Fundamental Toxicological Sciences (Fundam. Toxicol. Sci.)
Vol.5, No.2, 71-73, 2018

The Japanese Society of Toxicology

Fundamental Toxicological Sciences
URL : http://www.fundtoxicolsci.org/index_e.html

Toxicomics Report

Putrescine selectively alleviates methylmercury toxicity in C17.2 mouse neural stem cells

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(Received April 9, 2018; Accepted April 13, 2018)

ABSTRACT — We previously reported fluctuating levels of many metabolites in the brain of mice administered methylmercury. In this study, addition of putrescine, a polyamine, to the medium was found to confer methylmercury resistance to C17.2 mouse neural stem cells with regard to metabolites increased by methylmercury in the mouse brain. However, putrescine had little effect on the cytotoxicity of heavy metals such as cadmium and inorganic mercury. These results suggest that putrescine may selectively alleviate methylmercury toxicity.

Key words: Methylmercury, Toxicity, Putrescine

INTRODUCTION

Methylmercury can cross the blood–brain barrier and cause severe central nervous system disorders such as sensory paralysis, language disorders, ataxia, deafness, and narrowing of the visual field (Clarkson, 1997; Grandjean and Herz, 2011). In recent years, developmental disorders in the brain of the fetus have been reported in pregnant women who ingested methylmercury via fish and shellfish (Grandjean *et al*., 1997; Murata *et al*., 2004). However, the defense mechanism against methylmercury toxicity has not yet been clarified.

We previously reported varying levels of many metabolites in the mouse brain in response to methylmercury (Hwang *et al*., 2013). Among them, metabolites produced by amino acids and purine metabolism were included, but most had no previous report suggesting a relationship to methylmercury toxicity. In this study, we investigated the relationship between methylmercury toxicity and metabolites fluctuated by methylmercury using C17.2 mouse neural stem cells.

MATERIALS AND METHODS

Cell culture

Mouse neural stem cells (C17.2 cells) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 100 IU/mL penicillin, and 100 mg/mL streptomycin in a humidified 5% CO_2 atmosphere at 37°C.

Cell viability assay

C17.2 cells (10^4 cells/well) were seeded into 96-well plates for 24 hr, and treated with various heavy metals and/or putrescine at the indicated concentrations for 48 hr. Cell viability was measured using a 10% alamarBlue® (Invitrogen, Carlsbad, CA, USA) solution using an excitation wavelength of 544 nm and an emission wavelength of 590 nm.

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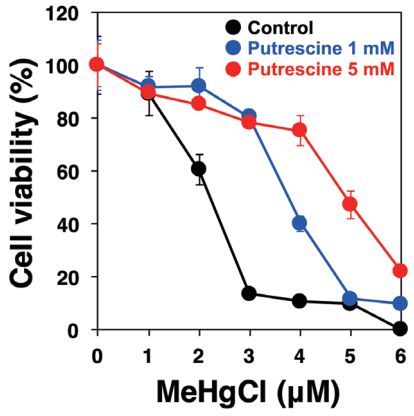

Fig. 1. Effect of putrescine addition on sensitivity to methylmercury in C17.2 cells. C17.2 cells (10^4 cells/well) were plated into 96-well plates and cultured in 100 μL of medium for 24 hr. Putrescine was then added to the cells, and 1 hr later cells were exposed to methylmercuric chloride (MeHgCl) at the indicated concentrations for 48 hr. Cell viability was determined using the alamarBlue® assay. Data represent mean ± S.D. of three replicates.

RESULTS AND DISCUSSION

Six metabolites (putrescine, hypotaurine, uric acid, γ-Glu-2-aminobutyric acid, cystathionine, and carnosine) whose level in the mouse brain was increased to 1.5 times or more by methylmercury, and four metabolites (homovanillic acid, N^5-ethylglutamine, 1-methylhistidine, and trans-glutaconic acid) whose level was reduced to 0.5 times or less were added individually to the medium, and the sensitivity to methylmercury in C17.2 cells was subsequently investigated. Among the ten metabolites, addition of polyamine putrescine to the medium conferred resistance to methylmercury in C17.2 cells (Fig. 1). However, metabolites other than putrescine had little effect on sensitivity to methylmercury in C17.2 cells (data not shown). Next, the effect of putrescine on sensitivities to various heavy metals in C17.2 cells were investigated. As a result, the addition of putrescine to the medium had little effect on the sensitivity to cadmium, inorganic mercury, arsenate, or tributyltin in C17.2 cells (Fig. 2). These results suggest that putrescine may exhibit selective alleviation activity on methylmercury toxicity.

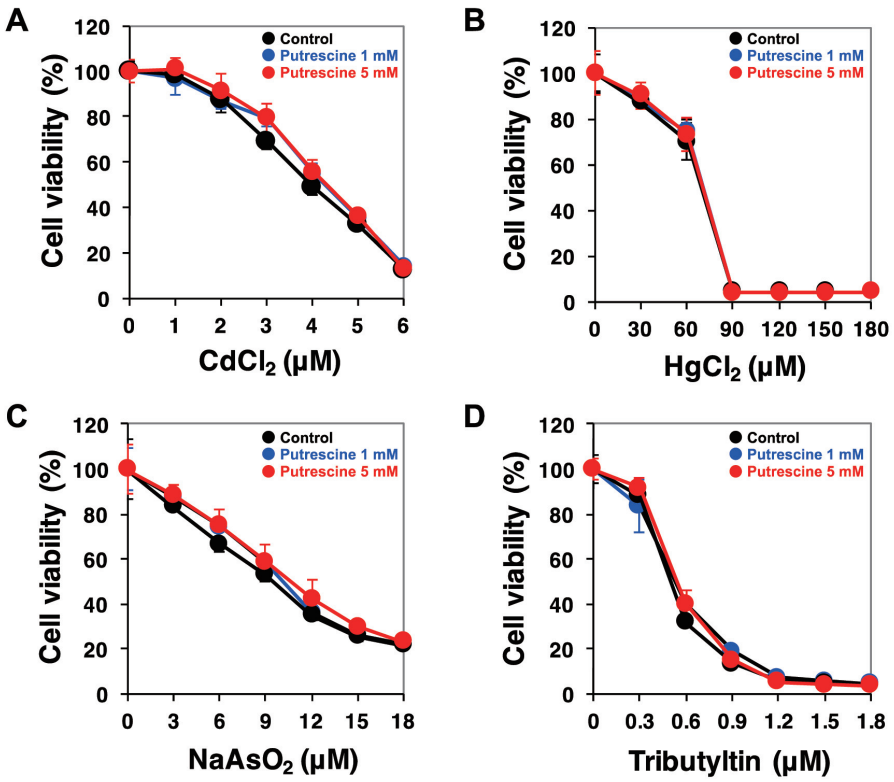

Fig. 2. Effect of putrescine addition on sensitivities to various heavy metals in C17.2 cells. C17.2 cells (10^4 cells/well) were plated into 96-well plates and cultured in 100 μL of medium for 24 hr. Putrescine was then added to the cells, and 1 hr later cells were exposed to various heavy metals at the indicated concentrations for 48 hr. Cell viability was determined using the alamarBlue® assay. Data represent mean ± S.D. of three replicates.

Polyamines are low-molecular-weight metabolites with two or more primary amino groups, and the main ones are putrescine, spermidine, and spermine, which present in most organisms. The roles of polyamines in the methylmercury toxicity has not been known until now. Methylmercury is known to induce neuronal cell death by activating *N*-methyl-*D*-aspartate (NMDA) receptors (Park *et al*., 1996; Rajanna *et al*., 1997). Spermidine and spermine reportedly have an inhibitory effect on NMDA receptors (Benveniste and Mayer, 1993; Ndountse and Chan, 2008; Tomitori *et al*., 2012). However, C17.2 cells exposed to each of these polyamines in the medium did not exhibit methylmercury resistance (data not shown). This suggests that putrescine may alleviate methylmercury toxicity through an unknown action, rather than inhibit NMDA receptors. In the future, it is expected that a novel defense system against central nervous system disorders caused by methylmercury will be clarified by investigating the molecular mechanism involved in alleviation of methylmercury toxicity by putrescine.

ACKNOWLEDGEMENT

This work was supported by a Grant-in-Aid for Scientific Research by the Japan Society for the Promotion of Science (KAKENHI 15H05714, 16H02961).

Conflict of interest---- The authors declare that there is no conflict of interest.

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