (IL-1, IL-6, TNF, CSF/colony stimulating factor) which increases production of neutrophil and stimulates production of antimicrobial peptide (AMP). IL-22 increases barrier function and stimulates production of AMP. APC also synthesizes IL-1 and IL-6 which together with growth factor TGF-β (transforming growth factor-β) activate transcription factor STAT3 and RORγT that stimulate differentiation of T cell CD4+ into Th17. Figure 3 shows collaboration between non specific and specific immune system against bacterial infection on skin[7].

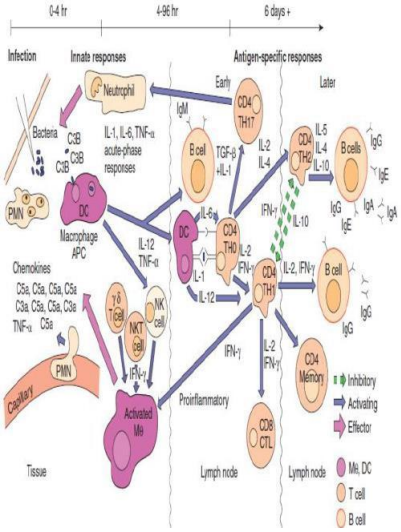

Figure 3 : Immune response to MRSA infection on skin wound

Components of +dalethyne Compound and Its Function in MRSA Infected Wound Healing Process

+dalethyne comes from ozonized olive oil, first introduced in India in 2015 by a plant based skin care company. Ozonation is done by infusing ozone using a cold plasma ozone generator[8]. Test by research center in Pharmacy Faculty, Airlangga University, showed that +dalethyne consist of fatty acids (oleic acid, palmitic acid, stearic acid, linoleic acid), essential oil (aldehyde group), iodine, and peroxide.

Unsaturated fatty acid has antibacterial activity against MRSA. Unsaturated fatty acid inhibits enzymatic activity in bacterial cell, inhibits bacterial nutrition intake, triggers peroxidation, and triggers auto oxidation which directly lyse bacterial cell. Essential oil also has antimicrobial activity. Its mechanism affects bacterial membrane structure and cell wall, pH homeostasis in bacterial cell, chaperone expression, and over production of cell surface protein. Essential oil increases permeability of bacterial cell which results in the loss of essential nutrition from cell (causing trauma to cell structure). It causes proton influx that goes beyond cytoplasmic capability to hold, decrease of pH, and disruption in cell function. Essential oil also causes over expression of chaperone protein (DnaK, GroEL, HtpG and Trigger factor Tf) and surface protein (OmpX and OmpA) which disrupt bacterial cell metabolic pathway. Essential oil is effective in inhibiting biofilm formation of MRSA by using quorum sensing mechanism which inhibits production and secretion of signal molecules necessary for biofilm formation. Multiple drug resistant MRSA is found to be still susceptible to essential oil[10]. Iodine and peroxide is topical antiseptic which kill, inhibit, and decrease the number of bacteria on the wound. This antiseptic has broad spectrum antimicrobial activity[9].

EXPERIMENTAL SECTION

The research is true laboratory experimental research because controlled outside variables intervention is applied to this research, or it uses Post Test Only Control Group Design. The object is *Rattus norvegicus* strain Wistar, aged around 3 months, weighing around 200 – 250 gram.

There are 6 groups of experiment object, which are O1 (negative control, observed on day 4), O2 (negative control, observed on day 6), OK1 (positive control, observed on day 4), OK2 (positive control, observed on day 6), OP1 (treatment group, observed on day 4), OP2 ((treatment group, observed on day 6). Negative control is wound on rat without MRSA infection, positive control is wound on rat with MRSA infection, and treatment group is wound on rat with MRSA infection and +dalethyne application. Each group has 6 rats (36 rats in total) every 6 rats are given 1 – 6 numbering by attaching a paper on the tail. The rat is then separated randomly to 6 groups until each group has 6 rats[11-20].

Each rat is weighed and kept in a 20 x 15 x 15 cm cage to adapt. Rats are anesthetized using ketamine solution. Ketamine solution is made of 3 mL ketamine and 1 mL xylazine, diluted with aqubidest for 6 mL injection, then injected to rats (0.1 mL per rat) until rats is in calm state and shows abdominal breathing only. Anesthetized rats are shaved 3 x 3 cm wide, disinfected with betadine, and incised ±2 cm on the back as deep as subcutaneous layer using scalpel[21-30].

In positive control group and treatment group, wound on rat skin is infected with 50 µL MRSA from 0.5 mcFarland bacterial suspension by using micropipette on the wound. Bacterial inoculation is done after bleeding stops in the wound. Application of cream containing active ingredient +dalethyne is done in treatment group. Application of cream containing active ingredient +dalethyne is done using cotton bud 2 days after infection. Application is done every day. On day 4 and day 6 after treatment, each group, including negative control group, is killed by using ketamine as anesthetics. Then, incision is applied to skin with normal skin range of 0.5 – 1 cm from side of wound. Skin tissue is put in fixation buffer formalin 10% for 15 – 24 hour. Deceased rats are buried[31-40].

Preparative of skin tissue is made and HE staining is done. Then, epithelial cell length is observed and measured using Optilab that's applied to microscope ocular lense with 40x magnification[41-50].

RESULT AND DISCUSSION

Mean and deviance (SD) of epithelial cell length in negative control, positive control, and treatment group are shown in Table 1, showing the increase of epithelial cell lengths in treatment group both in day 4 and day 6 compared to positive control group (Figure 4).

Comparison of epithelial cell length between treatment group and negative control group (Figure 5) shows no significant difference both on day 4 and day 6[51-60].

Table 1: Means and deviance of epithelial cell length (conversion from µm to mm) on wounded Wistar rat skin in negative control, positive control, and treatment groups

Group		Day 4	Day 6
Negative control	**Mean**	0,59	0,73
	SD	0,711505	0,0083666
Positive control	**Mean**	0,21333	0,42
	SD	0,162682	0,301993
Treatment	**Mean**	0,46	0,638333
	SD	0,194422	0,761326

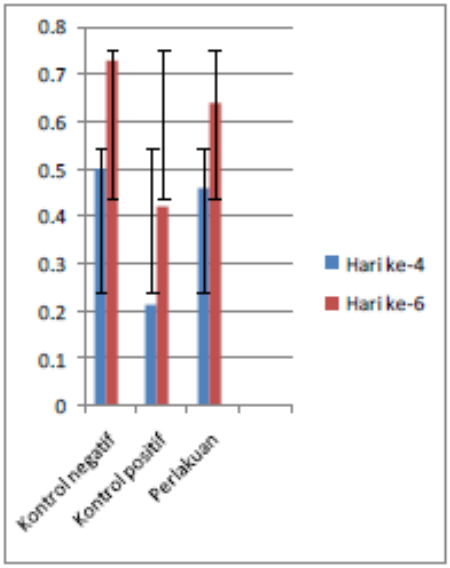

Figure 4 : Distribution and means of each group epithelial cell length

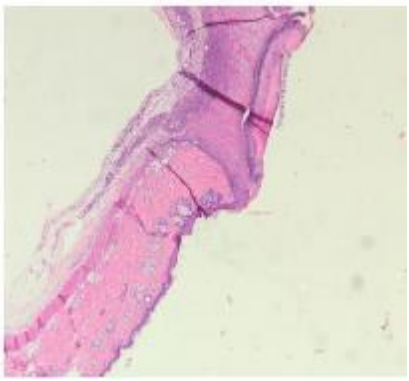

Figure 5 : Microscopic observation of epithelial tissue on day 4 after wounding in negative control group (40x magnification)

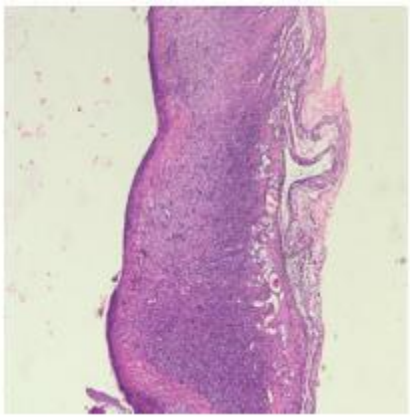

Figure 6 : Microscopic observation shows no epithelial cell formation on day 6 after wounding in positive control group (40x magnification)

In this research, means of epithelial cells length in negative control, positive control, and treatment groups show increase from day four to six day. It is as sign of epithelialization in all three groups. It's in accordance with theory of wound healing process, which states that epithelialization starts on day 3 when epithelial layer covers the base of wound. Keratin can be observed in both negative control and treatment groups, but can't be observed in positive control group (Figure 6). Within 24 hour of wounding, keratinocyte migrates to lateral and regenerates basalis membrane. After new basalis membrane is formed, keratinocyte stops migrating and continues with proliferation until its peak on day 4.

Epithelial layer keeps growing longer and thicker, then newly formed epithelial layer undergoes maturation which results into new corneum layer. With regeneration of basalis membrane, keratinocyte returns to its former shape and re-adhesion of hemidesmosome and lamina basalis happens. Presence of rete pegs shows that epithelialization is ongoing to form a normal epithelial tissue. Migrating epithelial cells will be connected to each other and closes the wound. After reaching normal epithelial thickness, migrating epithelial cells stops.

Control positive groups shows the lowest means of epithelial cells length. It's caused by longer inflammation process due to MRSA infection. Fibroblast still actively forms inflammasome complex to produce pro-inflammation cytokine IL- 1β. Fibroblast also proliferates and differentiates to synthesize granulated tissue components (collagen, elastin, and proteoglycan)[61-65].

Mean of epithelial cell length in treatment group is almost the same as in negative control group. It shows that +dalethyne can kill MRSA and induce epithelialization which results in wound healing time almost similar with physiological wound healing time. Unsaturated fatty acid, essential oil, iodine, and peroxidase can kill antibiotic resistant MRSA. Essential oil also reduces pH in wound micro environment which triggers migration and proliferation of keratinocyte. With maturation of new epithelial tissue, epithelial cells migrate to the side of wound and proliferate until it's connected with each other and closes the wound.

CONCLUSION

Based on the result of this research, it's concluded that topical application of +dalethyne accelerates epithelialization in wound healing process on skin of MRSA infected Wistar rats.

REFERENCE

[1] Tortora GJ; Funke BR; Case CL. Microbiology An Introduction 12th Edition. Pearson Education Inc, USA,**2016.**

[2] Abbas AK; Andrew H; Pillai S. *Cellular and Molecular Immunology 8th Edition*. WB Elsiver Company , Philadelphia. **2015**. 35-168.

[3] Robert L Modlin; Jenny Kim; Dieter Maurer; Christine Bangert; Georg Stingl. Innate and Adaptive Immunity in the Skin in Klaus Wolff et al (ed.), Fitzpatrick's Dermatology in General Medicine seventh edition, The McGraw-Hill Companies, Inc, USA**. 2008,** 95-114.

[4] David H Chu. Development and Structure of Skin in the Skin in Klaus Wolff et al (ed.), Fitzpatrick's Dermatology in General Medicine seventh edition, The McGraw-Hill Companies, Inc, USA. **2008,** 57-72.

[5] Madeleine Flanagan. Wound Healing and Skin Integrity, John Wiley & Sons Ltd, USA. **2005,** 33-48 .

[6] Ryu Sunhyo; Peter I Song; Chang Ho; Seo Hyeonsook; Cheong; Yoonkyung, Park. *Int J Mol Sci*. **2014,** 15, 8753-8772.

[7] Ranzato Elia; Burlando Bruno. Signalling Pathways in Wound Repair in Wound Healing: Process, Phases, and Promoting, Jane E. Middleton (ed.), Nova Science Publishers, New York. **2011,** 123-135.

[8] Edward. The Top Benefits of Ozonated Olive Oil. *http://www.globalhealingcenter.com/natural-health/ozonated-olive-oil/*. **2009.**

[9] Atiyeh Bishara S; Dibo Saad A; Hayeh Shady N. Wound Cleansing, *Int Wound J*. **2009,** 6, 420-438.

[10] Faleiro ML; Miguel MG. Use of Essential Oils and Their Components against Multidrug-Resistant Bacteria in Fighting Multidrug Resistance With Herbal Extracts, Essential Oils and Their Components (Mahendra Rai and Kateryna Kon editor). Elsevier Inc, USA. **2013,** 65-86

[11] Aller MA; Arias JI. Oxygen-Related Inflammatory Wound utama Process, Phases, and Promoting, Jane E. Middleton (ed.). Nova Science Publishers Inc, New York. **2011**, 25-48

[12] Brittany Busse. *Wound Management in Urgent Care*. *Springer International Publishing, Switzerland*. **2016,** 1-55
[13] Bergsson Gudmundur; Hilmarsson Hilmar; Thormar Halldor. Antibacterial, Antiviral and Antifungal Activities of Lipids in Lipids and Essential Oil as Antimicrobial Agents (Halldor Thormar ed), John Wiley & Sons Ltd, USA. **2011**, 48-75
a. Badiu, Diana. Vasile, Monica. Teren, Ovidiu. Regulation of Wound Healing by Growth Factor and Cytokines in Wound Healing: Process, Phases, and Promoting, Jane E. Middleton (ed.) Nova Science Publishers Inc, New York. **2011,** 73-93
[14] Bergstresser Paul R. Basic Science Approaches to the Pathophysiology of Skin Disease in the Skin in Klaus Wolff et al (ed.), Fitzpatrick's Dermatology in General Medicine seventh edition, The McGraw-Hill Companies, USA. **2008,** 87-92.
[15] Berger Timothy G. General Considerations of Bacterial Diseases in the Skin in Klaus Wolff et al (ed.), Fitzpatrick's Dermatology in General Medicine seventh edition. The McGraw-Hill Companies, Inc. USA. **2008,** 1689-93.
[16] Cassat James E; Smeltzer Mark S; Lee, Chia Y. Investigation of Biofilm Formation in Clinical Isolates of *Staphylococcus aureus* in Yinduo Ji (ed.), Springer Science+Business Medi, **2014,** 195-200
[17] Cordeiro JV; Jacinto A. *Nat Rev Mol Cell Biol.* **2013,**14, 249-262
[18] Choi Jin Kyui; Jang Ji-Hye; Jang Won-Hee; Kim Jaekwan; Bae Il-Hong; Bae Joonho; Park Young-Ho. Kim Beum Joon; Lim Kyung- Min; Park Jin Woo. *Biomaterials*. **2012,** 33(33), 8579-90.
[19] Carson Christine F; Hammer Katherine A. Chemistry and Bioactivity of Essential Oil in Lipids and Essential Oil as Antimicrobial Agents, Halldor Thormar (eds), John Wiley & Sons Ltd, USA**. 2011,** 204-223
[20] Chin GA; Diegelmann RF; Schultz GS. Cellular and Molecular regulation of Wound Healing. In Falabella AF, Krisner RS, eds. Taylor&Francis Group, New York. **2005,** 17-29.
[21] De Masi; Elen CDJ; Campos Antonio CL. De Masi; Flavia DJ; Ratti Marco AS; Ike Isabela S; Roberta DJ. *Braz J Otorhinolaryngol.* **2015,** 1-10.
[22] Di Ciccio; P Vergara; A Festino; AR Paludi; D Zanardi; E Ghidini; Ianieri A. *Food Control*. **2015,** 50, 930-936.
[23] Delves Peter J; Martin Seamus J; Burton Dennis R; Roitt, Ivan M. Roitt's Essential Immunology. John Wiley & Sons Ltd, United Kingdom. **2011,** 3-273.
[24] Djunaedi Djoni. *Jurnal Kedokteran Brawijaya*, **2006,** 97-100.
[25] Fitriyanti Sepvi. *Jurnal Berkala Epidemiologi*. **2015,** 3, 217-229.
[26] Bénédicte Fournier; Dana J Philpott . *Clin Microbiol Rev*. **2005**, 18, 521-540.
[27] Gomes F Leite; B Teixeira; Oliveira R. Strategies to control Staphylococcus epidermidis biofilms, Science against microbial pathogens: communicating current research and technological advances, A. Mendez-Villas (Ed.). Institute for Biotechnology and Bioengineering, Portugal. **2011,** 843-852.
[28] Galkowska H; Podbielsk A; Olszewski Waldemar L; Stelmach E; Luczak M; Rosinski G; Karnafel W. *Diabetes Res Clin Pract.* **2009,** 84(2), 187- 193.
[29] Gaynes Robert; Edwards JR. *Clin Infect Dis*. **2005,** 41, 848-54.
[30] Marta Zapotoczna; Hannah McCarthy; Justine K Rudkin; James P; O'Gara; Eoghan O'Neill. *J Infect Dis*. **2015,** 212(12), 1883-1893
[31] Mills Charles D; Lenz Laurel L; Ley Klaus. *Front Immunol***. 2015,** 6, 7-10.
[32] Mills Charles D; Thomas Anita C; Lens Laurel L; Munder Markus. *Front Immunol***. 2014,** 5, 42-46
[33] Marra Andrea. Animal Models in Drug Development for MRSA, In Yinduo Ji (ed.), Methicillin-Resistant Staphylococcus aureus (MRSA) Protocol, Methods in Molecular Biology, *Springer Science+Business Media*.**2014,** 333-344.
[34] Mogensen Trine H. *Clin Microbiol Rev.* **2009,** 22, 240-273.
[35] McGrath John A; McLean WH; Irwin. Genetics in Relation to the Skin in the Skin, In Klaus Wolff et al (ed.), Fitzpatrick's Dermatology in General Medicine seventh edition. The McGraw-Hill Companies, Inc, USA. **2008,** 73-86.
[36] Mille Stanley J; Sun Tung-Tien; Coulombe Pierre A. Epidermal Growth and Differentiation in Klaus Wolff et al (ed.), Fitzpatrick's Dermatology in General Medicine seventh edition.The McGraw-Hill Companies, Inc, USA. **2008,** 375-382.
[37] Michalik Liliane; Wahli Walter. *J Clin Invest.* **2006,** 116, 598-606
[38] Nan Wang; Hongwei Liang; Ke Zen. *Front Immunol.* **2014,** 5, 230-236.
[39] Nugraheni Ratna; Suhartono; Winarni S. *Media Kesehatan Masyarakat.* **2012** ,1 1 , 95-100

[40] Nabavian Reza; Garner Warren L.. Normal Wound Healing, Textbook of Burn Reconstruction. **2002,** 1-19
[41] Orstead HL; Keast David; Lalande LF; Francoise Marie. *Wound care Canada*. **2011,** 9 (2), 4-12
[42] Otto Michael. Nat Rev Microbiol. **2009,** 7(8), 555-567.
[43] Portou MJ; Baker D; Abraham D; Tsui J. *Vascul Pharmacol*. **2015,** 31-36
[44] Palavecino Elizabeth L. Clinical, Epidemiologic, and Laboratory Aspect of Methicillin-Resistant *Staphylococus aureus* Infections, in Yinduo Ji (ed.), *Methicillin- Resistant Staphylococcusaureus* (MRSA) Protocol, Methods in Molecular Biology second edition, *Springer Science+Business Media, LLC*. **2014,** 1-19.
[45] Proksch Ehrhardi. Jensen Jens-Michael. Skin as an Organ of Protection in Klaus Wolff et al (ed.), Fitzpatrick's Dermatology in General Medicine seventh edition. The McGraw-Hill Companies, Inc, USA. **2008,** 383-394
[46] Petzelbauer Peter; Peng Lisan S; Pober Jordan S. Endothelium in Inflammation and Angiogenesis in Klaus Wolff et al (ed.), Fitzpatrick's Dermatology in General Medicine seventh edition. The McGraw-Hill Companies, Inc. USA **2008,** 1585-97
[47] O'Gara James P; Humphreys Hillary. *J. Med Microbiol*. **2001,** 50, 582-587
[48] Rath Meera. Mulle Ingrid; Kropf Pascale; Closs Ellen I; Munder Markus. *Fron Immunol*. **2014,** 5, 13-19
[49] Reddy GAK; Priyanka B; Saranya ChS; Kumar CKA. *Int J Pharm Sci Rev Res.* **2012**, 2, 58-75
[50] Rodero Mathieu P; Khosrotehrani Kiarash. *Int J Clin Exp Pathol.* **2010,** 3(7): 643-653
[51] Rupp Mark E; Fey Paul D; Heilmann C; Gotz F. *J Infect Dis.* **2001,** 183, 1038-42.
[52] Sirokmany G; Pato Anna; Zana Melinda; Donko Agnes; Biro Adrienn; Nagy Peter; Geiszt Miklos. *Free Radic Biol Med*. **2016,** 97, 204-211.
[53] Sowash Madeleine G; Uhlemann Anne-Catrin. Community-Associated Meticillin- Resistant *Staphylococcus aureus* Case Studies in Yinduo Ji (ed.), *Methicillin- Resistant Staphylococcus aureus* (MRSA) Protocol, Methods in Molecular Biology second edition. Springer Science+Business Media, LLC. **2014,** 25-60.
[54] Salgado-Pabon Wilmara; Case-Cook Laura C; Schlievert Patrick M.. Molecular Analysis of Staphylococcal Superantigens in Yinduo Ji (ed.), *Methicillin-Resistant Staphylococcus aureus* (MRSA) Protocol. Methods in Molecular Biology second edition. Springer Science+Business Media, LLC. **2014,** 169-193
[55] Soepribadi I. Regenerasi dan Penyembuhan. Jakarta, Penerbit CV, Sagung Seto, Hal. **2013** 62- 67
[56] Scemons D; Elston D. Nurse to Nurse Wound Care. The McGraw-Hill Companies Inc. USA .**2009,**21-55.
[57] Tisserand Robert; Young Rodney. Essential Oil Safety: a Guide for Health Care Professionals second edition. Churchill Livingstone Elsevier, UK. **2014,** 5-90
[58] Turgeon Mary Louise. Immunology and Serology in Laboratory Medicine Fifth Edition . Elsevier Mosby Inc., Missouri. **2014,** 30-99.
[59] Tang Ling; Kirsner Robert S; Li Jie. Extracellular Matrix Molecules in Skin Wound Repair in in Wound Healing: Process, Phases, and Promoting, Jane E. Middleton (ed.). Nova Science Publishers Inc., New York. **2011**, 49-71.
[60] Thormar Halldor. Antimicrobial Lipids and Innate Immunity in Lipids and Essential Oil as Antimicrobial Agents (Thormar Halldor ed). John Wiley & Sons Ltd, USA. **2011,** 124- 144
[61] Takeuchi Osamu; Akira Shizuo. Pattern Recognition Receptors and Inflamation. **2010,** 805-820.
[62] Travers Jeffrey B. Mousdicas Nico. Gram- Positive Infections Associated with Toxin Production in Klaus Wolff et al (ed.),Fitzpatrick's Dermatology in General Medicine seventh edition. The McGraw-Hill Companies, Inc., USA. **2008,** 1710-19
[63] Traversa B; Sussman G. *Prim Inten*. **2001,** 9, 161-167
[64] Yang Junshu; Ji Yinduo. Investigation of *Staphylococcus aureus* Adhesion and Invasion of Host Cells in Yinduo Ji (ed.), *Methicillin-Resistant Staphylococcus aureus* (MRSA) Protocol, Methods in Molecular Biology second edition. Springer Science+Business Media, LLC. **2014,** 187- 192.
[65] Zhihong Yang; Xiu-Fen Ming. *Front immunol.* **2014,** 5, 178-184.