Reproductive Toxicology 69 (2017) 265–275

Contents lists available at ScienceDirect

Reproductive Toxicology

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

Oral exposure of pregnant rats to toxic doses of methylmercury alters fetal accumulation

Cláudia Oliveira[1], Lucy Joshee, Hannah George[2], Sanya Nijhara[2], Christy Bridges*

Division of Basic Medical Sciences, Mercer University School of Medicine, Macon, GA, USA

ARTICLE INFO

Article history:
Received 8 November 2016
Received in revised form 30 January 2017
Accepted 20 March 2017
Available online 21 March 2017

Keywords:
Methylmercury
Mercury
Placenta
Fetus
Metals

ABSTRACT

Methylmercury (CH_3Hg^+) is an environmental toxicant that may lead to significant pathologies in exposed individuals. The current study assessed the disposition and toxicological effects of 2.5 or 7.5 mg kg^{-1} CH_3Hg^+, conjugated to cysteine (Cys; Cys-*S*-CH_3Hg) and administered orally to pregnant and non-pregnant Wistar and TR^- rats. Rats were euthanized on gestational day 20 and the content of mercury in each fetus, amniotic sac, and placenta was determined. The brain, liver, and kidneys were removed from each fetus for estimation of mercury content. From the dams, a sample of blood, kidneys, liver, and brain were removed at the time of euthanasia. The findings from this study indicate that pregnancy leads to significant changes in the handling of mercuric ions, particularly in the liver. Furthermore, there are significant differences in the handling of non-nephrotoxic and nephrotoxic doses of Cys-*S*-CH_3Hg by maternal and fetal organs.

1. Introduction

Methylmercury (CH_3Hg^+) is an environmental toxicant that may lead to significant pathological effects in exposed individuals. In the United States, exposure to CH_3Hg^+ occurs primarily via the consumption of contaminated fish, although other routes of exposure have also been documented [1]. Despite guidelines from the United States Environmental Protection Agency (USEPA), certain populations continue to consume more than the recommended amount of fish [2–5]. Indeed, a recent study found that Asians, males, and older individuals tend to have blood concentrations of CH_3Hg^+ that meet or exceed the maximum blood concentration of CH_3Hg^+ recommended by the USEPA [6–8]. Consumption of more than the recommended amount of fish may be due to the absence of fish consumption advisories for contaminated waters [9] and/or a lack of awareness regarding fish consumption advisories [10]. Pregnant women, in particular, should be aware of guidelines regarding fish consumption in that exposure to methylmercury during pregnancy can lead to significant detrimental effects in the fetus [1].

CH_3Hg^+ has been shown accumulate in and exert significant toxicological effects in multiple organs [1]. In the brain, CH_3Hg^+ appears to readily cross the blood-brain barrier and can lead to neurotoxicological effects [11–14]. Owing to the role of the kidney in the excretion of metabolic wastes and xenobiotics, renal cells are also sites of CH_3Hg^+ accumulation [15]. In addition, mercuric ions have been shown to cross the placenta and accumulate in fetal organs following prenatal exposure to CH_3Hg^+ [16–18]. Interestingly, the concentration of mercuric ions has been shown to be greater in cord blood and the placenta than in maternal blood [19,20]. Thus, the concentration of CH_3Hg^+ presented to the fetus is likely higher than that present in the maternal circulation. Indeed, CH_3Hg^+-induced fetotoxicity results from plasma concentrations of CH_3Hg^+ that do not lead to pathological alterations in the mother [19]. Prenatal exposure of fetuses to CH_3Hg^+ has been linked to numerous neurological impairments such as reduced cognitive function, reduced motor activity, speech disorders, and cerebral palsy [1,21,22]. Since the developing fetus is especially sensitive and susceptible to the effects of CH_3Hg^+ [21,23], understanding the way in which this metal is transported by placental syncytiotrophoblasts and fetal organs is particularly important.

Despite the clinical importance of this area, little is known about the molecular mechanisms involved in the handling of CH_3Hg^+ by placental and fetal tissues. Recently, the multidrug resistance-

Abbreviations: CH_3Hg^+, methylmercury; Cys-*S*-CH_3Hg, cysteine conjugate of methylmercury Cys cysteine; USEPA, United States Environmental Protection Agency; GSH, glutathione; Mrp, multidrug resistance-associated protein; Bcrp, breast cancer resistance protein; P-gp, P-glycoprotein; OSOM, outer stripe of the outer medulla.

* Corresponding author at: Mercer University School of Medicine, Division of Basic Medical Sciences, 1550 College St., Macon, GA 31207, 478-301-2086, USA.
E-mail address: Bridges_cc@mercer.edu (C. Bridges).
[1] Present address: Post-Graduate Course in Biological Science—Toxicological Biochemistry, Federal University of Santa Maria, Santa Maria, RS, Brazil.
[2] These individuals contributed equally to this work.

http://dx.doi.org/10.1016/j.reprotox.2017.03.008
0890-6238/

associated protein (Mrp) 2 was implicated in the transport of mercuric ions from the fetal circulation into maternal blood following intravenous administration of a non-toxic dose of CH_3Hg^+ [17]. This previous study did not provide data regarding the handling of mercuric ions following oral exposure to CH_3Hg^+, which is the primary route of human exposure. Furthermore, the previous study did not assess the effects of exposure to a potentially toxic dose of CH_3Hg^+. Therefore, the current study was designed to test the hypothesis that oral administration of a toxic dose of CH_3Hg^+ alters the transport and disposition of mercuric ions in dams and fetuses.

2. Materials and methods

2.1. Animals

Female Wistar and TR$^-$ rats, weighing 250–275 g, were obtained from our colony in the Mercer University School of Medicine animal facility. Breeder pairs of TR$^-$ rats were originally obtained from the laboratory of Dr. Kim Brouwer at the University of North Carolina. TR$^-$ rats, which are in a Wistar background, possess a spontaneous mutation that results in an early stop codon in the Mrp2 (*Abcc2*) transcript. Therefore, Mrp2 in these rats is nonfunctional [24]. There were no significant differences in body weight among the animals used for these studies. Female rats of each strain were mated for 40 h with male rats of the same strain from our colony. Pregnancy was verified by weight gain and abdominal palpation.

Animals were provided a commercial laboratory diet (Tekland 6% rat diet, Harlan Laboratories) and water *ad libitum* throughout all aspects of experimentation. All animals were maintained on a 12 h dark: 12 h light cycle. Animals were handled in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health. All protocols using animals were approved by the Mercer University Institutional Animal Care and Use Committee.

2.2. Oral exposure to methylmercury

A study using X-ray absorption spectroscopy showed that the primary form of CH_3Hg^+ found in muscle of fish is a conjugate of cysteine (Cys; Cys-*S*-CH_3Hg) [25]. Therefore, in the current study, rats were exposed orally to Cys-*S*-CH_3Hg in order to mimic the ingestion of fish tissue. Beginning on gestational day (GD) 10, rats were exposed daily to 2.5 or 7.5 mg kg^{-1} 10 mL^{-1} Cys-*S*-CH_3Hg (in normal saline). Based on unpublished findings from our laboratory, we conclude that the 2.5-mg kg^{-1} dose is not nephrotoxic to dams while the 7.5-mg kg^{-1} dose is expected to lead to nephrotoxic effects. Cys-*S*-CH_3Hg was formed by mixing CH_3Hg^+ (Sigma) with Cys in a 1:1.25 ratio. Radioactive CH_3Hg^+ ($CH_3[^{203}Hg]$) was added to the solution so that each rat received a total of 2 μCi $CH_3[^{203}Hg]$. $CH_3[^{203}Hg]$ (6–12 mCi/mg) was generated at the University of Missouri Research Reactor using a method described previously [26]. Cys-*S*-CH_3Hg, containing $CH_3[^{203}Hg]$, was administered orally to rats using 15 gauge x 100 mm disposable plastic feeding tubes (Instech, Plymouth Meeting, PA). On GD 10, rats were placed in individual plastic metabolic cages. Feces and urine were collected daily from GD 10 until GD 20, at which time rats were euthanized.

Following oral exposure to Cys-*S*-CH_3Hg, it should be considered that thiol exchange likely occurs whereby the mercuric ion releases Cys and becomes bound to other available thiol-containing molecules [27,28]. In addition, previous studies have shown that following absorption/ingestion of CH_3Hg^+, a fraction of CH_3Hg^+ is biotransformed to inorganic mercury (Hg^{2+}) [29,30]. Identification of the exact forms of mercury taken up by various organs and tissues was outside the scope of the current study. Therefore, in the current study, we use "Hg" as a generalized term to represent the various potential species of CH_3Hg^+ and Hg^{2+} that may be taken up into cells.

2.3. Collection of fetuses, tissues, organs, and urine

Pregnant dams were euthanized on GD 20. At the time of euthanasia, dams were anesthetized with an intraperitoneal (i.p.) dose of ketamine and xylazine (70/30 mg kg^{-1} in 2 mL saline). Each fetus and placenta was removed carefully from the uterus. The amniotic sac was punctured and the amniotic fluid was collected on a piece of Whatman paper (Thermo Fisher), which was placed in a tube to measure the content of $CH_3[^{203}Hg]$. Each placenta was separated from the fetus, weighed, and placed in a tube for estimation of $CH_3[^{203}Hg]$ content. In addition, each fetus was weighed, decapitated, and placed in 3 mL of 80% EtOH in a glass scintillation vial. The whole fetus was counted in a gamma counter, following which the brain, kidneys, and liver were dissected from each fetus. Each organ was weighed and placed in separate tubes for the determination of $CH_3[^{203}Hg]$ content. After each placenta and fetus was removed from the dam, the uterus was removed, placed in a scintillation vial and counted in a gamma counter. The number of fetuses and placentas harvested from each dam was 11–18.

Following removal of placentas and fetuses, a 1-mL sample of blood was obtained from the inferior vena cava of dams and was saved for estimation of $CH_3[^{203}Hg]$ content. Total blood volume was estimated to be 6% of body weight [31]. The liver and kidneys were also removed from each dam. Each kidney was weighed and cut in half along a transverse plain. One-half of the right kidney was frozen immediately in liquid nitrogen for future PCR analyses. The remaining half of the right kidney was utilized for estimation of $CH_3[^{203}Hg]$ content. One-half of the left kidney was placed in fixative (40% formaldehyde, 50% glutaraldehyde in 96.7 mM NaH_2PO_4 and 67.5 mM NaOH) as preparation for histological analyses. A 3-mm transverse slice of the remaining half was utilized for separation of cortex, outer stripe of outer medulla, inner stripe of outer medulla and inner medulla. Each zone of the kidney was weighed and placed in a separate tube for estimation of $CH_3[^{203}Hg]$ content. The liver was then excised, weighed, and a 1-g section of liver was removed for determination of $CH_3[^{203}Hg]$ content. The brain was also removed, weighed, and placed in a glass scintillation vial for estimation of $CH_3[^{203}Hg]$ content.

Urine was collected daily, beginning on GD 11. At the time of collection, a 1-mL sample of urine was weighed and placed in a tube for estimation of $CH_3[^{203}Hg]$ content. Samples were counted in a Wallac Wizard 3 automatic gamma counter (Perkin Elmer, Boston, MA) in order to estimate the content of $CH_3[^{203}Hg]$ in each sample.

2.4. Quantitative PCR

At the time of euthanasia, one-half of the right kidney from each Wistar and TR$^-$ rat was cut into sections and frozen in liquid nitrogen. In order to isolate RNA, frozen kidney sections were pulverized with a mortar and pestle, TRIzol Reagent (Life Technologies, Grand Island, NY) was added, and RNA was extracted according to the manufacturer's protocol.

Reverse transcription of 1 μg of RNA was carried out using reverse transcriptase and random hexamers (Life Technologies). For real-time PCR analyses, 2 μL of the reverse transcriptase reaction were utilized. Analysis of kidney injury molecule-1 (Kim-1) and breast cancer resistance protein (Bcrp) was performed with an ABI Prism 7000 detection system using a Gene Expression Assay (Rn00597701_m1 and Rn00710585_m1, respectively, Life Technologies). Glyceraldehyde 3-phosphate dehydrogenase (Gapdh; Rn01775763_g1) was used as a reference gene.