

ESR spin trapping analysis of lipid radicals formed in PC-3 cells during hyperthermia treatment

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Lipids play a dual role in cell biology. They are essential components of cell membranes helping to control a majority of cellular activities. However, they are also the major determinants of many pathologies. In order to understand the radical mechanism by which the cells are damaged, its important to determine the radical species formed during cellular lipid peroxidation. ESR is a prime tool to study such short-lived radicals using spin traps. Our goal was to examine the mechanism behind the oxidative stress associated with hyperthermia. We hypothesized that lipid peroxidation is induced during hyperthermia. If lipid peroxidation is an important factor in hyperthermia, then increased levels of the antioxidant enzyme MnSOD should provide protection against hyperthermia. PC-3 cells (WT) and cells stably transfected with MnSOD (PC-3 MnSOD) were subjected to 43 degrees C for 1 h and incubated at 37 degrees C for 0-24 h. To test our hypotheses we used Folch extraction to isolate the lipid derived radical adducts of DMPO and analyzed them with ESR. The spectra were simulated and the radical species were identified. We found that cells with higher MnSOD expression had significantly lower levels of lipid radicals than WT cells as analyzed with ESR spin trapping. This observation is supported by independent measurement of steady state levels of LOOH. The detection of lipid-derived radicals using our approach clearly demonstrates that free radical-mediated lipid peroxidation is enhanced in cells subjected to hyperthermia.