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Effects of 50 Hz sinusoidal electromagnetic fields of different intensities on proliferation, differentiation and mineralization potentials of rat osteoblasts ☆

Jian Zhou [a], Lei-Guo Ming [a], Bao-Feng Ge [a], Jia-Qi Wang [a], Rui-Qing Zhu [a], Zhe Wei [b], Hui-Ping Ma [c], Cory J. Xian [d], Ke-Ming Chen [a,*]

[a] Institute of Orthopaedics, Lanzhou Command of CPLA, Lanzhou 730050, People's Republic of China
[b] Department of Medical Engineering, Lanzhou Command of CPLA, Lanzhou 730050, People's Republic of China
[c] Department of Pharmacy, Lanzhou General Hospital, Lanzhou Command of CPLA, Lanzhou 730050, People's Republic of China
[d] Sansom Institute for Health Research, School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, SA 5001, Australia

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ABSTRACT

Electromagnetic fields (EMFs) have been used clinically to slow down osteoporosis and promote fracture healing for many years. However, the underlying action mechanisms and optimal parameters of the EMF applications are unclear. In this study, we investigated the effects of treatment for different durations with 50 Hz sinusoidal electromagnetic fields (SEMFs) at different intensities on proliferation, differentiation and mineralization potentials of rat osteoblasts. Osteoblasts isolated from neonatal rats were treated with SEMFs (50 Hz at 0.9 mT–4.8 mT, 0.3 mT interval, 30 min/day up to 15 days). Compared to untreated control, SEMFs inhibited osteoblast proliferation (after 3 days' treatment) but increased alkaline phosphatase (ALP) activity (after treatment for 9 days) from 0.9 mT to 1.8 mT, declined from 1.8 mT until 3.0 mT, and then increased again from 3.0 mT to 3.6 mT and decreased once again from 3.6 mT to 4.8 mT. Numbers of colonies stained positive for ALP after 8 days and mineralized nodules stained by Alizarin red after 10 days showed the same bimodal tendency as with the ALP activity, with two peaks at 1.8 mT and 3.6 mT. SEMFs also bimodally increased *Runx-2*, *Col1α2* and *Bmp-2* mRNA expression levels in osteoblasts at 12, 24 and 96 h after exposure. The results indicated that while exposure to 50 Hz SEMFs inhibits the osteoblast proliferation, it significantly promotes differentiation and mineralization potentials of osteoblasts in an intensity-dependent manner with peak activity at 1.8 mT and 3.6 mT.

Introduction

Electromagnetic fields (EMFs) have been widely used in treatment of osteoporosis in recent years [1]. EMFs can improve bone mineral density (BMD) and biomechanical properties and decelerate the bone resorption process in vivo [2–4]. Several studies and randomized controlled trials have demonstrated that most patients with primary osteoporosis can get pain alleviation after 30–60 days of treatment of EMFs [5–7]. EMFs enhanced osteogenesis and bone healing in patients with bone non-unions, and were used as an adjunct to assist postsurgical healing for spinal fusion [8]. The Food and Drug Administration of USA approved EMFs as a safe and effective method for treating osteoporosis and as a therapy for bone non-unions [9,10].

Despite a long history of clinical use of EMF and some mechanistic studies, the underlying action mechanisms of EMFs and their optimal parameters for usage remain poorly understood. It has been found that EMFs modulate proliferation and differentiation of human oral keratinocytes and rat glioma cells [11–13], and alter morphology and intracellular calcium levels of human astrocytoma cells and rat calvarial osteoblasts [14–16]. While pulsed electromagnetic fields (PEMFs) were reported to cause decreased proliferation of human osteoblast-like cells (MG63) [17], it appeared to induce cell proliferation and promote extracellular matrix production and mineralization [18]. The reasons for the conflicting results are currently not clear. We found that different results are usually obtained by EMFs at different frequencies or intensities, and some previous studies had observed nonlinear window effects with respect to the waveform, frequency, amplitude, duration, cell type, cell age and treatment [19–21]. To obtain the best therapeutic effects of EMFs, the optimal parameters should be defined and used. The current study sought to determine the optimal intensity for 50 Hz sinusoidal electromagnetic fields (SEMFs) by analyzing their effects on proliferation and differentiation of primary osteoblasts.

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* Corresponding author. Fax: +86 931 8994950.
E-mail addresses: zhoujian715412@126.com (J. Zhou), mingleiguo8307@163.com (L.-G. Ming), dibbie@126.com (B.-F. Ge), wangjiaqi0506@126.com (J.-Q. Wang), darqing_zhu@yahoo.com.cn (R.-Q. Zhu), mahuipingcxr@yahoo.com.cn (H.-P. Ma), cory.xian@unisa.edu.au (C.J. Xian), chkeming@yahoo.com.cn (K.-M. Chen).

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Materials and methods

Reagents

Minimal essential medium (MEM) was obtained from Invitrogen (Auckland, Scotland, UK). Fetal bovine serum (FBS) was the product of Lanzhou National Hyclone Bio-Engineering Co. (Lanzhou, China). Collagenase II and trypsin were purchased from Gibco BRL (Gaithersburg, MD, USA). MTT (3-(4, 5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide), β-glycerophosphate, dexamethasone, and ASAP (ascorbic acid 2-phosphate) were all from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade.

EMF device

The device producing EMFs was self-made and consists of three main parts (Fig. 1): program control computer (Fig. 1A), power amplifier (Fig. 1B) and solenoid (Fig. 1C). The operation principle is shown in Fig. 1D. The program control computer generates digital signals (magnetic wave, intensity, and frequency). The digital-to-analog converters (USB4711A, Yanhua, Taiwan, China) convert digital signals to analog signals. The power amplifier amplifies the signals. The coils producing EMFs (solenoid) were placed in a CO_2 incubator (5% CO_2, 37 °C and 100% humidity) and connected to the power amplifier outside the cell incubator. The solenoid was composed of three cascade connected coils with a common core cylinder of acrylic tube (inner diameter 18.0 cm, height 21.0 cm). The winding number of enamel copper wire in the middle coil was less than those of the bilateral coils so that the EMFs produced by the solenoid have good equitability from either horizon (area from 30 mm to −30 mm error <1%) or vertical (area from 20 mm to −20 mm error <1%) axis. The device can produce a magnetic flux density range of 0.0–9.0 mT and a frequency range of 25–200 Hz. The EMFs could be sinusoidal, triangular, sawtooth and square wave. The device was calibrated by the Medical Measuring Station of Lanzhou. The 60-mm culture dishes containing osteoblasts (Nunc, Roskilde, Denmark) were placed in the area with good equitability within the solenoid (Nunc, Roskilde, Denmark) (Fig. 1C).

Osteoblast isolation and culture

Primary osteoblasts were isolated from skull bones of neonatal rats as described [22]. Briefly, the skull bone was rinsed by sterile phosphate buffered saline (PBS pH 7.4) containing antibiotics, minced to small pieces, and then digested by 5 mg/ml trypsin (twice, 10 min each) and then 1 mg/ml collagenase II (4 times, first time 10 min, other times 20 min), with the cells released in the second to fourth collagenase digestions being collected and supplemented with 2–3 ml medium (MEM, plus 10% heat-inactivated FBS, 100 U/ml penicillin and 1000 U/ml streptomycin). After the cell suspension was filtered through a 200-μm sieve to remove bone debris, the collected cells were seeded in 60-mm culture dish at a density of 3×10^3 cells/cm^2 with growth medium (the above medium plus 2 mM L-glutamine). Cells were cultured at 37 °C in 5% humidified CO_2 atmosphere. When the cells reached 70–80% confluence, they were washed with PBS, detached by 0.25% trypsin/1 mM EDTA and then sub-cultured at 3×10^3 cells/ml in 60-mm dishes and used for differentiation and proliferation assays.

SEMF exposure

The sub-cultured osteoblasts were randomly divided into 15 groups. Fourteen groups were exposed to SEMFs (50 Hz frequency) for 30 min/day under the following intensities: 0.9, 1.2, 1.5, 1.8, 2.1, 2.4, 2.7, 3.0, 3.3, 3.6, 3.9, 4.2, 4.5 and 4.8 mT respectively (with a 0.3 mT increment interval). One group was used as control cultured in same condition without SEMF treatment (0.0 mT). There were 6 parallel cultures for each intensity when cell proliferation was assayed. When osteogenic markers were analyzed, triplicate cultures were used for each intensity in every analysis.

MTT proliferation assay

To examine SEMF treatment effects on cell proliferation, the sub-cultured osteoblasts were seeded for 12 h in 60 mm culture dish and then treated with the SEMFs as above (n = 3/group). Cell proliferation was assessed by incorporation of MTT (3-(4,5-dimethyl-thiazol-2-yl)-

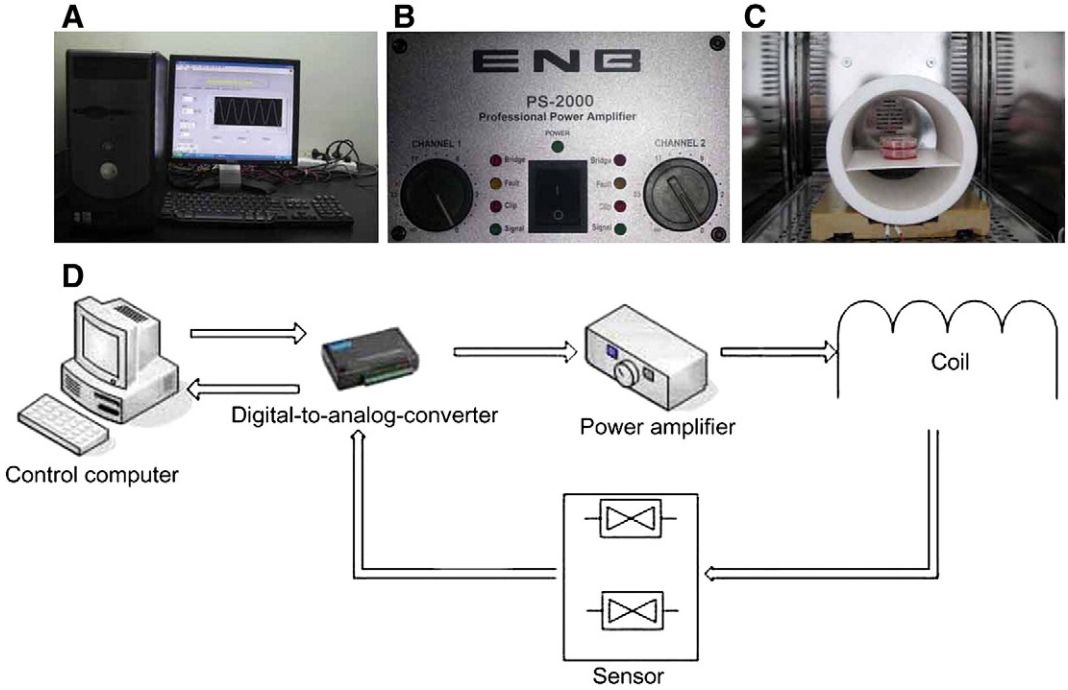

Fig. 1. Representation of the device used to generate the electromagnetic fields (EMFs). The device consists of three main parts: program control computer (A), power amplifier (B) and coils (C). The solenoid producing EMFs was placed in a 5% CO_2 incubator. (D) Diagram of the operation principle.