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Original article

The effects of sodium usnic acid by topical application on skin wound healing in rats

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ABSTRACT

Wound healing is the process of repairing and remodeling damaged tissue. This is a public health problem that can influence the survival rate and quality of life of injured people. This attracts the attention of the medical community because it has high health care costs and there is presently a lack of successful therapy. Thus, the application of natural ingredients and medicinal plants has become a focus of research. The purpose of this study is to investigate the effectiveness of topically-applied sodium usnic acid on macroscopic and microscopic changes under dermal injury. These effects were measured using wound contraction experiments, histological analysis, and immunohistochemistry analysis, and gentamicin was used as a positive control medicine. Our results revealed that wound healing rates were higher and re-epithelialized times were shorter with topical application of sodium usnic acid, as compared to the negative control group. Histological results showed treatment with sodium usnic acid caused a reduction in inflammatory cells and an increase in fibroblast proliferation, granulation tissue, vascular regeneration. Sodium usnic acid treatment also resulted in earlier complete re-epithelialization, formation of well-organized bands of collagen, and epidermal keratinization. Furthermore, the levels of VEGF were significantly higher at day 1 post-wounding in those treated with sodium usnic acid. In conclusion, our results indicate that the topical use of sodium usnic acid could promote skin wound healing, and this mechanism might be related to anti-inflammatory effects at the wound site.

1. Introduction

Wound healing is a complex process that can be divided into four progressive phases: coagulation and haemostasis, inflammation, proliferation, and scar tissue formation [1]. There are two important functions of the coagulation and haemostasis phase. The first function is to protect the vascular system and maintain the function of the vital organs by preventing exsanguination when the organism is injured. The second function of the coagulation and haemostasis phase is to provide a matrix for invading cells, which is needed for later phases of healing [2–5]. The next phase is inflammation, which functions to establish an immune barrier against invading micro-organisms [2]. The phase of proliferation begins on the third day after wounding. It presents as significant formation of granulation tissue with angiogenesis, fibroblast migration, and deposition of newly synthesized extracellular matrix [6,7]. The tissue remodeling phase results in the formation of new epithelium and scar tissue [8].

Skin wounds are a public health issue that can influence the survival of injured people and decrease their quality of life. It attracts the attention of the medical community because it is expensive and, thus far, therapy has been unsuccessful [9]. The goal of clinical treatment is to have a fast wound recovery time to properly restore structure and function [10].

Recently, treatment with natural ingredients and medicinal plants has become a research focus because of the limitations and adverse effects of chemical and synthetic drugs [11]. Usnic acid is a type of lichen metabolite that was first isolated in 1844. It possesses high commercial value and has been studied in depth [12]. The unique biological and physiological activities of this compound have a potential application in pharmacology and clinics [13]. This is because usnic acid has antioxidant, antimicrobial, antiviral, and anti-inflammatory activity [14]. In most research, usnic acid is regarded as an antibiotic with antibacterial activity against gram positive bacteria and drug-resistance bacteria [15,16]. Previous research has noted a protective effect of usnic acid during LPS-induced acute lung injury in mice through the attenuation of inflammatory responses and oxidative stress [17]. It was also reported that reconstituted bovine type-I collagen-based films containing usnic acid could be used as a wound dressing for dermal burn healing in rats [18]. Additionally, usnic acid enamines demonstrated wound-healing properties in both *in vitro* and *in vivo*

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experiments [19]. Furthermore, sodium usnic acid is synthesized by a chemical reaction of usnic acid and sodium salt. It has a higher solubility than usnic acid but still has similar effects to usnic acid [20]. It has been reported that sodium usnic acid might promote migrating and secreting growth factors of fibroblasts *in vitro* [21], and it accelerates the process of wound healing of a skin injury [22]. However, the optimization of the duration of treatment with sodium usnic acid for skin wound healing is still needed.

Thus, the current study further investigates these previously reported effects of sodium usnic acid on inflammatory responses and various types of wound healing. However, there is insufficient evidence to support these pathological effects of sodium usnic acid on skin injury, and its potential as a topical agent to accelerate the stages of the wound healing process hasn't been confirmed. Therefore, the current study investigates the topical effectiveness of sodium usnic acid on macroscopic and microscopic changes from dermal injury using wound contraction experiments, histological analysis, and immunohistochemistry analysis.

2. Materials and methods

2.1. Animals

1.1 Sixty-four healthy adult male Wistar rats were procured from Harbin Medical University, Harbin, China. They weighed between 180 g and 220 g, and their average age was 8 weeks. The animals were housed individually in polyethylene cages in an experimental animal room with a 12 h light/dark cycle. They were maintained at a room temperature of 22 ± 2 °C and humidity at 55% ± 15%. The rats were fed a standard diet and water ad libitum, and they were acclimatized for one week before the experiment. All experiments were carried out in accordance with the guidelines of the China Ethical Committee for Animal Experiments.

2.2. Full-thickness dermatic wound model

All rats were anesthetized with ketamine hydrochloride (50 mg/kg) and xylazine (10 mg/kg) by intramuscular injection (IM) [23]. After the shaving the hair on the back of each rat, the skin was sterilized with iodine three times followed by 70% alcohol to remove the iodine. Two circular incisions of 10 mm diameter and 2 mm depth were excised from full-thickness skin wounds located on the dorsal line of each animal. Postoperative animals were housed individually in properly disinfected cages to prevent infection or further damage to the wounds after recovering from anesthesia.

2.3. Determination of the optimal concentration of sodium usnic acid

AR Sodium usnic acid (Wuxianfeng technology Co, Ltd, Harbin, China) was dissolved in dimethyl sulfoxide (Sigma Chemical Co, St. Louis, Mo., USA) in several concentrations (300, 600, 1200, 2400, 4800, 9600, 19200, 38400, and 76800 μg/L). Twenty-seven rats were used to find the optimal concentration for wound healing. Wound healing rates indicated that the optimal healing effects were found at a concentration of 38400 μg/L sodium usnic acid in DMSO at (Fig. 1).

2.4. Grouping

After surgery, the rats were randomly divided into four groups with an n = 16. The negative control group (NC) was untreated. The vehicle control group (DMSO) was administered AR DSMO. The gentamicin treatment group (GA) was administered 0.01% gentimicin sulfate. The usnic acid treatment group (SUA) was administered 38400 μg/L sodium usnic acid in DMSO. Local drug delivery was applied once a day to every wound at a volume of 50 μL for 21 days.

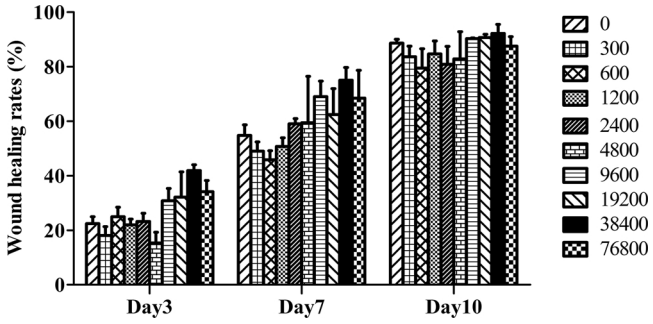

Fig. 1. Optimal concentration of sodium usnic acid. Wound healing rates of different concentrations of sodium usnic acid (0, 300, 600, 1200, 2400, 4800, 9600, 19200, 38400, 76800 μg/L) were administered on day 3, day 7, and day 10.

2.5. Wound healing analysis

Wound areas were recorded on days 3, 7, 10, and 14 post-wounding using transparency paper and a permanent marker. The wound area was measured by coordinate paper. The following formula was used to calculate the wound healing rates [24,25]: (Area of original wound − Area of remaining wound)/Area of original wound × 100. Wound closure time was defined as when the wound bed completely re-epithelialized and filled with new tissue.

2.6. Wound tissues collection

Four rats from each group were euthanized on days 3, 7, 14, and 21 post-wounding via ketamine hydrochloride overdose (200 mg/kg) administered intramuscularly. Full-thickness skin wound and subcutaneous tissue, with approximately 5 mm of the surrounding unwounded skin, was excised as wound samples. These samples were fixed in 10% buffered formalin solution (pH 7.4) for 48h for histopathological and immunohistochemistry examination.

2.7. Histological analysis

Five μm-thick slices were made from the wound samples embedded in paraffin, and then H & E staining was performed for histological analysis. All slides were examined under 40 × to 200 × magnifications, and three visual fields were randomly chosen under 200 × magnifications for histological analysis. As an evaluation method of histology, each visual field was given a histological score ranging from 1 to 15, according to the method of Greenhalgh et al. and Kant V et al. [26,27]. Fields showing no to very low accumulation of inflammatory cells and granulation tissue were scored from 1 to 3. A score of 4–6 was given to fields with thin immature granulation tissue dominated by inflammatory cells, few fibroblasts, blood vessels, and collagen deposition. A score of 7–9 was assigned to fields with moderate thick granulation tissue dominated by more fibroblasts and collagen deposition, more neovascularization, minimal to moderate epithelial layer formation, and few inflammatory cells. A score of 10–12 was given to thick, vascular granulation tissue dominated by fibroblasts with extensive extracellular collagen deposition and enveloped by partially immature to completely formed epithelial layer. A score of 13–15 was given to fields with thick, mature granulation tissue dominated by compact collagen depositions parallel to a well-formed, complete epithelial layer, decreased fibroblasts, and normal blood vessels.

2.8. Immunohistochemistry analysis

The slices were immersed in a covered plastic container with the target retrieval solution and placed in the autoclave at 121 °C for 15 min. Endogenous peroxidase was blocked with a peroxidase-