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RESEARCH ARTICLE

Influence of Electroacupuncture on the Soft Tissue Healing Process

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Abstract
The aim of this study was to determine the effect of bipolar electroacupuncture (EA) on a soft tissue defect in rabbits. Ten clinically healthy New Zealand white rabbits were divided into two groups: the control group (Group C, $n = 5$) and the experimental (EA) group (Group T, $n = 5$). During neuroleptanalgesia, defects of soft tissue (skin and muscle) were made at the dorsum site on the rabbits in both groups, and those defects were stimulated using EA. The biopsy samples were collected on Day 2, Day 4, and Day 6, prepared for histology, and examined microscopically. On the 2nd day, in Group C, the inflammatory degree was higher than it was in Group T; on subsequent days, low or identical degrees of inflammation were observed in both groups. Proliferative fibrous activity was increased on Day 4 for Group T and identical for both groups on Day 6. The dynamics of the epidermal thickness were characterized by a high rate on Day 2, Day 4, and Day 6 for Group T. EA facilitates a low tissue mechanical stress and has a positive effect on the healing of muscular defects. EA enhances the healing process, with no side effects.

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1. Introduction

The use of acupuncture in medical therapy is mostly based on the analgesic and anti-inflammatory effects accomplished while the soft tissues are being stimulated by using needles [1,2]. Soft tissues represent one of the most important "bridges" between effects triggered by the acupuncture needles and the nervous system.

Experimental studies on the use of electroacupuncture (EA) in the healing process for tissues show contradictory results with regard to improving and accelerating soft tissue healing [3—6]. EA carried out on the soft tissues of dogs immediately after surgical intervention, had no influence on the degree of recovery [7]. The use of an EA stimulus on different types of tissue was found to produce an unsatisfactory outcome [8]. EA has been shown to have a significant influence experimentally on the regeneration and the reconstruction functions of nerves and muscles [9]. Current studies show that the initial inflammatory response leads to an influx of neutrophils and macrophages, which play a crucial role in supplying cytokines of the growth factor and nitric acid, thus determining the migration of keratinocytes to the afflicted epithelium [10].

In this study, the authors used bipolar EA stimulation in 20-minute sessions; that is, the flow of electrical charge was induced in two different predominant directions, by manually switching the polarity. Even though modern EA apparatuses are designed to emit a bidirectional stimulus during the stimulation to avoid electrolysis, in some EA apparatuses, the intensity of the electric stimulus is higher for one direction of the electric charge, which causes more charge to move in one direction. The higher motion of the electric charge in one direction is responsible for the preferential electric stimulation for that direction. The effect of bipolar electric stimulation applied to a tissue defect has not, to the best of the authors' knowledge, been mentioned in any other studies involving tissue defects.

This study involves the effect of bipolar EA in an experimental study of soft tissue defects (skin and muscles) in rabbits. The essential aim of this study was to demonstrate the influence of bipolar EA on the skin and the superficial muscular layer involved in the healing of a tissue defect, by analyzing the importance of the inflammatory process, fibrosis proliferation, and epithelial proliferation in wound management.

2. Material and methods

This study was performed according to the guidelines on the use of living animals in scientific investigations. All experiments were approved by the Ethical Committee of the Faculty of Veterinary Medicine, Cluj-Napoca, Romania (No. 10473/24.07.2012).

The experiments were conducted using 10 2-year-old New Zealand white rabbits (Micro-farm rabbits, Mr. Petru Pestean, Cluj-Napoca, Romania) weighing between 2.5 kg and 2.8 kg. The rabbits were separated into two groups: five rabbits in the experimental (EA) group (test group) and five rabbits in the control group (NLA). Using rabbits ensured a large skin area for the placements of both of the acupuncture needles with a deep insertion (2 cm depth) and the electrodes to monitor the physical parameters. Also, the results obtained in rabbits can be extrapolated to other species (e.g., horses). In rabbits, we managed to form homogenous groups based on genetic origin, age, weight, etc.

The clinical functions, including internal temperature (t, °C), heart activity (HR), and respiratory activity (R) were recorded using an Infinity Delta system (Draeger Medical Systems, Inc., Telford, USA). The EA device was an AWQ-104E multipurpose electronic acupunctoscope (T.E.N.S. (TENS PLUS IND. CO., Kln., Hong Kong)). Other equipment required in this experiment included acupuncture needles (Natural 0.2/25 mm (Shanghai Xinhua E-General Merchandise Co., Ltd, Shanghai, China)), a biopsy instrument 4 mm Φ (Integra Miltex, Plainsboro, USA), anesthetics (halothane, ketamine 50 mg/kg, intramuscular, and xylazine 5 mg/kg, intramuscular), an antibiotic (enrofloxacin 5%, 5 mg/kg, subcutaneous (SC Pasteur, România)) and a formaldehyde solution to preserve the biopsy samples.

The experimental plan was designed to ensure that general anesthesia could be used to form tissue defects and to collect biopsy samples in three different stages of the wound healing (Day 2, Day 4, and Day 6). It also allowed multiple tissue defects (3/rabbit), including skin and muscle defects, to be formed in the rabbits and those defects to be stimulated by EA treatments at the sites of the tissue defects. Under the plan, biopsy samples were collected on the 2^{nd} day, 4^{th} day, and 6^{th} day of EA stimulation; two histological staining techniques could be used to obtain broad outlines of the cell structures and histopathological examinations (skin and muscle) could be conducted to assess the inflammatory degree, subepithelial proliferation, and epidermal thickness.

For both groups (control and test), neuroleptanalgesia was essential for forming the tissue defects on the initial day of the experiment, after the rabbits had been generally anesthetized and for stimulating the defects by using EA. Halothane as a general anesthetic was used in order to collect the biopsy samples on Day 2, Day 4 and Day 6.

The experimental soft tissue defects were formed by using a biopsy instrument to cut a round fragment of skin and muscle tissue (4 mm diameter, 5 mm depth) (Fig. 1A). The topographies of the soft tissue defects were established paravertebrally (1.5 cm to the midline) on the dorsal thoracic area between T1 and T8. An imaginary line through the needles inserted at the three defects was parallel with the midline. Soon after the defects had been formed, all defects were flanked by two acupuncture needles. The settings of the EA device and the local stimulation procedure for the soft tissue were designed especially for this study (Table 1).

Tissue defects were not sutured. In both groups on the same day, an antibiotic (enrofloxacin 5%) was administered to prevent local infection. The biopsy samples were collected on Day 2, Day 4, and Day 6 of EA stimulation. Biopsy samples obtained from cutaneous defects were fixed in 10% phosphate-buffered formalin solution for 24 hours, embedded in paraffin wax, cut into 4 μm sections, and stained with hematoxylin and eosin. Masson's trichrome staining was also used for collagen fiber evaluation. Three slides from each biopsy sample were collected randomly, and the cuttings involved intact tissue areas, including the defect or only the intact tissue, from an area with a radius of 0.3 cm surrounding the defect.