REAL-TIME MEASUREMENT OF CYTOSOLIC FREE CALCIUM CONCENTRATION IN DEM-TREATED HL-60 CELLS DURING STATIC MAGNETIC FIELD EXPOSURE AND ACTIVATION BY ATP

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1. Objectives

Studies have shown that static magnetic fields (SMF) can exert effects on biological systems [1]. One proposed method of SMF coupling with biological systems is through an action on free radical reaction kinetics [2]. Elevated intracellular free radical concentration can lead to oxidative stress, which can result in a variety of biological effects including DNA damage [3], cell dysfunction, and accelerated cell aging [4]. To blunt the impact of free radicals, cells have evolved buffering mechanisms, the most important of which is the glutathione disulfide-glutathione (GSSH/2GSH) couple [5]. Perturbation of this buffering mechanism results in altered intracellular free radical concentration, and so may affect the cellular response to a SMF. Cytosolic free calcium concentration ($[Ca^{2+}]_c$) is a potentially sensitive measure of cellular responses to external stimuli since it is involved in many biological functions including differentiation and proliferation [6]. Using $[Ca^{2+}]_c$ as a readout, this study investigated whether manipulation of radical buffering through depletion of GSH affected the sensitivity of HL-60 human leukemia cells to SMF exposure.

2. Methods

Cytosolic free calcium concentration was measured in real-time using a ratiometric fluorescence spectroscopy system. Human leukemia HL-60 cells were loaded with fura-2AM, placed in a cuvette, and then incubated in a circulating water bath at 37 ^{o}C. Cells were acclimated for 1200 s, at which time 8 mM of diethyl-maleate (DEM) was added to cells to deplete GSH. At 2700 s, cells were exposed to a SMF of 0 mT (sham) or 100 mT. Cells were then activated with 1 μM ATP at 3000 s. The field was return to sham levels at 3480 s and a fluorescence calibration was performed to facilitate calculation of $[Ca^{2+}]_c$. The potential systematic error related to sample order and simulated turn-on of the magnetic field was also investigated. Five $[Ca^{2+}]_c$ related measurements were obtained for each experiment: Pre-DEM exposure, Post-DEM/Pre-Field exposure, Post-DEM/Field exposure, peak $[Ca^{2+}]_c$ following ATP activation, and the full width at half maximum (FWHM) of the peak $[Ca^{2+}]_c$ response. Statistical analysis of data included a paired t-test and 2-way ANOVA.

3. Results

A systematic error was observed for the Peak-ATP metric, where the peak $[Ca^{2+}]_c$ depended on consecutive cell sample removal from one flask ($P<0.05$). Comparison of calcium related metrics between sham and 100 mT experiments revealed the following results: post-DEM/Field $[Ca^{2+}]_c$ was 53±2 nM and 58±2 nM for sham and 100 mT groups. Peak $[Ca^{2+}]_c$ was 189±10 nM and 185±9 nM for sham and 100 mT groups. FWHM was 51±3 s and 54±3 s for sham and 100 mT groups. There was no statistically significant difference between sham and 100 mT groups for any of the five calcium related metrics.

4. Conclusions

Based on the results from this study, it was observed that $[Ca^{2+}]_c$ did not change during SMF exposure in DEM-treated HL-60 cells either at rest or after activation with ATP. The finding supported the hypothesis that a 100 mT SMF had no effect on resting or activated $[Ca^{2+}]_c$ in HL-60 cells even when the intracellular free radical concentration was manipulated. There are other possibilities however. For example, (1) an effect of SMF might have been present but not measurable with the $[Ca^{2+}]_c$-dependent metrics measured in the study, and (2) potential effects of SMF on HL-60 cells may not be influenced by the presence or absence of DEM. Two streams for future work can be suggested from these possibilities. The experiments could be repeated over a greater range of magnetic field strengths above and below 100 mT. Also, repetition of the experiments at a variety of doses of DEM and with GSH potentiators such as L-NAC and glutathione diesters would allow for a greater range of GSH levels to be tested.

5. References:

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