Myocardial Function Improved by Electromagnetic Field Induction of Stress Protein hsp70

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Studies on myocardial function have shown that hsp70, stimulated by an increase in temperature, leads to improved survival following ischemia-reperfusion (I-R). Low frequency electromagnetic fields (EMFs) also induce the stress protein hsp70, but without elevating temperature. We have examined the hemodynamic changes in concert with EMF pre-conditioning and the induction of hsp70 to determine whether improved myocardial function occurs following I-R injury in Sprague-Dawley rats. Animals were exposed to EMF (60 Hz, 8 mG) for 30 min prior to I-R. Ischemia was then induced by ligation of left anterior descending coronary artery (LAD) for 30 min, followed by 30 min of reperfusion. Blood and heart tissue levels for hsp70 were determined by Western blot and RNA transcription by rtPCR. Significant upregulation of the HSP70 gene and increased hsp70 levels were measured in response to EMF pre-exposures. Invasive hemodynamics, as measured using a volume conductance catheter, demonstrated significant recovery of systolic contractile function after 30 min of reperfusion following EMF exposure. Additionally, isovolemic relaxation, a measure of ventricular diastolic function, was markedly improved in EMF-treated animals. In conclusion, non-invasive EMF induction of hsp70 preserved myocardial function and has the potential to improve tolerance to ischemic injury.

Extensive reports in the literature have shown that elevated levels of hsp70 improve cardiac function after hypoxic stress and ischemia-reperfusion (I-R; Suzuki et al., 1997, 2000, 2002). In this set of experiments, we evaluated whether pre-treatment with EMF-induced levels of hsp70 can preserve myocardial function after ischemia-reperfusion.

**Methods**

**Animal care**

Male Sprague-Dawley rats (250–400 g) were selected as the experimental species. To assure their health, the animals were examined by the animal facility veterinarian upon arrival. Animals were allowed to acclimate for at least 2 days and adjust to being handled before randomization into the study. Animals were housed in cages in an environmentally controlled room within the animal care facility at Columbia University. Care and management of rats was conducted according to facility standard operating procedures. At the conclusion of the acclimation period animals judged to be suitable for testing were assigned sequentially to either treatment or control. This investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). This study was approved by the Institutional Animal Care and Use Committee of Columbia University.

**Electromagnetic field exposures**

All EMF exposures were performed prior to induction of ischemia. The exposures described here are pre-treatment exposures. In previous studies, we tested a variety of field strengths and frequencies (Goodman et al., 1989; Wei et al., 1990; Jin et al., 1997), and eventually determined that a 60 Hz frequency and a field strength of 8 µT consistently induced the highest level of transcriptional activation of the HSP70 gene and the highest hsp70 protein levels (reviewed in Goodman and Blank, 2002). In the studies reported here, animals were exposed to 60 Hz/8 µT EMFs in a plastic exposure cage (16 cm x 24 cm) surrounded by Helmholtz coils (19 gauge copper wire, 164 turns, 1.5 inches thick covered with electrical tape). The system was designed and calibrated (R. Cangialosi, Electro-Biology, Inc., Fairfield, NJ) to our specifications for comfortably holding a large rodent; the cage holding the rats was suspended in a plastic enclosure (Fig. 1). EMF conditions were set using a function generator (BK Precision 4011A 5 MHz, Yorba Linda, CA) and digital multimeter (BK Precision 2706A). Rectal temperature was continuously monitored with a thermocouple probe (±0.1°C resolution; PhysiTemp., Cliffsde Park, NJ). The digital multimeter was used to measure the field intensity and verify the systems operation. Field parameters were monitored with a Hitachi V-106S 100 MHz oscilloscope and a calibrated inductive search coil. Exposure conditions were monitored with a Sypris triaxial magnetic field meter (Model 4080, Bell Laboratories, Orlando, FL). Experiments were carried out at room temperature (approx. 25°C).

**Anesthesia**

Rats were anesthetized with 2% isoflurane and mechanically ventilated via a tracheostomy (Harvard rodent ventilator, model 683, South Natick, MA) throughout the duration of the experiment.

**Surgical Procedures**

**Blood collection for hsp70 determination**

The left femoral vein was cannulated with polyethylene tubing (OD 0.965 mm, Becton Dickinson, Franklin Lakes, NJ) for repeated blood draws. Rats were then randomized to EMF exposure (60 Hz 8 µT) for 30 min (n = 6) or Control (no EMF exposure, n = 6). Blood was collected at baseline (pre-exposure) and after EMF exposure every 30 min up to 120 min. Serum was spun down at 1,200 rpm for 10 min; packed red blood cells (RBCs) were flash-frozen for hsp70 protein analyses, and stored at −80°C.

**Terminal tissue for HSP70 RNA determination**

Heart tissue (left ventricle; LV) was flash-frozen for analysis of HSP70 RNA by reverse-transcriptase polymerase chain reaction (rtPCR). GAPDH confirmed loading concentrations.

**Ischemia-reperfusion protocol**

The hemodynamic effects of EMF exposure on myocardial function after I-R (EMF n = 10, Control n = 10) were measured using the following protocol. Pre-anesthetized animals were randomly assigned to EMF pre-treatment and underwent EMF exposure (60 Hz, 8 µT) for 30 min. During this time period, each individual animal was allowed to rest comfortably in the...
exposure device under quiet conditions. No medications were administered prior or during this time. Animals designated as controls received no exposure and their cages were maintained in an EMF-free area and monitored with a Gaussmeter. At the conclusion of 30 min, all rats (both exposed and control) were anesthetized with 2% isoflurane and mechanically ventilated on 2% isoflurane after a tracheostomy. A 2 Fr volume conductance catheter (Millar Instruments, Houston, TX) was inserted into the left ventricle (LV) for continuous-pressure-volume (PV) tracings immediately following right carotid artery cannulation. After left thoracotomy, coronary ischemia was created by ligating the left anterior descending (LAD) coronary artery with a 5-0 polypropylene suture for a total of 30 min before release of the suture. Reperfusion was monitored for an additional 30 min. Hemodynamics (Chart for Windows v5, ADI Instruments, Colorado Spring, CO) were recorded at baseline (pre-ischemia), 30 min of ischemia, and at 1, 10, 20, and 30 min after reperfusion. Upon completion of the experiment, the heart was excised, and the LV was dissected for histology. Ischemic and non-ischemic portions of the LV were flash-frozen for extraction of protein for Western blot analysis of hsp70 levels and RNA extraction for reverse-transcriptase polymerase chain reaction (rtPCR) to determine upregulation of the HSP70 gene.

Hemodynamic and pressure-volume analysis
Hemodynamic determinations were made on all rats undergoing I-R (n = 20). LV end-systolic pressure (LVSP), LV end-diastolic pressure (LVEDP), and LV volume were measured using the Millar conductance catheter placed into the LV across the aortic valve. End-systolic volume (ESV) and end-diastolic volume (EDV) were measured using standard techniques (Ito and Bassett, 1983). Cardiac output (CO), arterial elastance (Ea), and preload-adjusted maximum power (P-A Max Power) were computed using a pressure-volume analysis program (PVAN v. 3.2, Millar Instruments). The time constant of LV isovolumetric pressure relaxation (τ) was calculated using the logarithmic method described by Raff and Glantz (1981). In experiments where animals had undergone ischemia-reperfusion, the hemodynamic and pressure-volume analyses indicated no adverse events or mortality associated with EMF exposure.

Protein sample preparation. Protein was extracted from myocardial tissue and packed RBCs using methods previously described (Lin et al., 1996, Carmody et al., 2000). Protein concentrations were determined by Bradford assay (Bio-Rad Laboratories, Hercules, CA).

Western blot. Equivalent (30 µg) amounts of protein were separated by gel electrophoresis on 10% polyacrylamide gels using appropriate molecular weight markers and transferred to PVDF membrane for immunoblotting. Blots were probed with anti-hsp70 antibody (1:10,000; kindly provided by Dr. Richard Morimoto, Northwestern University). The blots were then stripped and reprobed with anti-β actin (1:10,000, Sigma-Aldrich, St. Louis, MO) to confirm equivalent loading. Visualization was by the ECL detection system as previously described (Lin et al., 1998).

Reverse-transcriptase polymerase chain reaction (rtPCR). Total RNA was extracted from non-ischemic and ischemic left ventricular (LV) heart tissue using Trizol reagent (Life Technologies, Inc., Rockville, MD). Total RNA (0.1 µg) was processed directly to cDNA synthesis using the TaqMan® Reverse Transcriptase Reagents kit (Applied Biosystems, Foster City, CA). All PCR primers and TaqMan probes were designed using PrimerExpress software (Applied Biosystems) and published sequence data from the NCBI database (Lin et al., 2001).

Quantification of bands on films from Western blots and rtPCR. The films from Western blots and rtPCR analyses were scanned into a computer. The density of the bands was measured using image analysis software (ImageJ v. 1.38, NIH).

Statistical analysis. Continuous variables are expressed as mean ± standard error and compared using two-tailed independent t-testing with Levene’s Test for Equality of variances. Categorical variables were compared by χ² tests. Paired t-testing was used to evaluate significance within groups at multiple time points. For all analyses, a P-value of less than 0.05 was considered statistically significant. All analysis was performed using SPSS software (v. 11.5, Chicago, IL).

Results
EMF-induced hsp70 levels
As described above, rats were exposed to EMF (60 Hz, 8 µT) for 30 min, blood samples were collected from the left femoral vein from both EMF-pre-treated and unexposed (control) rats just before (baseline) and immediately after EMF exposure (time 0) and at 30, 60, 90, and 120 min. Blood samples were prepared for protein extraction and subsequent Western blot analyses of hsp70. As seen in Figure 2A, the levels of hsp70 protein were significantly elevated following 30 min of EMF pre-exposure, and the increase in protein level was sustained for 120 min post-exposure (P < 0.05 vs. Control, n = 6 per group, Fig. 2A); peak levels were reached at 30 min. Low baseline (constitutive) levels of hsp70 protein exist under non-stressed conditions. After EMF exposure, these levels were significantly elevated (≥40%).

EMF-induced transcript levels for HSP70 RNA
Terminal myocardial tissue was collected for determination of HSP70 RNA by rtPCR. Transcript levels of HSP70 RNA were significantly increased in terminal myocardial tissue extracts in response to EMF pre-exposure as compared to controls (P < 0.05 vs. Control, n = 6 per group), demonstrating upregulation of the HSP70 gene (Fig. 2B). There was no increase in HSP70 transcript levels in Control animals during the same time period. These results confirm previous literature that EMF induces upregulation of the HSP70 gene and increases hsp70 protein levels (Lin et al., 1997, 1998, 1999, 2001).

Ischemia-reperfusion: hsp70 levels
Levels of hsp70 were increased in both non-ischemic (N) and ischemic (I) left ventricle (LV) tissue after the termination of I-R in EMF-exposed samples as compared to Control animals (P < 0.05 vs. Control, n = 10 per group; Fig. 3A). rtPCR analysis of terminal LV tissue from ischemic (I) and non-ischemic (N), showed increased RNA transcript levels in EMF pre-exposed tissue as compared to controls (P = 0.053 vs. Control, n = 10 per group; Fig. 3B). The current results confirm that, in this ischemia-reperfusion model, EMF exposure upregulates the HSP70 gene and significantly increases hsp70 levels.

Ischemia-reperfusion: Hemodynamics
No adverse events or mortality were associated with EMF exposure. The mean temperatures before and after 30 min of EMF exposure were 36.1 ± 0.2°C and 36.0 ± 0.2°C, respectively, versus 36.0 ± 1.1°C in Control rats (EMF vs. Control, P = NS). The hemodynamics at baseline, after 30 min of coronary ischemia and after 10-30 min of reperfusion, are summarized in Table 1. LVSP was significantly improved beginning at 10 min of reperfusion until 30 min in EMF-treated animals versus Control (Fig. 4A). No significant differences were seen in LVEDP, ESV, and EDV. Coronary ischemia produced a significant reduction in LV dp/dt max (a measure of ventricular contractility) after 30 min. Recovery of LV dp/dt max
ventricular wall compliance, allowing faster and greater diastolic filling. Greater diastolic filling and relaxation of the ventricular wall improves cardiac output, lessens myocardial oxygen consumption, and increases the overall efficiency of the heart. Although no significant changes in arterial elastance were seen, a strong recovery trend of cardiac output to 71% of baseline with EMF versus only 43% in Control was observed. Adverse changes in arterial elastance would potentially affect long-term remodeling of central arteries, leading to multi-organ dysfunction (most notably, renal failure).

In summary, a clear effect on systolic contractile function in EMF-treated animals was found after reperfusion, as shown by increases in global indices of contractile function (LV dP/dt max and pre-load adjusted maximum power). These changes occurred without evidence of concurrent LV hypertrophy or at
the expense of reduced diastolic function, and without increased ventricular dimensions, which would have been expected with pressure-volume overload seen after ischemic injury. The changes in diastolic function may also reflect an effect of hsp70 on stabilization of cellular structure.

Discussion

The cytoprotective effect of stress response proteins, specifically hsp70, as a cellular defense mechanism, has been well described in the literature (Westerheide and Morimoto, 2005; Liu et al., 2007; Zhao et al., 2007; Brocchieri et al., 2008). Myocardial preservation after HSP70 gene transfection was first reported by Suzuki et al. (1997) who showed that increased hsp70 protein resulted in improved recovery of coronary flow, maximum LV dP/dt, and LV developed pressures. In isolated rat hearts undergoing 30 min of ischemia and 30 min of reperfusion, post-ischemic mitochondrial respiratory control indices (NAD' - and FAD-linked respiration) were improved in HSP70-transfected animals (Jayakumar et al., 2001). In a similar study, HSP70 gene transfection attenuated creatinine release and preserved coronary endothelial response to vasodilatory agents (Jayakumar et al., 2001). Finally, infarct reduction of almost 50% was demonstrated in HSP70-transfected rabbit hearts after ischemia-reperfusion (Okubo et al., 2001).

In the experiments reported here, we have applied 60 Hz, 8 μT EMFs to elevate hsp70 levels. Based on prior studies, we hypothesized that EMF exposures would increase levels of hsp70 and be cytoprotective after ischemia-reperfusion. Following ischemia-reperfusion after EMF exposure, we found significantly increased expression of the HSP70 gene and elevated hsp70 protein levels, and myocardial function (both systolic and diastolic) was significantly improved.

EMF-exposure improves contractile function and reduces reperfusion injury

The hemodynamic changes due to EMF exposure prior to ischemia are slight. However, our hemodynamic findings after ischemia-reperfusion demonstrate that EMF-induction of elevated hsp70 levels significantly reduced cardiac injury. Systolic contractile function, measured by LV dp/dt max and left ventricular systolic pressure (LVSP), was significantly increased in exposed animals compared to control animals, despite reductions in all groups after 30 min of ischemia. Pre-load adjusted power, a pre-load and rate-independent measure of systolic function, was also improved throughout the reperfusion period. Although LVEDP did not change, isovolumic relaxation was substantially improved after EMF exposure, suggesting that diastolic function was not adversely affected in order to maintain hemodynamics.

The changes in ventricular function appear to relate primarily to the ability of the myocyte to tolerate ischemia and prevent injury after reperfusion. After reperfusion, in addition to molecular mechanisms that permanently and directly cause cell death, cardiomyocytes face secondary insults in the form of inadequate coronary perfusion as a result of impaired hemodynamics (i.e., lower systolic pressures, decreased cardiac output, reduced contractility). The degree of ischemic tolerance displayed by EMF-exposed animals to resist the usual hemodynamic derangement after reperfusion is a likely factor in both cell survival and augmentation of existing cell functional units. Subsequently, reduction of infarction, salvage of underperfused myocytes at risk for necrosis, and increased myocardial perfusion are all expected cellular changes (Heads et al., 1995; Mestrel et al., 1996; Plumier and Currie, 1996; Suzuki et al., 1997; Benjamin and McMillan, 1998; Chong et al., 1998; Cornellussen et al., 1998; Jayakumar et al., 2000, 2001; Okubo et al., 2001). Improvements in systolic function can serve to interrupt the cycle of death which normally follows impaired contractility and prolonged hypoperfusion. Finally, subtle changes in ventricular compliance (i.e., a reduction in τ) may be critical for diastolic filling after reperfusion, especially in the setting of ischemia and increased myocardial oxygen consumption/wall stress.

Possible mechanisms for improved myocardial function by EMF

Recent studies have shown strong correlation between myocardial calcium handling to the function of hsp70—for example, deleting the inductive 70 kDa heat shock genes impairs cardiac contractile function and calcium handling associated with hypertrophy (Okubo et al., 2001; Kim et al., 2006). The Na/Ca exchanger, known to play an important determinant of myocardial contractility in heart failure, is acted on by hsp70, leading to desensitization by a reduction in V_{max} (Kiang et al., 1998). Hsp70 may also alter protein kinase C, and phospholipase A_2 (Ding et al., 1998): it is conceivable that hsp70 also modulates other components of calcium-handling, such as SERCA2a and phosphorylation of the ryanodine receptor (Marx et al., 2000). These results suggest interaction of hsp70 with elements of the calcium release mechanism that result in enhanced myocardial contractility.

Induction of hsp70 levels by rapid elevation of temperature also improves cytoskeletal-based cell survival pathways (Wei et al., 2006). Maintenance of cell architecture associated with hsp70 has been reported in the literature previously (Wei et al., 2006), and anti-apoptotic mechanisms of stress response

<table>
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<th>TABLE 1: Hemodynamics and pressure-volume analysis</th>
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<td>Ea (mmHg/ml)</td>
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EMF, electromagnetic field; LVEDP, left ventricular end-diastolic pressure; Ea, arterial elastance.

*P < 0.05 vs. Normal.

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EMF and biological interactions

EMF interaction with cells and tissues has been extensively studied in vivo and in vitro (reviewed in Goodman and Blank, 2002; Blank and Goodman, 2004, 2007). EMF is known to induce elevated DNA transcript levels of several genes including HSP70 as well as elevated levels of hsp70 protein in the absence of increased temperature (reviewed in Goodman and Blank, 1998). The interaction mechanism of EMF with DNA in cells and tissues to stimulate protein synthesis remains unknown. Several theoretical approaches to EMF mechanism have been proposed including cyclotron resonance (Liboff, 1985; Lednev, 1991) and forced vibration of ions (Panagopoulos et al., 2002). There is evidence from biochemical reactions that EMFs can accelerate electron transfer and move within DNA (Blank and Soo, 2001; Goodman and Blank, 2002, 2007).

An important clue to EMF stimulation of biosynthesis comes from identification of a specific EMF-sensitive DNA sequence on both the c-myc and the HSP70 gene promoters (Lin et al., 1999, 2001). The HSP70 promoter has three nCTCTn recognition motifs/response elements (−158 to −203 relative to the transcription initiation site) that are EMF-sensitive (Lin et al., 1999). The heat shock element (HSE), lying between −180 and −203, is required for induction of HSP70 gene expression by EMFs (Lin et al., 1999, 2001). Mutation of the nCTCTn sequences (EMRE, electromagnetic response elements) eliminates the EMF sensitivity of the HSP70 promoter (Lin et al., 2001). The EMF domain and the heat shock domain function independently. This is an HSF-1 dependent process (Lin et al., 1998, 1999; Lin et al., 2001). Furthermore, phosphorylation and activation of the upstream kinase SEK, or by inhibition of stress-induced suppression of JNK dephosphorylation (Merin et al., 1999; Yaglom et al., 1999; Park et al., 2001). A secondary effect of HSP70 gene upregulation is increased manganese superoxide dismutase activity, which serves to limit mitochondrial apoptosis in ischemia-reperfusion (Suzuki et al., 2002). Interestingly, MDA and LDH levels, markers of cardiac injury, were unchanged in our experiments (data not shown); this suggests that EMF does not diminish the extent of cardiac injury but rather enhances the contractile function of remaining myocytes.

![Fig. 4. Ischemia-reperfusion: Contractile function was significantly improved in response to EMF exposure. Contractile (systolic) function was significantly increased after ischemia-reperfusion in response to 30 min of EMF exposure, as measured by ventricular systolic pressure (LVSP; A), left ventricular dP/dt (LV dP/dt,max; B), and preload adjusted maximum power (P-A Max Power; C).](image)

![Fig. 5. Ischemia-reperfusion: diastolic function was significantly improved in response to EMF exposure. Diastolic function, or isovolumetric ventricular relaxation as measured by Tau (τ), was significantly improved throughout Reperfusion after 30 min of EMF exposure (\( P < 0.05 \) vs. Baseline, \( P < 0.05 \) vs. Control).](image)
the HSE in the heat shock domain is not interchangeable with the HSE in the EMF domain (Lin et al., 2001). nCCTn sequences, placed upstream of CAT or luciferase reporter constructs (that were otherwise unresponsive to EMFs) were transfected into HeLa cells and exposed to EMFs. Protein extracts from EMF-exposed transfectants had significant increases in both CAT and luciferase activity, as compared with identical transfectants that were sham exposed (Lin et al., 2001).

Interaction with electrons could account for activation of DNA by both low and high frequency EMFs. An EMF sensitive DNA sequence suggests that EMFs may interact both directly and indirectly with DNA. The initial interaction could involve the displacement of electrons in the H-bonds that hold DNA together, thereby causing chain separation and initiating transcription and translation. Blank and Goodman (2007), using experimentally observed processes as links in a causal chain, have proposed that DNA activation of transcription is based on EMF's displacement of electrons in DNA by the EMF and that this causes transient charging of small groups of base pairs (e.g., nCCTn). As the charged sites disaggregation forces overcome the H-bonds, and this disaggregation of the two chains at those sites permits transcription.

Clinical use of EMF technology

An abundance of hsp70 is clearly important to limit myocardial injury, following coronary occlusion by reducing infarct size and by increasing contractile function. Modulation of hsp70 levels in the heart, using heat stimulation, is currently problematic from a temporal standpoint. It is known that a twofold induction of hsp70 improves heart muscle cell resistance to oxidation, ischemia and hypoxia (Heads et al., 1995; Mestril et al., 1996; Chong et al., 1998). Endogenous hsp70 doubles after only 1 h following coronary artery occlusion (Loncar et al., 1998). It may take up to 24 h for hsp70 levels to reach a four- to fivefold level. The four- to fivefold level has historically been required, as a minimum, to provide improved ischemic tolerance. This is outside the "golden window of opportunity" to protect the myocardium, which is at the greatest risk in the first 6 h after occlusion. Lower levels of hsp70 have not provided sufficient ischemic tolerance to prevent permanent myocardial damage. In our experiments, augmented hsp70 levels occur as early as 30 min after exposure, and last up to 3 h. This is well-within an average door-to-intervention period for an acute coronary syndrome. The importance of this specificity is that EMF exposure produces hsp70 without the untoward downstream effects of ubiquitous stress response activation.

It is clear that significant potential exists for this technology, it is most applicable to the coronary revascularization population, consisting of patients undergoing coronary artery bypass grafting and PCs. With open-heart surgeries and PCI being performed for expanding indications of revascularization, the need for protective strategies is even more pressing. Both therapies aim to restore coronary flow to underperfused myocardium after periods of critical or sub-acute hypoperfusion. Any portion of myocardium that faces hypoperfusion followed by reperfusion faces the hazards of reperfusion injury (i.e., hemodynamic dysfunction, infarct expansion, or arrhythmias).

In the controlled setting of cardiopulmonary bypass in the operating room or in the angiography suite, EMF exposure can easily be incorporated into the clinical care protocols. Exposure times of 30 min prior to intervention can easily be coordinated with ischemia and timed reperfusion. An EMF device for use in the operating room or the emergency room would be uncomplicated. For example, in current EMF devices used for bone non-union and wound healing, coils are within lightweight binding strap that are placed on the patient before, during and/or following surgery. The exposure technology is entirely non-invasive. This device is portable, weighs about one pound, and can be easily applied by any technician.

The use of EMFs for the induction of hsp70 for post-ischemia-reperfusion treatment has clear advantages over the invasive elevated temperature treatment efforts tested to date. Non-ionizing EMF induction of hsp70 is safe, efficient and practical. These methods can be administered and readministered prior to and during coronary interventions. Furthermore, hsp70 levels can be increased repeatedly with EMF versus the limited single use of the thermal method.

Summary and Conclusion

In these experiments, we report a novel non-invasive technique to increase hsp70 levels using exposure to low energy, low frequency EMF. While stress proteins in cells and tissues have been previously utilized as diagnostic markers and prognostic indicators, a safe, non-invasive method of augmenting endogenous defense mechanisms as a therapeutic tool, such as EMF exposure, has significant clinical potential. Our data indicate that pre-exposure with EMF prior to ischemia and reperfusion, in a mammalian model, induces upregulation of the HSP70 gene, subsequently increased levels of hsp70 protein, and, most importantly, improved ventricular function after ischemia-reperfusion.

Acknowledgments

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