Original Article

Enhanced wound healing by topical application of ointment containing low concentration of povidone-iodine

Osamu Mitani[1, 3], Atsushi Nishikawa[1], Ichiro Kurokawa[2], Esteban C Gabazza[4], Masahiro Ikeda[1], Hitoshi Mizutani[3]

1: Central Research Laboratory, Maruishi Pharmaceutical Co., Ltd., 2-2-18 Imazu-naka, Tsurumi-ku, Osaka, 538-0042, Japan.

2: Department of Dermatology, Meiwa Hospital, Nishinomiya, 663-8186, Japan

3: Department of Dermatology, Mie University Graduate School of Medicine, 2-174, Edobashi, Tsu, 514-8507, Japan.

4: Department of Immunology, Mie University Graduate School of Medicine, 2-174, Edobashi, Tsu, 514-8507, Japan.

Correspondence to: Osamu Mitani

Central Research Laboratory, Maruishi Pharmaceutical Co., Ltd., 2-2-18 Imazu-naka, Tsurumi-ku, Osaka, 538-0042, Japan

Phone: +81-6-6962-1783

FAX: +81-6-6962-2948

E-mail: osamu_mitani@maruishi-pharm.co.jp

Abstract

Objective

To investigate the effect of a novel topical wound-healing agent, low-concentration povidone-iodine ointment (LPIO) with a hydrophobic white petrolatum-rich base, on skin-wound models in rats and rabbits.

Methods

We investigated the therapeutic efficacy of topically applied LPIO compared to that of standard-concentration povidone-iodine ointment (SPIO) and non-treatment control, using a full-thickness skin-wound model in hairless rats and a full-thickness skin-defect model in rabbit earlobes. Therapeutic efficacy was evaluated based on macroscopic wound-size reduction, as well as histopathological and immuno-histochemical examinations.

Results

LPIO enhanced wound healing in rat full-thickness skin ulcers, reducing wound size and inflammation, when compared with that in SPIO and non-treatment control. LPIO also markedly improved wound healing in rabbit earlobe ulcers by significantly improving re-epithelialisation, compared with that in SPIO.

Conclusions

The results of this study suggest that LPIO is a useful topical therapy for ulcerative lesions.

Key words

Wound healing, PVP-I, Wound model, Ointment, White petrolatum

Introduction

Several factors, including reduced blood flow, infection, moisture, temperature, metabolic conditions, and other exogenous stimuli, can affect the re-epithelialisation and wound contraction that ultimately can retard lesion recovery and prolong the time of therapy. . Following an open wound a hard crust is formed by a fibrin-platelet coagula, which protects the injured area from exogenous stimuli and infection. However, this initial process can also inhibit the growth of epidermal keratinocytes from dermal appendages and surrounding skin areas.[1-5] Topical skin therapy enhances wound healing by preventing infection, irritation, further traumatic injury and tissue ischemia. Wet dressing was recently recommended for the therapy of clean and fresh wounds.[1, 5] However, the exact mechanism by which skin wound healing is enhanced by moist conditions is not fully understood. Various cytokines, chemokines, and growth factors in the wound exudate can regulate and enhance intra-lesional cellular proliferation and contribute to rapid wound healing.[2, 3] These factors are released from activated platelets, proliferating keratinocytes, fibroblasts, and vascular endothelial cells.[4, 6] Wet dressings are believed to retain these locally released growth factors in the lesions,[1, 5] thus preventing wound drying.

Infection is a risk factor for retardation of wound healing, particularly for wet and closed wounds.[7] Therefore, topical therapies for skin wounds should also contain anti-septic reagents. However, topically applied antiseptic drugs may be toxic for skin cells including keratinocytes, fibroblasts, and vascular cells.[8] To resolve this problem, sterile, less irritant, non-adhesive, and moisture-controllable dressing materials are needed.

Iodine has been used for topical wound therapy especially for wounds with a high risk of infection. Free iodine is toxic at high concentrations, but it exerts cell proliferative effects[9] and has been used in various formulations including polyvinylpyrrolidone and dextrin polymer mixtures. These formulations have primarily antiseptic effects due to their iodine content and

high capacity for absorbing wound effusion and pus.[10-14] Therefore, development of topical wound-healing materials, containing iodine at non-toxic concentrations and in a less irritant form by using a more hydrophobic base is required.

In the present study, we developed a novel topical therapeutic material containing low-concentration povidone-iodine ointment (LPIO) in a hydrophobic base, investigated its therapeutic efficacy using a full-thickness skin-wound model in hairless rats and a full-thickness skin-defect model in rabbit earlobes, and compared its efficacy with that of a topical standard-concentration povidone-iodine ointment (SPIO).

Material and Methods

Animals

Twenty-four male hairless Wistar Yagi rats weighing 199.5–232.8 g were purchased from Japan SLC, Inc. (Hamamatsu, Japan), and six Japanese white male rabbits weighing about 3.5 kg were purchased from Kitayama Labes Co. Ltd. (Nagano, Japan). The animals were kept under standardised conditions (12-h light/dark cycle, 40–70% relative humidity, 20–24°C room temperature range) at the Central Research Laboratory of Maruishi Pharmaceutical Co. Ltd. (Osaka Japan), with access to water and standardised food. This study was reviewed and approved by the Animal Ethics Committee of Maruishi Pharmaceutical Co., Ltd (Approval Number: rat, 6014; rabbit, 08007).

Test materials

The LPIO contains 1% (w/w) povidone-iodine (PVP-I; BASF Japan Ltd., Tokyo, Japan), and the base component consists of white petrolatum, macrogol 400 (NOF Corporation., Ltd. Tokyo, Japan), macrogol 4000 (Maruishi Pharmaceutical Co., Ltd. Osaka, Japan), macrogol 6000 (Sanyo Chemical Industries Co., Ltd. Kyoto, Japan), sodium citrate and water. The SPIO contains 3% PVP-I (U-PASTA KOWA Ointment; KOWA Pharmaceutical Co., Ltd. Tokyo, Japan).

Experimental Design

Full-thickness skin-wound model in hairless rats

One week before topical therapy, skin flaps were prepared in 24 rats as preparative surgery. After shaving the hair with an electric clipper, a full-thickness skin flap (35 mm × 27 mm) including the panniculus carnosus was made in the back of the rats, using surgical scissors under sodium pentobarbital anaesthesia (40 mg/kg, i.p.). The flap was then returned to the raw

surface, and nine portions of the flap were stitched to create a three-sided flap.[15] One week after the preparative surgery, a full-thickness skin wound (10 mm × 10 mm) was made in the centre of the flap (Figure 1), under sodium pentobarbital anaesthesia. The wound extended through the panniculus carnosus to the muscular fascia. The 24 rats were randomly divided into three groups: control (control, n = 8), LPIO-treated (LPIO, n = 8) and SPIO-treated (SPIO, n = 8) groups. The wounds were treated with either 1 g of LPIO or SPIO spread on a sterile gauze dressing, or gauze dressing alone as control. The dressings were fixed with elastic bandages. The dressing was changed every day until day 14. The wounds were measured and recorded thrice a week, using a fixed camera with a scale. The sizes of the wounds were calculated using image analysis software (Lumina Vision®: Mitani Co., Tokyo, Japan). Changes in wound size were corrected by the wound size on day 0.

Skin-defect model in rabbit earlobes

Three rabbits were anaesthetised with intravenous sodium pentobarbital (40 mg/kg, i.p.). The hairs of the ventral side of the earlobes were removed by clipping and using topical depilatory cream (Veet: Reckitt Benckiser Japan Ltd. Tokyo, Japan). After cleaning the earlobes, 10 mm × 10 mm of full-thickness skin was surgically removed from each ear, without injuring the auricular perichondrium (Figure 2A, 2B).[16, 17] After surgery on day 0, the right and left ears were randomly divided into two groups: LPIO-treated (LPIO, n = 3 × 2 parts) and SPIO-treated (SPIO, n = 3 × 2 parts). Each rabbit was treated with 0.5 g of either LPIO or SPIO once a day. The wounds were wrapped with gauze and fixed with elastic bandages. Topical therapy with LPIO or SPIO was daily performed for 11 days. The wounds were recorded with a camera thrice a week as described above. The sizes of the wounds were calculated using image analysis software (Lumina Vision®: Mitani Co., Tokyo, Japan).

Histological examination

The day after the final treatment, skin samples were obtained under sodium pentobarbital anaesthesia. Samples were fixed with 10% neutral-buffered formalin and processed for preparations of paraffin-embedded sections. The histopathology sections were prepared along the long axis of the wounds. Sections of 3-μm thickness were stained with haematoxylin and eosin (H&E).

Immuno-histochemical examination

Paraffin-embedded sections were immuno-histochemically processed by the labelled streptavidin biotin (LSAB) technique. Sections on glass slides were subjected to heating at 90°C while immersed in citric acid buffer (pH 6.0) for 30 min for antigen retrieval. After incubating for 20 min at room temperature, the sections were treated with 3% H_2O_2 in a solution of methanol for 30 min at room temperature, in order to inactivate endogenous peroxidase. The sections were subsequently blocked with serum protein (Dako Japan Co., Ltd., Tokyo, Japan) for 10 min at room temperature, and then the samples were incubated overnight with primary antibodies at 4°C. Primary antibodies included mouse anti-proliferating cell nuclear antigen (anti-PCNA, PC10, Dako Japan Co. Ltd., Tokyo, Japan), mouse anti-p63 (4A4, Nichirei Biosciences Co. Ltd., Tokyo, Japan), mouse anti-keratin 14 (K 14: LL002, Thermo Fisher Scientific, CA, USA), and mouse anti-keratin 16 (K 16: LL025, Thermo Fisher Scientific, CA, USA). The sections were rinsed with PBS and subsequently reacted with biotinylated anti-mouse immunoglobulins (Dako Japan Co., Ltd., Tokyo, Japan) as secondary antibodies, for 30 min at room temperature. Subsequently, the samples were treated with horseradish peroxidase (HRP)-conjugated streptavidin (Dako Japan Co. Ltd. Tokyo, Japan) for 30 min at room temperature. The immune complexes were visualised using 3, 3′-diaminobenzidine tetrahydrochloride (Liquid DAB substrate kit, Dako Japan Co. Ltd., Kyoto, Japan).

Evaluation of wound healing histopathology

For the histopathological evaluation of wound healing in the H&E-stained sections, the following six findings were considered: cellular infiltration, oedema, haemorrhage, horny layer formation of the epithelial edge (HLF), fibrin deposition, and vascular proliferation. Cellular infiltration, oedema, and haemorrhage were graded as follows: grade 3 (present throughout the wound area and spreading into the surrounding normal skin), grade 2 (present throughout the wound area), grade 1 (partially present in the wound area), or grade 0 (scarce presence). Fibrin deposition and vascular proliferation were graded as follows: grade 2 (present throughout the wound area), grade 1 (partially present in the wound area), or grade 0 (scarce presence). HLF was graded as follows: grade 2 (HLF growth in the vertical direction), grade 1 (HLF growth in the horizontal direction), or grade 0 (no HLF).

The H&E-stained sections of the rat and rabbit wound models were photographed using a digital camera connected to a microscope (DP-70: Olympus Co., Tokyo, Japan). The re-epithelialisation rate was semi-quantitatively evaluated from the images, using image analysis software (Lumina Vision®: Mitani Co. Tokyo, Japan) and the following formula:

Re-epithelialisation rate (%) = 100 × (1 - length of non-epithelialisation/length of primal wound)

Cells stained immuno-histologically with either anti-PCNA or anti-p63 antibodies in the epidermis (1/2 specimen) of the rats were also evaluated.

Statistical analysis

The data were expressed as the mean ± the standard deviation (S.D.). The relative wound area was calculated from the following formula:

relative wound area (%) of day X = absolute wound area (day X)/absolute wound area (day 0) × 100.

Significance was analysed using SAS System Release 8.2 (SAS Preclinical Package, Version 5.0, SAS Institute Japan Ltd., Tokyo, Japan). *P* values lower than 0.05 were considered statistically significant. For evaluation of the parametric data (wound size and re-epithelialisation ratio) in rats, Bartlett's analysis for homogeneity of variance was performed. When the variance was homogeneous, the Tukey's test for multiple group comparisons was performed to compare the mean value in each group (control vs LPIO, control vs SPIO, and LPIO vs SPIO). For evaluation of the parametric data in rabbits, F-test for homogeneity of variance was performed. When the variance was homogeneous, the Student's *t*-test for two-group comparison was performed (LPIO vs SPIO). For non-parametric data (histopathological grades) in rats, Kruskal‐Wallis test for analysis of variance was performed. Tukey's test for multiple-group comparison was performed when significant results were obtained (control vs LPIO, control vs SPIO, and LPIO vs SPIO).

Results

Macroscopic findings

In LPIO-treated rats, the wounds were maintained under wet conditions throughout the treatment period. In contrast to the control group, no obvious crust formation was observed in the LPIO-treated wounds (Figure 3A). The wounds of the SPIO group were covered with a hard crust from day 1. Depression of the wound at the edge of the ulcer, observed as a groove with poor proliferation of granulation tissue, and over-proliferation of granulation tissue at the centre of the ulcer was observed in some rats after day 7. On day 7, wound size in the LPIO group (44.5 ± 11.5%) became significantly smaller than that in the control group (65.0 ± 16.1%, $p < 0.05$). On day 14, wound size markedly decreased to 9.1 ± 3.0% in the LPIO-treated group, while it was 18.3 ± 8.3% in the control group. Wound size did not significantly differ between the SPIO and control groups on day 14. Wound size in the LPIO group was significantly smaller than that in the SPIO group on day 14 (19.0 ± 6.7%, $p < 0.05$) (Figure 3B). No obvious sign of infection was identified in any of the rats throughout the experiment.

In rabbits, LPIO treatment successfully maintained the wounds under wet conditions, without hard crust formation until day 11. Re-epithelialisation of the wounds improved significantly in the LPIO group than in the SPIO group (Figure 4A). Notably, there were some animals that completed re-epithelialisation before the end of the study (day 11). In contrast, the wound surface in the SPIO-treated group was covered with a crust from day 1, and the wound caved in at the junction of the surrounding normal skin. Although wound healing from the edges was observed from day 9 in the SPIO-treated group, none of the animals showed complete wound healing throughout the treatment period. On day 7, the relative wound area in the LPIO group (50.6 ± 15.0%) was significantly smaller than that in the SPIO group (82.1 ± 9.7 %, $p < 0.01$). On day 11, the relative wound area in the LPIO group (5.3 ± 5.1%) was significantly smaller than that in the SPIO group (56.5 ± 8.6%, $p < 0.001$) (Figure 4B). No obvious sign of wound

infection was identified in any of the rabbits throughout the experiment.

Histopathological evaluation

In the rat study, complete histopathological re-epithelialisation of the wound was not observed in any of the three groups until day 14 (Figure 5A, 5B). Cellular infiltration, oedema, and fibrin deposition, but not haemorrhage and vascular proliferation, were significantly decreased in the LPIO-treated wounds than in the wounds of control rats. Cellular infiltration, oedema, fibrosis, crust formation, haemorrhage, and vascular proliferation, but not fibrosis, were significantly increased in the SPIO group than in the LPIO-treated and control rats. Infiltration of mononuclear cells and neutrophils, and hard crust formation significantly increased in all SPIO-treated samples. In addition, histopathological wound healing in the SPIO group was the poorest among the three groups (Figure 6). No obvious presence of bacterial colonies was identified in any of the rats throughout the experiment.

In rabbits, the LPIO-treated wounds showed rapid re-epithelialisation with granulation tissue formation, without obvious inflammatory changes. Surprisingly, complete re-epithelialisation before the end of the experiment was observed in 50% of the samples. In the SPIO-treated group, there were samples with only partial granulation tissue formation, and regeneration of epidermis was limited to the edge of the wounds (Figure 7). No obvious presence of bacterial colonies was identified in any wound of the rabbits throughout the experiment.

The re-epithelialisation rate was semi-quantitatively calculated in the rat and rabbit tissue specimens. In the rat model, the re-epithelialisation rate was significantly lower in the SPIO group (66.0 ± 7.7%) than in both the control (75.1 ± 7.2%) and LPIO-treated (82.1 ± 5.4%, $p < 0.05$) groups. In the rabbit model, the re-epithelialisation rate was significantly lower in the SPIO-treated wounds (24.3 ± 6.9%) than in the LPIO-treated wounds (92.8 ± 8.2%, $p < 0.001$) (Figure 8).

Immuno-histochemical evaluation

PCNA-positive cells were identified in basal keratinocytes and in cells of the underlying connective tissue. These cells were distributed mainly in the regenerated epithelium and the surrounding epidermis of the wounds in both rats and rabbits. The PCNA-positive cell number in the epidermis of the LPIO group (485.0 ± 99.0 cells, $p < 0.05$) was significantly higher than that in the SPIO (322.7 ± 48.7 cells) and control (315.7 ± 109.9 cells) groups in the rat model. In the rabbit model, the number of PCNA-positive cells in LPIO-treated wounds tended to be higher than that in the SPIO-treated wounds. Immuno-reactive p63- and PCNA-positive cells were detected in the basal keratinocytes of rat specimens. However, no significant difference in the number of p63-positive cells in the epidermis was detected between groups. In rabbit specimens, p63 was detected in basal keratinocytes in the regenerated epidermis and, to a lesser extent, in the surrounding epidermis of the wounds. K14 expression in specimens from rabbits treated with LPIO was clearly detected in the regenerated and surrounding epidermis. However, K14 expression in SPIO-treated lesions was limited to the subcorneal areas of the surrounding skin. In rat specimens, K14 staining was weak, and was mostly observed in the surrounding skin and regenerated epidermis of the LPIO-treated lesions. Immuno-reactive K16 was in general weakly detected in rat specimens. However, K16 stained clearly through the spinous layer in LPIO-treated wounds, and was limited to the subcorneal epidermis in SPIO-treated wounds. In general, the immuno-histological findings in the rabbit wound model were similar to those observed in the rat models. However, it is difficult to evaluate differences in re-epithelialisation between both groups by immuno-histological methods because wound healing in the SPIO-treated group was significantly delayed compared to that in the LPIO-treated group, and also because the area of regenerated epithelium was too small for evaluation (Figure 9A, 9B, 10).

Discussion

The ideal wound care requires keeping the wounds under wet and sterile conditions, and avoiding irritation by and adhesion of topical therapeutic materials.[18] However, these are difficult conditions to achieve. To address these issues, we investigated the effect on wound healing of a topical preparation containing a white petrolatum-based LPIO, and compared with a commonly available topical therapeutic material containing higher concentration of povidone-iodine and control in two different experimental models of full-thickness skin-wounds.

LPIO treatment accelerated wound healing in rats and rabbit and it was associated with rapid formation of dermal granulation tissue and enhanced re-epithelialisation without crust formation. The lack of crust formation was a characteristic finding in LPIO-treated wounds. The scab or crust on the wounds prevents the loss of fluids and infection, but keratinocytes must migrate under the tightly adhered crust.[6] Crust on wounds may prevent rapid re-epithelialisation. Impairment of crust formation is therefore an important advantage of LPIO.

The acute cutaneous wound healing process has three phases: (1) haemostasis and inflammation, (2) proliferation with skin resurfacing and dermal restoration, and (3) remodelling.[18] These three phases develop sequentially and switch appropriately during the wound healing process. The coagulation phase ends within a couple of days. In well-conditioned wounds, the inflammatory phase rapidly advances to the proliferative phase. Histopathological examination of LPIO-treated wounds showed less cellular infiltration, oedema, haemorrhage, and vascular proliferation, all of these findings reflecting improved wound healing compared with SPIO-treated and control wounds. Excess inflammatory infiltrates in chemokine IP-10 transgenic mice impaired neovascularization and appropriated granulation tissue formation.[19] Conversely, there are reports of improved healing in the absence of inflammatory infiltrates.[20] In well-controlled wounds, the proliferative phase leads to the remodeling phase with epidermis and dermal collagen maturation.[18] In-

terestingly, the collagen tissue under the newly recovered epithelium matured without excess cellular infiltration or vasculature in LPIO-treated wounds, which strongly suggests rapid tissue repair during maturation. The present study used acute models of cutaneous wound to study the therapeutic effect of LPIO; the effect of LPIO on chronic wound models such as diabetic ulcer and infected wound model was not assessed. Human wound repair is a complex biologic process that is impaired in numerous disease states. The process of chronic wound healing in humans such as the mechanism of wound re-epithelialisation is not clear.[21-23] We will focus on the potential beneficial effect of LPIO on chronic wound healing in future studies.

Reduction of the cytotoxic effects of ointment components is also important. Previous reports demonstrated that p63and K14 is located in the basal keratinocytes of slow-turning normal epidermis, and that K16 is present in the epidermal tongue during the rapid proliferation phase of wound healing. The expression of these proteins is enhanced during wound healing, and subsequently, is expanded to the suprabasal and spinous layers.[24-28] In the present study, simultaneous enhanced expression of these proteins in the wound healing areas was observed in the rat wound model, suggesting enhanced epidermal proliferation and increased epidermal layer. Both epithelial regeneration and dermal granulation tissue proliferation were poor, and expression of p63 and K14 was limited to the edge of the surrounding epithelium in the rabbi-ear wounds treated with non-wet dressings. No expression of these proteins was found in the ulcerative areas. Consistent with previous reports, [29, 30] high expression of p63 and K14 was detected in the rapidly regenerated epidermis from LPIO-treated wounds, indicating the beneficial effects of LPIO. No re-epithelialisation and poor dermal granulation tissue regeneration with a groove-like depression were observed in wound samples from SPIO-treated rats. Rapid and effective recovery of dermal granulation tissue is important to support epidermis regeneration. In the rabbit model, LPIO treatment rapidly improved granulation tissue regeneration, followed by epithelial regeneration. In the rat model, staining of keratinocytes in each sample was as stable as staining in the control.

However, the number of PCNA-positive cells in dermal granulation tissue of wounds treated with SPIO was decreased compared to LPIO-treated and control, indicating impaired granulation tissue in SPIO-treated wounds. These results indicate that dermal tissue regeneration is critical for wound healing, and the beneficial effect of LPIO therapy on dermal tissue regeneration. The precise mechanism of the rapid re-epithelialisation in LPIO-treated wet wounds is not clear, but it appears that LPIO treatment enhances re-epithelialisation by maintaining wet conditions in the wounds. Further investigation is required to clarify the precise biological mechanism of LPIO treatment advantages. Although this study demonstrated advantages of LPIO treatment on wound healing produced in experimental animal models, its effect on human wound healing is unknown. However, based on the significant beneficial effects of LPIO in experimental models, similar clinical efficacy can be expected in clinical settings.

In conclusion, LPIO treatment enhanced wound healing in rat-skin ulcers by reducing inflammation, irritation, bleeding, and crust formation, compared with SPIO and control. In rabbit-earlobe ulcers, LPIO markedly improved wound healing with significant grade of re-epithelialisation, compared with SPIO. LPIO consists mainly of povidone-iodine and hydrophobic white petrolatum, which are well-recognized safe materials. The present iodine-based topical material may be a potent therapeutic tool for accelerating the wound healing process.

Conflict of interest

Osamu Mitani, Atsushi Nishikawa and Masahiro Ikeda are employees of Maruishi Pharmaceutical Co., Ltd. This study was funded by Maruishi Pharmaceutical Co., Ltd., and was performed under a collaborative agreement between Maruishi Pharmaceutical Co., Ltd. and Mie University. Maruishi Pharm Co. Ltd. and Mie University hold the basic patent of the reported topical therapeutic material.

Acknowledgements

We are grateful to acknowledge Ms. K Hyakutake for preparing and providing the LPIO. We also thank Mr. M Kumagai, Mr. S Baba, Mr. Y Miyamoto, Mr. Y Watanabe, Dr. Y Yamamura, Dr. K Taya and Mr. K Masunomi for their helpful advice and cooperation.

References

1. Winter GD. A note on wound healing under dressings with special reference to perforated-film dressings. J. Invest. Dermatol. 1965; 45: 299-302.
2. Christopher P, Kiritsy AB and Samuel EL. Role of growth in cutaneous wound healing: A review. Critical Reviews in Oral Biology and medicine. 1993; 4: 729-760.
3. Matsuoka J and Gary RG. Two peptides related-derived growth factor are present in human fluid. Proc. Natl. Acad.Sci. U.S.A.1989; 86: 4416-4420.
4. Singer A and Clark RA. Cutaneous wound healing. N Eng J Med. 1999; 341: 738-746.
5. Shigeyama M, Ogaya T, Yoneyama T, et al. Preparation of a gel-forming ointment base applicable to the recovery stage of bedsore and clinical evaluation of a treatment method with different ointment bases suitable to each stage of bedsore. Yakugaku zasshi. 2001; 121: 441-450 (In Japanese).
6. Gurtner GC, Werner S, Barrandon Y, et al. Wound repair and regeneration. Nature 2008; 453: 314-321.
7. Kitayama Y, Nakatani T, Sanada H, et al. The effects on wound healing by cleaning the skin surrounding wounds on mice. Jpn J PU. 2005; 7: 818-826.
8. Tvedten HW and Till GO. Effect of povidone, povidone-iodine, and iodide on locomotion (*in vitro*) of neutrophils from people, rats, dogs, and rabbits. Am J Vet Res. 1985; 46: 1797-1800.
9. Hirsch T, Koerber A, Jacobsen F, et al. Evaluation of toxic side effects of clinically used skin antiseptics *in vitro*. J Sur Res. 2010; 164: 344-350.
10. Nakao H, Yamazaki M, Tsuboi R, et al. Mixture of sugar and povidone-iodine stimulates wound healing by activating keratinocytes and fibroblast functions. Arch Dermatol Res. 2006;298:175-82.

11. Gilmore OJA, Reid C and Strokon A. A study of effect of povidone-iodine on wound healing. Postgraduate Medical Journal. 1977; 53: 122-125.

12. Lamme EN, Gustafsson TO and Middelkoop E. Cadexomer-iodine ointment shows stimulation of epidermal regeneration in experimental full-thickness wounds. Arch Dermatol Res. 1998; 290: 18-24.

13. Yamazaki N, Iwama A, Igarashi M, et al. Acceleration of wound healing by cadex ointment 0.9%. Jpn Pharmacol Ther. 2001; 29: 827-837 (In Japanese).

14. Kanebako M and Inagi T. Absorption of water by U-pasta kowa. Jpn J Med Pharm Sci. 1994; 31: 1159-1162 (In Japanese).

15. Wong VW, Sorkin M, Glotzbach JP, et al. Surgical approaches to create murine models of human wound healing. J Biomed Biotechnol. 2011; 2011: 1-8.

16. Satsuki R. Treatment of intractable ulcers using artificial dermis and basic fibroblast growth factor- scanning electron microscopic observation of molded blood vessels. J. Kanazawa Med. Univ. 2004; 29: 163-172 (In Japanese).

17. Mustoe TA, Pierce GF, Morishima C, et al. Growth factor-induced acceleration of tissue repair through direct and inductive activities in a rabbit dermal ulcer model. J. Clin. Invest. 1991; 87: 694-703.

18. Korting HC, Schollman C and White RJ. Management of minor acute cutaneous wounds: importance of wound healing in a moist environment. J Eur Acad Dermatol Venereol. 2011; 25: 130-137.

19. Luster AD, Cardiff RD, MacLean JA, et al. Delayed wound healing and disorganized neovascularization in transgenic mice expressing the IP-10 chemokine. Proc Assoc Am Physicians. 1998; 110: 183-196.

20. Heber-Katz E and Gourevitch. The relationship between inflammation and regeneration

in the MRL mouse. Potential relevance for putative human regenerative (scarless wound healing) capacities? Ann N.Y. Acad Sci. 2009; 1172: 110-114.

21. Martin P. Wound healing- aiming for perfect skin regeneration. Science. 1997; 276: 75-81.

22. Henry G, Li W, Garner W, et al. Migration of human keratinocytes in plasma and serum and wound re-epithelialisation. Lancet. 2003; 361: 574-576.

23. Patel GK, Wilson CH, Harding KG, et al. Numerous keratinocyte subtypes involved in wound re-epithelialization. J Invest Dermatol. 2006; 126: 497-502.

24. Watanabe S, Osumi M, Ohnishi T, et al. Changes in cytokeratin expression in epidermal keratinocytes during wound healing. Histochem Cell Biol. 1995; 103: 425-433.

25. Parsa R, Yang A, McKeon F, at al. Association of p63 with proliferative potential in normal and neoplastic human keratinocytes. J Invest Dermatol. 1999; 113:1099-1105.

26. Kurokawa I, Mizutani H, Kusumoto K, et al. Cytokeratin, filaggrin, and p63 expression in reepithelialization during human cutaneous wound healing. Wound Rep Reg. 2006; 14: 38-45.

27. Moll R, Franke WW, Schiller DL, et al. The catalog of human cyokeratins: patterns of expression in normal epithelia, tumors and cultured cells. Cell. 1982: 31: 11-24.

28. Paladini RD, Takahashi K, Bravo NS, et al. Onset of re-epithelialization after skin injury correlates with a reorganization of keratin filaments in wound edge keratinocytes: defining a potential role for keratin 16. J. Cell Biol. 1996; 132: 381-397.

29. Hosaya A, Lee JM, Cho SW, et al. Morphological evidence of basal keratinocyte migration during the re-epithelialization process. Histochem Cell Biol. 2008; 130: 1165-1175.

30. Senoo M, Pinto F, Crum CP, et al. p63 is essential for the proliferative potential of stem cells in stratified epithelia. Cell. 2007; 129: 523-536.

Figure legends

Figure 1

The full-thickness skin wound model in hairless rats. 35mm x 27mm of full-thickness skin flaps (3-sided flap) were made in the back of rats, and the flaps were sutured in nine parts. After one week, 10mm × 10mm of full-thickness skin was removed from the centre of the flap.

Figure 2

The skin defect model in the rabbit auricle. (A) 10 mm×10mm of the full thickness skin was surgically removed from each ear without injuring the auricular perichondrium. (B) Histological cross-section.

Figure 3

Effects of LPIO and SPIO on the rat full-thickness skin wound model. (A) Representative photographs of each wound on days 0, 7 and 14. (B) Relative wound area after treatment. Values are expressed as the mean ± S.D. *$p<0.05$; Significantly different from the control group. #$p<0.05$, compared to SPIO.

Figure 4

Effects of LPIO and SPIO on the skin defect model in rabbits auricles. (A) Representative photographs of each wounds on days 0(pre), 7 and 14 are shown. (B) Relative wound area after treatment. Data are expressed as the mean ± S.D. ** $p<0.01$, *** $p<0.001$, compared to SPIO.

Figure 5

(A) Histopathological findings (low magnification) of the full-thickness skin wound model in rats. Arrows indicate wound edges. The interval between both edges in LPIO is narrower than in control and SPIO-treated groups. Scale bars indicate 500μm.

(B) Histopathological findings (high magnification) of the full-thickness skin wound model in rats. The one wound edge of control (a) and LPIO (b) and cells are shown in wounds of SPIO-treated group (c). Scale bars indicate 200μm.

Figure 6

Histopathological examination showing cellular infiltration, oedema, haemorrhage, horny layer formation of the epithelial edge (HLF), fibrin deposition and vascular proliferation in the control, LPIO and SPIO groups. Data are expressed as the mean ± S.D. * $p<0.05$, *** $p<0.001$, compared to; control. # $p<0.05$, ## $p<0.01$, ### $p<0.001$, compared to LPIO-treated group.

Figure 7

Histopathological finding (low magnification) of the skin defect model in rabbits auricles. There were clear differences in wound healing between LPIO and SPIO groups. Bars = 1000μm.

Figure 8

Re-epithelialisation ratio of wound healing. LPIO group has higher ratio than SPIO group in both rats and rabbits. Data are expressed as the mean ± S.D. * $p<0.05$, compared to control. ### $p<0.001$, compared to LPIO group.

Figure 9

Immuno-histochemical staining of PCNA, p63 and CK14 in rats. (A) Representative micrograph in control, LPIO and SPIO groups during re-epithelialisation of wound edges. Bars = 100μm. (B) The number of cells positive for PCNA and p63 staining. * $p<0.05$, compared to control and SPIO groups.

Figure 10

Immuno-histochemical staining of PCNA , p63, CK14, and CK16 in the rabbit of re-epithelized wound edge in NSC (normal skin control), LPIO and SPIO groups. Scale bars indicate 100μm.