

Reduced tubulin tyrosination as an early marker of mercury toxicity in differentiating N2a cells

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Abstract

The aims of this work were to compare the effects of methyl mercury chloride and thimerosal on neurite/process outgrowth and microtubule proteins in differentiating mouse N2a neuroblastoma and rat C6 glioma cells. Exposure for 4 h to sublethal concentrations of both compounds inhibited neurite outgrowth to a similar extent in both cells lines compared to controls. In the case of N2a cells, this inhibitory effect by both compounds was associated with a fall in the reactivity of western blots of cell extracts with monoclonal antibody T1A2, which recognises C-terminally tyrosinated α -tubulin. By contrast, reactivity with monoclonal antibody B512 (which recognises total α -tubulin) was unaffected at the same time point. These findings suggest that decreased tubulin tyrosination represents a neuron-specific early marker of mercury toxicity associated with impaired neurite outgrowth.

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1. Introduction

Increased worldwide industrialisation has led to higher levels of pollution by potent neurotoxins such as methyl mercury. This compound has been linked with numerous toxic episodes in man, which were invariably associated with disturbed motor function and mental impairment, causing symptoms such as fever, tiredness, tremors and delusions in severe cases (Castoldi, 2001; Jacobson, 2001). Of particular concern is the fact that methyl mercury can cause congenital poisoning via transplacental transfer (Jacobson, 2001), accounting for some of the reported cases of infant poisoning and raising awareness of its developmental toxicity.

The organic form of mercury is considered to be more toxic than inorganic mercury, presumably due to differences in its uptake and chemical reactivity (O'Kusky,

1992). However, although widespread exposure to methyl mercury is rare nowadays, aquatic microorganisms can convert inorganic mercury into organic mercury, which may then be ingested by larger species and eventually work its way up the human food chain, affecting both adults and children (Atchison and Hare, 2004; Counter and Buchanan, 2004). There is also concern over the use of ethyl mercury thiosalicylate (thimerosal) as a preservative in certain vaccines and topical medications, some of which are administered to infants, as the very young are believed to be more sensitive to mercury toxicity (Goldman and Shannon, 2001). Thus, the risk of mercury toxicity remains a cause for concern in today's society.

It has been suggested from cell culture studies that the neurotoxicity of methyl mercury is linked to its ability to inhibit axon outgrowth and to disrupt microtubules in developing neurons (Graff et al., 1997; Miura et al., 1999; Heidemann et al., 2001; Parran et al., 2003). Indeed, subpopulations of dynamic microtubules enriched in C-terminally tyrosinated α -tubulin were shown to be more sensitive to disruption by methyl mercury (Graff et al., 1997). By

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