論 文 要 旨

Suppression of methylmercury-induced pro-inflammatory cytokine expressions by *N*-acetylcysteine in astrocytes

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【Backgrounds and Study Purpose】

Methylmercury (MeHg) exposure is a public health concern due to its high potential for bioaccumulation through the food chain and its toxicological effects on the central nervous system. In the pathophysiology of neurodegenerative diseases, inflammation is known as a crucial regulating factor, and MeHg exposure at non-toxic dose can activate pro-inflammatory cytokines such as interleukin (IL)-6 and monocyte chemoattractant protein (MCP)-1 *in vivo* and *in vitro* studies. However, detailed mechanisms for MeHg-induced cytokine expressions are not identified.

The aim of this study is to clarify the underlying mechanism of non-cytotoxic MeHg-induced inflammation in U-87MG human astrocytoma cell line, the suppressive effects of *N*-acetylcysteine (NAC) on MeHg-induced cytokine expressions among different timing of NAC treatment were examined.

【Materials and Methods】

U87-MG astrocytoma cell line was stimulated with MeHg in range dose of 1-4 μM in different time (3, 6, 12, and 24 h). To investigate the NAC suppressive effects on MeHg-induced cytokines expression, the cells were treated with NAC in dose of 0.5-5 mM in pre-, co-, and post-MeHg exposure. The cytokine expressions at mRNA and protein levels were determined by real-time PCR and ELISA, respectively. Reactive oxygen species were examined using flow-cytometry.

【Results】

Exposure to MeHg at 4 μM, a non-cytotoxic concentration, significantly induced the mRNA

expression of MCP-1 and IL-6 at 6 h, and subsequently MCP-1 and IL-6 protein secretion at 12 h in the U-87MG cells. Flow-cytometry analysis showed a significant increase of hydrogen peroxide (H_2O_2) production but not superoxide anion (O_2^-) by 4 μM MeHg-exposure.

MCP-1 and IL-6 mRNA expressions with 5 mM NAC in the presence of MeHg were reduced by 10-20% of those expressions without NAC (co-treatment experiment). Pre-treatment of cells with 0.5 or 5 mM NAC at 0.5 or 1 h and its subsequent washout before MeHg addition suppressed those cytokine expressions. Post-treatment of cells with NAC at 1 or 3 h after MeHg addition also suppressed the cytokine expressions. However, the magnitude of suppression was evidently lower than that in the co-treatment while H_2O_2 was almost completely suppressed by NAC.

【Discussions and Conclusions】

Non-cytotoxic levels of MeHg exposure activated astrocytes and significantly induced IL-6 and MCP-1 expressions. Activation of these cytokines potentially recruits macrophages/microglia and changes the MeHg behaivior and neurotoxicity in the brain. MeHg exposure also induced H_2O_2 production but ROS might not be the only factor mediating the induction IL-6 and MCP-1 in astrocytes because pre-treatment of cells with NAC suppressed cytokine expressions by 50-60% although H_2O_2 generation was almost completely suppressed.

These results suggest that NAC may effectively suppress the MeHg-induced cytokine productions through both inhibition of reactive oxygen species and extracellular chelation of MeHg in astrocytes.