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Disposition of inorganic mercury in pregnant rats and their offspring

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ABSTRACT

Environmental toxicants such as methylmercury have been shown to negatively impact fetal health. Despite the prevalence of inorganic mercury (Hg^{2+}) in the environment and the ability of methylmercury to biotransform into Hg^{2+}, little is known about the ability of Hg^{2+} to cross the placenta into fetal tissues. Therefore, it is important to understand the handing and disposition of Hg^{2+} in the reproductive system. The purpose of the current study was to assess the disposition and transport of Hg^{2+} in placental and fetal tissues, and to test the hypothesis that acute renal injury in dams can alter the accumulation of Hg^{2+} in fetal tissues. Pregnant Wistar rats were injected intravenously with 0.5 or 2.5 $\mu mol\,kg^{-1}$ $HgCl_2$ for 6 or 48 h and the disposition of Hg^{2+} was measured. Accumulation of Hg^{2+} in the placenta was rapid and dose-dependent. Very little Hg^{2+} was eliminated during the initial 48 h after exposure. When dams were exposed to the low dose of $HgCl_2$, fetal accumulation of Hg^{2+} increased between 6 h and 48 h, while at the higher dose, accumulation was similar at each time point. Within fetal organs, the greatest concentration of Hg^{2+} (nmol/g) was localized in the kidneys, followed by the liver and brain. A dose-dependent increase in the accumulation of Hg^{2+} in fetal organs was observed, suggesting that continued maternal exposure may lead to increased fetal exposure. Taken together, these data indicate that Hg^{2+} is capable of crossing the placenta and gaining access to fetal organs in a dose-dependent manner.

1. Introduction

There is significant risk of humans being exposed to inorganic (Hg^{2+}) and/or methylmercury (CH_3Hg^+) through environmental, occupational or dietary means. Exposure to Hg^{2+} and CH_3Hg^+ can lead to serious toxicological consequences in the renal, hepatic, cardiovascular, reproductive, and nervous systems (ATSDR, 2008; Bridges and Zalups, 2010). Of particular concern is the effect of mercuric species on the reproductive system and the developing fetus. Despite guidelines from the Environmental Protection Agency (EPA), certain populations of pregnant women continue to consume more than the recommended amount of seafood (Nair et al., 2014; Soon et al., 2014; Xu and Newman, 2014). Interestingly, the content of CH_3Hg^+ in certain species of fish is increasing (Drevnick et al., 2015), which further increases the risk of mercury (Hg) exposure in fish-eating human populations.

Numerous studies have shown that following ingestion of CH_3Hg^+, mercuric ions can readily cross the placenta and accumulate in the fetus (Bridges et al., 2009, 2012; Sakamoto et al., 2013; Yorifuji et al., 2009). In contrast, little is known about the ability of Hg^{2+} to cross the placenta despite evidence that CH_3Hg^+ can be biotransformed to Hg^{2+}, either in plasma or target cells (Lorscheider et al., 1995; Norseth and Clarkson, 1970a; Norseth and Clarkson, 1971). It has been suggested indirectly that Hg^{2+} is unable to gain access to fetal tissues even though Hg^{2+} has been shown to accumulate in the placenta (Ask et al., 2002; Chehimi et al., 2012; Feng et al., 2004; Oliveira et al., 2012; Yang et al., 1996; Yoshida, 2002). Considering the placental accumulation of Hg^{2+}, it seems possible that Hg^{2+} may also gain access to fetal tissues and organs. Therefore, one aim of the current study was to determine the nature and pattern of accumulation and disposition of Hg^{2+} in placental and fetal tissues.

Given that the biotransformation of CH_3Hg^+–Hg^{2+} probably occurs primarily in maternal blood and organs, it is important to understand how Hg^{2+} is handled in maternal organs, as well those of the fetus. In adults, the primary site of Hg^{2+} accumulation and toxicity is the kidney, specifically the epithelial cells of the proximal tubule (Zalups, 2000). In fact, in as little as three hours after intravenous exposure to Hg^{2+} (as $HgCl_2$), approximately 55% of the administrated dose can be detected in the kidneys (Zalups, 1993). In animals exposed to nephrotoxic doses of $HgCl_2$,

Abbreviations: Hg^{2+}, inorganic mercury; $HgCl_2$, mercuric chloride; [$^{203}Hg^+$], radioactive mercury; OSOM, outer stripe of the outer medulla; ISOM, inner stripe of the outer medulla; Kim-1, Kidney injury molecule-1.

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pathological changes such as cellular necrosis, tubular dilatation and atrophy, proteinaceous casts, inflammation, and interstitial collagen deposition have been identified in and around proximal tubules (Bridges et al., 2014; Favero et al., 2014b). Increases in blood urea nitrogen (BUN) and plasma creatinine levels have also been reported, which suggests that glomerular filtration rate (*e.g.*, renal function) is reduced following exposure to highly nephrotoxic doses of $HgCl_2$ (Bridges et al., 2014; Zalups et al., 2014). When maternal exposure to $HgCl_2$ is great enough to cause reductions in renal function, it is possible that the maternal burden and corporal disposition of Hg^{2+} is altered because of a reduced ability to eliminate mercuric ions in urine. Consequently, it is possible that the placental and fetal burden of Hg will also be altered, leading to greater toxicological consequences in the fetus. Therefore, a second aim of this study was to test the hypothesis that acute renal injury in pregnant dams alters the fetal accumulation of Hg^{2+}.

In the present study, we exposed pregnant Wistar rats to either a non-nephrotoxic or a nephrotoxic dose of $HgCl_2$ and assessed the disposition and toxicity of mercuric ions not only in placental tissues, but also in fetal organs, either six or 48 h after exposure to Hg^{2+}. Understanding how mercuric ions accumulate in the placenta and fetus will provide insight into the toxicity and the mechanisms by which mercuric ions are handled fetuses.

2. Materials and methods

2.1. Animals

Male and female Wistar rats were obtained from our breeding colony housed in the Mercer University School of Medicine animal facility. Female Wistar rats, weighing 275–300 g, were mated with male Wistar rats in our facility for 36 h in order to obtain pregnant dams. All animals were provided a commercial laboratory diet (Tekland 6% rat diet, Harlan Laboratories) and water *ad libitum* throughout all aspects of experimentation. The animal protocol for the current study was reviewed and approved by the Institutional Animal Care and Use Committee. Animals were handled in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health.

2.2. Exposure of animals to $HgCl_2$

Four groups of pregnant dams were injected intravenously with $HgCl_2$. Dams in Group A were injected intravenously (i.v.) with a non-nephrotoxic dose of $HgCl_2$ (0.5 μmol kg^{-1} 2 mL 0.9% NaCl containing 1 μCi of [$^{203}Hg^{2+}$] per rat) (Zalups, 1997) while dams in Group B were injected with a nephrotoxic dose of $HgCl_2$ (2.5 μmol kg^{-1} 2 mL saline containing 1 μCi of [$^{203}Hg^{2+}$] per rat) (Zalups et al., 1991). Groups A and B were injected with $HgCl_2$ on day 20 of gestation (ED 20) and were euthanized 6 h later in order to assess the disposition of Hg^{2+} prior to or near the time of the induction of renal injury. Dams in Group C were injected intravenously (i.v.) with a non-nephrotoxic dose of $HgCl_2$ (0.5 μmol kg^{-1} 2 mL 0.9% NaCl containing 1 μCi of [$^{203}Hg^{2+}$] per rat) while dams in Group D were injected with a nephrotoxic dose of $HgCl_2$ (2.5 μmol kg^{-1} 2 mL saline containing 1 μCi of [$^{203}Hg^{2+}$] per rat). Rats in groups C and D were injected on ED 18 and were euthanized on ED 20 in order to assess the disposition of Hg^{2+} in dams with acute nephrotoxic injury. There were no obvious physiological or pathological changes in any of the rats at the time of injection.

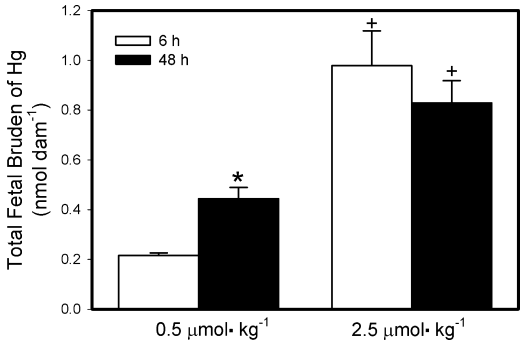

Fig. 1. Total fetal burden of Hg^{2+} 6 h or 48 h after intravenous injection of pregnant Wistar dams with 0.5 μmol $HgCl_2$ kg^{-1} 2 mL or 2.5 μmol $HgCl_2$ kg^{-1} 2 mL. Data represent mean ± SE of three or six dams. *Significantly different ($p < 0.05$) from the mean for the group of rats exposed to the same dose for 6 h. +Significantly different ($p < 0.05$) from the mean for the corresponding group of rats exposed to 0.5 μmol $HgCl_2$ kg^{-1}.

At the time of injection, each animal was anesthetized with 2–5% isoflurane and a small incision was made in the skin in the mid-ventral region of the thigh to expose the femoral vein and artery. The dose of $HgCl_2$ was administered into the femoral vein and then the wound was closed with two 9-mm wound clips. Subsequently, all animals were housed individually in plastic metabolic cages.

2.3. Radioactive Hg [$^{203}Hg^{2+}$]

Radioactive Hg [$^{203}Hg^{2+}$] was produced by neutron activation of mercuric oxide (enriched with Hg^{202}) at the Missouri University Research Reactor (MURR) facility as described previously (Belanger et al., 2001; Bridges et al., 2004). Briefly, a 3-mg sample of mercuric oxide was irradiated for 4 weeks at MURR. Following irradiation, the sample was dissolved in 1 mL of 1 N HCl and the activity was measured using a Fluka ion chamber. The specific activities ranged from 10 to 15 mCi/mg.

2.4. Collection of tissues and organs

At the time of euthanasia, rats were anesthetized with an intraperitoneal (i.p.) injection of ketamine and xylazine (70/30 mg kg^{-1} in 2 mL saline). A 3-mL sample of blood was first obtained from the inferior vena cava and 1 mL was placed in a polystyrene tube for estimation of [$^{203}Hg^{2+}$] content. Approximately 0.5 mL of blood was placed in a blood separation tube in order to separate plasma from the cellular contents of blood. Total blood volume was estimated to be 6% of body weight (Lee and Blaufox, 1985).

Right and left kidneys were then removed and each kidney was trimmed of fat and fascia, weighed, and cut in half along the mid-traverse plane. One-half of the right kidney was placed in fixative (40% formaldehyde, 50% glutaraldehyde in 96.7 mM NaH2PO4 and 67.5 mM NaOH) in preparation for histological analyses. The remaining half was frozen in liquid nitrogen for future RNA analyses. A 3-mm transverse slice of the left kidney was utilized to obtain samples of cortex, outer stripe of outer medulla (OSOM), inner stripe of outer medulla (ISOM) and inner medulla. Each zone of the kidney was weighed and placed in a separate polystyrene tube for estimation of [$^{203}Hg^{2+}$] content. The liver was then excised, weighed, and a 1-g section was removed for determination of [$^{203}Hg^{2+}$] content.

In groups A and B, urine and feces were collected 6 h after injection with $HgCl_2$. In groups C and D, urine and feces were collected for 24-h periods with the first collection taking place 24 h after the injection with $HgCl_2$. The second 24-h collection occurred 48 h after the injection with $HgCl_2$. For all groups, urine from each animal was mixed and a 1-mL sample was weighed and placed in a polystyrene tube for estimation of [$^{203}Hg^{2+}$] content. All of the feces excreted by each animal during each collection period were counted to determine accurately the total fecal content of [$^{203}Hg^{2+}$]. The content of [$^{203}Hg^{2+}$] in each sample was determined by counting in a Wallac Wizard 3 automatic gamma counter (PerkinElmer).