Environmental Research 152 (2017) 446–453

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Stable and episodic/bolus patterns of methylmercury exposure on mercury accumulation and histopathologic alterations in the nervous system

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ARTICLE INFO

Article history:
Received 5 January 2016
Received in revised form
6 May 2016
Accepted 20 June 2016
Available online 21 July 2016

Keywords:
Methylmercury
Exposure pattern
Stable
Bolus
Mercury accumulation
Neuronal alteration

ABSTRACT

The main purpose of the present study was to compare the blood and brain mercury (Hg) accumulation and neurological alterations in adult male and pregnant female/fetal rats following stable and episodic/bolus patterns of methylmercury (MeHg) exposure. In addition, MeHg accumulation in the human body was estimated by a one-compartment model using three different patterns of MeHg exposure. In the adult male rat experiment, doses of 0.3 and 1.5 mg MeHg/kg/day were orally administered to the stable groups for 5 weeks, while 7-fold higher doses of 2.1 and 10.5 mg MeHg/kg/once a week were administered to the bolus groups. The blood Hg levels increased constantly in the stable groups, but increased with repeated waves in the bolus groups. At completion of the experiment, there were no significant differences in the brain Hg concentrations or neurological alterations between the stable and bolus groups, when the total doses of MeHg were the same. In the pregnant female rat experiment, a dose of 1 mg MeHg/kg/day was administered orally to the stable group for 20 days (until 1 day before expected parturition), while a 5-fold higher dose of 5 mg MeHg/kg/once every 5 days was administered to the bolus group. In the brains of the maternal/fetal rats, there were no significant differences in the Hg concentrations and neurological alterations between the stable and bolus groups. The mean Hg concentrations in the fetal brains were approximately 2-fold higher than those in the maternal brains for both stable and bolus groups. Using the one-compartment model, the Hg accumulation curves in humans at doses of 7 μg MeHg/day, 48 μg MeHg/once a week, and 96 μg MeHg/once every 2 weeks were estimated to be similar, while the bolus groups showed dose-dependent amplitudes of repeated waves. These results suggest that stable and episodic/bolus patterns of MeHg exposure do not cause differences in Hg accumulation in the blood and brain, or in neurological alterations, when the total doses are the same.

1. Introduction

Methylmercury (MeHg) is a well-known neurotoxicant (WHO, 1976) that caused Minamata disease in Japan (Takeuchi et al., 1962). In the environment, the main chemical form of mercury (Hg) emitted from anthropogenic and natural sources is elemental Hg (Hg^0). Hg^0 is oxidized in the environment, and becomes divalent Hg (Hg^{2+}). A part of the Hg^{2+} is then transformed into MeHg by certain microorganisms and sunlight, leading to its bioaccumulation in fish and marine mammals, and producing food-web-dependent MeHg levels (UNEP, 2008).

The Governing Council of the United Nations Environment Programme (UNEP) started a global Hg assessment in 2003. Because the global Hg levels were estimated to have been increasing since the industrial revolution, reflecting anthropogenic increases in Hg emission (UNEP, 2013), it was agreed in January 2013 to develop a legally binding global instrument on Hg for global control of Hg pollution. The final impact of Hg pollution should appear as increased MeHg levels in fish and marine mammals such

Abbreviations: Hg, Mercury; MeHg, methylmercury.

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http://dx.doi.org/10.1016/j.envres.2016.06.034
0013-9351/

as seals and toothed-whales. Moreover, most human exposure to MeHg occurs through consumption of fish and marine mammals (NRC, 2000; WHO, 1976). Therefore, the UNEP and World Health Organization (WHO) are planning to start environmental and human Hg monitoring, especially in fetuses that will bear the next generation.

The outbreak of fetal-type Minamata disease patients with severe neurological symptoms, resembling those of cerebral palsy, was caused by extraordinarily high maternal exposure to MeHg during gestation, while the mothers themselves showed no or mild symptoms of MeHg intoxication (Harada, 1978). Historically, the occurrence of fetal-type Minamata disease patients was a landmark event that brought worldwide attention to their high risk. The developing brain is sensitive to MeHg (Rice and Barone, 2000). Furthermore, MeHg accumulates at higher concentrations in fetuses than in mothers, as we previously reported in various human and animal studies (Sakamoto et al., 2002a, 2002b, 2004, 2007, 2015). Following the experience of the fetal-type MeHg intoxication in Minamata, many cohort studies addressing the neurodevelopmental effects of MeHg exposure during gestation have been conducted. Birth cohort studies in the Republic of Seychelles (Myers et al., 1995b, 2009) and Faroe Islands (Grandjean et al., 1997, 2005; Murata et al., 1999) have attracted special attention.

Although the MeHg exposure levels estimated from blood and hair Hg concentrations were similar in the above-mentioned birth cohort studies, different conclusions were reached (NRC, 2000). In a report by The National Toxicology Council (NRC, 2000), a number of possibilities were discussed to explain the different conclusions. One possibility was differences in MeHg exposure patterns between the Seychelles and the Faroe Islands (Grandjean et al., 1997; Myers et al., 1995a; NRC, 2000). Seychellois people were constantly exposed to MeHg through their high fish consumption (12 fish meals/week). Meanwhile, Faroese people consumed not only fish with low Hg concentrations, but also occasionally pilot whales containing 10–20-fold higher MeHg concentrations (Grandjean et al., 1992). It was suggested that Faroese people were intermittently exposed to relatively high concentrations of MeHg from pilot whale consumption. Therefore, the NRC (2000) report pointed out that the episodic/bolus exposure pattern in the Faroe Islands, with heavier doses per occasion, could lead to more adverse impacts on neuronal development than the constant exposure in the Seychelles (Stern et al., 2004). These two different patterns (stable vs. episodic/bolus) of MeHg exposure can also occur in general populations of other countries. However, the effects of these two MeHg exposure patterns on Hg accumulation in the blood and brain have not yet been validated.

Many previous studies have used physiologically based pharmacokinetic (PBPK) models and other models to extrapolate rat data to humans for MeHg distribution to tissues (Carrier et al., 2001; Farris et al., 1993; Verger et al., 2007; Young et al., 2001). However, PBPK models require many values such MeHg and inorganic Hg concentrations in blood and organs at various times following a known exposure (Young et al., 2001). Therefore, to estimate the Hg body burdens after stable and bolus MeHg exposure in humans, we adopted a one-compartment model as a reliable and versatile model that has been basically employed by many regulatory authorities such as the WHO (WHO, 1976, 1990), Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2012) and US Environmental Protection Agency (EPA, 2001). EPA's current MeHg exposure reference dose (RfD) is 0.1 μg/kg/day and which is equivalent to 7 μg/70kg human body weight/day. Accordingly, 7 μg MeHg/day was chosen as a basic virtual MeHg daily dose in the present study.

In rat experiments, we measured blood Hg concentrations during the experimental period and brain Hg concentrations in adult male and pregnant female/fetal rats at completion of the experimental period in stable and bolus MeHg exposure groups. Using a one-compartment model, we also estimated the Hg accumulation curves in humans at doses of 7 μg MeHg/day, 48 μg MeHg/once a week, and 96 μg MeHg/once every 2 weeks.

2. Materials and methods

2.1. Adult male rats and MeHg administration procedure

Figure 1 shows the protocol for MeHg administration and blood sampling in adult male rats. Male Wistar rats supplied by CLEA (Tokyo, Japan) were maintained on a 12-h/12-h light/dark cycle at 23 °C with free access to rat chow (CLEA) and tap water. At the beginning of the study, 30 rats aged 7 weeks were divided into stable (10 rats) and bolus (20 rats) groups. For the MeHg solution, 25 mg MeHg chloride (98% purity; Tokyo Kasei Kogyo Co. Ltd., Tokyo, Japan) and 73.8 mg L-cysteine (Sigma, St. Louis, MO), corresponding to a molecular ratio of 1:1, were dissolved in 25 mL of distilled water. Two stable groups (5 rats/group) were orally administered 0.3 and 1.5 mg MeHg/kg/day for 5 weeks at predetermined time interval of 24 h (Fig. 1a). Two bolus groups (10 rats/group) received 2.1 and 10.5 mg MeHg/kg (7-fold higher doses) once a week at predetermined times (Fig. 1b). Approximately 10 μL of blood was collected from the tail vein. The peak (highest value) blood Hg concentration was reported to appear at approximately 24 h after a single dose of MeHg (on day 1) in rats (Norseth and Clarkson, 1970). Therefore, we selected the periods for blood collection corresponding to the peak and the trough (lowest value obtained at 1 week after MeHg administration) of the blood Hg concentrations for bolus groups (Fig. 1b). For the stable groups, blood was collected once a week on days 4, 11, 18, 25, and 32 (Fig. 1a), corresponding to the middle day between the peak and the trough of the blood Hg concentrations in the bolus groups. In the bolus groups, blood was collected from 5 different rats to avoid any surplus stress created by repeated withdrawal of blood from the same rats. The rats in the stable groups received 0.3 and 1.5 mg MeHg/kg/day and were dissected on day 36. Meanwhile, the rats in the bolus groups received 2.1 and 10.5 mg MeHg/kg/once a week, and were dissected on day 32 (4 days after final bolus doses of MeHg), since Hg concentration in the brain of rats has been reported to reach almost a plateau approximately 4 days after a single dose of MeHg (Norseth and Clarkson, 1970). All rats were deeply anesthetized by intraperitoneal injection of pentobarbital, and blood samples were taken by cardiac puncture. The rats were then euthanized by transcardiac perfusion with 0.9% saline for 5 min to flush out the remaining blood from the organs. Their brains were removed and the samples were kept at −80 °C until Hg analysis.

2.2. Pregnant rats and MeHg administration procedure

The studies in the Faroe Islands and Seychelles were birth cohort studies. For this reason, we also compared the effects of stable and bolus exposures on pregnant rats to reveal the MeHg concentrations in the maternal and fetal brains and the neurological effects in the fetal brains. Ten 9-week-old female Wistar rats (CLEA) were mated with male rats of the same age in the late afternoon. Vaginal smears were recognized in six female rats at 09.00 a.m. on the following morning. The day when vaginal sperm was observed in a smear was considered to be pregnant day 1. The pregnant rats were randomly assigned to two groups (3 rats/group). The rats in the stable group were orally administered 1 mg MeHg/kg/day for 20 days at predetermined times. The rats in the bolus group were orally administered 5 mg MeHg/kg/once every 5 days (5-fold higher dose) on days 3, 8, 13, and 18 at