

Effects of Manganese on Oxidative-Stress in CATH.a Cells

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ABSTRACT

The present study addressed the effects of Mn on oxidative stress in a catecholaminergic CATH-a cell line. Oxidative stress was measured with the fluorescent dye, 2',7'-dichlorofluorescein (DCFH). In the diacetate form the dye is taken up by the cells and cleaved by esterases, effectively trapping it within the intracellular space. DCFH is subsequently oxidized in the presence of reactive oxygen species (ROS) to the fluorescent 2',7'-dichlorofluorescein. The fluorescence was analyzed on an ACAS 470 Interactive Laser Cytometer. Treatment of CATH-a cells with MnCl₂ (up to 10 mM) from 10 min. up to 48 hrs. was not associated with increased intracellular ROS formation. While Mn did not significantly increase the rate of ROS formation, when Mn exposure was followed with an additional 5 min. treatment with H₂O₂, Mn (at concentrations > 5 mM) significantly increased (p<0.05) the effect of H₂O₂ on ROS generation. Prolonged (24 hr.) Mn treatment prior to exposure to H₂O₂ was associated with a statistically significant (p<0.05) reduction in ROS generation compared with cells treated with H₂O₂ alone. This statistically significant decrease (p<0.05) in ROS generation was preserved in CATH-a cells that were treated for 48 hrs. with 10 and 100 μM Mn followed by H₂O₂ exposure. Although the trend for diminished ROS generation was also apparent with 500 μM and 750 μM Mn (48 hrs.), the decrease did not attain statistical significance. Combined these results suggest that Mn can act as both pro- and antioxidant, and that oxidative stress-related effects of Mn are likely dependent not only on the intracellular concentrations of the metal, but also exposure duration, secondary oxidative challenges, and the overall oxidant "buffering" capacity of the cells (The study was supported by the International Manganese Institute, Paris).