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Original Research

Wound healing property of milk in full thickness wound model of rabbit

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ABSTRACT

Objective: Wound healing consists of several continuous phases in which various cells and chemical intermediates are involved. Milk as a rich source of nutritional elements has proved to have potential benefits for treatment of various diseases. The present study was designed to investigate the healing effect of low-fat cow's milk on an open skin wound model in the rabbit.
Methods: The 2%, 5%, and 10% (w/w) ointments of lyophilized powder of low-fat milk were prepared in the eucerin base and were applied twice daily in the treatment groups. Phenytoin 1% ointment was used as a standard control. The healing effect of the milk ointment (MO) was evaluated through the measurement of wound surface area, the extent of tissue tension, and the content of hydroxyproline. Histological evaluation of skin tissue specimens was also performed using hematoxylin and eosin staining.
Results: The results showed that the healing rate in the treatment group was significantly higher than that of untreated group and eucerin group ($p < 0.01$). The best healing effect was seen in 5% milk ointment with the shortest healing time (15 days) and the highest tissue tension in comparison to other groups. Although the tissue hydroxyproline content in this group was slightly lower than that of the phenytoin group, this difference was not significant. Histologic, findings indicated increased collagen fibers, increased fibroblasts and an evident decrease in inflammatory cells in that group.
Conclusion: It can, therefore, be concluded that low-fat cow's milk has significant beneficial effects on skin wound healing. Therefore, it may be used as a healing agent in different types of the wound in humans after certain clinical trials.

1. Introduction

The wound is defined as the rupture of the continuous structure of tissue resulted from physical, chemical, and biological damage. Wound healing is a complex and organized process which occurs in four sequential, overlapping phases, including hemostasis, inflammation, proliferation, and remodeling [1]. The hemostasis phase begins within the first few minutes following adhering, activation, and aggregation of platelets in the damaged area [2]. Activation of the coagulation cascade results in the formation of a clot of aggregated platelets in a mesh of cross-linked fibrin protein. This clot stops bleeding (hemostasis) [3]. The inflammatory phase is the body's natural response to injury [4]. After establishing homeostasis, the permeability of blood vessels significantly increases in the wound area; this feature causes the cells, antibodies, growth factors, enzymes, and nutrients reach the wound area [5]. In third phase fibroplasia and granulation tissue formation, fibroblasts proliferation forms a new provisional extracellular matrix (ECM) by secreting collagen and fibronectin. Establishment of new granulation tissue composed of collagen fibers and extracellular matrix and development of a new network of blood vessels within the tissue called angiogenesis which leads to wound remodeling. Formation of normal and healthy granulation tissue depends on a sufficient supply of

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oxygen and nutrients by the blood vessels. Healthy granulation tissue has a rough, reddish pink structure [3]. The epidermis is finally remodeled through the proliferation of epithelial cells; a process called epithelialization [6]. The wound edges are approximated through the specific function of myofibroblasts and contraction of the wound surface [7]. Maturation, in which the wound is completely closed, is the final phase of wound healing [3]. It consists of remodeling of collagen type III to collagen type I and alignment of collagen fibers along the lines of tissue stress, as well as removal of excess cells by apoptosis [8]. A variety of cells are involved in cutaneous wound healing, including fibroblasts, inflammatory cells, and epithelial cells (keratinocytes). Growth factors and cytokines play an important role to repair the wounded area. Among them; epidermal growth factor (EGF), transforming growth factor (TGF) β, platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) can be notified [5]. A number of other mediators have been identified in the process of wound healing. Different animal models have been used for the study of wound healing. Rabbit, rat and mice have been widely employed for the study of wound healing and effect of different treatments [9]. In this study regarding the wound size, location and analytical methods that were required rabbit was selected for full thickness wound model.

Numerous agents are being used to promote wound healing among them phenytoin, zinc, nifedipine, vitamin C, and some herbal products such as aloe vera are widely used [10,11]. However; mechanism of phenytoin has widely been studied and proliferation of fibroblasts and promotion of collagen production in wound site has been attributed to this drug [10]. Therefore, in many studies phenytoin is used as a standard healing agent [12]. In this work, phenytoin has also been used in a standard healing group.

Wound healing, to which humanity is faced from the beginning of creation, is one of the most important issues in medical science [12–14]. Clinically, various remedies and drugs, mainly chemically-derived, have been introduced to facilitate and accelerate the wound healing process, but their use has been limited due to side effects or low efficiency. In the traditional medicine, the milk of human and other animals have been used in different illness, especially the healing of soft tissue injuries. For example, nursing mothers with sore nipples are recommended to rub the milk on the wound [14]. It seems that milk is a rich source of nutrients and biological substances which can significantly help to build new tissue at the wound site. Due to the fact that milk is an emulsion of fat-soluble and water-soluble substances, it can be expected that this hydrophilic and hydrophobic dual-nature provides a unique feature to milk and facilitate its surface absorption. In addition, a part of milk protein known as Whey protein has an extremely absorption potential on the skin. As an important constituent of milk, whey contains a high level of amino acids, which support protein synthesis. Despite the numerous health benefits attributed to milk, the healing effect of cow's milk on skin wound healing has not been studied yet; therefore, we decided to study the impact of cow's milk with 1.5% fat on an open skin wound in rabbits.

2. Material and method

2.1. Animals

The study was carried out on male New Zealand white rabbits weighing 1.5–2.5 kg. The animals were kept individually in special cages and received a standard diet with fresh vegetables and water ad libitum. They were maintained on a 12 hours light-dark schedule at a temperature of 23 ± 2 °C and humidity of 45%–55%.

All animal procedures were performed in accordance with the guidelines laid by the committee for the purpose of control and supervision of experimental animals (CPCSEA) of Jundishapur University of Medical Sciences. This research was a blinded study. Investigators who applied the drugs and measured the area were blinded on the composition of ointments and the histologist who performed the histological examination on wound specimen was blinded to the type of treatments.

2.2. Preparation of 1.5% fat milk

Pasteurized and homogenized long-life cow's milk with 1.5% fat (low-fat) produced by Iran Dairy Industries Co. (Pegah) in 1-liter package was used.

2.3. Preparation of milk powder by freeze drying method

A freeze dryer was used to prepare the milk lyophilized powder. In this method, water is removed from biological, sensitive and valuable materials which are prone to damage or loss of their quality due to heat. One liter of 1.5% fat milk was equally divided into four flasks of the freeze dryer (250 ml each). A thin aluminium sheet was wrapped around the glass containers of the device in order to protect milk from light. Once the milk powder was completely dried, the product was removed from flasks and were ground using a mortar until a smooth, uniform powder was obtained.

2.4. Preparation of milk ointment

The lyophilized, smoothed powder of milk was added to eucerin as the base of ointment to prepare 2%, 5%, and 10% (w/w) low-fat milk ointments. The desired amount of milk powder was added to eucerin and was mixed to obtain a uniform ointment. This can be an acceptable standard to evaluate the effects of low-fat milk on wound healing, and thus, the effect of milk can be analyzed based on dose-response.

2.5. Preparation of 1% phenytoin ointment

Phenytoin was prescribed for the positive control group. The pure powder of phenytoin was purchased from DarouPakhsh pharmaceutical company (Tehran, Iran). It was then used to prepare 1% (w/w) eucerin-based ointment as described for milk ointments.

2.6. Experimental design

Animals were randomly divided into 6 groups of 6-member. The first group had no treatment as a negative control group. The second group received eucerin base as the sham group. In the positive control group, phenytoin ointment 1% was used as the standard compound for wound healing. Test groups were treated with 2%, 5% and 10% milk ointment. The wound of animals in each treatment group was dressed twice daily with the treatment compound. To avoid wound infection, animals' beds in cages were changed daily.

2.7. Creation of full thickness wound

The method of Hemmati and Mohammadian (2000) [15] was used to create a model of full thickness wound. The hair of the desired location at the back near the spine was shaved with an electric shaver. Then, it was washed with tap water and disinfected with povidone-iodine solution (Betadine®). The animal was placed in a crouching position. Then, a square of $20 \times 20\ mm^2$ was drawn using a metal sterile template and a fine tip color-stable marker. Local anesthesia was induced through subcutaneous injection of lidocaine to the 4 corners of the square. Scalpel blade no. 15 was used to deeply cut the skin along the square sides up to the thickness of dermis and hypodermis. To create a full-thickness wound, the skin at the wound site was separated from the underlying layers using surgical scissors and forceps. Bleeding was then controlled by applying sterile sponges, and the wound surface was irrigated gently with normal saline. The wound outline in each animal was drawn on a transparent plastic sheet sterilized with 70% alcohol using a fine tip color-stable marker; this was considered as the