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# Proteomic investigations on the mechanism of neurotoxicity in low-dose chronic methylmercury intake

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**TITOLO**
Analisi proteomica sul meccanismo di neurotossicità dell'assunzione cronica in basse dosi di metilmercurio

**KEY WORDS**
Methylmercury, brain, proteomics

**PAROLE CHIAVE**
Metilmercurio, cervello, proteomica

**Summary**
Contamination of seafood and especially fish with methylmercury is becoming a global issue. We fed rats with a low dose methylmercury (40 ug/kg body weight/day) for 3 months in order to see if the protein expressions in their brains were affected. With proteomic technologies, we found that the expression of about 100 proteins in the somatic sensory cortex was altered. A significant portion of these proteins are enzymes of the metabolic pathways. This finding is in line with the hypothesis that methylmercury accumulation in the brain caused decreased energy supply which eventually impaired ability for auto-repair in the brain.

**Riassunto**
La contaminazione dei prodotti ittici e soprattutto del pesce con metilmercurio sta diventando un problema globale. Abbiamo nutrito ratti con basse dosi di metilmercurio (40 ug/kg di peso corporeo/die) per 3 mesi per analizzarne l'influenza sull'espressione proteica nei loro cervelli. Con tecnologie proteomiche, abbiamo scoperto che era stata alterata l'espressione di circa 100 proteine nella corteccia sensoriale somatica. Una significativa porzione di queste proteine sono enzimi di pathways metabolici. Questo risultato è in linea con l'ipotesi che l'accumulo di metilmercurio nel cervello causi una diminuzione dell'approvvigionamento di energia che col tempo ridurrebbe la sua capacità di auto-riparazione.

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According to the International Energy Outlook 2010 compiled by the US Energy Information Administration, world marketed coal was 130.26 quadrillion BTU. It will rise to 167.76 quadrillion BTU by 2025 and 206.26 quadrillion BTU by 2035 (1). China burnt the largest amount of coal worldwide. It was estimated that China consumed 1.56 billion tons of coal in 2009, including 1.4 billion tons of power coal and 160 million tons of coal for heating. By 2035, these figures will increase by 55% (2). Coal burning emits significant amounts of inorganic mercury into the air which is eventually collected in our waterways through wind and rain. Sulphate- and/or sulphur-containing

bacteria in the sediment convert this inorganic mercury into methylmercury (MeHg, organic) through their metabolism (3, 4). This MeHg starts to accumulate and bio-magnify up the food chain (5, 6). A recent review summarized the formation and pathways in the environment (7). Methylmercury mostly accumulates in the muscles of fishes, and it is also known that fishes are a very important part of our daily diet. Regarding methylmercury, there is a constant debate on the risks and benefits of fish consumption (8). Local government experts reported that some fishes sold in the Hong Kong and Chinese markets had high levels of MeHg (9, 10). Further, Fok et al. measured the mercury levels of 1057 sets of maternal and cord blood samples obtained from a pediatric clinic in a local hospital. Median maternal hair mercury concentration was 1.7 ppm. They also reported that among the 1057 cord blood samples, only 21.6% had mercury concentrations lower than 29 nmol/L (11). It is known that fetal exposure to more than 29 nmol/L concentration of mercury affects fetal brain development (12-15). Since acute and higher doses of MeHg intake causes severe neurotoxicity, the possible harmful effects of chronic and lower doses of MeHg intake from contaminated fishes is becoming a cause for a public concern!

In cases of acute and high dose of MeHg poisoning, one of the first signs of intoxication is paresthesia (numbness of the skin), indicating that the somatosensory cortex is affected. Other neurological symptoms include loss of physical coordination, difficulty in speech, narrowing of the visual field, and ultimately death. It is known that once entered into the body, methylmercury will be absorbed through the gut before being bound to cysteine to form cysteine-methylmercury. It will then be carried throughout the body and eventually cross the blood-brain barrier and placenta (16). However, why does MeHg preferentially deposit in specific parts of the brain, such as the somatosensory cortex and cerebellum, remains unknown. Exact details of the molecular mechanisms of how chronic low-dose MeHg intake causes neurotoxicity are unclear.

As we are exposed to a low-dose but persistent environment of MeHg, we were very interested to study the effects of chronic low-dose intake of MeHg in a rat model. After dissolving in corn oil as a vehicle, we fed a group of rats (n=6) with 40 g/kg body weight/day for 12 weeks. Parallel to this experimental group, there were 2 other groups: the vehicle group (with corn oil only, n=6) and the normal control group (n=6). After 12 weeks, rat brains were harvested and dissected into different parts. Amounts of methylmercury present in these samples were measured with cold-vapour atomic fluorescence spectrometry (CVAFS). Besides measuring MeHg concentration, proteomic changes in these samples were also studied using isotope-coded protein labelling (ICPL) coupled with the analysis of LC-ESI-ion-trap-MS/MS (LC-ESI) and LC-MALDI-TOF/TOF-MS (LC-MALDI). We found that about 100 proteins were differentially expressed in the somatosensory cortex of the MeHg intoxicated rats. Pathway analysis of these differentially expressed proteins using Kyoto Encyclopedia of Genes and Genomes (KEGG) found that about 60% of these proteins are related to metabolism. It is predicted that decreased expression of these proteins invariably led to shutting down the metabolic pathways, and hence energy supply to the brain cells. Hence, we hypothesize that low-dose chronic intake of MeHg, which may shut down energy supply to the brain cells, will eventually contribute to neuronal death.

Further studies to investigate if, or confirm that the MeHg-intoxicated rats have decreased metabolism in their brain tissues, not just the somatosensory cortex, are currently in progress in the authors' laboratory. We aim at documenting the total effects of MeHg intoxication affecting all regions of the brain.

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