Potential role of heme oxygenase-1 in neuroinflammation caused by manganese

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In humans, excess exposure to manganese (Mn) is associated with a Parkinsonian type neurological disorder. While Mn can be directly neurotoxic to neurons (dopaminergic and GABAergic) in the midbrain, it can also increase glial (microglia and astrocytes) derived inflammatory products that contribute to the development of Mn neurotoxicity. Several studies report that Mn potentiates the production of inflammatory cytokines, reactive oxygen species, nitric oxide, and prostaglandins induced by inflammagens, such as lipopolysaccharide (LPS) in microglia cells. Inducible heme oxygenase (HO-1), an enzyme responsible for the cleavage of the oxidant heme into biliverdin, carbon monoxide, and iron (Fe), is increased in response to oxidative stress caused by various toxicants, including metals, and has been reported to play a role in the regulation of inflammation. Expression of HO-1 is increased in response to LPS. However, at present, the effect of Mn on HO-1 has yet to be determined. The first study was designed to examine the effect of Mn on HO-1 induction with and without the presence of LPS in microglia (N9) and dopaminergic neuronal (N27) cells and to uncover the role of HO-1 on cytokine potentiation by Mn. N9 microglia and N27 neuronal cells were exposed to either LPS (100 ng/ml), Mn (100 μM), or combined Mn+LPS for 24 hours. In microglia, while Mn had minimal effects on its own, induction of HO-1 (protein and mRNA) by LPS was potentiated by Mn. The increase in HO-1 was not due to increased intracellular Mn, but was accompanied by a small but significant increase in Fe concentration and an increase in mRNA and protein for iNOS, and the inflammatory cytokines TNF-α and IL-6. Mn also potentiated the increase in COX-2 levels caused by LPS. In contrast, neither LPS nor Mn had any effect on HO-1 in the N27 neuronal cells. Moreover, combined Mn+LPS treatment caused a small, but significant decrease in HO-1. These results indicate that Mn potentiates the induction of HO-1 by LPS in microglia, but not in neuronal cells. Because the microglial increase in HO-1 is accompanied by an increase in inflammatory mediators as well as Fe, there is a possibility that in the presence of Mn, HO-1 acts as a pro-oxidant and is involved in the enhanced cytokine production by Mn-exposed activated microglia that has been reported previously. To further investigate, N9 cells were pretreated (3 hrs) with an HO-1 inhibitor (tin protoporphyrin;SnPP), prior to Mn, LPS, and Mn+LPS exposure. In the presence of SnPP, Mn caused even greater potentiation of TNF- α and IL-6 levels in the culture medium, suggesting that increased HO-1 by Mn+LPS is not contributing to the potentiating effects of Mn on cytokine production. In fact, it has an anti-inflammatory role. Because H2O2 is a possible inducer of HO-1 and it has been reported to be increased in microglia exposed to Mn, we looked at the role of H2O2. As reported, Mn exposure increased H2O2 (6h post treatment). However this effect was not potentiated by LPS. Furthermore, the presence of the HO-1 inhibitor further increased H2O2 output by microglia exposed to Mn, as well as from control and LPS exposed microglia. Thus it appears that H2O2 is not responsible for the increased HO-1. On the contrary, HO-1 serves in an antioxidant capacity in part by controlling the redox status of microglial cells. Supported by: R01ES016965 (NIEHS)