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Research Report

Effects of low level of methylmercury on proliferation of cortical progenitor cells

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ABSTRACT

Methylmercury (MeHg) is a potent environmental neurotoxin that shows toxicity to developing central nervous system (CNS), causing brain damage in children even at low exposure levels. However, the mechanisms for its effect on CNS are not well understood. In current study, primary cultures of progenitor cells from embryonic cerebral cortex were used as a model system to study the potential effect and the underlying mechanism of MeHg on neural progenitor cells. Results showed that, in cultured cortical progenitor cells, 48-h exposure to low-level of MeHg (at 2.5 nM, 5 nM and 50 nM, respectively) caused G1/S cell cycle arrest in a dose-dependent manner without inducing cell death. Interestingly, the expression of cyclin E, which promotes G1/S transition, but not cyclin D1 and CDK2, was selectively downregulated by exposure of MeHg. In addition, low-level of MeHg inhibited the maintenance of ERK1/2 phosphorylation, possibly by abolishing the late phase ERK1/2 activation induced by bFGF. Thus, MeHg may induce proliferation inhibition and cell cycle arrest of neural progenitor cells via regulating cyclin E expression and perturbing a pathway that involves ERK1/2.

1. Introduction

Methylmercury (MeHg) is an environmentally persistent pollutant. Humans typically encounter MeHg from eating fish and seafood which contain trace amount of MeHg, and low-level exposure is essentially ubiquitous. Clinical findings in victims of the Japanese and Iraqi outbreaks have disclosed pronounced susceptibility of the developing central nervous system to this environmental pollutant (Bakir et al., 1973; Eto, 1997). Previous laboratory studies have also demonstrated that the developing brain is particularly vulnerable to MeHg toxicity (Ferraro et al., 2009; Onishchenko et al., 2007; Stringari et al., 2008).

The mechanisms underlying the sensitivity of developing brain to MeHg exposure can be attributed to perturbation of the highly regulated processes associated with brain development, including the rapid and coordinated cell proliferation, differentiation and migration. Progenitor cell proliferation is a key stage for neurogenesis, and cell-cycle parameters also affect rates of neuronal generation. Extrinsic factors that affect progenitor proliferation may interfere with cortical cytoarchitecture. Neuroanatomical study revealed that high dose of

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MeHg prenatally exposure can cause decreased cell number and abnormal brain cytoarchitecture (Faustman et al., 2002). However, it is shown by numerous studies that MeHg levels in the cord blood of pregnant women is fairly low nowadays (Bjornberg et al., 2005a), and this raises a concern about the effect of very low levels of MeHg, particularly in nanomolar range, on neurogenesis.

Recent studies have pointed to the selective detrimental effects of MeHg on neurogenesis (Burke et al., 2006; Falluel-Morel et al., 2007). These studies showed that MeHg induced acute inhibition of neuronal proliferation and disturbed the cell cycle progression. However, the molecular mechanisms of the chronic effect at sub-toxic exposure levels remain unknown. Cell cycle progression can be blocked at the G1 checkpoint in response to both the intracellular and extracellular cues. The transition from G1 into the S phase of the cell cycle is controlled by cyclin-dependent kinases (CDKs) complexes. In mid/late G1 stage, cyclin E is induced and forms complexes with CDK2, whose activity appears to be essential for entrance of the S phase (Dehay and Kennedy, 2007). Previous studies demonstrated that MeHg exposure acutely disrupted G1/S transition through reducing cyclins such as cyclin E, cyclin D1and cyclin D3 in hippocampus cells (Falluel-Morel et al., 2007).

Extracellular signal-regulated kinase (ERK), a member of the mitogen-activated protein kinase (MAPK) family, is also one of key molecules in growth factor signaling. The p42 and p44 ERKs of MAPK family have been shown to regulate a wide range of cellular responses and have particularly well-defined roles in cell proliferation. ERK activation acts at several levels to increase the activity of CDKs in late G1 phase and sustained ERK activation is required for cell proliferation (Chambard et al., 2007). Interestingly, previous study showed that MeHg significantly decreased NGF-induced ERK1/2 phosphorylation after 2.5-min exposure in PC12 cells (Parran et al., 2004).

In this study, we investigated the chronic effects of low-level MeHg on cerebrocortical neurogenesis using a primary culture of cortical progenitor cells derived from embryonic rats. We analyzed particularly the consequences of MeHg exposure on the proliferation of these cultured progenitor cells and signaling pathways that are essential for cell proliferation, in the hope of elucidating the mechanisms by which MeHg causes neurodevelopmental damage. We found that low levels of MeHg suppressed the proliferation of neuronal precursors without affecting their survival. Moreover, MeHg inhibited the ERK signaling pathway and cyclinE was a sensitive target of MeHg.

2. Results

2.1. *Effects of low-level MeHg exposure on cell viability*

To determine whether low-level MeHg affects cell viability, cortical progenitor cultures were incubated with 2.5 nM–50 μM MeHg for 48 h. Cell death induced by MeHg in the culture was evaluated by MTT assay. We observed a dose-dependent decrease in cell viability (Fig. 1). The increase in the cell death upon exposure to MeHg at higher concentrations (500 nM, 5 μM and 50 μM) was statistically significant ($p<0.01$) compared to the negative (untreated) control or MeHg at lower concentrations (2.5 nM, 5 nM and 50 nM). Thus, low-level MeHg (2.5 nM, 5 nM and 50 nM) did not cause significant occurrence of cell death.

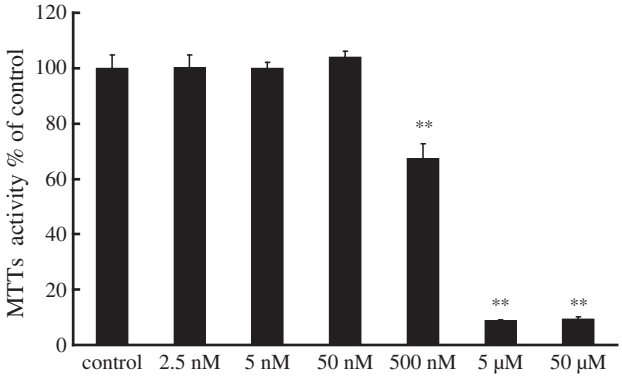

Fig. 1 – Cell viability measurement using MTT assay. Cultures of neural progenitor cells were treated with MeHg (2.5 nM, 5 nM, 50 nM, 500 nM, 5 μM and 50 μM) for 48 h. Cells were then incubated for 4 h with 0.5 mg/ml MTT at 37 °C. The value of the untreated sample was regarded as 100% ($n>3$).

2.2. *Effects of low-level MeHg exposure on cell apoptosis*

Cortical progenitor cultures were treated with 2.5 nM–50 μM MeHg for 48 h and cell apoptosis was analyzed by immunochemistry. The levels of activated (cleaved) caspase-3, a major mediator of cell apoptosis, and Hoechst/PI double staining were assessed. A statistically significant increase ($p<0.01$, $p<0.05$) in the percentage of activated capase-3-positive cells was observed upon treatment with 500 nM, 5 μM and 50 μM MeHg. These increases were statistically different from responses to low concentrations (2.5 nM, 5 nM, 50 nM). In contrast, low-level MeHg did not induce significant increase in cell apoptosis (Fig. 2A, B, C).

2.3. *Low-level MeHg exposure reduces proliferation of cortical progenitor cells and induces cell cycle arrest*

To directly evaluate the effect of MeHg on the proliferation of cortical progenitor cells, we assayed the cell incorporation rate of BrdU, a thymidine analog that could be incorporated into the genomic DNA as cells progress through S phase and was thus widely used for evaluating cell proliferation. The cultures were incubated with BrdU during the last 4 h of MeHg treatment to label proliferating progenitor cells. The result showed a significant reduction ($p<0.01$) in the proportion of BrdU-incorporated cells with 48 h exposure of low-level MeHg (Fig. 3), suggesting a reduction in cell-cycle entry in these cells. The decrease in the number of BrdU-positive cells suggests that MeHg may induce cell cycle arrest and inhibit cortical progenitor cells to enter the S-phase.

2.4. *MeHg selectively reduces the expression of cyclin E*

Considering the decrease in BrdU labeling, we assessed the effects of MeHg on cell cycle regulatory proteins involved in