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Microcirculatory effects of pulsed electromagnetic fields

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Abstract

Purpose: Pulsed electromagnetic fields (PEMF) are used clinically to expedite healing of fracture non-unions, however, the mechanism of action by which PEMF stimulation is effective is unknown. The current study examined the acute effects of PEMF stimulation on arteriolar microvessel diameters in the rat cremaster muscle. The study hypothesis was that PEMF would increase arteriolar diameters, a potential mechanism involved in the healing process.

Methods: Local PEMF stimulation/sham stimulation of 2 or 60 min duration was delivered to the cremaster muscle of anesthetized rats. Arteriolar diameters were measured before and after stimulation/sham stimulation using intravital microscopy. Systemic hemodynamics also were monitored during PEMF stimulation.

Results: Local PEMF stimulation produced significant ($p < 0.001$) vasodilation, compared to pre-stimulation values, in cremasteric arterioles in anesthetized rats ($n = 24$). This dilation occurred after 2 min of stimulation (9% diameter increase) and after 1 h of stimulation (8.7% diameter increase). Rats receiving "sham" stimulation ($n = 15$) demonstrated no statistically significant change in arteriolar diameter following either "sham" stimulation period. PEMF stimulation of the cremaster ($n = 4$ rats) did not affect systemic arterial pressure or heart rate, nor was it associated with a change in tissue environmental temperature.

Conclusions: These results support the hypothesis that local application of a specific PEMF waveform can elicit significant arteriolar vasodilation. Systemic hemodynamics and environmental temperature could not account for the observed microvascular responses.

Keywords: PEMF; Microcirculation; Rats; Vascular mechanisms

Introduction

Pulsed electromagnetic fields (PEMF) promote fracture healing in non-unions and have significant biological effects in other tissues such as the heart [2], connective tissue [1], and the growth rate of the chick embryo [22]. The molecular mechanism of action of PEMF stimulation has not yet been determined. Several studies have suggested that PEMF elicits vascular changes in vivo [2,12,25]. A recent study by Roland et al. demonstrated significant neovascularization of an arterial loop created subcutaneously in the rat in response to chronic PEMF stimulation [21].

The current investigation examined the effect of a PEMF waveform that has been shown to be clinically effective in healing fracture non-unions. The microvasculature of the striated cremaster muscle of the rat was studied before and after localized PEMF stimulation to examine whether or not acute (2 min) or short-term (1 h) PEMF stimulation affected microvascular diameters. PEMF exposure was restricted to the cremaster microvasculature to limit systemic cardiovascular effects. Because one mechanism for eliciting an angiogenic response is associated with vasodilation, the study hypothesis was that PEMF stimulation localized to the cremaster muscle would elicit a measurable change in arteriolar diameter.

Materials and methods

Pulsed electromagnetic field

The PEMF waveform used clinically in the Physio-Stim PEMF device (Orthofix, McKinney Texas) was replicated using a Hewlett Packard waveform generator (model 33120A; Hewlett Packard, Loveland, CO, USA) connected to a pair of parallel Helmholtz coils 30 mm in diameter. The coils were placed 14 mm above and below the isolated cremaster muscle of the rat (Fig. 1). The PEMF waveform is illustrated in Figs. 2 and 3. PEMF field strength measured in the

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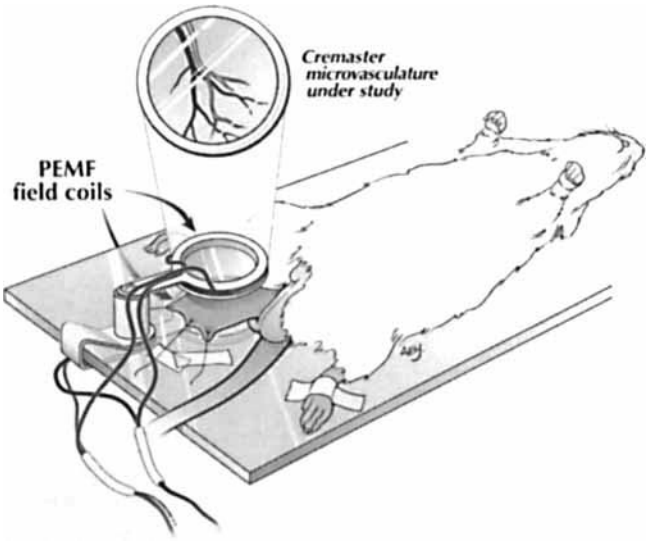

Fig. 1. Illustration of the rat cremasteric preparation used for studying the microvascular effects of PEMF. The location of the Helmholtz field coils above and below the cremaster muscle also is illustrated. The acrylic plastic board upon which the specimen is studied is placed on the stage of a Leitz LaborLux 12 compound microscope for intravital microscopic measurement of arteriolar diameters. Used with permission, Wake Forest University School of Medicine Orthopaedic Manual. Winston-Salem, NC: Orthopaedic Press.

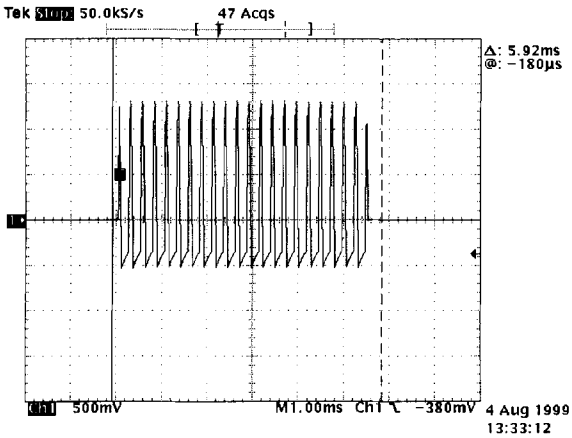

Fig. 2. Burst of Physio-Stim PEMF as measured within the field stimulation coils.

apparatus was a positive amplitude of 18.8 T/s and a negative amplitude of 8.0 T/s. The waveform produced by this system was verified *at the location of the cremaster* in each experiment using an electromagnetic field probe and a oscilloscope (model TDS 220; Tektronix, Wilsonville, OR, USA).

PEMF does not create any heat within the target tissue because it is not a radiofrequency and falls into the electromagnetic spectrum below the range of infrared radiation. The PEMF coils per se, however, have the potential to generate heat during activation since a electrical current is applied to a coil of wire with an inherent resistivity. The potential for heat generation by the coil apparatus used in the present experiment was tested using a two channel thermistor system (model 4000A, thermistor probes 427; Yellow Springs Instrument Co., Yellow Springs, OH) with one thermistor recording coil surface temperature and the other thermistor recording ambient temperature on the rodent surgery board 10 cm from the cremaster pedestal. The coil was tested

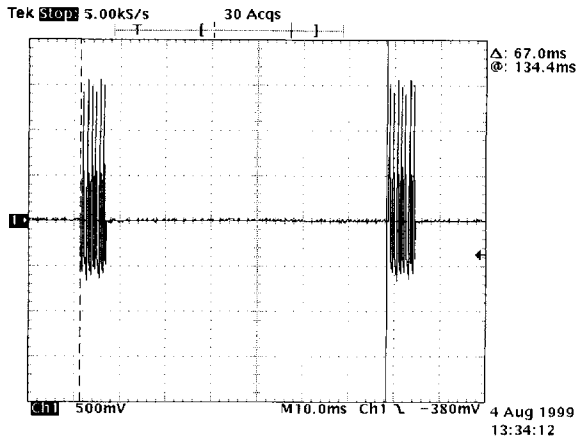

Fig. 3. Burst interval of Physio-Stim pulsed electromagnetic waveform as measured within the field coils.

over a 2 min activation period and a 4 min period immediately following activation with the Physio-Stim PEMF signal. An additional test was performed placing a thermistor on the pedestal used for microscopic observations with a ambient temperature reference thermistor placed on the rodent surgery board 10 cm from the pedestal. The temperature at both sites was measured before, during, and after coil activation of 2 or 60 min.

Animal model

All protocols were approved by the institutional animal care and use committee. Male Sprague–Dawley rats (Zivic Miller, Zelienople, PA, USA; $n = 43$) weighing 98–160 g were studied. The animals were anesthetized with an intraperitoneal injection of urethane (1000 mg/kg body weight). This anesthetic is used because it maintains microvascular tone. The right cremaster muscle was prepared for microscopic evaluation according to the method of Dusseau et al. [5]. Briefly, the right scrotum was opened, the cremaster muscle was dissected from the testicle, and the testicle was extirpated. The cremaster then was spread over an 18 mm diameter pedestal and covered with an 18 mm coverslip. The rat was placed on a compound microscope stage (LaborLux 12; Leitz, Wetzlar, Germany) and the cremasteric microvasculature was examined using a 10× objective (Wild, Heerbrugg, Switzerland; n.a. = 0.4) and a 40× objective (Zeiss, Jena, Germany, n.a. = 0.65). Following surgical preparation of the cremaster muscle, every animal was allowed to stabilize for 1 h before the experimental protocol was begun. Microvascular dimensions were measured from video images captured with a high resolution black and white video camera (Pulnix TM745E, Subtechnique, Alexandria,VA) using a calibrated video dimension analyzer (*CIM Solutions, Advance, NC*). This instrument uses an adjustable video cursor with a digital readout of the measurement. The operator aligns the video cursor with the inner lumen of the blood vessel and records the digital measurement displayed.

Experimental protocol

Two arterioles (third or fourth order) of each cremaster muscle were studied. These arterioles were selected based upon their size and optical clarity. Baseline measurements of arteriolar diameter were performed using the measurement techniques noted above followed by exposure to PEMF or sham-PEMF. For sham-PEMF exposure, the animal was prepared for study as described previously; however, the electromagnetic coils were not connected to the waveform generator.

The experiments were performed in the following manner. Measurements of microvascular diameter were measured before PEMF stimulation. These pre-stimulation values were recorded as initial baseline diameters. The electromagnetic coils then were activated for 2 min, and measurements of arteriolar diameter were made immediately after cessation of stimulation, at 2 min post-stimulation, and at 5 min post-stimulation. After an additional 5 min, the stimulation/recording

paradigm was repeated. The animal then was allowed to rest for 30 min, after which new measurements of pre-stimulus diameter were performed. PEMF stimulation or sham-PEMF stimulation then was initiated for 1 h. Post-stimulation measures of diameter were performed immediately and at 10 and 20 min post-stimulation. At the end of the experiments, the rat was euthanatized.

An additional study was performed to examine whether or not PEMF stimulation of the cremaster muscle elicited any systemic hemodynamic response in systemic arterial pressure or heart rate. Rats ($n = 4$) were anesthetized and prepared for experimental observations as above. In addition, the femoral artery was cannulated with a polyethylene catheter (PE50, Clay Adams, Sparks MD) to monitor arterial pressure using a recording oscillograph (model WR7700, Western Graphtech, Irvine, CA). The arterial pressure and heart rate were monitored before and after PEMF stimulation of the cremaster muscle using the stimulation waveform parameters noted above for a period of 2 min.

Data analysis

The microvascular effects of PEMF stimulation were studied in 24 rats; the microvascular effects of sham-PEMF were studied in an additional 15 rats. The maximal arteriolar diameter changes associated with PEMF stimulation or sham-PEMF stimulation were compared to pre-stimulation values in each animal using Student's t-test for paired data. The systemic hemodynamic effects of cremaster PEMF stimulation were detailed before and after PEMF stimulation using descriptive statistics.

Results

PEMF stimulation of the cremaster muscle in the rat resulted in significant changes in arteriolar diameters compared to baseline values. Conversely, sham-PEMF stimulation did not elicit changes in arteriolar diameter in the rat cremasteric arterioles. The mean values of the arteriolar diameters and the standard error of the mean index of variability (SEM) for both PEMF and sham-PEMF stimulation are presented in Table 1.

The vasodilatory microvascular effects appeared to be cumulative because the arteriolar diameters at the end of the PEMF sessions were significantly larger than the baseline diameters. The sham stimulation did not produce any long-term change in arteriolar diameters over the same time period. An analysis of vessel size-dependent changes was performed because of a possibility of a size-dependent response to the PEMF. This analysis revealed no differences in response to PEMF between small (less than 30 μm diameter) and large (greater than 30 μm diameter) arterioles.

Table 1
Mean values of cremasteric arteriolar diameter (in μm), reported with standard error of the mean, recorded before and after 2 min or 1 h of PEMF ($n = 24$ rats) or sham-PEMF ($n = 15$ rats) stimulation

	2 min of exposure		60 min of exposure	
	Before	After	Before	After
PEMF	32.0 ± 2.3	34.9 ± 2.7***	36.7 ± 2.7	39.9 ± 2.7***
Sham-PEMF	48.3 ± 3.0	48.8 ± 3.0	47.9 ± 3.2	47.8 ± 3.4

*** $p < 0.001$.

Table 2
Mean values of mean systemic arterial pressure and heart rate reported with standard error of the mean, before and after 2 min of PEMF stimulation of the cremaster muscle of the rat ($n = 4$ rats)

	Before PEMF	After PEMF
Heart rate (beats per min)	234 ± 17.9	235 ± 20.5
Mean arterial pressure (mmHg)	83.0 ± 2.6	83.8 ± 3.1

Two minutes of PEMF stimulation of the cremaster muscle did not elicit a noticeable change in systemic hemodynamics, either heart rate or mean arterial pressure, after 2 min of exposure. These results are summarized in Table 2.

There were no measurable temperature changes on the PEMF Helmholtz coil or on the cremaster pedestal during periods of coil excitation equivalent to experimental exposure with the PEMF signal. This suggests that excitation of the Helmholtz coil does not result in the production of thermal energy that could alter the microvascular dimensions under observation.

Discussion

PEMF is a therapeutic agent that elicits a biological effect independent of any thermal influence or observable physical interaction with the tissue. Earlier studies suggest that PEMF induces weak ionic currents in the tissue [3], possibly leading to stimulation of signaling pathways at the cell membrane level [1], a concept supported by the observation that PEMF can alter cAMP metabolism via alterations in calcium flux in osteoblast cell culture [4]. PEMF promotes healing in fresh fractures [11] and fracture non-unions [9] and stimulates skeletal development in the developing chick embryo [22]. PEMF has been demonstrated to enhance osteointegration of hydroxyapatite into cancellous bone following 6 weeks of once-daily stimulation [6] and facilitate soft tissue healing in injured ligaments [8,17], tendons [24], and cartilage [13,20], as well as overall wound healing [18]. PEMF also stimulates the growth of vascular endothelial cells in culture [26]. Greenough et al. [12] have demonstrated that a pulsed burst PEMF waveform increases the rate of capillary growth in chronically implanted rabbit ear chambers. This observation supports studies that demonstrated an increased rate of epithelialization of open wounds following treatment with PEMF [23]. However, the absolute rate of wound healing was not significantly different between treated and control groups, a finding similar to that reported by Glassman et al. [10] for PEMF treatment to promote wound healing in rats.

Pre-treatment with PEMF results in enhanced regeneration of the sciatic nerve in an experimentally injured rat model [15]. The mechanism of this effect could

be mediated through potentiation of the effects of insulin-like growth factor-one (IGF-1), because IGF-1 has been shown to stimulate nerve regeneration in a similar model [16]. Fitzsimmons and Baylink [7] have demonstrated that PEMF increases IGF-II receptor numbers.

The present study demonstrated that the Physio-Stim PEMF waveform elicits a physiologic response of microvascular dilation in the cremaster muscle of the rat. This response is rapid in onset (following only 2 min of stimulation) suggesting that it does not require the expression of a gene for elaboration of a specific protein to produce an effect, at least for the response noted in the present study. Although the percent increase in diameter following PEMF stimulation appears modest, it is important to recall that resistance to blood flow, as defined by Poiseuille, is inversely proportional to the radius of the vessel, to the fourth power (Eq. (1)).

$$\text{Resistance} = \frac{8\mu \text{ length}}{\pi \text{ radius}^4} \quad \text{where } \mu \text{ is viscosity} \qquad (1)$$

Therefore, because arterial flow to a tissue is determined by arterial pressure divided by resistance, a small increase in the vessel radius translates into a large decrease in resistance and a dramatic increase in flow. The PEMF signal was applied only to the cremaster muscle. Additional studies demonstrated that the PEMF signal did not produce any systemic effects on arterial pressure or heart rate. Furthermore, the effects of PEMF appear to be cumulative because the arterioles demonstrated an approximate 25% overall increase in diameter over the experimental time-course. This effect compares with no measurable change observed in microvessel diameter in the sham-PEMF group (48.3 ± 3.0 μm versus 47.8 ± 3.4 μm) over the same period. The cumulative effects of PEMF stimulation will require further study in order to clarify the mechanism(s) through which this cumulative effect occurs. The mechanism responsible for either the short term or long term effects is unknown. The possibility that PEMF, or the Helmholtz coils used to generate the PEMF signal, warms the tissue resulting in a temperature-related vasodilation is not supported by the temperature measurements of both the Helmholtz coil and the pedestal upon which the cremaster was studied. Temperature increases were not measured during or after PEMF activation at either location.

The present findings are relevant to the study by Roland et al. [21] who demonstrated a significant increase in neovascularization (angiogenesis) in an in vivo model of neovascularization in which rats received daily PEMF stimulation for 8–12 weeks. However, the acute vascular effects of PEMF were not investigated by these authors. Other microvascular studies suggest that persistent dilation of arterioles can lead to angiogenesis [14,27]. Milkiewicz et al. [19] have suggested that the transduction mechanism from arteriolar vasodilation to angiogenesis is modulated by alterations in shear force. A similar mechanism could account for the microvascular observations following acute and long term PEMF exposure.

The observations obtained in the microvascular model used in the present study necessarily are restricted to male rats. The cremaster model has been used extensively for study of the striated muscle microcirculation and is considered to be representative of striated muscle microvasculature in general. Gender differences in microvascular responses to PEMF stimulation were not examined.

Several PEMF signals are used clinically. Other PEMF signals must be tested in order to determine whether the vascular responses elicited by PEMF in the present experiment represent a generalized response to PEMF or a specific response to a selective waveform. It is likely that specific responses are associated with specific waveforms, as Saha et al. have suggested [22]. The elucidation of the mechanism(s) responsible for either response is crucial.

Summary: The current study supports the hypothesis that local application of a specific PEMF waveform can elicit significant arteriolar vasodilation. Systemic hemodynamics and environmental temperature could not account for the observed microvascular responses.

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