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The efficacy of povidone-iodine and normal saline on excision wound in mice

Supparit Pakdeethai[1], Watchara Chongsa[2], Tadsanee Punjanon[3], Suwichak Chaisit[3], Cherdsak Boonyong[3], and Pataweekorn Ketkomol[3*]

[1]Pathobiology Unit, Faculty of Science, Rangsit University, Pathum Thani 12000, Thailand
[2]Physiology Unit, Faculty of Science, Rangsit University, Pathum Thani 12000, Thailand
[3]Pharmacology and Toxicology Unit, Faculty of Science, Rangsit University, Pathum Thani 12000, Thailand

*Corresponding author; Email: pataweekorn.k@rsu.ac.th

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Abstract

In cleaning wounds, little attention has been given to the solutions, waiting for the natural process without the user agent, but these problems can cause chronic injuries and inflammation. Povidone-iodine is a tropical antiseptic agent that popularly used to prevent infection and aid healing of wounds. However, the effects of povidone-iodine on the histologic change of excision wound healing have not been reported. Therefore, the objective was to assess the histological influences of 10% povidone-iodine ointment on healing process of experiment excision wounds in mice. Albino ICR mice were randomly divided into two groups with six mice each; a normal saline-treated group and a povidone-iodine-treated group. Wounds were created in all mice and treated with normal saline or povidone-iodine once daily for 14 days. The wound areas were measured on days 2, 5, 8, and 14. Then, a percentage of wound contraction was calculated. The histoarchitecture of the wound was observed on days 14 by staining with hematoxylin-eosin and Masson's trichrome. The results showed that the average percentage of wound contraction of the povidone-iodine-treated group was significantly higher than the normal saline-treated group on days 5, 8, and 14. The histoarchitecture studies of the povidone-iodine-treated group showed a thickening of the epidermis and dense deposition of collagen fiber when compared to the normal saline-treated group. These findings indicated that povidone-iodine treatment could accelerate the wound healing processes.

Keywords: *collagen production; excision wound; epidermis; histoarchitecture; mice; povidone-iodine; wound healing.*

1. Introduction

A wound is an injury or damage in the continuity of any body tissues. It can occur as a result of trauma, infection, or some pathological process, such as inflammation. Many types of wounds include cuts, scrapes, puncture, burn, pressure sores, and excision (Takeo, Lee, & Ito, 2015). Once a wound occurs, the body immediately begins to heal itself to re-establishing tissue integrity (Abd Jalil, Kasmuri, & Hadi, 2017). Wound healing is the physiological process that involves a complex interaction of physical, chemical, and cellular events to replace and restore damaged tissue. It can roughly be separated into four overlapping phases, including homeostasis, inflammation, proliferation, and remodeling (Guo,

& DiPietro, 2010). The homeostasis phase begins immediately after wounding, with vascular constriction, platelet aggregation, and fibrin clot formation to stop the bleeding. Once bleeding is controlled, the inflammatory phase begins with vascular dilation to allow inflammatory cells to migrate into the wound area to destroy bacteria and remove debris. In this phase, the wound becomes slightly swollen, red or pink, and tender (Lux, 2022). The proliferative stage is characterized by the proliferation of granulation tissue, producing angiogenesis, fibroblast, collagen deposition, and extracellular matrix formation. In a healthy wound, fibroblasts begin to appear 3 days after the initial injury. These fibroblasts initially secrete ground substance and, later, collagen (Kumar, Katoch, & Sharma, 2006). Remodeling is the final and most important phase of wound healing as it determines the strength of collagen and the manifestation of wound healing. Generally, remodeling starts after up to 21 days of injury occurrence and can last for years (Nayak, Sandiford, & Maxwell, 2009; Landa, van Dishoeck, Steyerberg, & Hovius, 2016). However, the wound healing process can be impaired or delayed by multiple factors such as infection, ischemia, and foreign body. Infection is a significant factor causing inflammation, tissue damage, and a delayed healing process. Therefore, the appropriate management of the wound can accelerate wound healing.

Povidone-iodine is antiseptic, nontoxic to host, and has a lower risk of antibiotic resistance. It has been used for over a century (Polo, Fabri, & Apolônio, 2020) in treating wounds, and it is also used as a topical agent for skin sterilization, surgical scrubs, and washing hands (White, Cutting, & Kingsley, 2006; Bigliardi et al., 2017). Povidone-iodine is available as a 2.5% dry powder spray, a 7.5% surgical scrub, and a 10% ointment (Juhász, 2002). Previous studies demonstrated that povidone-iodine promoted wound healing process in a various wound types via multiple mechanisms such as the prevention of microorganism growth (antimicrobial) and the acceleration of granulation tissue in the inflammatory phase (Brånemark, Albrektsson, Lindström, & Lundborg, 1966; Lineaweaver et al., 1985; Rodeheaver et al., 1982; Raziyeva et al., 2021). However, the effects of povidone-iodine on the histologic state of excision wound healing have not been reported. Therefore, this study aimed to assess the histological influences of 10% Povidone-iodine ointment on wound healing process of experimental excision wounds in mice.

2. Objectives

The objective was to assess the histological influences of 10% Povidone-iodine ointment on the wound healing process of experimental excision wounds in mice.

3. Materials and Methods

3.1 Drugs and Chemicals

The drugs and chemicals used in the study were indomethacin (Sigma Chemical Co., USA), isoflurane (Abbott, France), 0.9% sodium chloride (Milimate Compay Limited, Thailand), and 10% povidone-iodine ointment (Rugby, USA).

3.2 Animals and Grouping

Healthy female Albino ICR mice weighing between 25 to 30 g were used in this study. The animals were purchased from Nomura Siam International Company Limited and were acclimatized for a week at the Laboratory Animal Centre, Thammasat University. Placed in separate cages at a temperature of (22±1) °C and 12-hour light: 12-hour dark cycle, the animals were fed with standard laboratory food pellets and water *ad libitum*. Mice were randomly segregated into two groups with individually caged six mice each (Group I and II). 0.9% sodium chloride (normal saline) solution and 10% Povidone-iodine ointment, respectively, were applied to Group I and Group II.

3.3 Ethical issue

All the procedures were performed under ethical principles with the approved guidelines of the Institutional Animal Ethical Committee of Thammasat University [Ethics certificate code: 022/2561 on Feb 12, 2019].

3.4 Excision wound Creation

All mice received indomethacin (IND) dissolved in normal saline solution (NSS) 10 mg/kg, i.p., body weight for 30 min and were, later, anesthetized with 3-4% isoflurane inhalation. The dorsal regions were shaved using an electric shaver, 1 cm away from the vertebral column and 2 cm away from the ear, then disinfected with 70% ethanol. After that, the excision wound was created by cutting the total thickness of the skin area to 314 cm^2 and left open. Mice were left undressed to the open environment. The normal saline solution and 10% Povidone-iodine were applied once daily in the morning from the day of the operation until day 14 (Park et al., 2017).

3.5 Wound contraction measurement

The wound area was measured using millimeter-scale graph paper on days 2, 5, 8, and 14. The percentages of wound healing were measured using the initial area, and the areas were determined using the following formula (Park et al., 2017):

$$\text{Percentage of a wound contraction rate} = \frac{\text{wound area on day 0-wound area on day N}}{\text{wound area on day 0}} \times 100$$

Where N = number of days (2, 5, 8, 14)

3.6 Histoarchitecture study

On day 14, mice were euthanized with 100% carbon dioxide exposure in a chamber. Gas is introduced through the tube at the top. Tissues were removed and fixed on 10% buffered formalin for the histopathology study. Tissue was embedded in a paraffin block and cut into a thickness of 5 µm per section. These tissue sections were stained with Hematoxylin and Eosin (H&E) for general morphological observation and Masson's Trichrome staining to detect collagen fibers. Histological analysis of the tissue was carried out using Nikon microscope digital slight (DS-L3, Japan) (El-Ferjani et al., 2016).

3.7 Statistical analysis

Data were presented as mean ± standard deviation (mean ± SD). The statistical significance of the difference was assessed by *t*-test. Values of $p<0.05$ were considered significant.

4. Results

4.1 Wound contraction

The excision wound model assessed wound healing after applying a normal saline solution and 10% povidone-iodine ointment. Wound healing effects were determined by taking photographs and measuring the wound contraction areas. The wound contraction areas were observed from days 2, 5, 8, and 14 after inducing open wounds shown in Figure 1. Group I treated with normal saline solution had a pinkish color of granulation tissue until day 5 and the normal saline was then replaced with the scab on day 14. Group II was treated with 10% povidone-iodine ointment; it dried on day 5 and was then replaced with a scab until day 14 after the skin fully closed. The percentage of the wound contraction rates in group II increased significantly on days 5, 8, and 14, compared to Group I as shown in Figure 2.

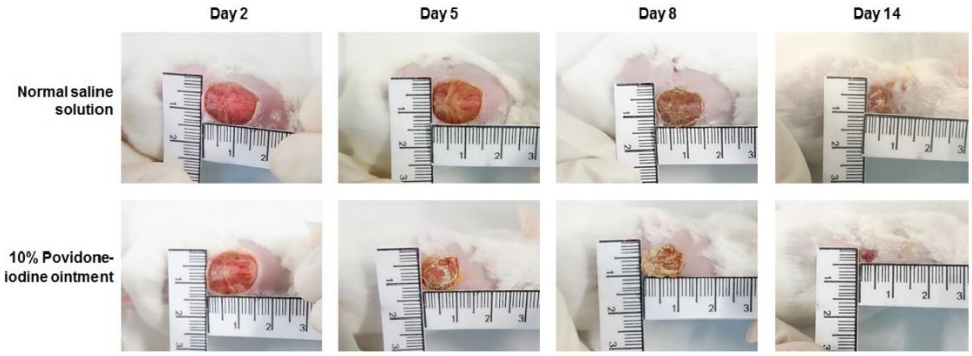

Figure 1 Photography of the wound contraction on days 2, 5, 8, and 14. Group I was treated with a normal saline solution, and group II was treated with 10% povidone-iodine ointment.

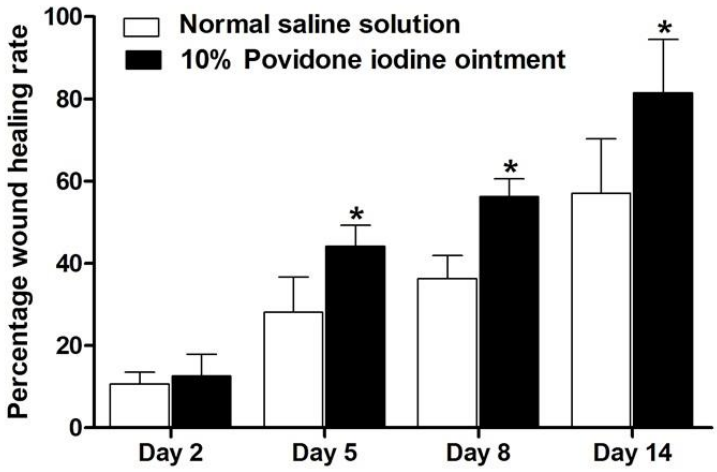

Figure 2 The percentages of the wound contraction rates on days 2, 5, 8, and 14, of which data was presented in the mean ± SD (N=6).

4.2 Histoarchitecture study

The histoarchitecture studies of the tissue sections were observed under microscopic images on day 14 by H&E stained and Masson's Trichrome as shown in Fig. 3 and 4. Histological section manifestation of the granulation tissue obtained from both groups showed a prominent epidermal and dermal layer. Group I treated with normal saline solution showed a thin layer of epidermal whereas the dermal layer showed many invasive inflammatory cells (e.g., mast cells), more minuscule fibroblast, and blood vessels and few signs of collagen. There was also a presence of a greater scar width as shown in Figures 3A and 4A. The histological section of Group II treated 10% povidone-iodine ointment showed that the epidermal layer is thick with more keratinocytes in the stratum spinosum. The keratinocyte at the stratum granulosum showed more accumulated keratohyalin granules. In contrast, the dermal layer showed a reduction in inflammatory cells, an increase in blood vessels and fibroblast cells, and a dense deposition of collagen fibers as shown in Figures 3B and 4B.

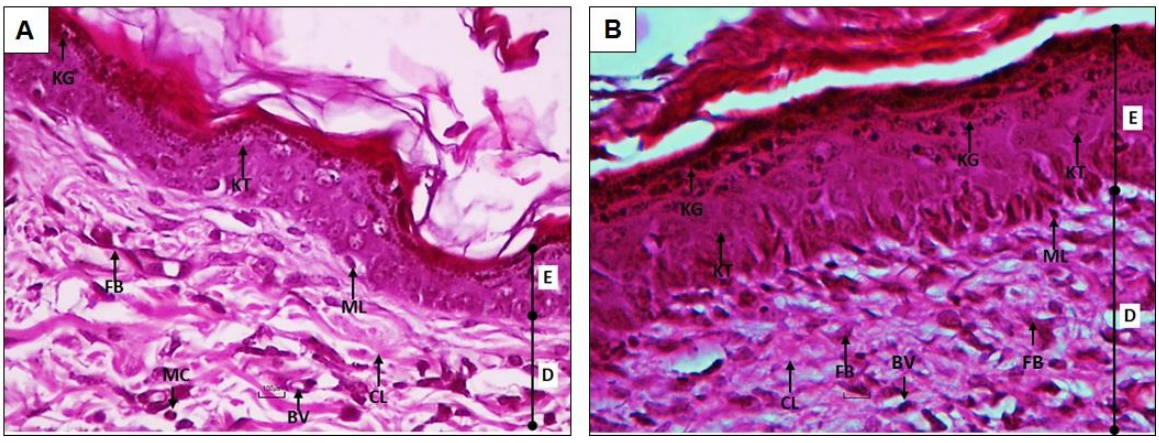

Figure 3 Light microscopic images (40x) of granulation tissue of Group I treated with normal saline solution, and Group II treated with 10% povidone-iodine ointment on day 14. (H&E stain, Bar = 100μm). E: Epidermis, D: Dermis, ML: Melanocyte, KT: Keratinocyte, FB: Fibroblast, CL: Collagen fiber, MC: Mast cell, BV: Blood vessel, KG: Keratohyalin granules in stratum granulosum.

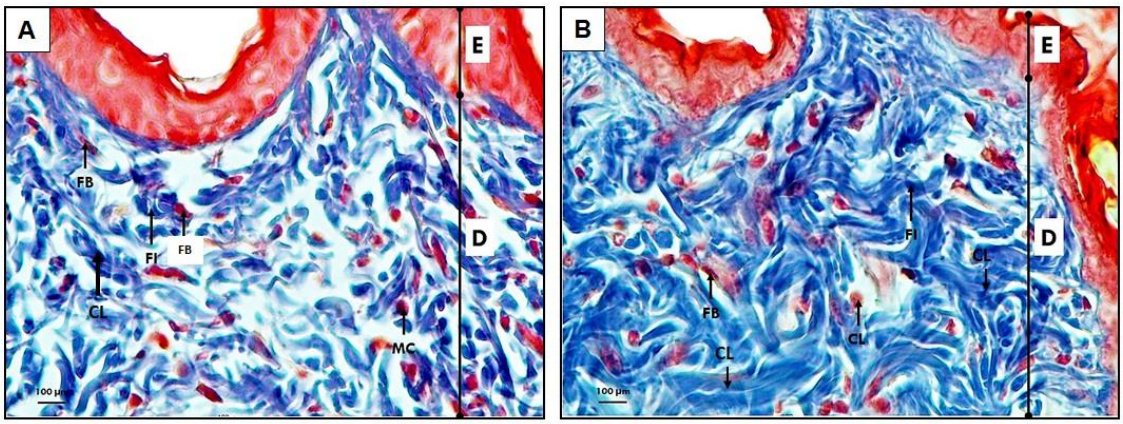

Figure 4 Light microscopic images (40x) of granulation tissue of Group I treated with normal saline solution, and Group II treated with 10% povidone-iodine ointment on day 14. (Masson's Trichrome stain, Bar = 100µm). E: Epidermis, D: Dermis, FB: Fibroblast, CL: Collagen fiber, MC: Mast cell, BV: Blood vessel

5. Discussion

A wound is a damage to the skin and soft tissue. Its healing process involves four phases; the homeostasis, inflammation, proliferation, and remodeling. General wound healing is completed within 4 to 6 weeks whereas chronic wounds fail to heal during that time. Many factors can lead to impaired wounds, such as infections, ischemia, and decreased collagen synthesis (Kuo et al., 2022; Khan, & Naqvi, 2006). Cleansing is a vital component of wound management; however, little attention has been given to the solutions used for cleansing purposes (Yalcınkaya et al., 2022). Also, sometimes, waiting for the wound to heal follows a natural process without the user agent or cleaning, but these problems can cause chronic injuries and inflammation. Therefore, applying topical agents that have a potent anti-infection and anti-inflammation could facilitate wound healing processes (Somboonwong, Kankaisre, Tantisira, & Tantisira, 2012). Povidone-iodine and normal saline have been used to manage wounds for a long time, but the effects of povidone-iodine on the histologic state of the excision wound healing have not been reported. The objective was to assess the histological influences of 10% Povidone-iodine ointment on the wound healing process of experimental excision wounds in mice. In this study, the normal saline-treated group was assigned as a control group to mimic the general wound management. The results revealed that an application of the 10% Povidone-iodine ointment increased the percentage of wound contraction rate indicating that 10% Povidone-iodine ointment could accelerate the wound contraction. Wound contraction generally occurs when the wound edges move towards each other in a centripetal fashion thus reducing the wound's dimension. Although the mechanism for wound contraction has been debated, at least it is mediated with the collagen deposition followed by packing closer together for generating force in wound contraction (Ehrlich, & Hunt, 2011). Our histoarchitecture studied on granulation tissue revealed that the dermal layer of the 10% Povidone-iodine ointment group showed more fibroblast cells together with dense and well-organized arrangement collagen fibers, more blood vessels, and a few inflammatory cells. This finding suggesting that 10% Povidone-iodine ointment could promote the collagen production and deposition, which in turn, facilitating wound contraction, reducing inflammation, and promoting angiogenesis. Furthermore, the granulation tissue of 10% Povidone-iodine ointment group also showed the thickening of an epidermal layer with more keratinocytes and more keratohyalin granules that are almost similar to normal skin. It indicated that 10% Povidone-iodine ointment could accelerate re-epithelialization process. Previous studies on wound healing effects of topical drugs, including

silver sulfadiazine, povidone-iodine, and 0.9% sodium chloride-induced by burn injuries in rats showed that the group treated with silver sulfadiazine and povidone-iodine had a better effect on wound healing than those treated with 0.9% sodium chloride (Yüksel, Yıldırım, Bal, & Kuloglu, 2014). Another comparative study between topical povidone-iodine and normal saline solutions for wound laceration in adult patients showed that adult patients treated with povidone-iodine revealed enhanced wound contraction (Gravett, Sterner, Clinton, & Ruiz, 1987). Moreover, data from a study showed that povidone-iodine enhanced wound contraction through the increased expression of transforming growth factor beta (TGF-β), that plays an important role in wound healing, angiogenesis, immunoregulation, and cancer (Wang et al., 2017). Moreover, the previous studies showed that antiseptics with iodine compounds, including cadexomer iodine and povidone-iodine have a better effect on chronic wound healing than the saline group. (Kashyap, Beezhold, Wiseman, & Beck, 1995; Holloway Jr, Johansen, Barnes, & Pierce, 1898). Our study, hence, was supported by all previously mentioned studies that showed 10% Povidone-iodine ointment was more effective than the normal saline solution on excision wounds.

6. Conclusion

Our results found 10% povidone-iodine ointment was more effective than the normal saline solution by increasing the re-epithelialization and collagen accumulation in a short period. Also, Povidone-Iodine was a widely used disinfectant and could effectively kill bacteria. Therefore, to prevent infection and promote wound healing should suitably manage injuries.

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