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Electromagnetic Field at 15.95–16 Hz is Cardio Protective Following Acute Myocardial Infarction

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Abstract—Previous studies have shown that pre-exposure of the heart to weak magnetic field reduces infarct size shortly after induction of myocardial ischemia. To investigate the role of AC magnetic field with a frequency of 15.95–16 Hz and 80 mT on left ventricular (LV) remodeling following chronic coronary occlusion and a short episode of ischemia followed by reperfusion (I/R). LV dimension and function were measured using echocardiography. Femur bone marrow was isolated and cells were phenotyped for endothelial linage and immuno stained for endothelial cells. The area at risk was measured using triphenyltetrazolium chloride staining. A significant reduction of 27% in shortening fraction (SF) was measured following acute myocardial infarction (AMI) compared with a 7% decrease in animals exposed to magnetic field ($p < 0.04$). A significantly higher number of colony forming units and endothelial progenitor cells were counted within the treated groups subjected to magnetic field ($p < 0.02$). Exposing the heart to magnetic field prior to reperfusion did not show any preservation either on SF or on infarct size. Magnetic field was protective in the AMI but not in the I/R model. The mechanisms underlying cardiac protection induced by AC magnetic field following chronic injury deserves further investigation.

Keywords—Ischemia/reperfusion, Echocardiography, Colony forming units, Endothelial progenitor cells, Rats.

INTRODUCTION

Electromagnetic fields are ubiquitous and are due to many sources. Manmade sources include power lines and power stations, while natural sources include static magnetic fields of the earth, solar activity, and more. Some studies have shown therapeutic properties while others claimed detrimental effects.[1,26] Magnetic fields have been used for more than 20 years in medicine, such as for skin lesions,[19] and was shown to improve the healing of bone fractures through upregulation of transforming growth factor $\beta 1$ (TGF-$\beta 1$) and favor osteoblast proliferation.[8] Ahmadian *et al*.[3] have shown enhancement of collagen synthesis. Other studies have shown improved blood flow through the activation of several angiogenic growth factors.[30] As far as we know, Albertini *et al*.[5] were the only investigators to show that pulse electromagnetic field is able to limit the area of necrosis after an acute ischemic injury caused by permanent ligation of the left coronary artery in rats. Albertini *et al*. have shown that 18 h of magnetic field exposure, with an intensity of 3.0 mT and a frequency of 75 Hz, significantly reduces necrotic area in rats subjected to acute myocardial infarction (AMI). However, they had a very high mortality rate; with only a 63% survival rate while 95 live animals were necessary to demonstrate significant changes in percent necrotic area. A second group of animals subjected to AMI was sacrificed 6 days' post-infarction. The mortality rate was high (74%) here as well despite the fact that smaller infarcts were created (distal coronary occlusions). Nevertheless, application of magnetic field for 6 days' post-infarction did not affect necrotic area. Their study suggested that magnetic field limits inflammatory responses, local edema, and favors microcirculation recovery; however, this was only speculated and not tested. We therefore aimed to re-evaluate the effect of magnetic field with a frequency of 15.95–16 Hz in a rat model of chronic coronary artery occlusion with large infarction and assess its effect on left ventricular (LV) remodeling. We also investigated cardiac function while using histological measures to explain our observation.

The above-mentioned studies utilized a wide range of frequencies and variable magnetic fields. Based on these data, we selected to test the effect of AC magnetic field on the healing post-acute infarction. In the present study, we investigated the protective effect of

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magnetic field with frequencies of 15.95–16.0 Hz on myocardial recovery from AMI or from reperfusion (I/R) insult. We chose our magnetic frequency based on a previous study by Aguilar-Bryan *et al.*[2] who found that the interburst interval for K_{ATP} channel is 62.7 ms, which equals 16 Hz. We hypothesized that a frequency of 15.95–16.0 Hz would induce K_{ATP} ion channel opening to mimic the pharmacological effect of potassium ion channel openers,[20] which are known to be cardiac protective in certain human clinical studies.[9,23,28] The opening of K_{ATP} ion channels would subsequently shorten action potential and decrease Ca^{2+} ion entry, which have negative inotropic effects on cardiac, as well as, smooth muscle cell (SMC) contractility. This change in ion efflux during myocardial ischemia reduces myocardial work (demand), which better matches the reduced oxygen supply (blood flow) when blood flow is limited.

METHODS

Animals

Two experimental protocols were executed consecutively: (1) AMI with short (1 week) and long-term (4 weeks) follow-up; and (2) brief episode of ischemia (45 min) followed by 120 min of reperfusion. Animal experiments conformed to the *Guide for the Care and Use of Laboratory Animals* and was approved by Tel-Aviv University Committee (approval nos. M-05-035 and M-07-043).

We chose rats as an acceptable and published model of chronic myocardial ischemia, and due to our own experience with that model.[10] In addition, we were able to house 4 animals per cage, thus only 1 magnetic field was required per cage. Male Sprague Dawley rats ($n = 36$) 3–4 months of age weighing 237 ± 14 g were used. Animals were housed in a climatized room at a 12/12 h-light/dark cycle, and under veterinarian supervision throughout the experimental period. The animals were not isolated from stray magnetic fields such as those generated by electronic equipment, lights, and air conditioning.

Weak Magnetic Field Device

The device was composed of a transducer emitting weak magnetic field from 8 plates of 7 cm × 14 cm each (Aerotel, Holon, Israel; Fig. 1). Each plate consisted of 69 spiral coils at a diameter of 1 cm each (Fig. 2). The cage was placed on the plate throughout the designated exposure time period. The field was calibrated to deliver 1.62×10^{-5} T/A, and delivered a frequency of 4–64 Hz, AC. Calibration of the device

FIGURE 1. An image of the magnetic device.

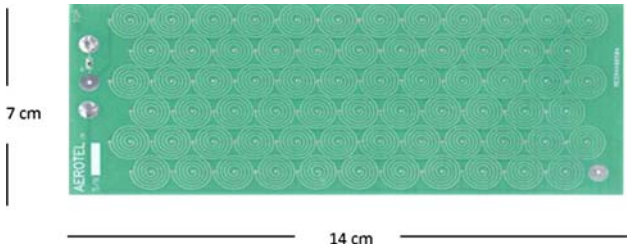

FIGURE 2. Graphic presentation of one plate consisting of 69 coils.

was performed with the use of a pick-up coil to determine magnetic flux. The pick-up coil consisted of 20 turns of wire and was connected to an Oscillator (Fluke, PM 3392A). A resistor of 20 Ω was connected to the generator (Newtronics, 200MSTPC). The magnetic field was monitored using a Gaussmeter (range 0.01–1999 mG; sensitivity 1 nT, Alphalab, Salt Lake City, UT, USA). When activated, the coil emitted alternating magnetic fields of 3–5 μT. At a depth (distance) of 3.5–4.0 cm, the measured fields (using the Gaussmeter) were 80–100 nT at the gross location of the radiated location of the volume-region of the heart (within the thoracic region of the rats in their cage).

In a preliminary unpublished experiment, we investigated the effect of different magnetic fields and frequencies on surface electrocardiogram (electrical changes) of anesthetized rats. We found that the most abundant changes occurred with magnet frequency of 15.95–16.0 Hz and magnetic field of 80 nT. We therefore decided to use these parameters for our current experiment and to investigate their effects on the remodeled myocardium.

AMI MODEL

Induction of AMI

Rats ($n = 48$) were divided into 2 groups. One group was subjected to AMI and another group

remained without MI (control). Half of each group was exposed to 1 or 4 weeks of magnetic field ($n = 12$/group) applied immediately following induction of the MI (or no-MI for controls). One group was sacrificed at the termination of exposure to magnetic field (1 week post-MI), and the second group was sacrificed 4 weeks after, during LV remodeling. Rats with no MI served as additional control group to test the magnetic field effects on non-infarcted myocardium for the assessment of untoward effects (mostly safety).

Rats underwent surgical ligation of the left anterior descending (LAD) coronary artery as previously described.[13] The experimental animals were anesthetized by intramuscular injection of Xylazine (10 mg/kg) and Ketamine (90 mg/kg). Following intubation and ventilation (Harvard respirator, 2.5 mL, and 75–78 strikes/min), the skin was cut and the chest muscles were separated to open the chest. A 5/0 silk thread was placed under the LAD and tied permanently. Thereafter, the muscles and the skin were sewn; the animals were extubated and allowed to recover for 1 or 4 weeks until sacrificed. The magnetic field-exposed rats were placed in their cages on the exposure plates.

Measurements of LV Dimensions and Function

In order to measure LV dimensions (volumes) and function (shortening fraction), transthoracic echocardiography was performed at baseline (before induction of MI), immediately after induction of MI, and before sacrifice. All measurements were repeated in the non-AMI (control) rats. Echocardiograms were performed with a commercially available echocardiography system (Sonos 7500; Phillips, Amsterdam, The Netherlands) equipped with a 12-MHz phased-array transducer (Hewlett Packard, Andover, MA, USA). All measurements were averaged over three consecutive cardiac cycles and were performed by an experienced technician who was blinded to treatment. The data were analyzed by another expert (M.S.F.) who was blinded to treatment. SF (%) was calculated as $[(\mathrm{LVIDd} - \mathrm{LVIDs})/\mathrm{LVIDd}] \times 100$, where LVID indicates LV internal dimension, s is systole, and d is diastole. LV fractional area change (%) was calculated as $[(\mathrm{EDA} - \mathrm{ESA})/\mathrm{EDA}] \times 100$, in which EDA indicates LV end-diastolic area and ESA end-systolic area.

Sacrificed, Tissue Harvest and Bone Marrow Collection

Upon sacrifice (1 and 4 weeks post-MI), hearts were removed for immuno-staining and for bone marrow (BM) collection to assess number and potency of endothelial progenitor cells (EPCs). BM was collected by flushing the femur bone with complete medium constituted of DMEM-LG (Bio-Whittaker, Verviers, Belgium), 10% fetal calf serum (FCS, Sigma, St. Louis, MO, USA), glutamine 2 mM (Bio-Whittaker), and penicillin/streptomycin (50 U/mL and 50 mg/mL) (Gibco-Invitrogen, Carlsbad, CA, USA). Cells were washed twice in a medium and plated in a Petri dish. Medium was supplemented with green florescent protein and PDGFAA 10 ng/mL (R&D Systems, Minneapolis, MI, USA). Cells were cultured and amplified on coated petri dishes in complete medium without growth factors. Medium was changed 3 times per week for 2 weeks.[25]

Colony Forming Units

The identification and quantification of hematopoietic stem cells and EPCs were examined using colony forming units (CFU) assay.[33] BM colony was added to methylcellulose medium, vortexed, distributed into Petri dishes and incubated in water—saturated, 5% CO_2 atmosphere. Colony size and number were measured. The quantitative evaluation of CFUs was performed under magnification of 125× using a Zeiss microscope (Axioskope 2, Feldbach, Switzerland). Petri dishes were projected onto a computer monitor. Nine fields were randomly chosen for evaluation and compared among the experimental groups.[33]

Confirmation of CFU Phenotype

For phenotyping of endothelial characteristics of the CFU, the following antibodies were used in immunofluorescence analyses: rabbit polyclonal anti-Tie-2 (C-20), mouse monoclonal anti-flk-1 (A-3), and goat polyclonal anti-CD31 (PECAM-1, M-20) (Santa Cruz Biotechnology, Santa Cruz, CA). Endothelial cell lineage was further confirmed by indirect immunostaining with the use of 1,10-dioctadecyl-3,3,30,30-tetramethylindocarbocyanine perchlorate-acetylated low-density lipoprotein (DiI-acLDL), and co-staining with BS-1 lectin (Sigma).[14,15]

Fibronectin Adhesion Assay

In order to investigate the adherence potential of EPCs, an adhesion test was performed. EPCs from rats were washed with phosphate-buffered saline (PBS) and gently detached with 0.5 mmol/L ethylenediaminetetraacetic acid (EDTA) in PBS. After centrifugation and resuspension in basal complete medium supplemented with 5% fetal calf serum (FCS), identical cell numbers were placed on fibronectin-coated culture dishes and incubated for 30 min at 37 °C. Adherent cells were counted by the investigator unaware to the treatment groups.

The quantitative evaluation of CFUs phenotype and fibronectin adhesion assays were performed under magnification of 400× using Olympus microscope (IX51 2, DUO controller, Hamburg, Germany). The slides were projected onto a computer monitor. Nine fields were randomly chosen for evaluation. The average number of positively stained cells were measured and averaged from each slide and the average experimental group was presented as mean of 9 fields ± SD.

Histology

We used an immunohistological approach to determine the number of blood vessels grown within the infarcted or remodeled myocardium, and the mitotic index (for proliferating nuclei/cells) as part of our explanations of the results. The paraffin-embedded heart samples were cut by microtome (5 μm thick), placed on histological slides and stained for: (1) hematoxylin and eosin (H&E); (2) SM α actin[7] to assess the number of endothelial cells; and (3) proliferating cell nuclear antigen (PCNA) to assess the number of proliferating endothelial nuclei.[6]

For alpha SM actin, slides were incubated with 10% H_2O_2, 80% methanol for 15 min and then incubated with trypsin, peroxide. Using the Histostain Plus Bulk kit (Zymed Laboratories, South San Francisco, CA, USA), the slides were incubated with non-immuno serum blocking solution, specific primary antibody (Sigma) diluted 1:200, biotinylated secondary antibody, and streptavidin enzyme conjugate (in this order). Finally, the detection was evaluated by the diaminobenzidine solution. SMCs were stained brown, myocardial muscle stained light violet, scar tissue stained gray, and cell nuclei stained blue.

For PCNA, paraffin embedded sections were deparaffinized in xylene and alcohol and placed for 15 min in alcohol–H_2O_2 for blocking endogenous peroxidase. Sections were incubated for 1 h with a primary non-diluted ready-to-use murine anti-p53 antibody (Dako, Carpentaria, CA, USA) or murine anti-PCNA antibody (Dako) diluted at 1:500 at room temperature in a humidified chamber. Slides were rinsed with phosphate buffered saline for 3 min and incubated first with the biotinylated linked goat anti-mouse antibody for 30 min and then with the peroxidase conjugated streptavidin, for 30 min. After the slides were rinsed, the peroxidase label was demonstrated using 3-amino-9-ethylcarbazol for 15 min and counterstained with Mayer hematoxylin.

Positively-stained slides were quantitatively using magnification of 400×. The number of endothelial cells and PCNA-stained cells within the infarction and the non-infarcted regions were counted. The slides were projected onto a computer monitor. Nine fields of 0.067 mm^2 each from the scar zone and 9 fields from the septum/non-infarcted zone were randomly chosen for evaluation. The average blood vessel number and diameter and the absolute numbers of proliferated cells were measured and averaged per each slide and zone. The average number for each experimental group was presented as mean of 9 fields ± SD.

I/R MODEL

Inducing Ischemia Reperfusion

Since most cardiac patients with AMI undergo a reperfusion procedure, we subjected rats to I/R model to mimic the clinical situation. Rats were divided into 2 groups. One group ($n = 6$) was subjected to 60 min of magnetic field (80 nT, 16 Hz) prior to I/R and another group ($n = 6$) were not exposed to the magnetic field (control). Both groups had ischemia for 45 min followed by 2 h of reperfusion. The same anesthetic method was applied to these animals as was applied to the AMI animals. The skin was cut and the chest muscles were separated to open the chest. A reversible coronary artery snare occluder 5/0 silk was placed around the left coronary artery. Thereafter, the muscles and the skin were sewn. Forty-five min later the chest was opened reperfusion was allowed for 2 h. In order to ensure the induction of ischemia, electrocardiogram (ECG, limb electrodes) was registered throughout the procedure using Biopac recorder (Biopac Systems, Goleta, CA). AC magnetic field was applied to the animals for 60 min prior to induction of the ischemia but not during reperfusion. The control group was not exposed to AC magnetic field.

Measurements of LV Dimensions and Function

Echocardiography was performed as previously described to measure SF (%) and fractional area change (FAC, %) at baseline, repeatedly at 45 min, following induction of the ischemia, and 120 min following reperfusion.

Sacrifice and Measurement of Infarct Size

Hearts were removed and 10% Evans blue dye (Sigma) was injected into the aorta to demarcate the area at risk. Hearts were perfused with PBS to wash out the excess Evans blue dye, and thereafter cut into 3 transverse slices, from apex to base, of equal thickness (1.5 mm apart). The slices were then incubated in 1% 2,3,5-triphenyl-tetrazolium chloride (TTC) solution (Sigma) in PBS at 37 °C for 20 min. Sections were

fixed overnight in 2% paraformaldehyde. The areas of irreversible injury (TTC negative) were quantified using Nikon camera (Coolpix 4500) and quantified with Sigma software and presented as a percentage of the entire area of the section.

Statistical Analysis

Echocardiographic analyses of each experimental model were compared between magnet exposed and non-exposed counterparts using un-paired Student's *t*-test and analysis of variance (ANOVA). CFUs area and stained and adhered cells at 1 and 4 weeks post-MI were compared using ANOVA test. Means ± SD were calculated for each parameter. A p value ≤0.05 was considered statistically significant.

RESULTS

AMI Model

Forty-eight of 53 rats (90%) survived the infarction. Five rats died within 24 h following surgery (3 were exposed to magnetic field and 2 were not).

LV Dimensions and Function

Application of the magnetic field for 1-week post-MI was associated with preserved LV function at both time points compared with controls (Fig. 3a). At one-week, SF was 36 ± 3% for WMF-exposed rats (white bar) and 29 ± 4% in the non-exposed MI rats (black bar). Animals without MI exhibited SF of 57 ± 13% for non-exposed and 43 ± 3% in WMF-exposed rats, $p = 0.04$.

In the 4-week group, SF during remodeling (post-MI) was 23 ± 3% in the non-WMF-exposed rats compared with 34 ± 4% for the exposed rats ($p = 0.02$). Rats without MI exhibited similar SF of 36 ± 3% and 38 ± 5% for the non-WMF-exposed and WMF-exposed rats (Fig. 3a).

FAC was similar post-MI in the 'one-week group': 31 ± 3% in the non-exposed MI rats and 33 ± 5% for the WMF-exposed rats. FAC of normal non-MI hearts showed significantly lower value in the WMF-exposed rats compared with non-exposed rats: 38 ± 3% and 52 ± 7%, respectively ($p = 0.05$, Fig. 3b).

In the 4-week group, FAC post-MI was 23 ± 3% in the non-WMF-exposed rats compared with 34 ± 4% for the WMF-exposed rats. FAC for the normal, no-MI hearts was 31 ± 5% for non-WMF-exposed

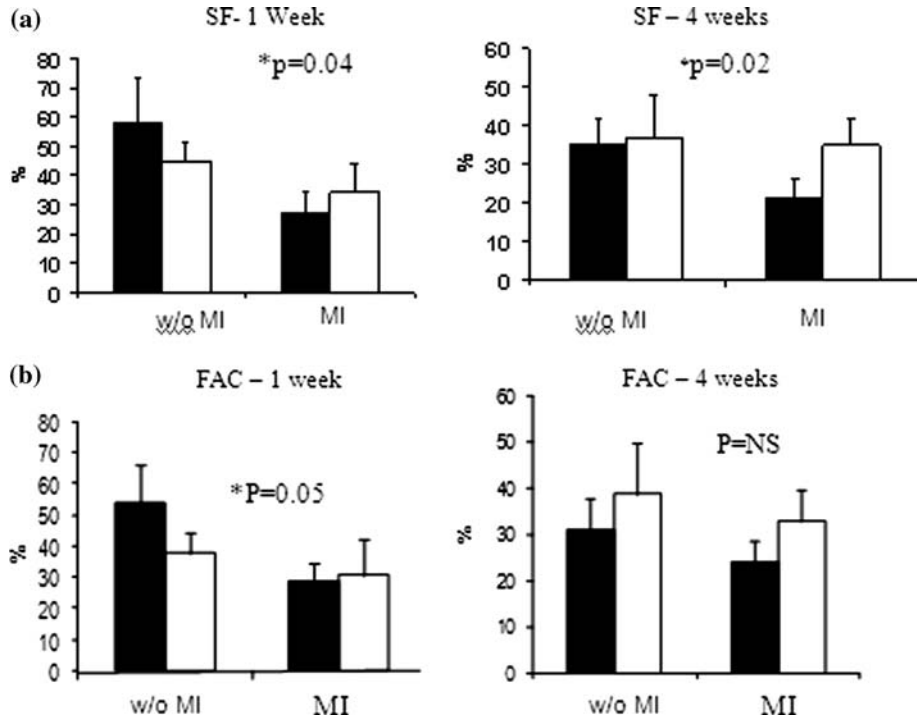

FIGURE 3. (a) Left ventricular shortening fraction (SF, %) and (b) fractional area change (FAC, %) 1 week (left) or 4 weeks (right) post-acute MI or controls (w/o MI). White bars: w magnetic field; Black bars: w/o magnetic field. SF: *$p = 0.04$ between magnet exposed and non-exposed rats in the 1-week group and *$p = 0.04$ in the 4-week group. FAC: *$p = 0.05$ between magnet exposed and non-exposed rats in the 1-week group and p = NS in the 4-week group. Between the magnet exposed and non-exposed rats without MI *$p = 0.04$ in SF and *$p = 0.03$ in FAC.

and 37 ± 8% for WMF-exposed rats ($p = 0.051$, Fig. 3b).

Histology

Number of Blood Vessels within the Scar Area

Histology demonstrated a significantly higher number of blood vessels within the scar area in both time points post-MI. In 1 week, 13.6 ± 5.4 blood vessels/mm^2 were counted for non-WMF-exposed rats and 17.7 ± 5.7 blood vessels/mm^2 were counted for WMF-exposed animals, $p = 0.002$. At 4 weeks post-MI, 19.1 ± 6.7 blood vessels/mm^2 were counted in the non-exposed animals compared with 24.1 ± 6.4 in WMF-exposed group, $p = 0.001$ (Fig. 4a).

Number of Blood Vessels within the Septum Area

At 1 week post-MI there were no significant differences in blood vessel count between the two groups in the surviving myocardium (Fig. 4b). However, a significantly ($p = 0.001$) higher blood vessel count was observed within the remodeled myocardium 4 weeks post-MI in the WMF-exposed group (12.7 ± 5.5 blood vessels/mm^2) vs. non-WMF-exposed rats (9.4 ± 5.2 blood vessels/mm^2).

Mitotic Index of Scar Area

One week post-MI, a significantly higher mitotic index (expressed as the number of proliferating cells) was measured for the WMF-exposed animals compared with their non-exposed counterparts: 17.2 ± 5.9 proliferated cells/mm^2 vs. 11.1 ± 3.6 proliferated cells/mm^2, respectively, $p = 0.001$. At 4 weeks post-MI the number of mitotic endothelial cells was the same (Fig. 5a).

Mitotic Index of the Septum Area

One week post-MI there were also higher number of proliferating cells in the septum in the magnetic field exposed animals compared to those non-exposed: 11.2 ± 4.7 proliferated cells/mm^2 vs. 8.6 ± 4.1 proliferated cells/mm^2, respectively, $p = 0.003$. At 4 weeks post-MI there were no differences between the two experimental groups (Fig. 5b).

Area of CFUs

Significantly larger CFU areas were evident following AMI in the magnetic field exposed animals compared with controls: $33.2 \times 10^6 \pm 5.2 \times 10^6$ μm^2 vs. $14.1 \times 10^6 \pm 3.8 \times 10^6$ μm^2, respectively, $p = 0.005$ (Figs. 6a–6c). At 4 weeks' post-MI, the CFU numbers were $21.3 \times 10^6 \pm 4.5 \times 10^6$ μm^2 in the magnetic field group and $1.04 \times 10^6 \pm 0.7 \times 10^6$ μm^2 in the non-magnet group, $p = 0.001$ (Fig. 6d).

Endothelial Phenotype

Staining for CD-31/FLK-1 indicated a significantly higher number of stained cells following AMI in the magnetic field exposed group compared with those

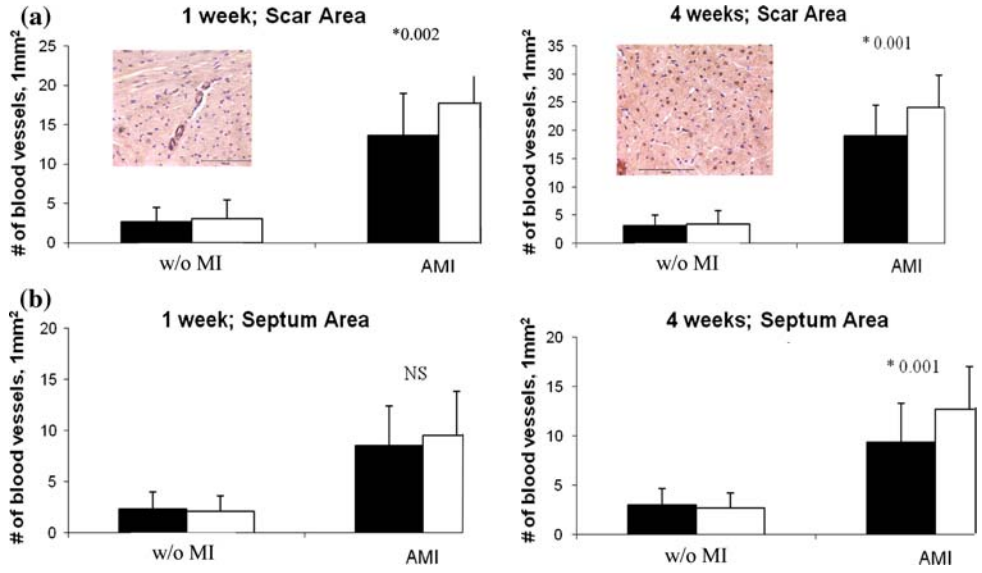

FIGURE 4. (a) Number of blood vessels (/mm^2) within the scar area and (b) the septum area 1 week (left) or 4 weeks (right) post-AMI or controls (with no MI). Black: w/o magnetic field; White: w magnetic field. Scar area: *$p = 0.002$ in the 1-week group and *$p = 0.001$ in the 4-week group. Septum area: p = NS in the 1-week group and *$p = 0.001$ in the 4-week group. Histological staining for alpha smooth muscle actin in the scar area (400× magnification). The arrows represent blood vessels. (a) Non-treated rat; (b) AC magnetic field treated rat.

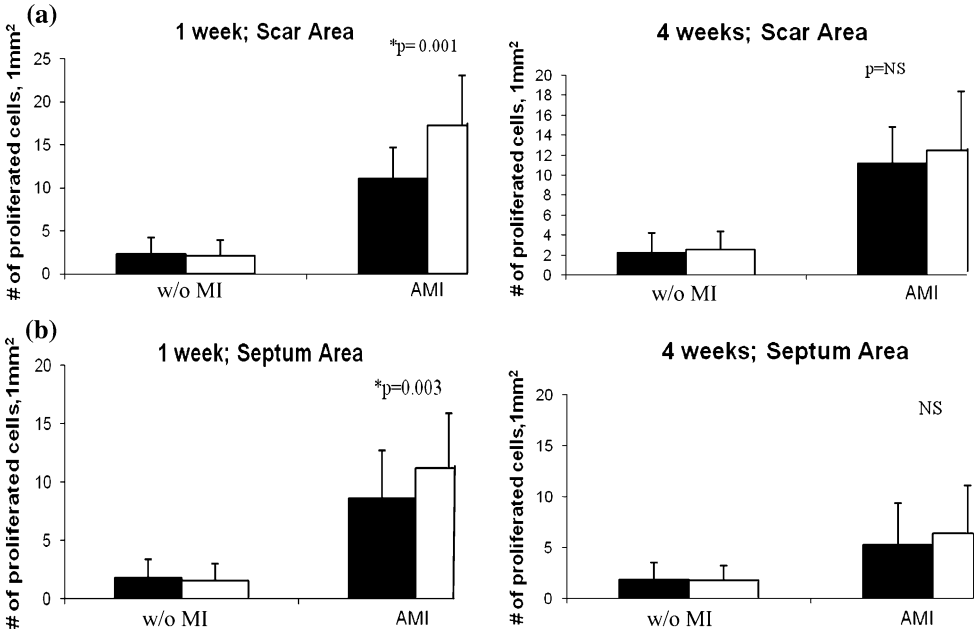

FIGURE 5. (a) Mitotic index expressed as the number of proliferating endothelial cells within the scar area and (b) the septum area 1 week (left) or 4 weeks (right) post-AMI or controls (with no-MI). Black: w/o magnetic field; White: w/magnetic field. Scar area: $*p = 0.001$ in the 1-week group and p = NS in the 4-week group. Septum area: $*p = 0.003$ in the 1-week group and p = NS in the 4-week group. Histological staining for PCNA at the scar area (400× magnification). The arrows represent proliferated endothelial cells. (a) Non-exposed rats; (b) Magnetic field exposed rats.

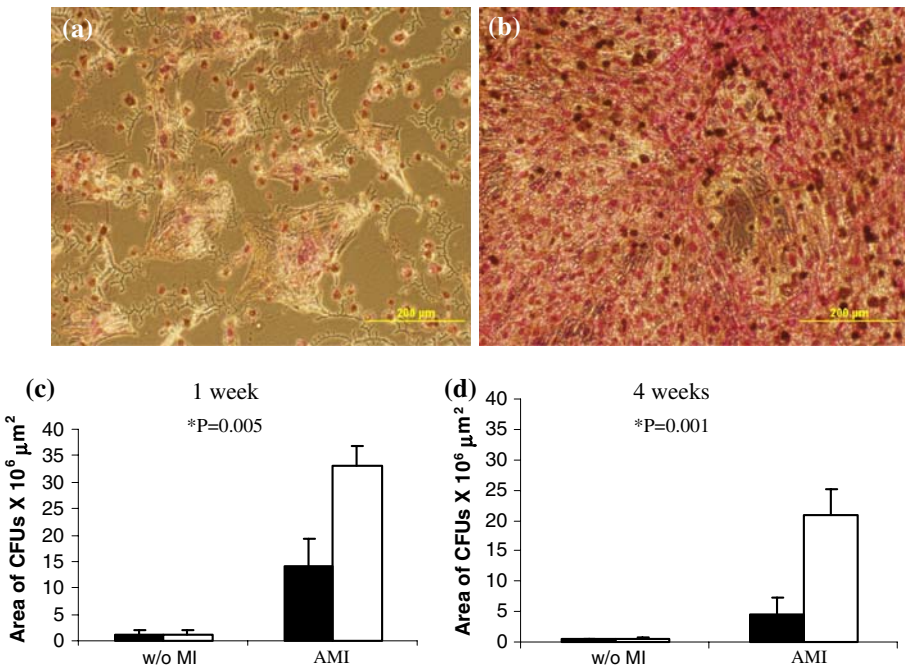

FIGURE 6. (a) A representative example of colony forming units stained with Giemsa of an untreated rat and (b) treated rat 1 week post-AMI. The average number of CFU area ($\times 10^6$ μm^2) is demonstrated in (c) 1 week post-AMI and (d) 4 weeks post-AMI. Black: w/o magnetic field; White: w magnetic field. $*p = 0.005$ and $*p = 0.001$, respectively.

non-exposed (11.2 ± 0.95 vs. 5.7 ± 1.2 (Figs. 7a–7c) and 19.3 ± 2.1 vs. 16.6 ± 3.6 (Fig. 7d) for 1 and 4 weeks, respectively, $p = 0.03$ (Fig. 7)). No differences were observed within the controls (no MI).

Significantly higher numbers of Di-LDL/BS-Lectin stained cells were evident following AMI in the magnetic field exposed groups compared with their non-exposed counterparts: 20.2 ± 1.07 vs. 4.8 ± 1.1 cells

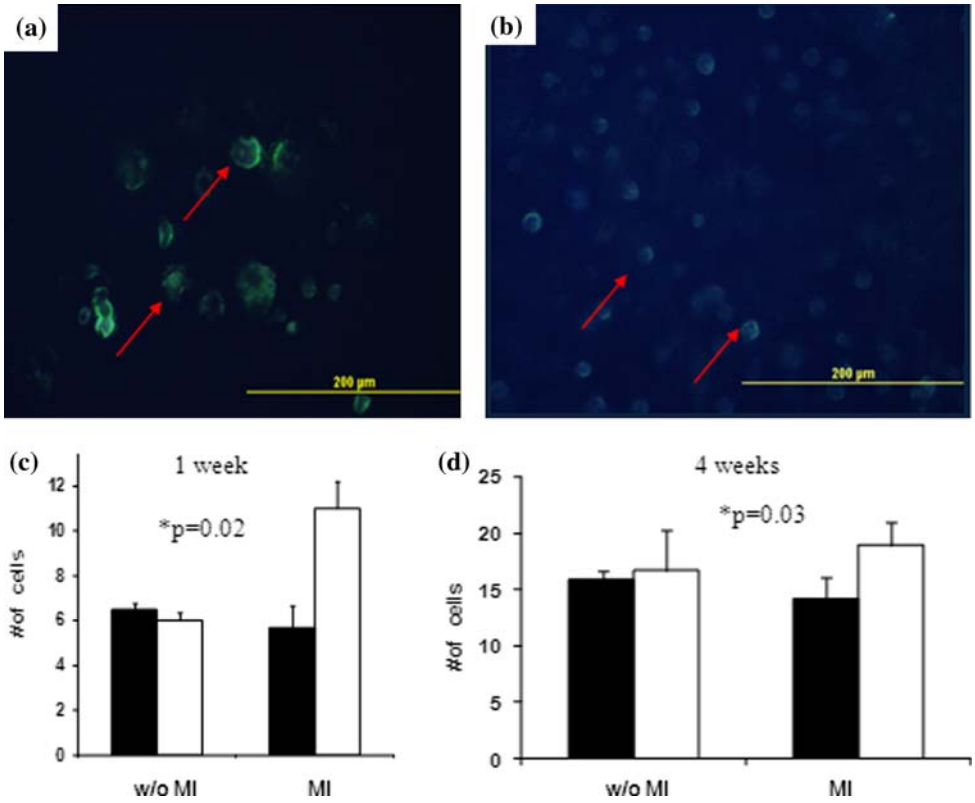

FIGURE 7. (a) Representative examples of CD-31/FLK-1 stained cells with MI untreated rat and (b) MI treated rat. The arrow represents stained cells. (c) The average number of cells 1 week post-AMI and (d) average number 4 weeks post-AMI. Black: w/o magnetic field; White: w magnetic field. *$p = 0.02$ and *$p = 0.03$, respectively.

(Figs. 8a–8c) and 22.5 ± 3.7 vs. 15.4 ± 3.5 cells (Fig. 8d), for 1 and 4 weeks, respectively, $p = 0.04$. The number of positive stained cells was not different among the non-MI animals exposed to magnetic field.

Fibronectin Adhesion Assay

Fibronectin adhesion assay indicated a significantly greater adherence potential of EPCs 1 and 4 weeks post-WMF application in the acute MI groups: 13.6 ± 5.2 cells vs. 4.2 ± 3.8 cells, for WMF-exposed and non-exposed animals, respectively, $p = 0.01$ at 1 week, and 22.2 ± 4.2 vs. 16.2 ± 3.3 at 4 weeks, respectively, $p = 0.002$ (Fig. 9). Here as well, no changes were observed in control, non-MI animals.

I/R Model

Twelve of 16 rats (75%) had survived the I/R insult. All animals died during coronary occlusion (before reperfusion). Three rats were exposed to the magnetic field and 1 was not exposed.

LV Dimensions and Function

Exposing the heart to magnetic field prior to reperfusion did not show any effect on SF or FAC following 120 min of reperfusion: $46.6 \pm 9.1\%$ vs. $45.1 \pm 7.4\%$ (Fig. 10a) and $42.2 \pm 8.2\%$ vs. $39.8 \pm 6.6\%$ (Fig. 10b), for magnet exposed and non-exposed animals, respectively, $p =$ NS.

Area at Risk

Magnetic field was not shown to reduce the region of irreversible ischemia in the treated group compared with controls: $16.1 \pm 6.4\%$ in the magnetic field exposed vs. $19.1 \pm 7.4\%$ in the non-magnet exposed groups, $p =$ NS.

DISCUSSION

In the present study we showed that 1 week of AC magnetic field at a frequency of 15.95–16.0 Hz and intensity of 80 nT preserved LV dimensions and function, and stimulated vascular growth and proliferation post-AMI. We also observed a significant increase in BM-derived EPCs and potency following exposure to magnetic field. No protective effect was noted on the reperfused myocardium following the brief episode of ischemia.

An early study by Albertini *et al.*[5] showed that 18 h exposure of magnetic field with the intensity of 3.0 mT

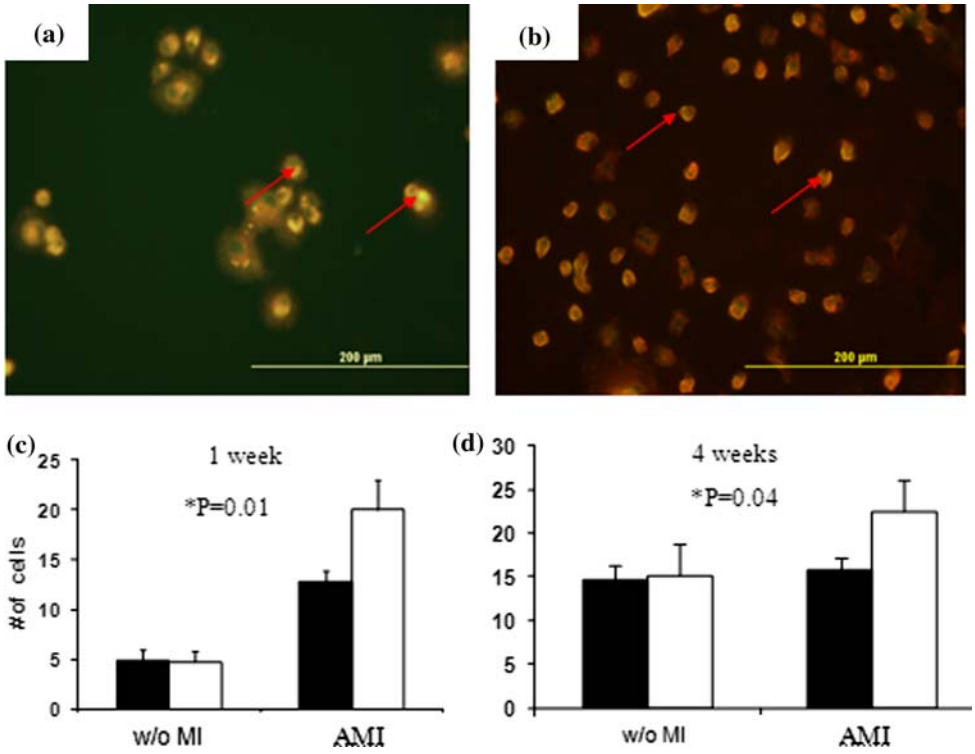

FIGURE 8. (a) Representative example of Di-LDL/BS-Lectin stained cells with MI untreated rat and (b) MI-treated rat (arrows represent stained cells). (c) Average number of cells 1 week post-AMI. (d) Average number 4 weeks post-AMI. Black: w/o magnetic field; White: w/magnetic field. *$p = 0.01$ and *$p = 0.04$, respectively.

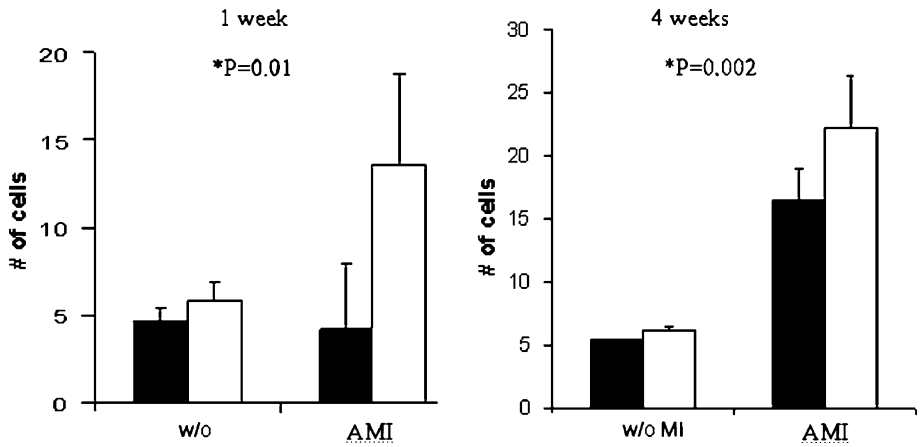

FIGURE 9. Fibronectin adhesion assay. In the left graph, average number of adherence EPCs 1 week post-AMI. In the right graph, average number 4 weeks post-AMI. Black: w/o magnetic field; White: w/magnetic field. *$p = 0.01$ and *$p = 0.002$, respectively.

and a frequency of 75 Hz significantly reduced necrotic area in rats subjected to AMI. In the same paper, however, application of alternating magnetic field for 6 days' post-infarction did not appear to affect the area of necrosis. Their study suggests that activation by magnetic field limits the inflammatory responses, local edema, and favors microcirculation recovery. They used a large number of rats and had a high mortality rate (165/257 rats survived the experiment). In the current experiment we used a far lower magnetic field intensity (80 nT vs. 3.0 mT) and frequency (15.95 Hz vs. 75 Hz) than Albertini *et al.* and found protective effects in animals subjected to AMI and exposed to magnetic field for 7 and 28 continuum days following induction of the ischemia. We found preserved LV dimensions and function which may be attributed to the increased number of newly grown capillaries (as evident from increased positive stained

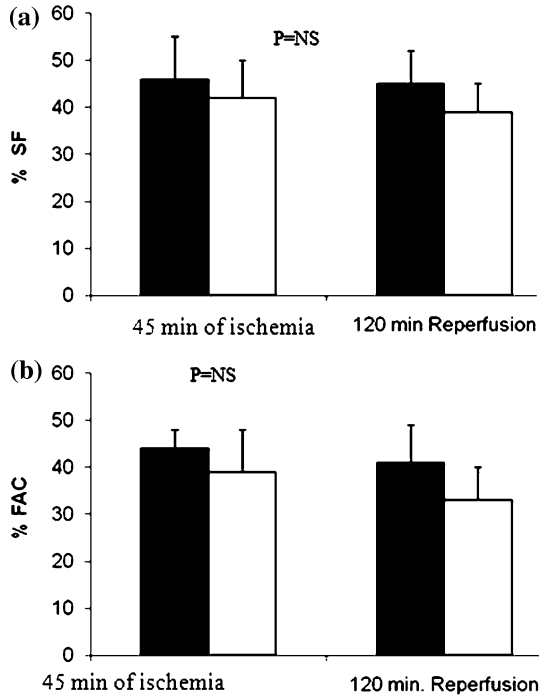

FIGURE 10. (a) Changes in shortening fraction (%) and (b) fractional area change (%) 45 min following ischemia and 120 min following reperfusion. Black: w/o AC magnetic field; White: w magnetic field. p = NS.

endothelial cell number and proliferation) and secondly, and most likely due to the increased number and function of endothelial progenitor cells.

Our present findings are in agreement with Roland *et al.*,[29] and Tepper *et al.*[31] who demonstrated increased neovascularization (angiogenesis) *in vivo* in rats subjected to daily magnetic field application for 8–12 weeks. We have demonstrated that 1 week of exposure to magnetic field is enough to produce angiogenic response. Tepper *et al.*[31] have shown that 15 Hz of magnetic field increased angiogenesis *in vitro* and *in vivo* through the release of a known mitogenic growth factor: fibroblast growth factor. Other microvascular studies suggested that persistent dilation of arterioles can also lead to angiogenesis.[18,24] Milkiewicz *et al.*[27] have suggested that the transduction mechanism from arteriolar vasodilation to angiogenesis is modulated by alterations in shear forces. DiCarlo *et al.*[11] have shown that chick embryo exposed to magnetic field significantly increased survival rates (68.7%) relative to the survival rates of control embryos (39.6%) subjected to cardiac anoxia. They used 60 Hz and 2 μT and suggested that the mechanism of protection relates to increased synthesis of stress protein.

Another possible mechanism by which the post-infarct myocardium induces angiogenesis is through the stimulation of BM-derived EPCs number and potency following AMI.[21] As in all tissues, ion channels present in mesenchymal stem cells[16]; it is most likely that magnetic field affects proliferation and differentiation of stem cells through modulation of these channels.

Our study is not the first to show that magnetic field has an effect on stem cells. Ventura *et al.*[32] have found that exposure of mouse embryonic stem cells to 0.8 mT and 50 Hz magnetic field stimulated the expression of GATA-4, Nkx-2.5, and dynorphin B, which act as cardiac lineage-promoting genes and play a major role in cardiogenesis. Their study suggests that magnetic field directs the differentiation process of stem cells into a specific cellular phenotype. Their study was performed *in vitro* while we investigated the *in vivo* effect of magnetic field by showing elevated numbers of EPCs in the BM and an elevated number of blood vessels in the heart. This could be explained by increased transcriptional activity of the cells and phenotypic differentiation of fibroblasts.[29] In cell culture, magnetic field application was shown to induce differentiation of chondrocytes[17] or to inhibit retinoic acid-induced differentiation of embryonal carcinoma cells.[4] Ahmadian *et al.* have concluded that the effect of magnetic field is limited to narrow biologically active windows in frequency and intensity and showed that a frequency of 25 Hz with peak intensity of 2 mT can significantly enhance collagen synthesis in rat skin when applied for 2.5 h/day for a duration of 8 days.[3]

Despite our documented results, further studies are needed to support such findings. Ion channels expression has been documented in the stem cells of rabbits, human, and rats.[21,22,24] Kawano *et al.*[22] first demonstrated ionic homogeneity in human mesenchymal stem cells and found that calcium ion oscillations were regulated by Na^{+}–Ca^{2+} exchanger. In addition, they found that intermediate calcium-activated potassium channels were present in most of the cells. Activation of these channels might be a possible mechanism responsible for the proliferation and differentiation of stem cells.

We hypothesize that magnetic field could be sufficient to produce a protective effect following AMI by opening adenosine-tri-phosphate (ATP)-dependent potassium channels and mimicking the ischemic preconditioning mechanism. Opening K_{ATP} channels is a fundamental physiological means for reversal or prevention of depolarization activity of the membrane. The activation of K_{ATP} channels causes marked shortening of the cardiac action potential. At the single cell level, K_{ATP} ion channel opening and the subsequent action potential shortening and membrane hyperpolarizing will, by decreasing calcium ion entry, have an inhibitory effect on the inotropic state of the heart. This may serve as a cardio protective effect for the ischemic myocardium following AMI. When entry

of calcium ion decreases, oxygen consumption of the contractile elements is reduced and drastic expression of calcium ion loading during the ischemic period and the subsequent recovery is expected to be reduced.

In the ischemia reperfusion model we have not observed any beneficial effect on LV dimension, function, or infarct size. This might be explained by the fact that the ischemia preconditioning process causes release of autacoids including adenosine, bradykinin, and opioids from the ischemic myocardial tissue.[12] These agonists occupy Gi-coupled receptors on the myocardial cell surface which, in turn, activate a protein kinase C isoform.[25] Bradykinin and opioids couple to protein kinase C by a tortuous pathway that includes activation of phosphatidylinositol 3-kinase, endothelial nitric oxide syntheses, and protein kinase G opening of mito K_{ATP} channels. In rats that were not exposed to magnetic field we assume that K_{ATP} channels had been opened by the induction of ischemia reperfusion insult. Therefore, rats that were exposed to magnetic field did not have any additional and specific effect on the opening of these ion channels.

CONCLUSION

AC magnetic field is expected to be cardio-protective following AMI but was not demonstrated in our study to have an effect following ischemia reperfusion injury. Yet it was found to preserve LV dimensions and function, and to induce angiogenesis and cell proliferation in the surviving myocardium. The increased number of EPCs may also participate in homing the cells into the remodeling myocardium, thereby producing favorable effects by participating in vascular growth.

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