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RESEARCH ARTICLE

Leech Therapy for Linear Incisional Skin-Wound Healing in Rats

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Abstract
Background: The aim of this study was to investigate the effects of leech therapy (LT), in comparison with topical phenytoin (PHT), on incisional skin-wound healing in animal models.
Methods: This experimental study included 15 male rats (5 animals in 3 groups) with approximately equal body weights (350 ± 10 g). Skin wounds with lengths of 20 mm and depths of 0.5 mm were made on the dorsolateral region of rats 4 cm from the spine. The first group (PHT group) was treated daily with topical PHT (1%) while the second group (LT group) received LT at the beginning of the experiment. The control group received neither the drug nor the therapy. Wound healing was evaluated every day, and the study was continued until the wound had completely healed. Changes in the areas and the appearances of the skin wounds and histological differences (at the end of the experiment) were used to investigate the differences in wound healing among the groups.
Results: The process of wound healing was significantly faster in the group treated with LT ($p < 0.05$) than in the group treated with the PHT.
Conclusion: The study results showed that LT improved incisional skin-wound healing in rats.

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1. Introduction

By definition, a wound is a physical injury that results in an opening or breaking of the skin. Cuts and bruises may occur as a result of objects impacting the skin [1]. There are several types of wounds, such as surgical, traumatic, and chronic. Two categories of wounds exist, namely, partial and full thickness. Full-thickness wounds involve a loss of deeper layers of skin and fat; they disrupt the blood vessels and produce a scar when healed [2]. Wound-care treatments are debridement, alleviation of weight-bearing wounds, compression therapy, antibiotics, hyperbaric oxygen therapy, whirlpool therapy, ultrasound treatment, electrical stimulation, and magnetic therapy. There are also many wound-care products such as alginates, antimicrobials, and hydrogels [2].

The use of a simple and reproducible model is a basic requirement for objective analysis of the effects of different external factors on skin-wound healing [3]. In clinical trials, wound area is the most commonly reported property of wounds. In an effort to provide a highly accurate and precise measure of wound area, numerous studies have compared different modalities of measuring this parameter [4]. Among the many possibilities for measurement of wound area are tracing the wound and recording the trace [5—8], tracing the wound and then cutting out the trace and weighing it [5], tracing the wound onto a paper block and then counting blocks [9], photographic studies [4], the use of a specifically designed coordinate measuring device [9], and planimetry [6—8,10]. The process of tracking and documenting changes in wound area is an important part of the overall treatment and assessment method [8,11,12].

Wound healing immediately starts after an injury and proceeds with a complicated but well-organized interaction among various types of tissues and cells [1]. Skin-wound healing consists of the inflammatory, proliferative, and maturation phases [1]. In the inflammatory phase, the recruitment of leukocytes such as neutrophils and macrophages into the wound site is characteristic. In the proliferative phase, the migration and proliferation of keratinocytes, fibroblasts, and endothelial cells result in re-epithelialization and tissue granulation. In the maturation phase, excess collagen in the wound site is degraded by several proteolytic enzymes, leading to the completion of tissue repair [2]. It is considered that perfusion is an important factor in accelerating the healing rate of skin wounds. The saliva of medicinal leeches demonstrated such an ability of perfusion.

Leech therapy (LT) has made resurgence in Russia for the treatment of hypertension, migraines, phlebitis, varicose veins, arthritis, hemorrhoids, and ovarian cysts. LT was a mainstay in conventional treatment of pain and inflammatory diseases since the ancient ages until the 20th century [13,14].

In the United States, plastic surgeons use leeches to drain blood from wounds after limb or tissue reattachment. The application of LT in modern medicine is greatly diverse [15]. The World Health Organization has recommended traditional medicines to be used more effectively in the health-care systems [13]. LT is a procedure that has been recommended in traditional Iranian medicine and traditionally used by native inhabitants and physicians (Hakims) for centuries in the treatment of hemorrhage, fever, etc. [16].

Medicinal leeches are used in reconstructive maxillofacial surgery [17] and in some other procedures such as wound and flap healing in the plastic and reconstructive surgery [18], as well as in the treatment of venous insufficiency, varicosities, hemorrhoids, and in various other diseases [19]. In addition to the application of medicinal leeches in plastic surgery to maintain blood flow in congested skin flaps [20], the application of LT has also been evaluated in several clinical studies on osteoarthritis of the knee [21] or thumb [22], or epicondylitis [23]. Moreover, many noncontrolled clinical studies and case reports refer to wound healing by applying LT [24—27]. Phenytoin (PHT) was first used clinically in the treatment of epilepsy in 1938 and it has been used in the healing of a variety of wounds since the 1950s [28—31]. In a clinical study, it was first shown that pretreatment with oral PHT enhanced healing of periodontal wounds [32]. In other investigations, acceleration of the healing of war wounds as well as diabetic and leprotic ulcers has been reported [33,34]. Despite these applications of the therapy, some contradictory results have also been reported; for example, administration of PHT *in vitro* had no effect on human dermal fibroblasts or epidermal keratinocytes [35]. However, so far no studies have been performed to assess the comparative effect of LT with any other therapeutic drug on skin-wound-healing parameters. To confirm and complete previous research findings, the comparative effects of topical PHT and LT on the rate of wound healing in an incisional skin-wound model in rats were investigated in this study.

2. Materials and methods

2.1. Animals

Locally produced 15 male Wistar rats (*Rattus norvegicus albinus*) weighing 350 ± 10 g (9—11 weeks old) were housed in groups of one in plastic solid-bottomed cages provided with bedding in an air-conditioned colony room on a 12-hour light/dark cycle at a constant temperature (21 ± 0.5 °C) with relative humidity (25—30%). The animals were fed with a standard pelleted diet and tap water *ad libitum*. Procedures involving animals and their care were conducted in accordance with the institutional guidelines of the AJA University of Medical Sciences Ethics Committee and conformed to the criteria outlined in the Guide for the Care and Use of Laboratory Animals [36].

2.2. Chemicals and reagents

Ketamine, xylazine, and phosphate-buffered saline (PBS) were obtained from Merck (Germany). The other chemicals used in this study were formalin (Merck, Germany) and 99% ethanol (Takestan, Iran).

2.3. Leeches

The medicinal leeches (*Hirudo orientalis* Utevsky & Trontelj, 2005) weighting 3—4 g had been kept in a clean plastic