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Acupuncture accelerates wound healing in burn-injured mice

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ABSTRACT

The effects of acupuncture on healing of deep second degree burns were compared to the conventional hydrocolloid dressing, Duoderm in mice. The expression level of inflammatory protein-1α (MIP-1α) was significantly reduced in the injured skin and the number of eosinophils in blood decreased significantly following the acupuncture treatment compared to the Duoderm dressing at 7 days after the burn. In addition, the acupuncture treatment was more effective in decreasing the wound size and inducing epidermal regeneration. Histological findings also revealed that there was less leukocyte infiltration and a greater reduction in the immunohistochemical reaction to MIP-2 in the wounds treated with acupuncture versus Duoderm. Furthermore, the numbers of BrdU- and basic fibroblast growth factor (bFGF)-positive cells were significantly increased by the acupuncture treatment, compared to the Duoderm treatment at 7 days. Moreover, in the acupuncture treated mice, the expression of fibronectin was increased and α-SMA was decreased at 7 days. Thus, this present study demonstrates that acupuncture accelerates the skin regeneration process following deep second degree burns.

1. Introduction

Wound healing involves an orchestrated series of overlapping processes resulting in a varying degree of functional and structural restoration. When successful, wound healing restores normal function with a well-organized, minimal scar. Removal or damage to the skin by trauma, surgical wounds, burns, or ulcers results in major functional and psychological problems for patients and can even cause death. In this respect, an adequate treatment is essential to permit maximal recovery of the dermis and epidermis. Traditional treatments include autologous, heterologous, and/or cadaveric skin transplantation, and more recently, the use of new wound dressings for dermal regeneration. These wound dressings are bioartificial matrices, composed mainly of collagen and polyvinyl alcohol. Two examples are Alloask D and Duoderm, with the former being an example of the use of lyophilized porcine dermis and the latter representing a wound dressing made of hydrocolloid [1]. These dressings have several advantages compared to skin transplantation, including the lack of donor site-related morbidity associated with autologous transplantation and a lower risk of immune rejection and transmission of diseases compared to heterologous transplantation strategies. Unfortunately, their clinical

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use is still limited due to low regenerative capacity and high infection risk for the patients [2,3]. These problems are mainly associated with the low vascularization capacity of these scaffolds which limits the presence of immune cells, oxygen, and nutrients in the wound area [2,3].

Basic fibroblast growth factor (bFGF) is a potent mitogen and chemoattractant for endothelial cells and fibroblasts [4–7]. The administration of recombinant bFGF to skin wounds has been shown to accelerate both dermal and epidermal wound healing in pigs and rats [8,9], and bFGF has shown to suppress scar formation by promoting an early and persistent increase in the rate of apoptosis of granulation-tissue cells [10]. In addition, bFGF-knockout mice had delayed healing of skin wounds [11]. These data suggest that bFGF plays significant roles in skin wound healing and scarless repair process.

Traditional Chinese medicine, especially acupuncture, has made many important contributions to medicine. In China and neighboring countries/regions, it has served as the basis of medical knowledge for thousands of years [12]. Although the mechanism of promoting functional recovery by acupuncture is not clear, several mechanisms have been suggested, such as the expression of c-*fos* and c-*jun* [13,14], synthesis of morphine [15], and the production of growth factors (e.g., brain-derived neurotrophic factor [BDNF], glial cell line-derived neurotrophic factor [GDNF] and bFGF) [16–18] in various disease models. However, the therapeutic effects of acupuncture stimulation have not been examined in burn wound healing.

In the present study, we investigated the effects of acupuncture stimulation in the healing of deep second degree wounds in mice compared with the Duoderm dressing.

2. Materials and methods

2.1. Animals

Six-week-old male BALB/c mice (total 110), weighing 20 g each, were purchased from Orient (Korea). The experimental procedures were performed in accordance with the animal care guidelines of the National Institute of Health (NIH) and carried out with a prior approval from the Institutional Animal Ethical Committee. The animals were housed under laboratory conditions at a controlled temperature ($20 \pm 2\,^{\circ}C$) and maintained under 12 h light–12 h dark cycles (lighting from 07:00 to 19:00 h) with food and water made available *ad libitum*. The animals were divided into four groups: Sham group (Sham, $n = 10$), Burn group with no treatment (Burn, $n = 30$), Duoderm CGF (11 mm in diameter; Convatec, USA)-treated group after burn (DT, $n = 30$), and acupuncture-treated group after burn (AT, $n = 30$). Each group was subdivided into three time point groups (3, 7 and 14 days after injury; $n = 10$ for each time point).

2.2. Experimental preparation of burn

The mice were anesthetized by intraperitoneal injection of pentobarbital sodium (50 mg/kg), and their dorsal hair was clipped and depilated with a hair removal cream (Shiseido Co., Tokyo, Japan). The dorsal skin was exposed for 10 s to water heated to 100 °C to produce a deep second degree burn of 11 mm in diameter that was measured using a caliper (Mitutoya, Japan) and confirmed by gross pathological change (Fig. 1A). The wounds of DT group mice were covered with Duoderm CGF and exchanged it daily for the experimental period.

2.3. Acupuncture treatment procedure

For the AT group, acupuncture treatment was given daily for 30 min at two opposing spots, each situated 1 cm away from the burn area, by using a stainless press-needle of 0.2 mm in diameter and 1.5 mm in length (Haenglimseowon, Seoul, Korea). Mice were lightly immobilized, and acupuncture needles were fixed with a bandage on the skin (Fig. 1B). The sticky needle devise was used to insert the needle to the targeted depth exactly (1.5 mm), and sustained the inserted needle for 30 min without any restraint stress or anesthetics. In a pilot study, we observed that applying sticky needle for 30 min did not induce any inflammatory reactions.

2.4. Eosinophil counts

At 7 days after injury, the animals were anesthetized by pentobarbital sodium. Whole blood was obtained by cardiac puncture and stained with Hinkelmann's solution (0.5% [wt/vol] eosin Y, 0.5% [wt/vol] phenol, and 0.185 [vol/vol] formaldehyde in distilled water), and eosinophils were counted.

2.5. Wound closure measurements

Immediately after creating the wounds, the initial wound sizes (diameter in mm) were measured using a caliper and the Optimas 5.2 software (Media Cybernetics; Bethesda, MD) analysis. Wound sizes were determined in the same manner at days 3, 7 and 14.

2.6. Histological examination

Mice were sacrificed at the indicated time points after surgery. The animals were fully anesthetized with pentobarbital sodium (50 mg/kg), transcardially perfused with phosphate buffered saline (PBS), and then fixed with a freshly prepared solution consisting of 4% paraformaldehyde in 100 mM phosphate buffer (pH 7.4). The skin samples of burn wounds were removed, post-fixed in the same fixative overnight, embedded in paraffin under vacuum, sectioned at 5-μm thickness, mounted on glass slides, and finally stained with hematoxylin and eosin. Macrophage inflammatory protein 2 (MIP-2)- and bFGF-positive cells in the burn wound area were examined by the immunoperoxidase technique using anti-mouse MIP-2 and anti-mouse bFGF antibodies, respectively (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The sections were incubated for another 1 h in the solution of biotinylated mouse secondary antibody. The bound secondary antibody was then amplified with a Vector Elite ABC kit® (Vector Laboratories, Burlingame, CA, USA). The antibody–biotin–avidin–peroxidase complexes were visualized using 0.02% 3,3-diamonobenzidine (DAB). The number of immune-positive cells in the wound area was counted from each section under