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Assessing pre/post-weaning neurobehavioral development for perinatal exposure to low doses of methylmercury

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ABSTRACT

Fetuses and neonates are known to be high-risk groups for Methylmercury (MeHg) exposure. MeHg can be transferred to the fetus through the placenta and to newborn offspring through breast milk. The aim of the present study was to investigate the neurotoxic effects of low doses of MeHg (1 and 5 μg/mL in drinking water) administration, from gestational day 1 to postnatal day (PND) 21, on the neurobehavioral development of rats. The results showed that the no-observed-effect level of MeHg is somewhere in the range of 1–4 μg/mL. Neurobehavioral development analysis revealed a delayed appearance of cliff drop and negative geotaxis reflexes in the 5 μg/mL MeHg exposure group. Developmental exposure to MeHg affected locomotor activity functions for the females, but not for the males, implying that the female pups were more vulnerable than the male pups. All pups exposed to 5 μg/mL of MeHg showed a significant deficit in motor coordination in the rotarod test compared with controls, and the highest accumulated concentrations of Hg were found in the cerebellum, followed by the hippocampus and cerebral cortex, indicating that the cerebellum is a possible target for MeHg toxicity. We demonstrated adverse effects of developmental exposure to MeHg associated with tissue concentrations very close to the current human body burden of this persistent and bioaccumulative compound.

Introduction

Methylmercury (MeHg) is an important environmental toxicant that causes neurological and developmental impairment in both humans and animals. Fetuses and neonates are known to be high-risk groups for MeHg exposure. MeHg can be transferred to the fetus through the placenta and to offspring through breast milk (Sakamoto et al., 2007; Montgomery et al., 2008; Nyland et al., 2011). Due to the recycling of mercury from the environment, it is common to ingest small quantities of mercury due to consumption of contaminated freshwater fish and seafood (Grandjean et al., 2001; Knobeloch et al., 2007; Ulrich et al., 2007; Chumchal et al., 2010). Of the different routes of exposure, most humans are exposed to Hg by ingestion of food and/or water contaminated with MeHg. According to Cheng et al. (2009) there is a wide variation in hair Hg concentrations between the fathers, mothers and children of the same household, which is probably related to the quantity, frequency and type of fish consumed. High levels of MeHg are found often in large predatory freshwater and saltwater fish, such as northern pike, salmon, swordfish, tuna, and shark (Simmonds et al., 2002). Accumulation of Hg in tissues of these fish is related primarily to the predatory nature and the longevity of these

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fish in contaminated waters. For example, previous studies estimated that as many as 500,000 babies born yearly in the U.S. have cognitive deficits that may be linked to perinatal MeHg exposure (Trasande et al., 2006; Montgomery et al., 2008; Dórea et al., 2011; Gao et al., 2007) and have found that there is a neurodevelopmental risk for males from perinatal MeHg exposure resulting from fish consumption. Therefore it is important to obtain more information regarding the neurotoxic effects of perinatal low dose MeHg exposure, particularly at dosages that approximate human consumption, on the development of offspring.

A number of experimental studies have been conducted in mice and rats, in order to evaluate the potential noxious effects of developmental exposure to MeHg on developing and adult organisms. The results indicate that MeHg can cause persistent disturbances in spontaneous motor behavior and dysfunction in learning and memory in adult mice and rats (Goulet et al., 2003; Grotto et al., 2011). However, the effects of MeHg on neurobehavioral development are not well defined. Therefore, the specific aims of this study were to investigate whether exposure to low doses of MeHg can induce developmental neurotoxic effects. The MeHg doses (1 and 5 μg/mL in drinking water) were chosen based on data showing that 5 μg/mL of MeHg did not induce any typical poisoning symptoms or histopathological findings in adult rats, even if they continued ingesting it for over 2 years (Eto et al., 1997; Yasutake et al., 1997). Therefore, we assumed that the dose level was moderate for adolescent rats in this experiment (Sakamoto et al., 2002). The mothers continued to be given the diet after parturition, and thereby their offspring were exposed to MeHg through breast milk until weaning. This study was designed mainly to determine the changes in MeHg levels along with pre/post-weaning neurobehavioral development of male and female pups. We also want to demonstrate adverse effects of developmental exposure to MeHg associated with tissue concentrations very close to the current human body burden of this persistent and bioaccumulative compound. The GD 1 to PND 21 period of administration was chosen because it spans from the formation of the first central nervous system areas to weaning (PND 21), when indirect exposure to the compounds through the mother ends (Rice and Barone, 2000). Furthermore, this prolonged developmental exposure is constant and maternally-mediated, like that occurring in human infants.

1. Materials and methods

1.1. Animals and treatments

All experimental procedures involving animals were performed in compliance with the National Institute of Minamata Disease (NIMD) on the care and use of laboratory animals. Wistar rats (9 females and 9 males, 10 weeks old) were supplied from Central Institute for Experimental Animals (CLEA) Japan. Amphimixis was allowed after 3 days of acclimatization. Females were inspected daily for the presence of the vaginal plug (gestational day, GD 0). On GD 1, the males were removed and 9 females were randomly divided in three treatment groups including 0 (control), 1 and 5 μg/mL MeHg. MeHg dissolved at concentrations of 1 and 5 μg/mL, respectively, was administered daily to rats in their drinking water, from GD 1 to postnatal day (PND) 21. On PND 22, the litters were culled to 10–12 pups and weaned.

On PND 40, two male and two female pups randomly selected from each litter of each group were euthanized for Hg analysis. The pups for Hg analysis were perfused *via* the ascending aorta with phosphate buffer after the blood sample was collected. All brains were immediately removed and dissected over ice-cold glass slides to remove the cerebellum, cerebral cortex and hippocampus. Liver and kidney samples were also collected and washed repeatedly in ice-cold physiological saline for Hg analysis. The tissues for Hg analysis were frozen at –20°C until assay.

1.2. Assessment of pre-weaning neurobehavioral development

Every 2 days, from PND 3 to 21, six pups randomly selected from each litter of each group were used for postnatal assessment of neurobehavioral development. The following reflexes were scored (Branchi et al., 2002): *Righting reflex*: pup was placed on its back on a flat surface and the time to turn over with all four paws was recorded, with a cut-off time of 2 sec. *Cliff avoidance*: pup was positioned on the edge of a bench, with its forepaws and nose just over the edge. The time of withdrawal of head and both forefeet was recorded, with a cut-off time of 2 sec. *Negative geotaxis*: pup was placed on a 45° angle slope with its head downwards, and the percent success rate and the time necessary to turn around 180° were recorded, with a cut-off time of 5 sec.

1.3. Assessment of post-weaning neurobehavioral development

1.3.1. Motor coordination

For the evaluation of coordination and balance, the rotarod test was performed with three trials per day for two consecutive days from PND 34–35. The apparatus (Natsume, Tokyo, Japan) consisted of a bar, 8 cm in diameter and 10 cm long, which rotated at 15 r/min. The duration time, that is, the time from when the pup was mounted on the rod until it fell off, was recorded in seconds. The individual performance was cut off at 60 sec (Sakamoto et al., 2002). All pups were tested.

1.3.2. Locomotor activity

The locomotor activity was assessed using an open field on PND 36. Briefly, each pup was moved from its home cage to the center square (5 cm × 5 cm) of the open field (15 cm × 15 cm), and covered with a Plexiglas box (15 cm × 15 cm × 15 cm) for 5 min. The number of line crossings and central square entries was scored. All pups were tested.

1.3.3. Spatial learning

To assess spatial learning and memory, all pups were tested in the Y-maze on PND 39. Each pup was placed at the end of one arm (arm A) facing the center of the maze, and allowed to move freely within the maze for a period of 3 min. The total number of arms entered and the order of arm entries were recorded. The total number of arms entered provides an indication of locomotor activity, and the order of arm entries provides a measure of spontaneous alternation behavior and thus working memory (Podhorna and Brown, 2002).