Effects of Low-Frequency Pulsed Electromagnetic Fields on Wound Healing in Rat Skin

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Abstract

Background—Pulsed electromagnetic field stimulators are used for promotion of healing in various conditions such as bone, cartilage and ligament injuries, but there are controversies about the use of these stimulators for skin wound healing.

Objective—The aim of this study was to investigate the effects of different pulse rates of pulsed electromagnetic fields (PEMF) on skin wound healing in rats.

Methods—Forty-eight male Wistar rats were used in this study. After anaesthetizing and shaving, a paired full thickness incision wound (35 mm in length) was made on each side of the dorsal midline of the rats. The animals were divided into eight groups (n=6) namely control, sham and six treatment groups which were exposed to 10, 20, 40, 50, 60, and 80 Hz pulse rates.

Treatment groups were exposed to PEMF (4 mT) for 30 minutes twice a day for ten days after surgery. Wound healing was evaluated by measuring the maximum length, the surface area of the wounds and the healing fractions every two-days. The full contraction period of the wounds and the tensile strength of scars were also measured.

Results—Results showed that: I -The absolute and normalized length of wounds in animals receiving PEMF (20 Hz) were significantly less than that of the sham (P<0.01). II -The wound healing duration in this group (12.7 days) was significantly shorter than that of the sham group (P<0.01). III - The wound tensile strength in this group was significantly greater than that of the sham group (P<0.01).

Conclusion—We can conclude that PEMF with 20 Hz pulse rate is effective in promoting of wound healing.

Keywords • PEMF • wound healing • electromagnetic field

Introduction

The process of regeneration and repair following injury represents one of the most fundamental defense mechanisms of an organism against environmental damage. Biological processes such as inflammation, proliferation, wound contraction and remodeling lead to the scar formation.1

Certain systemic diseases, such as injuries to the nervous system, metabolism and aging, have negative influence on the healing process, and lead to chronic wound formation. Measurements show that injured tissue is characterized by a higher healing potential as compared to intact skin.2 It is believed that this electric field plays its role in healing by guiding cellular movements that close wounds. It has been shown that they fields can affect the orientation, migration and proliferation of cells such as fibroblasts and keratinocytes, which are of key importance in healing.3

The use of low-frequency pulsed electromagnetic field (PEMF) for selective control of cellular function has given biology and medicine a new dimension. There are successful results in the use of low-frequency PEMF in the treatment of ununited fractures, 4 however, its effects on soft tissue have not yet been established. PEMF stimulators with different characteristics were designed to induce voltages similar to those produced normally, during mechanical deformation of connective tissue.5
Researchers have used PEMF stimulators to promote healing, but the results differ significantly from one another and there are discrepancies between the use of PEMF parameters.\(^6\) Further studies are needed to determine the optimal PEMF parameters for the acceleration of wound healing. This study was designed to compare the different PEMF pulse rates on skin wound healing in rats.

**Material and Methods**

**Animal Preparation**

Forty-eight Wistar male rats weighing 200 – 300 g were used. They were kept in standard cages, and food and water were available to them without any restriction. The room temperature was also kept constant at 20 ± 2 C\(^\circ\). Anesthesia was performed by the intra-peritoneal injection of ketamine hydrochloride, 100 mg/kg, (chemical works of Gedeon Richter, LTD) and Pompeum, 10 mg/kg, (Bayer, LTD), 10 minutes before surgery. After shaving and cleaning, a pair of full-thickness incisions (35mm in length) was made parallel to and at a distance of 1.5 cm on each side of the dorsal midline. The animals were then divided into eight groups; namely the control, sham and six treatment groups (6 rats/group). The treatment groups were exposed to PEMF (4 mT) pulse rates of 10, 20, 40, 50, 60 and 80 Hz.

The sham-exposed animals were fixed inside the restrainer kept inside an unenergized coil (the same condition as the experimental animals). The PEMF flux direction was parallel to the incisions. The animals in the control group were wounded like other groups but were kept as such throughout the experiment.

**PEMF Signal Generation and Exposure**

The system was fed by a function generator (FG- 330 Iwatsu, Japan), producing a square wave with 2 ms pulse width. The output of the function generator was amplified by a home made audio amplifier connected to a coil made of 8400 turns of copper wire (0.75 mm) with 8-cm (i.d.) and 3.5 cm length, producing 4 mT field effective intensity on the surface of rat skin. The field intensity was measured by a Hall Effect Teslameter (HI-3550 Holaday-Indus, Germany). The PEMFs were applied to the treatment groups for 30 minutes twice a day for 10 consecutive days.

**Evaluation of Wound Healing**

To evaluate wound healing, various parameters, such as duration of healing, absolute and normalized length, surface area of the wound, and tensile strength were used. The planimetry method was as follows. A trace of each wound surface was made with tracing paper, and then stuck on a millimeter paper and the squares located inside the trace were counted. In addition, the wounds with larger surface area were measured by a digital planimeter (KP-90 Placom, Japan).

The maximum length and surface area were measured on the second day after surgery. Thereafter, this measurement was carried out every two-days until full healing occurred. The healing percentage or the normalized values were calculated by dividing the maximum length or surface area of the wounds by that measured on the 2nd day after surgery. The "duration of wound healing" was the time taken for full contraction of the wound. Twenty days after the surgery, the animals were killed by an overdose of ether and the tensile strength of each scar was measured. For every wound, a rectangular segment of the skin (5× 1 cm), perpendicular to the incision, was removed and a one centimeter width from the center of incision was taken (to ensure the exclusion of unincised skin of the removed segment). The subcutaneous tissue was carefully removed from each skin sample, moistened with a saline solution (0.9 % NaCl) and immediately taken to the tensiometer. The skin sample was connected to a bag of water by a clip at one end and to the tensiometer by a second clip at the other end. Water was added to the bag at a rate of 2 g/s (regulated by a valve) until complete healing.
rupture of the wound. The weight of the bag and water was then measured and noted as the "tensile strength".

**Results**

The healing time of the sham and control groups did not differ significantly. Fig. 1a shows the effect of various pulse rates of PEMF (4 mT) on wound healing. The animals in the treatment groups, which were exposed to PEMF with 20 and 10 Hz pulse rates, were significantly different from that of the sham group (P<0.02 and P< 0.05 respectively).

Fig. 1b shows the effect of various pulse rates (4 mT) on the tensile strength of scar tissue measured 20 days after the surgery. As seen, the tensile strength of the 20 and 80 Hz pulse rate groups are much higher than that of the sham group (P<0.01 and P< 0.05 respectively).

Variation of the wound length is shown in Fig. 2a. Statistical data analysis (2-Sample Kolmogorov-Smirnov) shows significant difference between these two groups.

Wound healing percentage was calculated using the equation \([L_2 - L_1]/L_2 \times 100\), where \(L_2\) and \(L_1\) are the maximum wound lengths on the second and any other day respectively. Fig. 2b shows the variations of wound healing percentages per day for the 20 Hz and sham groups. These two distributions differ significantly (K=1.63, P<0.01). The variations of wound healing percentages per day were calculated based on wound surface areas, \([(S_2 - S_1)/S_2] \times 100\), where \(S_2\) and \(S_1\) are wound surface areas on the second and any other day respectively. The distribution of wound healing percentage for the 20 Hz group was not significantly different from that of the sham group.

In brief, although wound healing time for the 10 Hz group (as shown in Fig. 1a) was much shorter than that of the sham group, none of the distributions of wound length and healing percentage per day showed significant variation between these two groups.

**Discussion**

Published reports indicated that the PEMF stimulators may promote wound healing, but this depends on parameters such as frequency, intensity, exposure time and orientation of PEMF. It was suggested that the suitable dosage and optimum PEMF parameters involved in wound healing be found.7-9 The purpose of this study was to investigate the effects of pulse rates of PEMF on skin wound healing in rats.

The animals in the sham group were kept in the restrainer inside the unenergized coil. These conditions may increase stress or temperature of animals, they did not affect wound healing time significantly and it was seen that the animals adapted to these conditions very soon.

It has been shown that in vitro PEMF exposure favors collagen production by fibroblasts and in vivo it increases the tensile strength of scar tissue in the rat.10-12

Our results show that treatment with PEMF (4 mT) stimulators having 20 Hz pulse rate, decreases wound healing time and increases the tensile strength of scar tissue. This indicates a window in the range of 10 to 80 Hz pulse rates.

The increased tensile strength of the scar tissue is due to the increase in synthesis, alignment and maturation of collagen.13

Goldman et al.14 have shown enhancement of fibroblast proliferation in response to certain
conditions of the electric field (41 mv/m, 10 Hz) and Katsir et al.\textsuperscript{11} showed that chick embryo fibroblast proliferation was modified by EMF (60 Hz, 0.7 mT).

Berg et al.\textsuperscript{15} showed that the proliferatory response of yeast to electromagnetic field is frequency dependent and there is a window so that a high proliferation is detected at 15 Hz and a minimum proliferation at 40 Hz. These reports confirm our results in that there is a window between 10 to 80 Hz pulse rates (which is around 20 Hz). The significance in the absolute values and the normalized wound maximal lengths for the 20 Hz group compared to the sham group, verifies that the PEMF with 20 Hz pulse rate accelerates wound healing. Our results on the wound surface area indicate that PEMF stimulation does not affect wound contraction in rats which is in keeping with Scardino et al.\textsuperscript{5} that showed PEMF had a positive effect on epithelilization only.

Based on the results obtained in this study it could be concluded that PEMF stimulators (which could later become a practical tool for clinical use) are effective devices for the acceleration of wound healing but further studies are required to determine the optimal frequency, direction and intensity of the PEMF to be used in soft tissue healing and two clarify the exact mechanisms in involved in this phenomenon.

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**References**