Effect of a 100mT Static Magnetic Field on *Hsp70* mRNA in HL-60 Cells

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Abstract

Cellular stress response proteins like *Hsp70* may be affected by exposure to static magnetic fields (SMF). We examined whether exposure to a 100 mT SMF and GSH-depleting diethymaleate (DEM) would affect the levels of *Hsp70* mRNA in HL-60 cells. Cells were exposed to SMF, DEM, a combination of DEM and SMF as well as 42ºC. We found that only exposure to 42ºC significantly affected the levels of *Hsp70* mRNA. Exposure to a 100 mT SMF, DEM, or a combination of SMF and DEM must not affect protein stability thereby altering levels of *Hsp70* mRNA.

1. Introduction

The effect of magnetic fields on biological systems has been studied for a number of years. These studies have produced conflicting evidence on the influence of static magnetic fields (SMF) on systems such as calcium homeostasis and protein levels involved in cellular stress responses. Although follow-up reports from a variety of groups are not directly comparable due to differences in experimental variables including strength of the magnetic field, length of exposure, and organism, there has been consistent use of relatively weak magnetic fields (40-600μT), which correspond to occupational and residential situations

Heat shock proteins are involved in stress related cellular responses involving protein folding. The stressors that trigger this response vary widely and include both chemical and physical agents (e.g. heat and magnetic fields). The effect of magnetic fields on the expression of heat shock genes has been examined extensively since the discovery that weak electromagnetic fields increased the amount of *hsp70* transcript in human cell lines and yeast [1].

Since the observable effect of magnetic field exposure may be small under normal cellular conditions, manipulation of the cellular environment to amplify potential effects may allow for measurable and reproducible findings. Diethylmaleate (DEM) changes the cellular environment by decreasing the amount of reduced glutathione (GSH) in the cell. The decreased concentration of this free radical scavenger will theoretically increase the level of damaging radicals in the cell. This in turn may amplify the cell's response to magnetic field exposure.

2. Objective

The objective of this study was to examine the effect of a 100 mT SMF after treatment with DEM on the amount of *Hsp*70 mRNA in HL-60 cells.

3. Methods

Undifferentiated HL-60 cells were centrifuged, resuspended in a balanced saline solution, and placed in a disposable cuvette. The cuvette was placed between the poles of a magnet held within a circulating water bath. Cells were acclimated within the apparatus at 37ºC and 0 mT with constant stirring for 20 min. Sham-exposed cells remained under these conditions for an additional 30 min then immediately removed from the apparatus. Cells used for the positive control (heat) were exposed to 42ºC for 20 min. Experimental cells were exposed to A) 9 mM DEM at 20 min and 100 mT SMF at 25 min; B) 9mM DEM at 20 min, or C) 100 mT SMF at 25 min. All experimental

cells were removed from the apparatus at 50 min. Immediately following exposure, RNA was isolated from the cells to be used in reverse transcription-PCR. The PCR was done using multiplexed primers for *Hsp70* and the internal control GAPDH. The average integrated density value (AVG) of the band for each individual PCR product was determined using AlphaImager 2200 v5.5 gel doc software. To determine the size of any effect, the AVG value of each *Hsp70* specific PCR band was divided by the AVG value of its corresponding GAPDH specific band to obtain a normalized *Hsp70* value. The normalized values of SMF-exposed, DEM-exposed and SMF- plus DEM-exposed were divided by the control value to obtain a ratio of effect size.

4. Results

Analyses of the normalized AVG ratios of 11 to 12 replicates of each condition showed that there was an average 1.5X increase in the level of *Hsp70* mRNA in DEM-exposed cells as compared to control, a 1.6X increase in the SMF-exposed cells, a 1.8X increase in the SMF- plus DEM-exposed cells, and a 4.9X increase in heat-exposed cells (Figure 1). The observed differences in the DEM-, SMF-, and (SMF plus DEM)-exposed cells were not statistically significant as determined by a one-way ANOVA. The heat-exposed cells did show a statistically significant difference in mRNA levels compared to control cells.

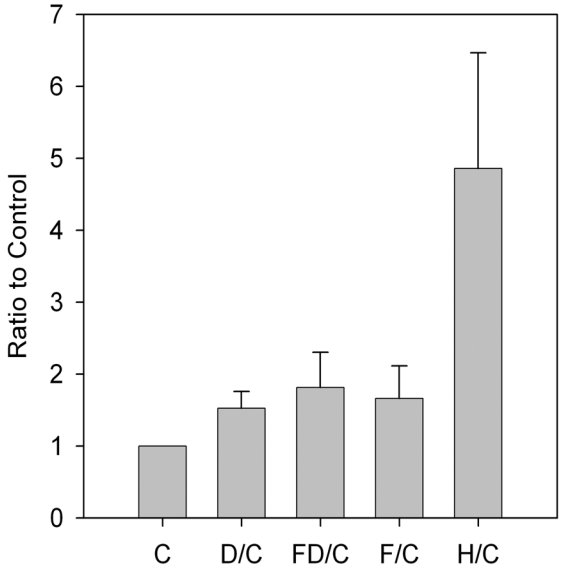

Figure 1: Normalized AVG ratios of 9 mM DEM (D), 100 mT SMF field and 9 mM DEM (FD), 100 mT SMF (F) and 42ºC exposure (H) as compared to the control (C). Each bar represents the average of 11-12 replicates. Error bars represent SEM.

5. Discussion

We found that there was no statistically significant effect on the amount of *Hsp70* mRNA when HL-60 cells were exposed to DEM, a 100 mT SMF, or a combination of DEM and SMF. In contrast, exposure to 42ºC (i.e. positive control) did lead to a statistically significant increase in the amount of *Hsp70* mRNA. Heat shock proteins are the hallmark of cellular stress; however, they also play an important role in the unstressed cell by assisting with the folding of newly synthesized proteins. Since most stressors act as protein denaturants it follows that such stress would induce the increased production of heat shock proteins. Exposure to a 100 mT SMF for 30 min may not be a severe enough condition to affect protein stability within the cell, which could explain why levels of *Hsp70* were not increased. Surprisingly, treatment of the cells with 9 mM DEM, which has been shown in our lab to significantly increase cytosolic free Ca^{2+} concentration, did not increase *Hsp70* mRNA levels, nor did the combination of DEM and SMF. Thus, despite the fact that DEM exposure depleted GSH levels enough to alter intracellular calcium homeostasis and presumably increased the levels of protein damaging radicals, the cells did not respond through increased levels of *Hsp70*.

It has also been proposed that magnetic fields exert their effect on the plasma membrane [2]. If this is the case, then a SMF exposure longer than 25 min may be required to affect the plasma membrane, which might in turn alter the levels of *Hsp70* mRNA [3].

Future work could include increasing the concentration of DEM until a measurable change in the cellular expression of *Hsp70* is observed followed by longer exposures to a SMF.

6. References

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7. Acknowledgments

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