Mercury Threatens Neurodevelopment

Methylmercury (MeHg) and Mercury Chloride (HgCl₂) are neurotoxic and neurodegenerative agents; exposures in Japan and Iraq have illustrated that they disturb fetal brain development at strikingly low doses. Both reactive oxygen-species (ROS) formation and the disruption of signaling networks are implicated in the toxicity of organic and inorganic forms of mercury; however, the time-, isoform-, and dose-dependent effects of these poisons are poorly understood. Previous work has suggested HgCl₂ and other metals may selectively inhibit Jak/Stat signaling1-3, an important modulator of neurodevelopment. Metal exposure can result from fish consumption and may be more relevant to human health. Therefore we replicated these studies to compare the toxicities of MeHg and HgCl₂ and their effects on CNTN-evoked Jak/Stat signaling. The involvement of ROS in mercury-induced changes in Stat Y705-phosphorylation was assessed in parallel with ROS-activated fluorescent dyes.

Materials and Methods

Full Cell Treatments

The human SH-SY5Y neuroblastoma cell line was a gift from Dr. Jan Nemeth (CWRU) and was maintained in a balanced DMEM, 10% FBS, and 0.1% L-glutamine. Cells were supplemented with 10% (v/v) fetal calf serum, 10000 U/ml penicillin and 10,000 mg/ml streptomycin, 5 mg/ml bovine FGF-β, 100 U/ml penicillin, 100 μg/ml streptomycin, and added at 1:3 to 1:1.5 split ratio to poly-D-lysine-coated culture plates (BD Falcon). After 24 h, cells were maintained in serum-free L15 media for 21 h before treatment with indicated for 3 h. Western blotting, histochemistry and qRT-PCR were performed on cells last harvested by harvest challenge with an unconfined culture neuroblastoma (CNS) Jak/Stat-like cell line, oclusum to examine. For the MTT assay, in its untreated condition. All was followed by addition of MTT.

Reactivity of Cytokines

Epidermal growth factor (EGF) and nerve growth factor (NGF) were obtained from Sigma-Aldrich (St. Louis, MO) and used as a source of cytokine stimulation. The Stat activation was assessed by a previously described Stat activation. The CNTN activation was assessed by a previously described CNTN activation. The CNTN activation was assessed by a previously described CNTN activation.

Conclusions

In agreement with previous reports, MeHg was 20-45 times more cytotoxic than HgCl₂ with acute exposures of 5h.

We also confirmed that ROS and HgCl₂ inhibit Stat activation in neuroblastoma cell lines.

MeHg does not catalyze ROS formation at the same cytotoxic doses as HgCl₂.

MeHg may enhance the Jak/Stat response to CNTF at subcytotoxic doses.

Enhancement and inhibition of Stat3 phosphorylation by MeHg are ROS-independent mechanisms.

Future directions for our research include determining outcomes on gene expression in the SH-SY5Y line and conducting homologous experiments in late neural precursor cells in order to delineate the effects of mercury isoform on Stat-dependent gene expression.

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References

