Mechanisms of Manganese-Induced Rat Pheochromocytoma (PC12) Cell Death and Cell Differentiation

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ABSTRACT

Our laboratory has been investigating the mechanism by which Mn induces rat pheochromocytoma (PC12) cell death and cell differentiation. We selected PC12 cell as our model system since it possesses much of the biochemical machinery associated with dopaminergic neurons. Mn-induced neuronal differentiation ofPC12 cells is dependent on the interaction of the cell surface integrin receptors with the basement membrane proteins vitronectin and fibronectin. Similar to NGF, Mn-induced neurite outgrowth is dependent on the phosphorylation and activation of the MAP kinases, ERK1 and 2 (P44/42). PC12 cell death induced by Mn is both time and concentration dependent with approximately 50% cell survival at 48 hrs in the presence of 0.3 mM Mn. Toxicity, most likely, is not caused by oxidative stress as no change in formation of the lipid peroxide product, 9-HODE, was observed in PC12 cells exposed to Mn. Mn was found to stimulate the activation of the apoptotic marker proteins, p38 and caspase-3 within 24 hrs of treatment. However, the selective inhibitor ofcaspase-3-like proteases, DEVD-CHO, and the nonselective caspase inhibitor, Z- V AD-FMK, fail to prevent Mn-induced PC12 cell death. The role of mitochondria in initiating Mn cytotoxicity was also investigated since prior studies demonstrate that Mn promotes changes in mitochondrial function and membrane permeability. ATP levels were decreased in PC12 cells exposed to Mn in both a time and concentration dependent manner. Based on our studies, we hypothesize that both apoptosis and necrosis contribute to Mn-induced PC12 cell death although the necrotic events prevail even when the apoptotic signaling is inhibited. Immunoblotting studies suggest that uptake ofMn into PC12 cells is regulated by the divalent metal transporter, DMT1. Consistent with