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Toxicomics Report

Metabolomic analysis of low molecular weight substances released into medium from HEK293 cells treated with methylmercury

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ABSTRACT — This study attempted to identify substances that are driven out of HEK293 cells by methylmercury. Metabolomic analysis revealed that the levels of 3-phenylpropionic acid, citrulline, lactic acid, ornithine, proline and beta-alanine in the cell culture medium were increased by the treatment of cells with methylmercury. Address to the mechanism underlying the release of these substances will provide useful information to elucidate the toxicity mechanism of methylmercury.

Key words: Methylmercury, Metabolomic analysis, Substances released from cells, HEK293 cells

INTRODUCTION

Methylmercury is a well-known environmental pollutant that induces disorders in central nervous system (Bakir *et al.*, 1973; Harada, 1995). It is presumable that methylmercury affect release of endogenous substances (e.g. amino acids, saccharides and nucleotides) from the cells (Aschner *et al.*, 1994), while there is little evidence. In this study, we performed metabolomic analysis to identify low molecular weight substances that are released into medium from HEK293 cells treated with methylmercury.

MATERIALS AND METHODS

Materials

Methylmercuric chloride was purchased from Sigma-Aldrich (St. Louis, MO, USA). InartSep C18 was obtained from GL Science (Tokyo, Japan). All other reagents used were of the highest grade available.

Cells and cell culture

Human embryonic kidney HEK293 cells were maintained in Dulbecco's modified Eagle's medium (10% fetal bovine serum, 0.3% L-glutamine and antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin)). HEK293 cells were grown at 37°C in a humidified incubator under an atmosphere of CO_2 (5%) and room air (95%).

Metabolomic analysis

HEK293 cells were seeded on a 10 cm dish at a cell concentration 6 x 10^6 cells/dish, and exposed to methylmercuric chloride (50 µM) for 2 hr. The cells were washed twice with PBS, transferred to fresh medium and incubated for additional 6 hr. The medium was collected and filtrated by syringe filter (0.2 µm pore size) (Corning, NY, USA). An aliquot of the medium (10 mL) was loaded onto a C18 (ODS) solid phase extraction column (GL Science, Tokyo, Japan), which was equilibrated by DDW (Toyama *et al*., 2015). ODS flow through fraction was collected and subjected to metabolomic analysis. Metabolomics was performed using CE-TOF/MS in both positive and negative modes as we previously reported (Hwang *et al.*, 2013). Briefly, CE-TOF/MS was performed using the Agilent CE Capillary Electrophoresis System and data analysis was performed using software from Human Metabolome Technologies Inc. (Yamagata, Japan). We detected 51 compounds (positive ion 36 and negative ion 15) from the Basic Scan library. The compounds increased more than 1.3 fold compare to control were listed in the table.

Statistical analysis

Statistical significance was assessed by the *t*-test. All *p* values are two tailed.

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RESULTS AND DISCUSSION

To identify the substances that are driven out of the cells by methylmercury, HEK293 cells were cultured in medium containing methylmercury for 2 hr. After washing with PBS, the cells were further cultured for 6 hr in fresh methylmercury-free medium. The medium was collected, passed through ODS column to eliminate ODS-binding compounds (Toyama *et al*., 2015) and subjected to metabolomic analysis. Metabolomic analysis of low molecular weight compounds in the fraction showed that the concentrations of 3-phenylpropionic acid, citrulline, lactic acid, ornithine, proline and beta-alanine increased 1.3 times or more if the cells were treated with methylmercury (Table 1). The fact that these six substances are driven out of the cell by methylmercury is interesting in terms of assessing the influence of methylmercury on cellular functions. Elucidation of the mechanism underlying the release of these substances will contribute to understanding of the effect of methylmercury on cellular functions.

Table 1. Compounds whose release into medium was increased by treatment of HEK293 cells with methylmercury.

Compound	Ratio (Control vs methylmercury)
Proline	1.85
Ornithine	1.59
β-Alanine	1.45
Lactic acid	1.43
Citrulline	1.32
3-Phenylpropionic acid	1.30

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

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