Non-thermal electromagnetic fields increase rate of hemoglobin deoxygenation in a cell-free preparation

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Abstract - The reducing agent dithiothreitol was used to deoxygenate human haemoglobin, and the time course observed via visible light spectroscopy. A decrease in the time required for deoxygenation was found for exposures to both a pulsed radiofrequency signal currently in clinical use for treatment of pain and edema, and a 150 mT static magnetic field.

INTRODUCTION

Previous studies have demonstrated EMF sensitivity of human hemoglobin in solution and erythrocyte suspension [1-6]. Radiofrequency mobile telephone signals have been shown to decrease hemoglobin oxygen affinity [7], and changes in infrared absorption have been reported for low-frequency EMF[8]. Results from this study, using a clinically effective non-thermal pulse-modulated radio frequency EMF signal, indicate an effect on Hb deoxygenation in aqueous solution. Hemoglobin deoxygenation was assessed using the reducing agent DTT in an assay that yielded complete oxy (HbO2) \(\rightarrow\) deoxy (Hb) conversion over a period of several minutes to several hours, dependent upon the relative concentrations of DTT and Hb. Exposure to a pulse-modulated PRF yielded increases in the rate of HbO2 \(\rightarrow\) Hb, several hours after EMF exposure was ended. Similar results were obtained for a 150 mT static magnetic field.

METHODS

120 mM Hbo2 obtained from fresh human blood was prepared in 50 mM Hepes buffer (pH 7.2), and HbO2 \(\rightarrow\) Hb conversion induced with 20-50 mM DTT at 22.1°C. Upon addition of DTT, the 10 mL reaction mixture volume was divided into 1 mL aliquots in sealed 1.5 mL Eppendorf tubes and exposed for 10-90 minutes to either the ambient geomagnetic field (null), or ambient plus a 0.1 G, 27.12 MHz pulse-modulated radiofrequency EMF (Ivivi Health Sciences, LLC, San Francisco) transmitted in 4 ms bursts, repeating at 5 Hz (PRF). Exposures were also made using permanent ceramic magnets to produce a 150 mT SMF. Triplicate samples were taken from each tube and HbO2 \(\rightarrow\) Hb conversion was measured spectrophotometrically at 540, 560 and 576 nm immediately post EMF exposure and at successive 1 min intervals, until maximal conversion was observed.

RESULTS

Immediately post-exposure, no differences were found between PRF, SMF and null exposures. In contrast, 150 min after PRF or SMF exposure, significant changes in visible light spectra were observed in the 540-576 nm region (Figure 1, upper panel). PRF-treated samples yielded a 14.7% increase in the ratio of Hb/HbO2 (p < 0.01), corresponding to a measured 4.21 \(\mu\)M (13.2%) decrease in HbO2 (p < 0.002) and a concomitant 4.34 \(\mu\)M (6.2%) increase in Hb (p < 0.005) (Figure 1, lower panel). For the ratio of DTT/HbO2 employed here, 150 min correspond to the time of most rapid deoxygenation. In preliminary SMF experiments, results obtained using a 150 mT exposure were similar in magnitude to those obtained with PRF exposure. Addition of 5M Urea to the reagent solution decreased the time to deoxygenate and increased substantially the difference in time to deoxygenate for EMF-treated vs. untreated samples (Figure 2, upper panel). Under these conditions, the SMF-treated samples undergo complete deoxygenation before the untreated samples begin to lose O2 (Figure 2, lower panel). Addition of 100 mM MgCl2 further decreased the time to deoxygenate. However, in the absence of urea, 100 mM MgCl2 did not alter the time required for deoxygenation.

CONCLUSIONS

Exposure to EMF can alter the rate of DTT-induced deoxygenation of human hemoglobin, resulting in more rapid conversion of HbO2 \(\rightarrow\) Hb. The rate of HbO2 \(\rightarrow\) Hb conversion is nonlinear in time and is dependent upon the ratio of DTT/HbO2. By reducing this ratio, EMF effects may be observed as long as 12 to 24 hours after PRF or SMF exposure. In the absence of urea, the effects of a clinically effective PRF signal and a 150 mT SMF were similar. However, in 5 M urea the effect of the SMF on time to
deoxygenate was greater than that due to PRF, suggesting that further work is required to assess possible differences between electric and magnetic field effects. The mechanism by which this EMF effect occurs is presently unknown. However, the observed effect occurs several hours after the EMF exposure is removed, suggesting that EMF modifies the protein tertiary structure in a manner that alters the energy required for the oxy-deoxy conformational change. The observation that differences in visible spectra are not apparent until the time of rapid deoxygenation suggests that the known cooperativity of Hb oxy-deoxy conversion contributes to the sensitivity of the DTT deoxygenation reaction to EMFs. If the increase in the deoxygenated species observed here can be shown to occur in vivo, then an increase in oxygen availability may be one means by which clinically relevant EMF-mediated enhancement of growth and repair processes can occur.

**Figure 1.** Upper panel: effect of pulsed radiofrequency (PRF) and static magnetic (SMF) fields on Hb deoxygenation at 150 minutes post EMF exposure. Deoxygenation occurs at an earlier time for EMF exposed samples and EMF effect is observable at time of most rapid deoxygenation. Lower panel: PRF treatment of Hb under deoxygenating conditions. PRF exposure resulted in 14.7% increase in the ratio of deoxy/oxy Hb, suggesting that PRF treatment alters Hb tertiary structure and energy required to bind oxygen.

**Figure 2.** Upper panel: Hb Deoxygenation after 10 minute exposure to 150 mT static magnetic field in 5M urea. No apparent change in oxy/deoxy ratio is visible until time of rapid deoxygenation. Lower panel: addition of 5M urea to reaction solution renders effect more apparent. Treated samples are completely deoxygenated before untreated begin to lose O₂.

**REFERENCES**


