The effect of ionic silver water (Ag⁺) toward TNF-α (Tumor Necrosis Factor-α) expression and epidermal thickness in open wound healing rat (Rattus norvegicus) model

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Abstract. Incision wounds are one type of open wounds that occur caused by a sharp instruments such as a result of medical procedure in surgery process. At the time of injury, body will naturally heal that wounds through three phases, inflammation, proliferation, and maturation (remodeling). Ag (silver) solution can accelerate wound healing because it has antibacterial properties and will increase fibroblast cells production. This study aims to determine the effect of Ag (silver) solution based on expression TNF-α (Tumor Necrosis Factor-α) and epidermal thickness in incision wound of the white rat (Rattus norvegicus) Wistar strain. The experimental animals were male white rat Wistar strains weighing 150-250 grams, aged 8-12 weeks divided into 4 groups. Negative control group without treatment. P1 group that is incised and treated by 10% Povidone Iodine with doses 0,5 ml. P2 group that incised and treated by Ag (silver) solution with doses 0,5 ml. P3 group that incised and treated by combination between 0,5 ml ag (silver) solution and 0,5 ml Povidone Iodine. All treatments was given for seven days. TNF-α expression was measured using the Flowcytometry method and epidermal thickness was observed histopathologically by Haematoxyllen Eosin (HE) staining. Data analysis TNF-α expression was performed by Kruskal Wallis test while epidermal thickness were analyzed by one way ANOVA test and tukey follow-up test with 95% confidence level (α = 0.05). The results of this study showed that combination therapy of Povidone Iodine 10% and Ag solution tends to decrease TNF-α expression and showed increasing cell regeneration of the epidermis in terms of epidermal thickness. The conclusion of this study is that combination therapy can accelerate the wound healing process.

1. Introduction
Animals and humans in daily work are always faced with certain hazards, such as the dangers of infectious, toxic reagents, electrical equipment and other equipment used daily so as to potentially suffer the risk of wounds [1]. Wound is a form of damage to skin tissues caused by contact with heat sources (such as chemicals, hot water, fire, radiation, and electricity), results of medical action, changes in...
physiological conditions, or animal bites [2]. Wounds cause impaired function and body anatomy structure, damaging the continuity of the skin, mucosa, membrane, or other organs [3].

The process of healing the wound physiological occurs through several phases, namely inflammation, proliferation, and remodelling [4]. The inflammatory process begins after the formation of blood clotting, this process occurs immediately after the incision of the skin. In this process required a pro-inflammatory mediator such as Tumor Necrotic Factor (TNF-α). TNF-α is a pro-inflammatory mediator stimulated by a neutrophils which are beneficial for stimulating inflammatory cells, fibroblasts, and epithelial cells. The higher the TNF-α expression on the wound, indicating that the inflammatory process is ongoing, while the TNF-α expression decreases, indicating the wound is getting better.

The goal of wound handling is to heal wounds faster by minimizing the pain, scars, and discomfort of the patient [5]. In cases of open wounds often occur in the presence of infections caused by the inclusion of bacteria on the wound, circumstances will be worse when not immediately given antiseptic immediately. Chemical antiseptics such as Povidone Iodine [1]. Povidone Iodine contains a free iodine and polyvinylpyrrolidone (PVP) which has a strong anti-microbial effect, but 10% Povidone Iodine can cause contact dermatitis in the skin, toxic to fibroblast and leukocytes, inhibit neutrophil and lowering the monocytes thus slowing the wound healing process [6].

The use of metal particles such as silver (Ag) in wound healing is still very little used, especially in Indonesia. Ag (silver) water can be used as an ingredient for wound healing, high antibacterial content on water Ag can be used to treat damaged epithelium tissue on the skin. The antibacterial activity produced by the Ag (silver) water simultaneously induces apoptosis in the inflamed cells so that the wound will continue in the next phase until the wound closes [7]. Silver water will also induce fibroblasts cell counts, reducing neutrophil infiltration and macrophages on incision wounds, in wound healing [8].

Based on the explanation above, this study aims to examine the therapeutic response of Ag (silver) on the healing of the incision wound based on the expression of TNF-α and the thickness of the epidermis in rats (Rattus norvegicus). The results of research are expected to be used as an alternative in wound healing using natural.

2. Materials and methods

2.1. Tools and materials
Rat cage, rat drinking bottle, chaff, feed box, Sonde tool, dissecting set, surgical board, glove, mask, blender, digital scales, object glass, cover glass, disposable syringe, micromote, mortar and pestle, spray bottle, glass bottle, microscope Light (Olympus BX51), digital camera, reaction tube, organ pot, freezer, ware, blue tip, Yellow tip, Centrifuge, Flowcytometry, Image Raster® software, and BD Cell Quest Pro™ software.

White Mouse (Rattus norvegicus) strains of Wistar age 8 – 12 weeks with a weight of 150-250 grams, feed, drinking water, water Ag (silver) ppm 20, aquades, ethanol 70%, ethanol 80%, ethanol 90%, ethanol 95%, absolute ethanol, physiological NaCl, Xylol, alcohol (70%, 80%, 90%, absolute), paraffin block, dye haematoxylin and eosin, anti-rat primary antibodies TNF-α, PBS, and 10% formalin.

2.2. Animals model preparation
The animal trials used in this study were 20 white Rat (Rattus norvegicus) male strains of Wistar aged 8 – 12 weeks with a rat weight between 150 – 250 grams obtained from the pharmacology Laboratory of the Faculty of Medicine University Brawijaya. Animal acclimatization for 7 days with the aim of adapting the animals to try with the new environment. At this stage observation of the general circumstances of the animal try. Feeding during the period of acclimatization is standard feed according to the requirement of 20 grams/tail/day in the form of pellets (10% of body weight) and drinking water is given ad libitum.

2.3. Preparation of Ag Water
Ag Water (silver) used in this study was processed in the Pharmacology Laboratory of Veterinary Medicine School, Brawijaya University using electrolysis method. The water content of Ag (silver) used in the treatment of 1 L solvent is 20 ppm, so that every 1 mL of solvent (aquabides) is 0.02 mg of silver, meaning in the administration of 0.5 mL of Ag water then contains 0.01 mg of silver.

2.4. Rat (Rattus norvegicus) as animal model
Rats (Rattus norvegicus) was 20 acclimatization in laboratory conditions first for 7 days, then divided into 4 groups with each group consisting of 5 tails. Each group enclosure is annotate using labels. Dorsal parts are wiped with alcoholic cotton 70% and performed anaesthesia by using a combination of ketamine dose 40-75 mg/kg and xylazine 5-12 mg/kg in intra-muscular[9], after that shaved part hair part dorsal until clean area of 5x5 cm and carried out the making of the incision along 2 cm in the median portion of the dorsal vertebrae with depth to the subcutaneous part until M. Panniculus carnosus using a sterile blade with a size of 24 G.

2.5. Administration of Ag (silver) water
Each of these animals is given twice as much therapy a day. Ag (silver) water is given topically, which is by dripping water to the wound location. Ag water as much as 0.5 ml. Group of animals try negative controls without any treatment. The P1 group of animals was imparted and was given a 10% Povidone Iodine of 0.5 ml. P2 group animals that were assigned and administered therapy Ag water rate of 20 ppm as much as 0.5 ml. P3 Group is given a 10% combination of Povidone Iodine therapy 0.5 ml and Ag (silver) water 20 ppm as much as 0.5 ml with a ratio of 1:1. After the treatment of the location of the wound immediately closed with sterile gauze to avoid contaminated. The therapy is administered for 7 days.

2.6. Data analysis
Epidermal thickness of the HE staining is measured perpendicular to the lining of the corneum to the basal layer (from the inside out) using a micro meter unit. The coating is the thickest to the thinnest[10]. Calculation of the epidermis thickness is done quantitatively which has been averaged using the Software Image Raster®. Quantitative data of the relative rate of TNF-α was analysed statistically with the crucial test of the Kruskal-Wallis with the equivalent significance of 0.05. The thickness of epidermis is analysed statistically with test one-way ANOVA followed by Tukey test with a confidence level of 95% (α = 0.05).

3. Result and discussion
Based on the macroscopic description of the four treatment groups (C(-), P1, P2, P3) can be seen in the P3 group which is given an incision treatment with a combination therapy of Air Ag (silver) and a 10% Povidone iodine showing the most rapid wound healing process of other treatment groups.

3.1. Levels of TNF-α post-wound open in the rat’s skin
The measurement results show the average TNF-α relative rate in the P1 group higher when compared to the negative control group. In general, the case of wounds often occur in the presence of infections caused by the inclusion of bacteria in wounds. Circumstances will get worse when bacterial contamination is increasing which will be directly proportional to the increase of TNF-α cells, therefore necessary antiseptics to help accelerate the wound healing process. Povidone Iodine has good antiseptic properties against Gram-positive and gram-negative bacteria. Povidone Iodine Administration is 10% effective to shut off microbial cells, but the administration of Povidone Iodine 10% also causes irritation to the wound because substances contained in antiseptic materials will be regarded as foreign bodies by the body because of components and the arrangement contained different body cells [10]. This causes the TNF-α levels of the incision group to be administered by a 10% Povidone Iodine to increase compared to the TNF-α levels under normal conditions.
### Table 1. Relative levels of TNF-α

| Treatment     | Average relative rate of TNF-α (µm) ±SD(%) |
|---------------|--------------------------------------------|
| Negative Control | 3.41±0.18                                   |
| P1 group      | 7.73±0.73                                   |
| P2 group      | 15.31±0.95                                  |
| P3 group      | 9.81±1.16                                   |

P2 group treatment obtained the results that the administration of Ag water therapy post incision affects the wound healing process, but showed higher results when compared to the treatment P1 group. Ion Ag is topically capable of killing bacteria, the minimum water concentration of Ag in the destruction of bacteria is between 5-50 ppm. Ion Ag + in the form of fluid produced after the process of making water Ag has several actions against bacteria, such as damaging the cell wall of bacteria, activating enzymes in the bacteria, and interacts the synthesis of DNA on bacteria [11]. The high relative rate of TNF-α in the P1 group treatment can also be influenced by several factors including the error of Flowcytometry test, so that the skin cells that are expected to come out are not able to be well-expression, but it can also be due to of chronic inflammation.

P3 group treatment is a group treatment given a combination therapy between 10% Povidone Iodine and Ag water, affects the wound healing process, in addition to the results of the measurement indicates that TNF-α average relative level is lower when compared to P1 and P2 groups. It can be interpreted that the combination of Povidone Iodine and Ag water is more quality in repairing wounds. Povidone iodine is composed of povidium ions and Iodine ions [12]. When combined with Ag ion, the ion I in Povidone Iodine will bind to the Ag ion in silver water. New compounds that result from these reactions are able to increase the bactericidal effect produced by each compound, thus causing the most maximal effect.

#### 3.2. Levels of epidermal histopathology in the open wound healing process

The significant difference between the negative control group and P1 group treatment is caused that negative control group is a group of rats that are not given any treatment, so no change occurs on the skin tissues, while In the P1 group treatment occur should cause damage to skin tissue. The treatment of Povidone Iodine in the wound is generally acceptable to the body even though it has side effects in the form of local stimuli or allergic reactions [13]. The increased thickness of the epidermis indicates that in a P1 group the epidermis cells are still in the inflammatory stage, characterized by still many inflammatory cells visible. Cells that are still in the inflammatory stage will experience swelling,
swelling in the epidermis cells due to the production of fluid to the interstitial tissue. So, in P1 there is an increase in the most high thickness of epidermis than neither negative nor P2 and P3.

Table 2. Relative levels of epidermal thickness

| Treatment   | Average relative rate of Epidermal Thickness (µm) ±SD(%) |
|-------------|---------------------------------------------------------|
| Negative Control | 74.67±2.02a                                            |
| P1 group     | 165.456±1.02b                                          |
| P2 group     | 154.318 ±1.15b                                         |
| P3 group     | 122.026 ±1.67ab                                         |

Figure 2. Histopathology of Rat’s skin tissue with HE staining 400x magnification. Remarks: A. Negative Control, B. P1 Group, C. P2 Group, D. P3 Group

P2 group was the rat group in the incision and given the water therapy Ag 20 ppm showed no significant results to the P1 group which signifies the effect caused by Ag water against the regeneration of epidermis cells having similarities such as the effects posed by Povidone Iodine 10%. Ag water proved to regulate the activity of zinc in the body to increase the epithelization of cells. It is evidenced by the immunocytochemical evaluation saw the metallothionesis bond indicating that silver induces proteins and adds Zinc and copper concentrations. Both metals function in increasing the proliferation of epithelial cells [11].

P3 group is a treatment group given therapy in the form of a combination of Povidone Iodine 10% 0,5ml and water Ag 0.5 ml showed no different results significantly either against the negative control group or with the P1 or P2 treatment, and showed an average lower thickness of the epidermis when compared to 1 treatment also 2. It is because the ion particles of the Povidone iodine will bind to the ions in the Ag water forming an AgI bond which will affect the process of proliferation and regeneration of skin cells. In addition, administration of Ag water also affects the neovascularisation process of wound tissue [14]. Neovascularisation is a new blood vessel of buds formed from blood vessels and develops into a new skin tissue. Neovascularisation process reaches the peak at 3-5 days after the wound and will decline on the 7th day [15]. P3 group treatment showed that the repair of the post-wound epidermis cells showed no significant results and approached the negative control group.

Based on the results of the description and analysis of the statistics Tukey can be concluded that the provision of a combination therapy Povidone Iodine 10% and Ag water shows the most effective result of the process of wound healing of the incision in white rats (Rattus norvegicus) be reviewed from the thickness factor of the epidermis. This is because the results of the epidermis thickness measurement in the 3 treatment group close to the normal indicator of the negative control group.
4. Conclusion
The therapeutic treatment of Ag water combined with 10% Povidone Iodine post wound incision is able to lower the relative rate of TNF-α and also can improve the regeneration of cells, reviewed by epidermis thickness damaged when compared to the P1 group treatment on rat (Rattus norvegicus).

References
[1] Purbani 2009 Menguat Khasiat Jarak Pagar (Jakarta: PT. Argo Media Pustaka).
[2] Reksoprodjo S 2012 Kumpulan Kuliah Ilmu Bedah (Jakarta: Fakultas Kedokteran Universitas Indonesia).
[3] Purnama H, Sribiwono and Ratnawulan S 2015 Farmaka Suplemen 15 1-9
[4] Sugianman V K 2011 JKM 11 70-79
[5] De Jong W and Samsudidayat R 2010 Buku Ajar Ilmu Bedah (Edisi 3) (Jakarta: Penerbit Buku Kedokteran EGCG).
[6] Zakariya M, Sudiana I K and Wahyuni E D 2009 Jurnal Ners 4 1-8.
[7] Nadworny P L, Wang J, Tredget E E and Burrell R E 2010 J. Inflamm. (Lond) 7 13
[8] Pereira R F and Bartolo P J 2016 Adv. Wound Care 5 208-229.
[9] Plumb D C 2008 Plumb’s Veterinary Drug Handbook Ed 6th (Ames: The IOWA State University Press).
[10] Katzung B G 2002 Farmakologi Dasar dan Klinik Ed III (Jakarta: Penerbit EGC).
[11] Atiyeh B S, Costagliola M, Hayek S N and Dibo S A 2007 Burns 33 139-48
[12] Tjay T H 2007 Obat-Obat Penting Khasiat, Penggunaan dan Efek-Efek Sampingnya (Jakarta: PT Elex Media Komputindo).
[13] Ocyana D 2001 Pelatihan Asuhan Persalinan Normal Bersih dan Aman (Jakarta: Bakti Usaha).
[14] Leaper D 2012 International Consensus : Appropriate Use of Silver Dressing in Wounds (London: Wounds International Enterprise House).
[15] Hibono M M 2017 E-Jurnal Indonesia Journal of Anti Aging Medicine 1 25-31