Effect of Weak Static Magnetic Fields on Endothelial Cells

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Pulsed electromagnetic fields (PEMFs) have been used extensively in bone fracture repairs and wound healing. It is accepted that the induced electric field is the dose metric. The mechanisms of interaction between weak magnetic fields and biological systems present more ambiguity than that of PEMFs since weak electric currents induced by PEMFs are believed to mediate the healing process, which are absent in magnetic fields. The present study examines the response of human umbilical vein endothelial cells to weak static magnetic fields. We investigated proliferation, viability, and the expression of functional parameters such as eNOS, NO, and also gene expression of VEGF under the influence of different doses of weak magnetic fields. Applications of weak magnetic fields in tissue engineering are also discussed. Static magnetic fields may open new venues of research in the field of vascular therapies by promoting endothelial cell growth and by enhancing the healing response of the endothelium. Bioelectromagnetics 31:296–301, 2010.

Key words: weak static magnetic fields; tissue engineering; endothelial cells

INTRODUCTION

There is a plethora of evidence of the successful use of pulsed electromagnetic fields (PEMFs) and its effects on biological systems. Most of the implementation of PEMFs occurs in the area of orthopedics and acute wound repair. PEMFs have been used clinically to accelerate bone growth of non-union fracture [Bassett et al., 1974, 1977, 1978]. In cellular studies, PEMFs stimulate the secretion of growth factors [Aaron et al., 2004a,b]. Electromagnetic fields have also found applications in wound healing [Yen-Patton et al., 1988; Callaghan et al., 2008]. Yen-Patton et al. reported an enhancement of growth rate on human umbilical vein cells (HUVECs) by weak pulsating electromagnetic fields. Greenbaum et al. [1991] showed an acceleration of endothelial cell migration to a wound in vitro. Tepper et al. [2004] demonstrated that PEMFs increased proliferation and tubulization in HUVECs in vitro. In vivo for an animal model, PEMFs increased angiogenesis. The authors suggested that PEMFs might accelerate bone growth by assisting the healing area with blood vessel growth. These reports and others on magnetic field effects on angiogenesis are discussed in detail in McKay et al. [2007]. The induced electric field was the source of the external stimulus in the studies mentioned above. Of all these reports, very few, if any, make reference to the magnetic fields established by the coils. This is common since it is accepted that the induced electric field is the dose metric and that the time varying magnetic field establishes the electric field. However, it is worth mentioning that the magnetic waveform in these studies is a square pulse of 5 ms duration and 20 G in amplitude modulated by a sawtooth wave.

On the other hand, Heermeier et al. [1998] reported an increase in collagen type I mRNA expressions in human osteoblastic cells when exposed to 20 Hz sinusoidal electromagnetic field. In that study, the coils generated a sinusoidal signal with magnetic field amplitude of 6 mT and a maximum induced
electric field of 0.113 mV/cm, 3 cm from the center of the Petri dish. In these studies, the induced electric field intensity was far below that used in the PEMFs studies. Rodemann et al. [1989] showed that normal human skin and lung fibroblasts in vitro exposed to the same 20 Hz sinusoidal signal induced the differentiation of mitotic to irreversibly postmitotic fibroblasts. These studies suggest that the dose metric is magnetic.

Although magnetic fields are more common in nature than electric fields, magnetic effects on biological systems are encountered less often in the literature compared to electric current-mediated responses. This is especially true for weak magnetic fields. Within this spirit, our work concentrates on weak static magnetic field effects on endothelial cells. The aims of this study are to evaluate the effectiveness of weak static magnetic fields on human umbilical vein endothelial cells (HUVECs) activity, and to examine the effects of the fields on functional parameters that are tied to key activities in the cells. A better understanding of the interplay between weak magnetic fields and endothelial cell function may contribute to the design of new vascular therapies or the prevention of undesired effects.

The rationale for pursuing this study stems from the possibility to broaden the scope of magnetic healing for the treatment of vascular diseases, which remain the leading cause of mortality and a heavy socioeconomic burden in westernized societies. Weak static magnetic fields may additionally provide a valuable tool for vascular tissue engineering where techniques based on the use of magnetic fields are increasingly gaining attention [Perea et al., 2006, 2007]. Beyond their function for cell delivery purposes, magnetic fields could help reduce the prolonged culture period of endothelial cells or promote the anti-thrombogenic function of the endothelium for successful use in tissue-engineered products [Ahsan and Nerem, 2005].

MATERIALS AND METHODS

Cell Culture

Human umbilical vein endothelial cells (passage 2–4, 2–4 weeks old) were cultured in endothelial growth medium (Promocell GmbH, Heidelberg, Germany) supplemented with 10% fetal calf serum (FCS), 0.004 ml/ml endothelial cell growth supplement/heparin, 0.1 ng/ml epidermal growth factor (EGF), 1 ng/ml basic fibroblast growth factor, and 1 μg/ml hydrocortisone at 37 °C with 5% CO₂. The cells were cultured in a 75 cm² flask to expand cell number. After reaching confluence, the cells were seeded in cell culture plates.

Magnetic Stimulating System

The study used Helmholtz coils to establish the magnetic fields. The coils were placed vertically on a Plexiglas shelf inside the incubator. The coils generating the static magnetic field were 18 cm in diameter, 10 cm height separation, and had 30 turns of 16 AWG magnetic wire and were driven by a DC power supply (HP 6205C Dual, Hewlett-Packard, Palo Alto, CA). The cells were placed in 6-well plates centered vertically between the coils. Six-well culture plates 8.5 cm × 12.5 cm were used throughout the experiment. The area of interest inside the coils consisted of a cube 8.5 cm × 12.5 cm × 6.5 cm; the area accommodated three 6-well plates stacked vertically. The intensity of the magnetic field within the volume of interest deviated up to 5% in the x–y plane as shown by a Matlab simulation. The magnetic protocol, unless otherwise stated, consisted of either 60 or 120 μT field intensity for 24 h per day while the control group was kept inside a μ-metal cylinder shielding the Earth’s magnetic field. The μ-metal cylinder contained holes allowing flow of air and CO₂ inside it. The magnetic field intensity inside the μ-metal cylinder registered from 0.2 to 0.5 μT by a gauss meter (FW Bell, Rochester, NY). The value of the background static magnetic field inside the incubator varied from 6 to 13 μT; these are the resultant fields in the x, y (plane of growth of cell), and z direction.

Cell Proliferation Assay

The effect of magnetic treatment on cellular proliferation was determined by direct count of cell numbers after 2, 3, and 4 days of stimulation for different experiments. For the cell counting assay, 6-well culture plates were seeded at a concentration of 8.0 × 10³ cells per well and incubated in 5% CO₂ at 37 °C for one day prior to subjecting the experimental group to weak static magnetic fields. After magnetic stimulation cycle, the cells in 3 wells per termination point were counted three times using a cell counter (CASY Model TT, Beilefeld, Germany).

FACS Analysis eNOS Expression

To study the impact of magnetic treatment on eNOS (endothelial nitric oxide synthase) expression of endothelial cells, the cells expressing eNOS after passage 6, with and without magnetic treatment, were quantified by flow cytometry. Trypsinized cells of both groups were fixed and permeabilized with cytostix/cytoperm reagent (BD, Heidelberg, Germany) for 20 min on ice and after, washed with Perm/Wash reagent (BD). Cells (1 × 10⁶) were incubated with 1 μg rabbit anti-human eNOS antibody (Dianova, Hamburg, Germany) for 30 min at 37 °C. For the secondary
antibody, 0.5 μg goat anti-rabbit Fluorescein (KPL, Wembley, Middlesex, UK) per 10^6 cells were applied after washout of primary antibody with phosphate-buffered saline (PBS). After incubation at 37°C for 20 min and a brief PBS wash, cells were immediately analyzed on a fluorescence-activated cell sorter (FACS Calibur, BD). Cells (2.0 × 10^6) were recorded and data analysis was performed with BD CellQuest Pro software.

**RT-qPCR for Vascular Endothelial Growth Factor (VEGF) Gene Expression**

Total cellular RNA was isolated from control and magnetic groups using Absolutely RNA Miniprep Kit (Stratagene, La Jolla, CA). Integrity of RNA was checked by agarose gel electrophoresis prior to cDNA transcription. Affinity Script QPCR cDNA Synthesis Kit (Stratagene) was used according to manufacturer’s instructions for reverse transcription. Every RT reaction was performed with Oligo (dT) primers and 250 ng of isolated total RNA and immediately stored at −20°C. Real-time PCR was carried out using Stratagene Real-time Cycler Mx 3000 with Brilliant SYBR Green QPCR Master mix (Stratagene) and Quantitect primer sets (Qiagen, Hilden, Germany) for target gene VEGFA and reference gene 18s. PCR efficiencies were determined by performing qPCR with log dilutions of cDNA of target gene or reference gene. The expression of target gene was normalized to the endogenous reference control gene 18s. Target gene expression was normalized to 18s gene expressions. The expression ratio of different exposure times to the magnetic field was determined by normalization to the corresponding control. All results were efficiency corrected.

**Nitric Oxide Concentration**

Total NO\textsubscript{2}/NO\textsubscript{3} were quantified as a measure of NO level. BioAssay Systems’ QuantiChrom\textsuperscript{TM} Nitric Oxide Assay Kit was used according to the manufacturer’s recommendations (BioAssay Systems, Hayward, CA). The kit measures NO production following the reduction of nitrate to nitrite using the Griess method.

**Statistical Analysis**

Statistical analysis was performed using the Student’s t-test with a minimal confidence level of 0.05 for statistical significance. Each experiment was performed at least three times with a minimum of three samples per termination point, resulting in a total number of six samples for each experiment. The data shown constitutes a representative sample of the experiments performed.

**RESULTS**

**Effects of Weak Static Magnetic Fields on Cell Number and Morphology**

HUVECs cell number increased during the culture period. Direct cell count of HUVECs showed an increase of 40% in cell number after exposure to 120 μT static magnetic fields compared to control (Fig. 1). A visual assessment of cell numbers obtained in Figure 1 is shown in Figure 2. Magnetic group displays a typical compact cobblestone packing (Fig. 2) of the cells compared to the untreated group.

**Endothelial Cells Show Sensitivity to Magnetic Treatment Near the Geomagnetic Field**

Endothelial cells were exposed to two distinctive magnetic treatments: 60 μT for 24 h per day, and 60 μT for 1 h per day. Endothelial cell number increased 40% by day 2 for the experimental group exposed to the treatment 24 h per day; 1 h treatment had no effect on cell number (Fig. 3).
Magnetic Treatment Upregulated eNOS Expression

Endothelial cells of passage P6 were subjected to magnetic treatment of 120 μT. After 3 days of treatment with weak static magnetic fields, the quantity of eNOS positive cells was 40% higher in the magnetic group compared to the untreated group (Fig. 4).

Magnetic Treatment Does Not Change VEGF Gene Expression

The VEGF expression ratio between magnetic field-treated cells and untreated cells was not altered after 24 or 48 h of exposure (data not shown).

Weak Static Magnetic Fields Does Not Change Nitric Oxide Concentrations for Distinctive Exposure Times

Two days after plating, HUVECs reached confluence and the magnetic stimulation of 120 μT static field began. In order to examine the effects of static magnetic fields on NO activity, a NO calorimetric assay was conducted. Figure 5 shows the results of the assay. On two separate experiments, HUVECs were stimulated for 30 min, 2 h, 5 h, and 24 h before collecting supernatants for the assay. The optical density for control and magnetic group did not differ after 30 min, 2 h, 5 h, and 24 h of stimulation. Figure 5 demonstrates that the magnetic treatment did not affect NO concentration.

DISCUSSION

Our present work demonstrates the effectiveness of weak magnetic fields on HUVECs proliferation and eNOS expression. Weak magnetic fields of 120 μT increased HUVECs proliferation by 40% over a period of 2 days. Endothelial cells showed sensitivity to both magnetic field intensity and exposure time; HUVECs responded to static fields as low as 60 μT, however, magnetic treatment for an exposure of 1 h had no effect on proliferation.
Endothelial cells functionality increased after magnetic treatment which upregulated eNOS expression. Weak static magnetic fields seem to have a rejuvenation effect on endothelial cells because 78% of endothelial cells expressed eNOS after treatment compared to 54% for control. Weak static magnetic treatment had no effect on VEGF gene expression, which indicates no increased potential of tumor development by overexpression of VEGF. Although weak magnetic treatment increased eNOS positive HUVECs by 40%, NO concentration remained unaltered throughout the culture period as measured by the Griess method. The results on NO concentration and increase in cell proliferation presented here contradict recent reports on the effects of transient NO release and cell proliferation [Fitzsimmons et al., 2008]. In contrast to our approach, where the dose metric is magnetic, the study by Fitzsimmons et al. implemented pulsing electric fields (PEF). However, it is possible that in this report the NO concentration was probed too late after the application of the weak magnetic fields. Future studies will address this point by measuring NO activity in 10-min intervals after exposure to magnetic fields.

A main result presented in this report is the observation of biological effects as a consequence of annihilating the geomagnetic field. Other reports have also shown that shielding the geomagnetic field influences animal behavior [Prato et al., 2005]. All controls in this report were subjected to a μ-metal cylinder which cancels the Earth’s magnetic field to tenths of a microTesla. One purpose for using the μ-metal cylinder was to eliminate fluctuations in the static magnetic field inside the incubator, thus providing a better control especially when comparing to low level magnetic fields of 60 μT. Proliferation was down-regulated by 37% for endothelial cells kept inside the μ-metal cylinder compared to the group treated with 60 μT static fields. This result may indicate the necessity of the geomagnetic field for proper biological activity. The apparent response of endothelial cells to the magnetic treatment to promote recovery was unanticipated. Although VEGF regulates many endothelial cell functions including proliferation, differentiation, and vascular tone, the magnetic exposure did not change its concentration. The mechanism of interaction between the biological system and the weak magnetic field remains unclear. Other reports deny the possibility of weak external magnetic fields to affect biological processes. Nevertheless, there is experimental evidence in which weak magnetic fields interact with biological systems. We look forward to continuing our work on low field effects on cells. Future works include change of proliferation as a function of magnetic field intensity and effects of combination of alternating and static magnetic fields. This work may provide an indication of the type of mechanism that is involved in the observed biological response.

CONCLUSION

Cellular activities of human umbilical vein endothelial cells under weak static magnetic fields were investigated. Our results demonstrate the application of static magnetic fields to effectively stimulate endothelial cells. Magnetic treatment of endothelial cells increased cell number for magnetic fields close to the geomagnetic field. eNOS expression also increased for endothelial cells after magnetic treatment. Future studies will focus on the investigation of the effects of magnetic intensity on cellular activities, functional properties, and expression of involved genes. Overall, the observations presented herein suggest that weak static magnetic field may play a significant role in the enhancement of the healing response of the endothelium. Furthermore, the magnetic treatment could be a useful tool in vascular tissue engineering by shortening the culture time of endothelial cells. The prevention of cell degradation and maintenance of functional characteristics by treatment with weak static magnetic fields may play an important role in the production of tissue engineered vascular grafts.

REFERENCES


