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Neuronal Studies of Manganese Toxicity

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Beamline(s): X9B

Introduction: Welders, metal workers, and manganese miners have developed neurological symptoms somewhat similar to those of Parkinsonism because of inhaling dust containing manganese. The primary effects relate to loss of dopamine in the striatum and cell death in the globus pallidum. MMT, a manganese compound, has been used as a gasoline additive in the US and Canada, exposing vast populations to Mn-containing dust. Inside neuronal tissue, manganese is sequestered by mitochondria. The oxidation state of intraneuronal and intramitochondrial manganese is an important factor in hypotheses concerning the mechanism by which manganese acts. The primary goal of this work was to determine the amounts of Mn^{2+} and Mn^{3+} present under a range of relevant conditions. A secondary goal was to determine the manganese complexes present in these samples.

Methods and Materials: During the period 10/01/01 to 9/30/02, we used the facilities of beamline X9B at the National Synchrotron Light source at Brookhaven National Laboratory to study manganese in neuron-like cells and astrocytes. The cells studied were PC12 cells, NT2 cells, PC12 cells induced to display a more neuronal phenotype using nerve growth factor, and cultured astrocytes.

Results: XANES spectra of cells of these types which had sequestered varying amounts of manganese under varying conditions were compared with the XANES spectra of model Mn compounds chosen to represent likely complexes found intracellularly or to be similar to such complexes. These model compounds included Mn^{2+} , Mn^{3+} and Mn^{4+} complexes. It has already been shown by others that Mn^{2+} can be oxidized to Mn^{3+} by superoxide radical and many hypothesize that Mn-induced damage at the cell level is caused by oxidation of important cellular components by Mn^{3+} . Our XANES results however, showed no evidence for formation of Mn^{3+} from Mn^{2+} , even after days of incubation of Mn in these cells and after days of incubation under the more pro-oxidant conditions induced by additions of tert butyl hydroperoxide. Through spectroscopic similarity, our data support the view that most of the Mn in these cells is in the Mn(II)ATP complex; however, evidence for other complexes is also seen in smaller amounts.

Conclusions: Our earlier results showed that even though mitochondria accumulate Mn^{2+} and produce the largest concentrations of superoxide radical in the cell, and even though superoxide radical has been shown to be capable of oxidizing Mn^{2+} to Mn^{3+} , no evidence was found for stabilization of Mn^{3+} in mitochondria. The work in the current period has extended these findings to show that there is no evidence for stabilization of Mn^{3+} in astrocytes or neuron-like cells. The XANES spectra of Mn in the cells studied varied somewhat from cell type to cell type but generally seemed most similar to Mn(II)ATP. This suggests that any damage caused by Mn^{3+} must be initiated during the brief period between the formation of Mn^{3+} by action of superoxide radical and its reduction to Mn^{2+} .

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