Chapter 2

The Physiological Roles of Leptin in Skin Wound Healing

Reiko Tokuyama-Toda and Kazuhito Satomura

Additional information is available at the end of the chapter

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Abstract

Leptin, a 16 kDa circulating anti-obesity hormone, has many physiological properties such as body weight homeostasis, lipid metabolism, hematopoiesis, thermogenesis, ovarian function, bone formation, and angiogenesis. Interestingly, a certain study showed that skin wound healing delayed in leptin deficient *ob*/*ob* mice. However, little has been known about the physiological role of leptin in skin wound healing. In this chapter, we introduce whether local and single-dose administration of leptin exerted a promotive influence on the skin wound healing. Immunohistochemical analysis revealed that leptin receptor was expressed in mouse epidermal cells. In addition, topical administration of leptin promoted the healing of chemical burn wounds created on the back skin of mice without any side effects. Then, the mechanisms of the promotive effect of leptin on the wound healing of the skin were demonstrated immunohistochemical and biological analysis; namely, leptin stimulated angiogenesis in the connective tissue beneath the wounded area and the cell proliferation, differentiation/function, and migration of human epidermal keratinocytes. These findings revealed the possible and promising usefulness of leptin as a new wound-healing promoting agent.

Keywords: leptin, skin, wound healing, new promoting agent, local administration

1. Introduction

Leptin, the product of *ob* (*obese*) gene, is a 16 kDa non-glycosylated polypeptide anti-obesity hormone mainly produced and secreted by adipose tissues [1]. Recent studies have demonstrated that leptin is also produced by placenta [2], stomach [3], skeletal muscles [4], brain, and pituitary gland [5, 6]. Leptin influences body weight homeostasis through its effects on food intake and

energy expenditure by negative feedback at the hypothalamic nuclei [7]. Leptin is also known to exhibit a variety of physiological actions on lipid metabolism [8], hematopoiesis [9], thermogenesis [10], ovarian function [11], bone formation [12, 13], and angiogenesis [14, 15]. The leptin receptor (Ob-R) is expressed in various tissues including the hypothalamus [16, 17], adipose tissue [18], skeletal muscle [19], and hepatocytes [18, 20]. The multifunctionality of leptin and the wide distribution of its receptor suggest that leptin plays a variety of physiological roles not only as a systemic hormone but also as a local growth factor.

The surface of the body is covered by skin to communicate with the external environment and to protect deeper tissues and organs by separating them from the external environment such as chemical, mechanical, and thermal stresses, infections, and dehydration [21, 22]. Normal dermal wound repair processes, such as inflammation, angiogenesis, contraction, deposition of extracellular matrix, granulation tissue formation, epithelialization, and remodeling, require various cellular and molecular signals [23]. In this biological process, skin fibroblasts interact with surrounding cells such as keratinocytes, inflammatory cells, and endothelial cells [21, 24]. Fibroblasts produce extracellular matrix, glycoproteins, adhesive molecules, and various cytokines [25, 26]. The lack of these signals may result in poor healing of wounds such as diabetic ulcers [27, 28].

A certain study showed that skin wound healing delayed in leptin deficient *ob*/*ob* mice and that exogenously administered leptin restored this delayed wound healing by enhancing re-epithelialization of the wound in these mice in diabetic condition [29]. Another some studies unveiled the effect of leptin on wound healing by demonstrating that leptin acted as an autocrine/paracrine regulator in the wounded site [30, 31]. These findings strongly suggest the possibility that leptin could be a potential medicine for promoting wound healing in skin. However, all previous studies refer to whole-body dosage administered intraperitoneally, and even when administered locally, the leptin must have been administered every day. So, we investigated whether local and single-dose administration of leptin exerted a promotive influence on the skin wound healing. Because, we thought that local and single administration of leptin could avoid the influence of its adverse effect such as metabolic disorders, hyper/hypoglycemia caused by the fact that leptin is a multifunctional and potent systemic hormone, and could be advantageous for the lowering of patients' distress in some cases in its clinical application.

2. Localization of leptin receptor in mouse skin

An immunohistochemical analysis of mouse skin using anti-leptin receptor antibody revealed that leptin receptor was expressed in prickle and granular cells of epidermis (**Figure 1**). These findings showed that epidermal cells are target of leptin.

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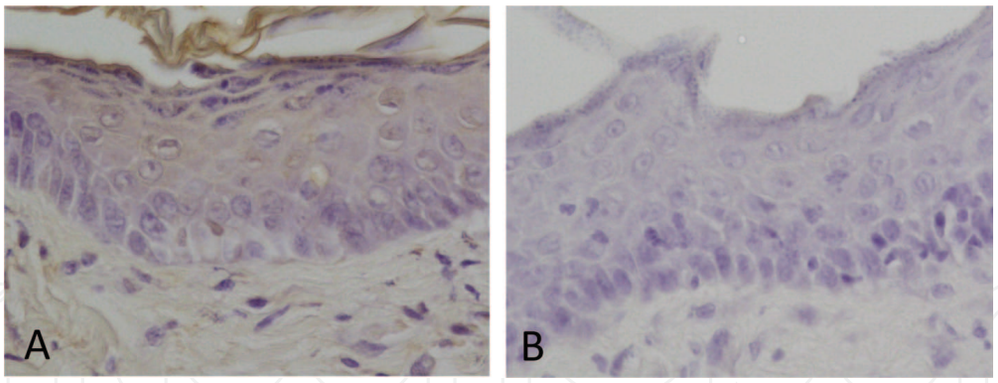

Figure 1. Immunohistochemical localization of leptin receptor in normal mouse skin. (A) Leptin, (B) negative control. Leptin receptor was expressed in prickle and granular cells of epidermis of mouse skin.

3. Effect of leptin on the wound healing of the skin

To elucidate the effect of leptin on the wound healing of the skin, mouse skin chemical burn model was used. Eighteen 6-week-old male ICR mice were fed a normal diet and maintained under a 12-h light/12-h dark cycle. Chemical wounds were created on the back skin by applying two pieces (12 × 12 mm) of filter paper soaked with 20% sodium hypochlorite for 5 min. Wound formation was verified next day, and the wounds were covered with 15 g (12 × 12 × 1 mm) of MedGel (Med GEL Corp., Tokyo, Japan) containing 10 μL of 100 ng/mL leptin (R&D Systems, Minneapolis, USA) or phosphate-buffered saline (PBS) as a control. This hydrogel-contained leptin or PBS was attached to the chemical burn site and dressed. The size of the ulcer was measured on day 4 and 8 after burn formation, and the skin tissue around the wound was obtained for histological analysis. In consequence, at day 4, slightly enhanced re-epithelialization was observed in leptin-treated group, but no significant difference was noted between leptin-treated and control group. In contrast, at day 8, significantly enhanced re-epithelialization was observed in leptin-treated group (**Figure 2**). These experiments showed that the wound area decreased much faster in the leptin-treated group compared with the control group. These findings demonstrated that single and local administration of leptin using bioabsorbable hydrogel promoted the wound healing of skin.

Meanwhile, body weight (BW), and levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) or blood sugar (BS) were not affected through experiment period, showing that topically administered leptin had no systemic adverse effects (**Figure 3**). These findings certify that topically administered leptin is capable of promoting wound healing of the skin without any systemic adverse effects in this period. However, unfortunately, we could not elucidate whether local and single administration of leptin could avoid or not the influence of its side effect over a long period. This issue should be elucidated in the future investigation.

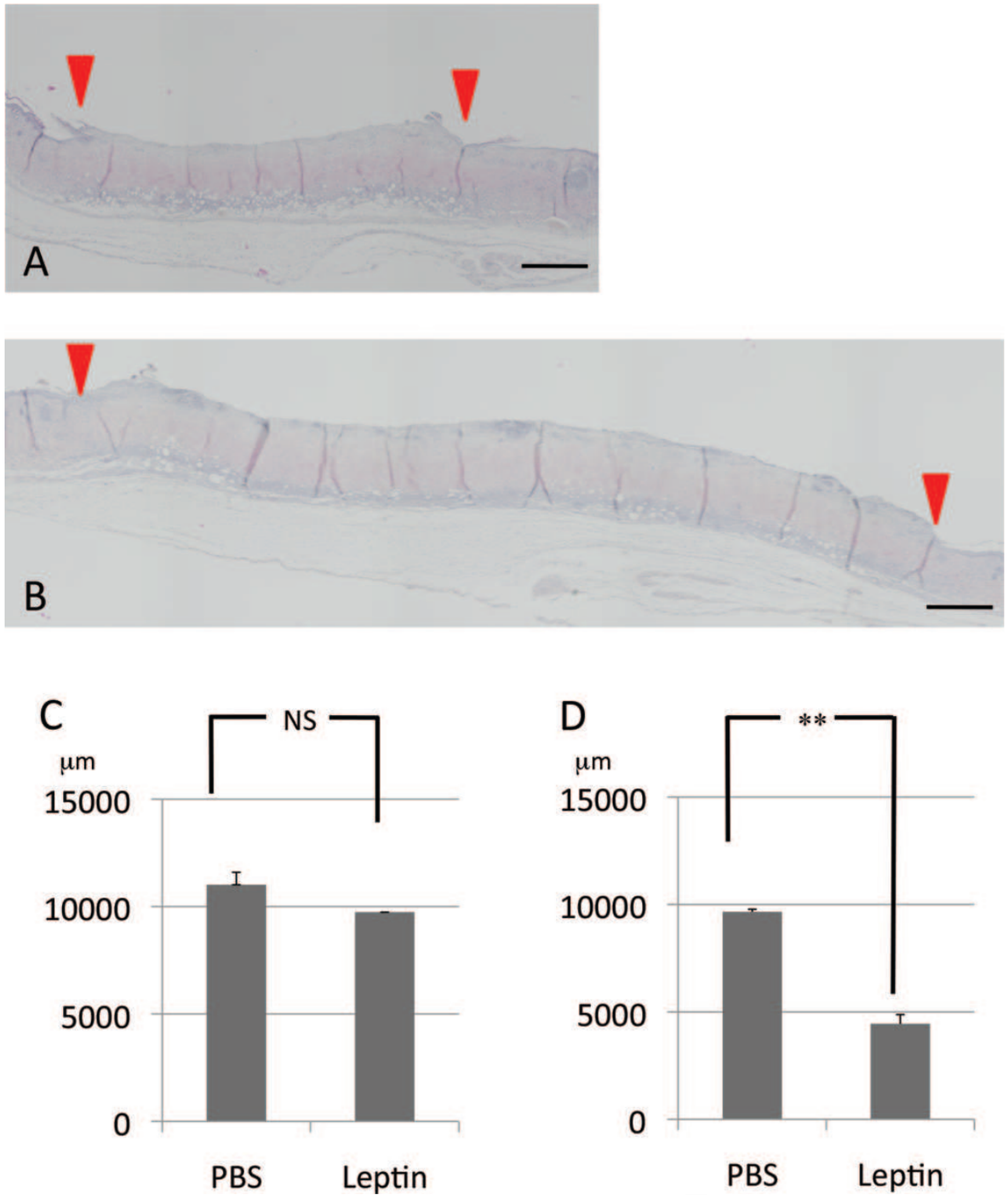

Figure 2. Effect of leptin on the wound healing of the mouse skin. (A) Histological findings of wound repair of skin at day 8 after initial wounding in leptin-treated group. (B) Histological findings of wound repair of skin at day 8 after initial wounding in control group. Spaces between arrow heads show ulcerative area without epithelium. Wound heal is significantly enhanced in leptin-treated group. (C) Skin wound healing at day 4 after wound creation. No significant difference in wound healing was noted between leptin-treated and control group. (D) Skin wound healing at day 8 after wound creation. Significantly enhanced re-epithelialization was observed in leptin-treated group. **P < 0.01. H-E staining. Bars: 500 μm.

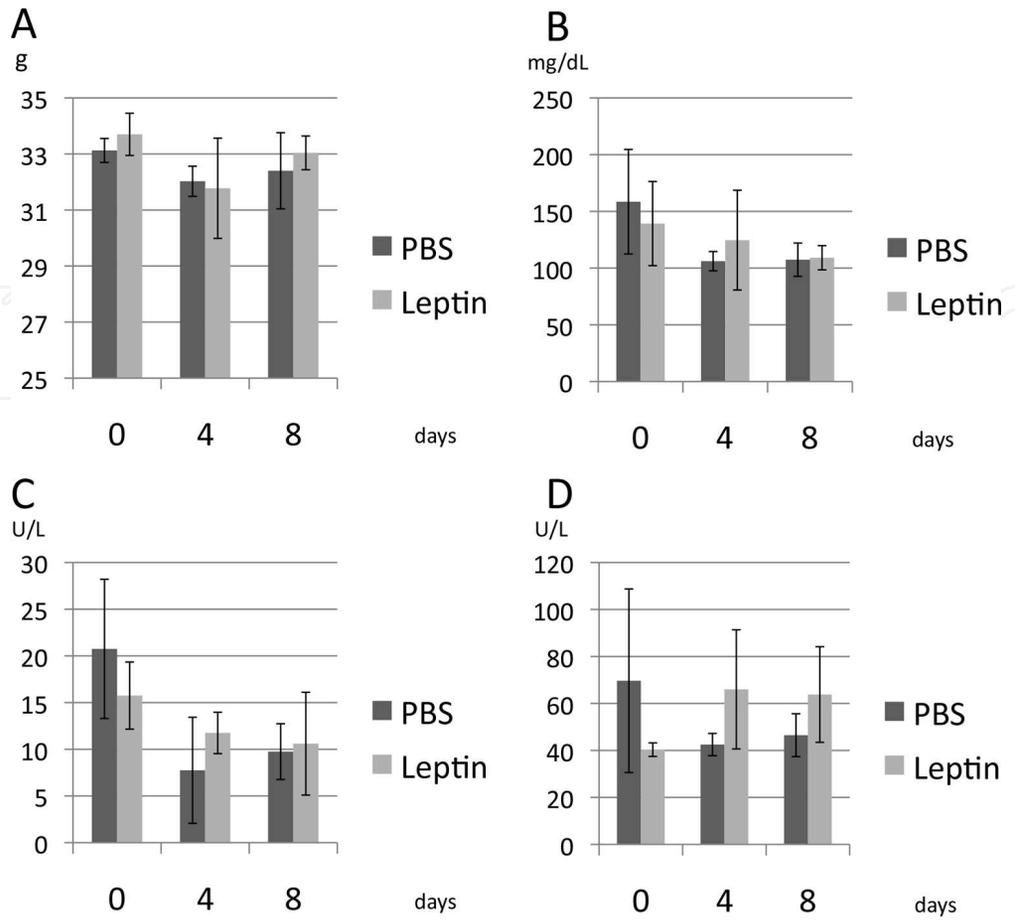

Figure 3. Changes in BW, AST, ALT, and BS. (A) BW, (B) BS, (C) AST, (D) ALT. None of these laboratory parameters were significantly affected by leptin application.

4. Mechanism of the promotive effect of leptin on the wound healing of the skin

4.1. Effect of leptin for angiogenesis on the wound healing of the skin

To elucidate the mechanism of the promotive effect of leptin on the wound healing of the skin, first, the influence of leptin on the angiogenesis in the connective tissues beneath the wound in the skin was revealed by histological analysis. The localization of blood vessels was analyzed by immunohistochemistry by using anti-CD31 antibody. Then, at day 4, after initial wounding, no significant difference on the number of CD31-positive cells was detected between leptin-treated and control group. However, at day 8, after initial wounding, the number of CD31-positive cells significantly increased in leptin-treated group (**Figure 4**). These findings demonstrated that leptin stimulates angiogenesis in the connective tissue beneath the ulcer, and promotes wound healing in the skin by accelerating the supply of nutritions, oxygen, and even some bioactive substances.

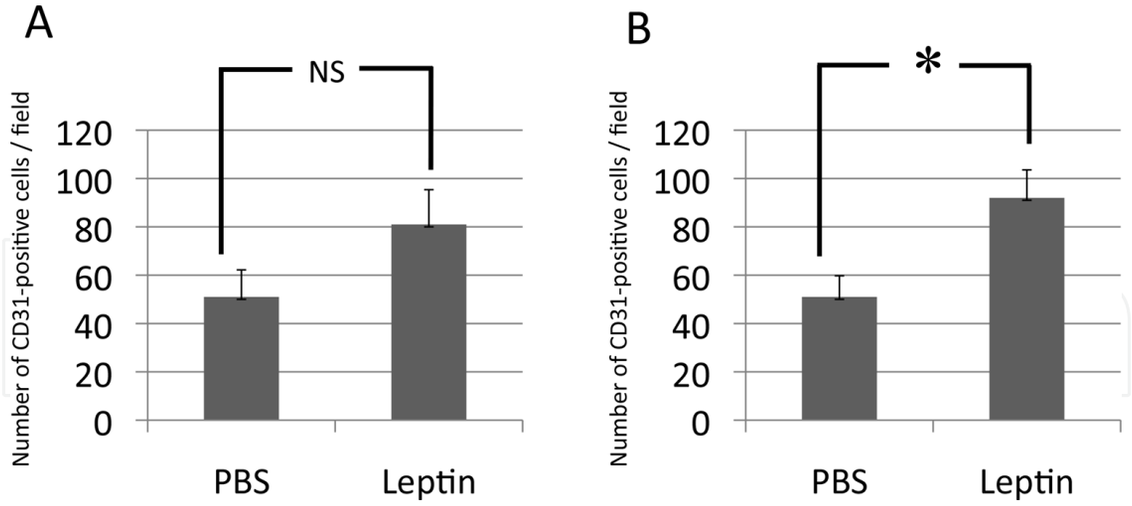

Figure 4. Number of vascular endothelial cells in the dermal connective tissue beneath the ulcerated area. (A) At day 4, after initial wounding, no significant difference in the number of CD31-positive cells between leptin-treated group and control group. (B) At day 8, after initial wounding, more vascular endothelial cells distributed in the connective tissue beneath the ulcer in leptin-treated group compared with control group. *P < 0.05.

4.2. Effect of leptin on the proliferation of human epidermal keratinocytes

To reveal another possible mechanism underlying the promotive effect of leptin on the skin wound healing, cell biological analyses were performed using human epidermal keratinocytes on the premise that the cells were proven to express the mRNA and protein of leptin receptor (*Ob-R*) (data not shown). To elucidate the effect of leptin on the proliferation of human epidermal keratinocytes, the cells were cultured in the absence or presence of various concentrations of leptin. The results indicated that the proliferation of human keratinocytes was significantly enhanced by leptin at a concentration equal to and more than 10 ng/mL

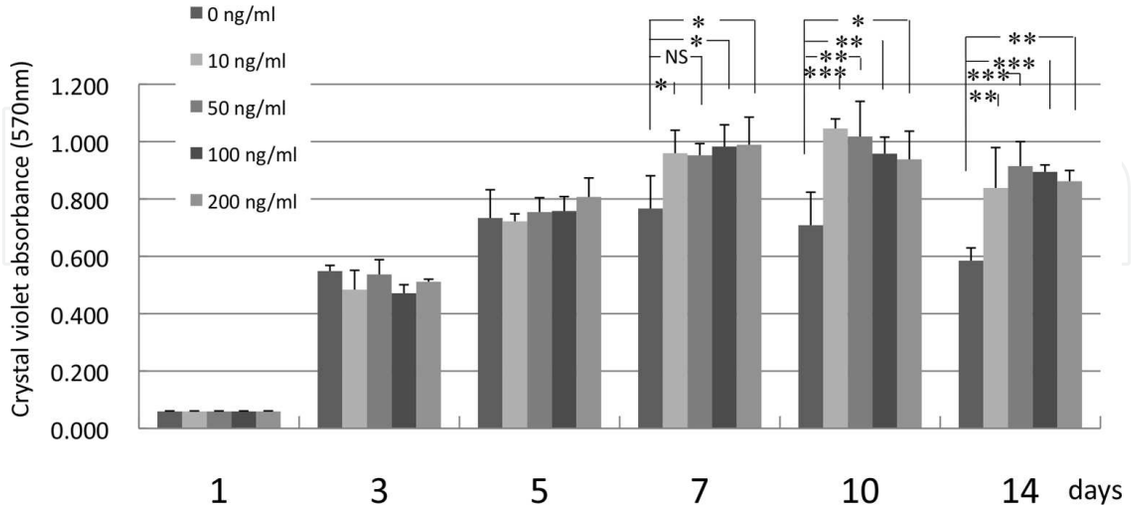

Figure 5. Effect of leptin on the proliferation of human epidermal keratinocytes. Leptin-enhanced cell proliferation at a concentration equal to and more than 10 ng/mL. *P < 0.05, **P < 0.01, ***P < 0.001.

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(**Figure 5**). These findings showed the modest stimulatory effect of leptin on the proliferation of human epidermal keratinocytes.

4.3. Effect of leptin on the differentiation/function of human epidermal keratinocytes

Next, the effect of leptin on the differentiation/function of human keratinocytes was demonstrated using quantitative RT-PCR analysis of the expression of mRNA encoding keratinocyte-related genes, that is, *Cytokeratin 13*, *Cytokeratin 14*, and *Transglutaminase I*. Accordingly, this analysis detected an elevation in expression levels of these gene expressions in the presence of 100 ng/mL leptin (**Figure 6**). These findings showed that leptin has a stimulatory effect on the differentiation/function of human epidermal keratinocytes.

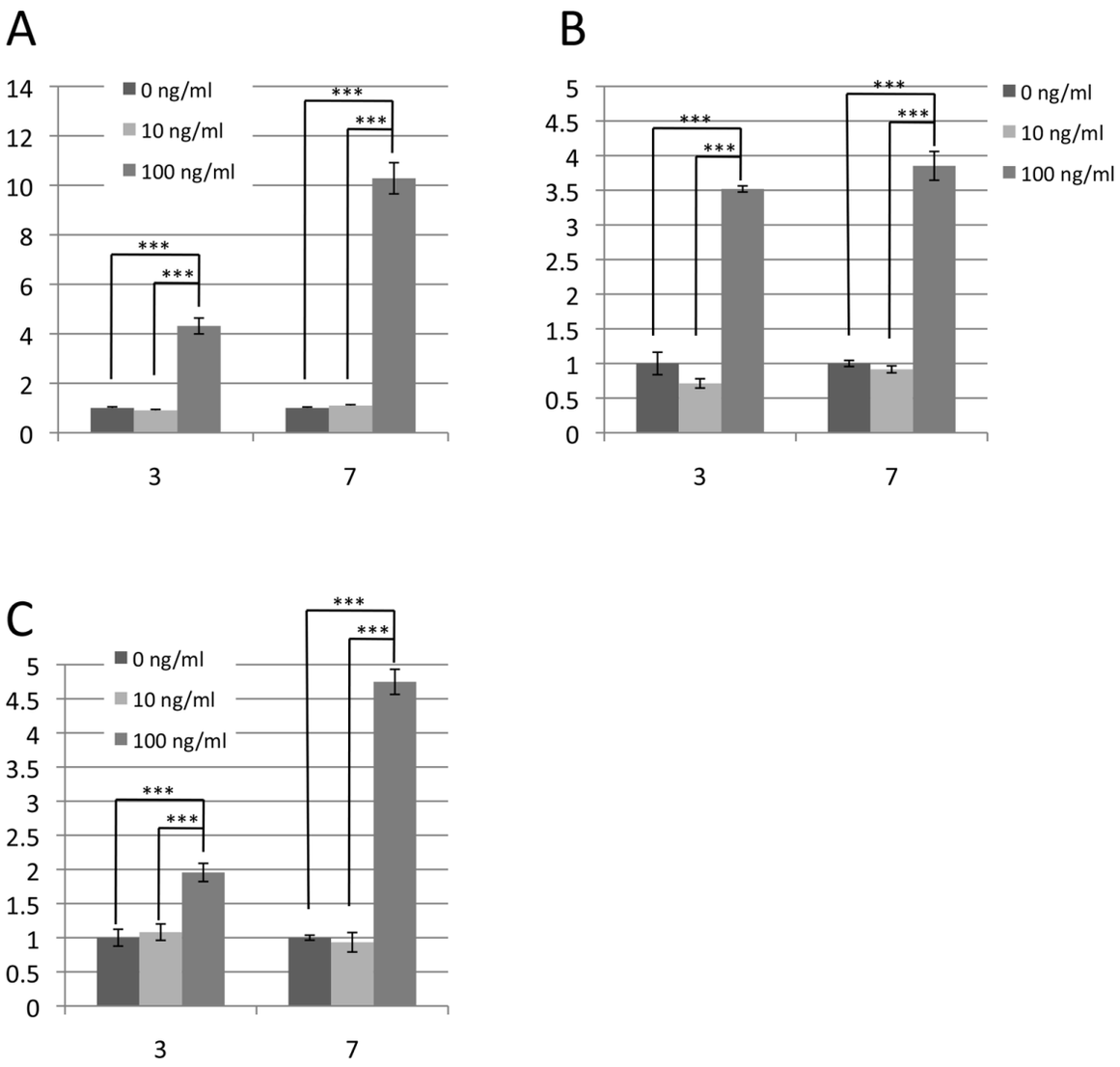

Figure 6. Effect of leptin on the expression of mRNA encoding *Cytokeratin 13*, *Cytokeratin 14*, and *Transglutaminase I* in human epidermal keratinocytes analyzed by quantitative RT-PCR analysis. (A) *Cytokeratin 13*, (B) *Cytokeratin 14*, (C) *Transglutaminase I*. Leptin exerted stimulatory effect on the gene expression of *Cytokeratin 13*, *Cytokeratin 14*, and *Transglutaminase I* at the concentration of 100 ng/mL. ***P < 0.001.

4.4. Effect of leptin on the migration of human epidermal keratinocytes

Moreover, to elucidate the effect of leptin on cell migration around the skin wounded area, scratch assay using human epidermal keratinocytes was performed. The assay was performed using CytoSelect Wound Healing Assay kit (Cell Biolabs Inc., San Diego, USA) according to the manufacturer's instructions. After preparation, the cells were treated with or without 100 ng/mL of leptin. Images of wound healing were captured using a phase-contrast microscope at 0, 3, 6, 9, 12, 18, and 24 h after the preparation. The area of open wound field was calculated by using ImageJ software [32]. Consequently, the significant effect was not observed during initial 12 h. However, the area without cells decreased significantly in leptin-treated group compared with control group from 18 to 24 h (**Figure 7**). This assay revealed that leptin significantly accelerated the migration of human epidermal keratinocytes.

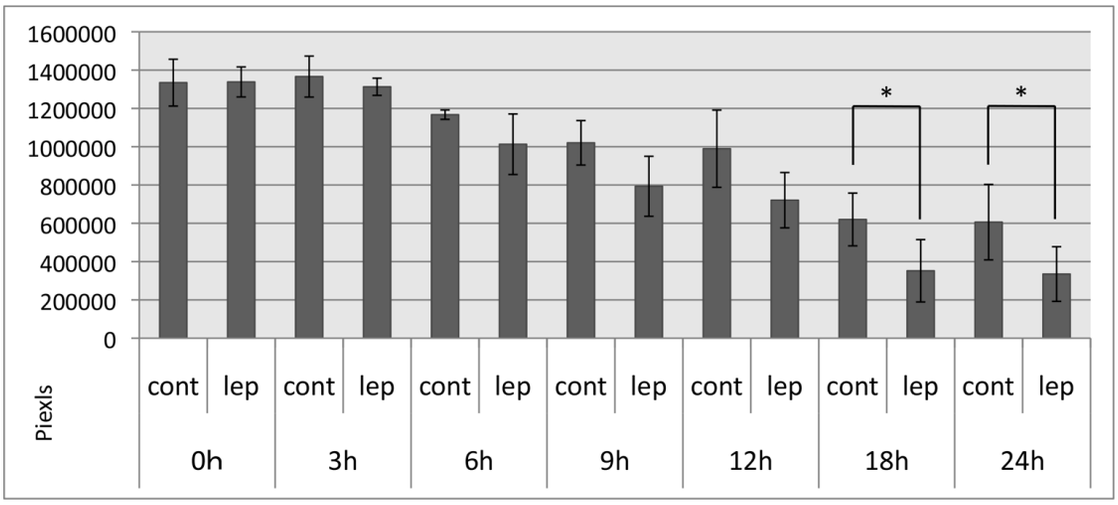

Figure 7. Effect of leptin on the migration of human epidermal keratinocytes. Leptin accelerated the migration of human epidermal keratinocytes, significantly. *P < 0.05.

5. Conclusion

Leptin is capable of promoting wound healing of skin by influencing epidermal keratinocytes proliferation, differentiation/function and migration, and angiogenesis in the connective tissue beneath the wounded area. Moreover, we showed that single dose and topically administration of leptin could promote wound healing in the skin without any side effects by using an adequate drug delivery system [33]. In addition to these findings, our previous study demonstrated that local administration of leptin could promote wound healing in the oral mucosa by enhancing epithelial cell migration and angiogenesis in the connective tissue beneath the wound [34]. Taken together, leptin is proven to play physiological roles in wounded area not only as a systemic hormone but also as a local growth factor. Importantly, these findings presented in this chapter declared the possible and promising usefulness of leptin as a new wound-healing promoting agent.

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