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Proteome profiling reveals regional protein alteration in cerebrum of common marmoset (*Callithrix jacchus*) exposed to methylmercury

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ABSTRACT

Methylmercury (MeHg) is known to selectively damage the calcarine and precentral cortices along deep sulci and fissures in adult cases, but the detailed mechanism is still unclear. This study aims to identify and analyze the differential proteome expression in two regions of the cerebrum (the frontal lobe and the occipital lobe including the calcarine sulcus) of the common marmoset exposed to MeHg using a shot-gun proteomic approach. A total of 1045 and 1062 proteins were identified in the frontal lobe (FL) and occipital lobe (OL), of which, 62 and 89 proteins were found significantly changed with MeHg exposure. Functional enrichment/depletion analysis showed that the lipid metabolic process and proteolysis were affected in both two lobes. Functional changes in FL were characterized in cell cycle and cell division, sulfur compound metabolic process, microtubule-based process and glycerolipid metabolic process. In comparison, proteins were enriched in the functions of transport, carbohydrate metabolic process, chemical caused homeostasis and regulation of body fluid levels in OL. Pathway analysis predicted that vasopressin-regulated water reabsorption was disturbed in MeHg-treated FL. Our results showed that MeHg induced regional specific protein changes in FL and OL but with similar endpoint effects such as energy diminish and disruption of water transport. APOE and GPX1 were shown to be possible key proteins targeted by MeHg leading to multiple functional changes in OL. This is the first report of the whole proteome changes of primate cerebrum for MeHg neurotoxicity, and the results will contribute to the understanding of molecular basis of MeHg intoxication in humans.

1. Introduction

Mercury as a global environmental toxicant posed a potential health risk for wild-life and humans (Driscoll et al., 2013). Mercury has been used for seed fungicide and as an industrial byproduct (acetaldehyde catalysts) in the last century. Fish and marine mammal consumption are the main sources of mercury exposure for humans (Clarkson and Strain, 2003). Since the first outbreak of mercury poisoning in Minamata, 1956s, mechanisms for MeHg toxicity effects in different species have been widely studied (Farina et al., 2011). Cerebrum and cerebellum are the main target regions for MeHg neurotoxicity. The typical symptoms of Minamata disease patients were ataxic gait, slow movement, vision deficit, and sensory disturbance (Eto, 1997). The autopsy of Minamata disease cases showed that in the acute phase, the level of mercury was found highest in cerebrum and cerebellum compared to the other regions in the nervous system (Okabe and Takeuchi, 1980). MeHg selectively induced brain morphology changes, including neuron loss, particularly occurred around the calcarine cortex and cerebellar cortex in adult cases (Oehmichen et al., 2006; Takeuchi et al., 1979). Brain edema happened in the perivascular space and neuron swollen in the cerebral cortex in Minamata acute cases (Eto, 1997). It was hypothesized that accelerated neuronal injury occurred following by the cerebral edema in the MeHg-exposed brain (Eto et al., 2001). Cerebrum has been documented as sensitive brain region for mercury

Abbreviations: MeHg, methylmercury; FL, frontal lobe; OL, occipital lobe; KEGG, Kyoto Encyclopedia of Gene and Genomes; GO, gene ontology; BP, biological process; GPX1, glutathione peroxidase; APOE, apolipoprotein E; AQP4, aquaporin 4.

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accumulation in different species, such as common marmoset (Yamamoto et al., 2012), ringed seals (Krey et al., 2014), beluga whales (Ostertag et al., 2013), and otters (Dornbos et al., 2013).

Despite numerous studies reporting neurotoxicity of MeHg in different species of mammals, there is little information on the neurotoxic mechanism of MeHg in different brain regions specifically. This is partly due to a lack of a relevant model and tool to systematically study the global mechanisms of MeHg toxicity in these regions. Due to the similar characters with human beings in anatomy and behavior, a non-human primate such as common marmoset (*Callithrix jacchus*) exhibit more advantages than the other primates and rodent species in biomedical research especially in neuroscience and toxicological screening (Kishi et al., 2014; Okano et al., 2012). We have recently shown that shotgun proteomic approach is an effective method to study global effects of MeHg in the cerebellum (Shao et al., 2015). The objective of this study was to determine the mechanisms of neurotoxic effects in different regions of the cerebrum of common marmosets with subacute MeHg exposure. The specific objective was to identify and compare the global proteome changes of FL and OL of the cerebrum induced by MeHg exposure. Using bioinformatic analysis including biological cellular function, pathway, and protein network analysis, the mechanisms underlying the phenotypic changes induced by MeHg were investigated. Western blot was used to validate the result from proteomic prediction for key proteins.

2. Material and methods

2.1. Experimental animals and methylmercury administration

Experimental conditions concerning MeHg exposure in common marmoset was previously described (Yamamoto et al., 2012). Briefly, six adult female common marmosets in 250–330 g body weight were divided into MeHg-treated and vehicle groups. Methylmercury chloride (MeHgCl: 1.5 mg/kg body weight) was administrated using an indwelling infant feeding tube with 3 marmosets for 14 days, following 14 days without treatments. MeHg chloride was dissolved in distilled water (Otsuka Pharmaceutical Factory, Tokushima, Japan) with L-cysteine (MeHgCl: L-cysteine = 1:1). The control group was given the same concentration of L-cysteine (7.5 mM) as the MeHg-treated group. This dosage was chosen based on results of our preliminary experiments with multiple time and doses, in order to mimic pathogenesis and symptoms of Minamata disease. This dosage regime resulted in akinesia, cerebral brain edema in this marmoset model, which have been observed in Minamata disease of acute adult cases (see details in Yamamoto et al., 2012).

All animal procedures were approved by the Animal Research Committee at the National Institute for Minamata disease, and all procedures conformed to the Guide for the Care and Use of Laboratory Animals by the Institute of Laboratory Animal Resources. The FL and OL regions (in the prefrontal cortex of the FL and OL of cerebrum around the calcarine sulcus) in the vehicle and MeHg-treated groups were dissected after anesthesia using ketamine hydrochloride (Daiichi Sankyo, Tokyo, Japan). Dissected tissue samples were immediately frozen in liquid nitrogen and stored at −80 °C for proteomic analysis.

2.2. Sample preparations and mass spectrometric analysis

FL and OL samples in triplicates from vehicle and MeHg-administrated groups were used for liquid chromatography-tandem mass spectrometry (LC–MS/MS) analysis. Tissue preparation and protein quantification used the same methodology and procedure as described previously (Shao et al., 2015). Briefly, proteins extracts (100 μg) were precipitated, reduced and alkylated, followed by trypsin digestion. The digested peptides were acidified with formic acid, and fractionated by StageTip strong cationic exchange fractionation into five fractions by five pH steps (pH3.0, 4.0, 6.0, 8.0, 10.0). Lastly, the peptides were desalted on Sep-Pak column (Waters Corp., Milford, MA) and lyophilized in a SpeedVac (ThermoFisher Scientific, San Jose, CA). An Agilent 1100 capillary-HPLC system (Agilent Technologies, Santa Clara, CA) coupled with LTQ-Orbitrap mass spectrometer (Thermo Fisher Scientific, San Jose, CA) was used to analyze the peptide mixtures.

2.3. Homo sapiens database search and quantitation

The human database search and protein identification procedure were used as described previously (Shao et al., 2015). The peak lists from raw data files were generated with software MaxQuant (Version 1.3.0.5) and searched against SwissProt protein database of human (version 20120711), including commonly observed contaminants. The following criteria were applied: cysteine carbamidomethylation was set as a fixed modification; the methionine oxidation (+15.99492 Da) and protein N-terminal acetylation were set as variable modification. Enzyme specificity was set to trypsin, not allowing for cleavage N-terminal to proline. Only two missing cleavages of trypsin were allowed. For MS/MS spectra, the precursor ion mass tolerances were 7 ppm, and fragment ion mass tolerance was set to 0.8 Da. Razor and unique peptides were applied for quantitation. The false discovery rate was limited to 0.01 on both protein and peptide levels. A minimum sequence length of six amino acids was required for peptide identification.

For protein identification, if the identified peptide sequence of one protein was equal to or contained in another protein's peptide set, these two proteins were grouped together by MaxQuant and reported as one protein group. For quantitation, a minimum of 2 spectra counts is needed.

2.4. Data analysis and cellular component distribution

All the contaminant and reverse data were removed, and then the identified proteins were converted to UniProt gene ID by

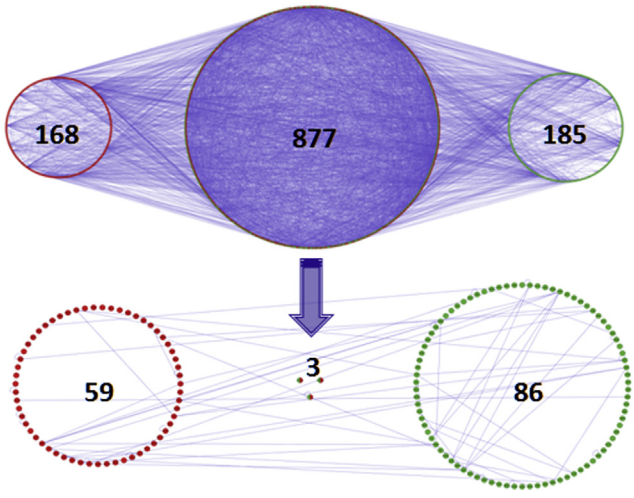

Fig. 1. Quantification analysis of identified proteins in frontal lobe and occipital lobe in MeHg exposed marmoset. A. Numbers and PPI network of total identified proteins in upper panel and differentially expressed (statistical difference) proteins in lower panel. A total of 1230 proteins were identified, and 877 overlapping proteins were detected from the frontal lobe and occipital lobe in triplicate runs (upper panel). 148 proteins are statistically different, and only 3 proteins shared between the two regions.