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Effect of DMPS and DMSA on the Placental and Fetal Disposition of Methylmercury

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ABSTRACT

Methylmercury (CH_3Hg^+) is a serious environmental toxicant. Exposure to this metal during pregnancy can cause serious neurological and developmental defects in a developing fetus. Surprisingly, little is known about the mechanisms by which mercuric ions are transported across the placenta. Although it has been shown that 2,3-dimercaptopropane-1-sulfonate (DMPS) and 2,3-dimercaptosuccinic acid (DMSA) are capable of extracting mercuric ions from various organs and cells, there is no evidence that they are able to extract mercury from placental or fetal tissues following maternal exposure to CH_3Hg^+. Therefore, the purpose of the current study was to evaluate the ability of DMPS and DMSA to extract mercuric ions from placental and fetal tissues following maternal exposure to CH_3Hg^+. Pregnant Wistar rats were exposed to CH_3HgCl, containing [^{203}Hg], on day 11 or day 17 of pregnancy and treated 24 h later with saline, DMPS or DMSA. Maternal organs, fetuses, and placentas were harvested 48 h after exposure to CH_3HgCl. The disposition of mercuric ions in maternal organs and tissues was similar to that reported previously by our laboratory. The disposition of mercuric ions in placentas and fetuses appeared to be dependent upon the gestational age of the fetus. The fetal and placental burden of mercury increased as fetal age increased and was reduced by DMPS and DMSA, with DMPS being more effective. The disposition of mercury was examined in liver, total renal mass, and brain of fetuses harvested on gestational day 19. On a per gram tissue basis, the greatest amount of mercury was detected in the total renal mass of the fetus, followed by brain and liver. DMPS and DMSA reduced the burden of mercury in liver and brain while only DMPS was effective in the total renal mass. The results of the current study are the first to show that DMPS and DMSA are capable of extracting mercuric ions, not only from maternal tissues, but also from placental and fetal tissues following maternal exposure to CH_3Hg^+.

1. Introduction

Methylmercury (CH_3Hg^+) is a reproductive toxicant that is prevalent in the environment. Humans are often exposed to CH_3Hg^+ through consumption of contaminated water and/or food, such as predatory fish [1]. Of particular concern is the exposure of pregnant women to CH_3Hg^+ and the potential deleterious effect(s) that this toxicant may have on the developing fetus. CH_3Hg^+ in maternal blood readily crosses the placenta and accumulates in fetal and placental tissues [2–7]. The neurological system is a major site of CH_3Hg^+ toxicity. Therefore, it is not surprising that the fetal brain and neurological system are particularly susceptible to the effects of CH_3Hg^+, with detrimental fetal effects occurring at concentrations that would not negatively affect the mother [8–10]. Prenatal exposure of fetuses to CH_3Hg^+ has numerous deleterious effects, including reduced cognitive function, dysarthria, strabismus, and cerebral palsy [11–14]. Given the prevalence of CH_3Hg^+ in the environment and its potential threat to fetal health, it is of significant clinical importance that we have a thorough understanding of the way in which mercuric ions are handled by the placenta.

Despite the clinical importance of this area, little is known about the molecular mechanisms by which CH_3Hg^+ crosses the placental barrier. Kijiwara et al. [15] showed that CH_3Hg^+, as a conjugate of cysteine (Cys; Cys-*S*-CH_3Hg), is transported from maternal blood across the placenta by a neutral amino acid carrier, which was hypothesized to be system L. More recently, in vivo studies in *Xenopus laevis* oocytes showed that Cys-*S*-CH_3Hg is a substrate of both isoforms of system L, Lat1 (*Slc7A5*) and Lat2 (*Slc7A8*) [16]. These findings are consistent with the theory that Cys-*S*-CH_3Hg acts as a molecular mimic of one or more endogenous substrates (e.g. methionine) at the docking site of one or more membrane transporters.

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The ability of mercuric ions to be extracted from placental and/or fetal tissues has not been examined thoroughly. Although it is well-established that the metal chelators, 2,3-dimercaptopropane-1-sulfonate (DMPS) and 2,3-dimercaptosuccinic acid (DMSA) can reduce the total body burden of CH_3Hg^+ [17–20], the ability of either chelator to extract mercuric ions from placental or fetal tissues has not been shown. Interestingly, administration of DMPS to pregnant mice exposed orally to CH_3Hg^+ appeared to protect fetuses from the toxic effects of methylmercury [21]. Similarly, a separate study in rats suggests that DMPS is capable of extracting mercuric ions from fetal and placental tissues following maternal exposure to inorganic mercury [22]. Currently, there is no evidence indicating that either, DMPS or DMSA is capable of extracting mercuric ions from placental and fetal tissues of dams exposed to a form of organic mercury, CH_3Hg^+.

The metal chelators, DMPS and DMSA, each possess vicinal thiol groups which facilitate the formation of highly stable DMPS and DMSA-*S*-conjugates of CH_3Hg^+. In kidney, it is thought that DMSA, and possibly DMPS, are taken up at the basolateral membrane of renal tubular cells by the sodium dicarboxylic transporter (NaC2; *Slc13a3*) and/or the organic anion transporter 1 (Oat1; *Slc22A6*), [23,24] following which, they likely form stable conjugates with intracellular mercury. These conjugates appear to be substrates for endogenous transporters, such as the multidrug resistance-associated protein 2 (Mrp2; *Abcc2*) [25–27], located on the apical plasma membrane of proximal tubular cells [28]. Given that placental trophoblasts are polarized cells similar to proximal tubular cells in the kidney, we hypothesize that DMPS and DMSA are each capable of extracting mercuric ions from fetal and placental tissues in a manner similar to that which occurs in proximal tubular cells. Therefore, the purpose of the current study was to evaluate the ability of DMPS and DMSA to extract mercuric ions from placental and fetal tissues following maternal exposure to CH_3HgCl.

2. Materials and methods

2.1. Animals

Pregnant Wistar rats weighing 250–275 g were purchased from Harlan Laboratories (Indianapolis, IN). There were no significant differences in body weight among the animals used for these studies. Animals were provided a commercial laboratory diet (Tekland 6% rat diet, Harlan Laboratories) and water *ad libitum* throughout all aspects of experimentation. 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. Intravenous injections

Rats were injected intravenously (i.v.) according to our previously published protocol [25,26,29,30]. Each animal was anesthetized lightly with ether 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 fascia around the femoral vein was trimmed and a non-nephrotoxic dose of CH_3HgCl (5 mg/kg in 2 mL normal saline containing 1 μCi of CH_3 [^{203}Hg] per rat) was administered into the vein. The wound was closed using two 9-mm stainless steel wound clips. CH_3 [^{203}Hg] (6–12 mCi/mg) was generated using a method described previously [26,31].

2.3. Experimental design

Two separate experiments were performed. For the first experiment, 15 pregnant rats were injected (i.v.) with CH_3HgCl on day 11 of pregnancy and were sacrificed on day 13 (E13). Animals at this stage of pregnancy were chosen because rapid brain development occurs at this point in the gestational period. For the second experiment, 15 pregnant rats were injected (i.v.) with CH_3HgCl on day 17 of pregnancy and were sacrificed on day 19 (E19). These animals were chosen in order to examine the disposition of mercuric ions in fetuses that were near the end of their gestational period, which is 20–21 days for rats. The steps following administration of CH_3HgCl were carried out identically in both sets of rats. Following injection with CH_3HgCl, each set of rats was divided randomly into three groups of five rats each. Animals were then placed in individual plastic metabolic cages. Twenty-four hours after the injection of CH_3HgCl, each of the five rats in group 1 was injected i.v. with a 200 mg/kg dose of DMPS (in 2 mL/kg normal saline). At the same time, each of the five rats in group 2 was injected i.v. with a 200 mg/kg dose of DMSA (in 2 mL/kg normal saline). Each of the remaining five rats (group 3) was injected i.v. with normal saline (2 mL/kg). Forty-eight h after injection with CH_3HgCl (either day 13 or day 19), rats were sacrificed.

2.4. Collection of fetuses, tissues, organs, urine and feces

At the time of sacrifice, pregnant rats were anesthetized with an intraperitoneal overdose of ketamine and xylazine (70/30 mg/kg in 2 mL saline). A 1-mL sample of blood was obtained from the inferior vena cava with a 3-mL syringe and a 20-gauge needle and placed in a polystyrene tube for estimation of [^{203}Hg] content. Total blood volume was estimated to be 6% of body weight.

The liver and kidneys were also removed from each pregnant rat. Each kidney was weighed and cut in half along a transverse plain. A 3-mm transverse slice of the left kidney 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 polystyrene tube for estimation of [^{203}Hg] content. The liver was then excised, weighed, and a 1-g section of liver was removed for determination of [^{203}Hg] content. The brain was also removed, weighed, and placed in a glass scintillation vial for estimation of [^{203}Hg] content.

Urine and feces were collected throughout the duration of each experiment. The urine excreted by each animal was collected 24 h and 48 h after injection with CH_3HgCl. Subsequently the urine from each animal was mixed and a 1-mL sample was weighed and placed in a polystyrene tube for estimation of [^{203}Hg] content. All of the feces excreted by each animal during each 24-h period were counted to determine accurately the total fecal content of [^{203}Hg]. The content of [^{203}Hg] in each sample collected was determined by counting the samples in a Wallac Wizard 3 automatic gamma counter (Perkin Elmer, Boston, MA).

The uterus of each anesthetized animal was removed and individual fetuses and placentas were extracted. The number of fetuses and placentas harvested from each dam was 11–18. Each placenta was weighed and placed in a polystyrene tube for estimation of [^{203}Hg] content. In addition, each fetus was weighed, decapitated, placed in 3 mL of 80% EtOH in a glass scintillation vial. In experiments where rats were sacrificed on day 19 of pregnancy, individual fetuses were dissected to remove the brain, kidneys and liver. Each organ was weighed and placed in separate polystyrene tubes for the determination of [^{203}Hg] content.

2.5. Data analyses

Data for each experiment were analyzed first with the Kolmogorov–Smirnov test for normality and then with Levene's test for homogeneity of variances. Data were then analyzed using a 2×2 two-way analysis of variance (ANOVA) to assess differences among the means. When statistically significant *F*-values were obtained with ANOVA, the data were analyzed using Tukey's *post hoc* multiple comparison test. A *p*-value of <0.05 was considered statistically significant. Each group of animals contained 5 rats, with 11–18 fetuses per rat.

3. Results

3.1. Content of mercury in maternal tissues

3.1.1. Renal burden of mercury

The amount of mercury in the total renal mass of pregnant Wistar rats exposed to 5 mg/kg CH_3HgCl on day 17 of pregnancy is shown in Fig. 1. Approximately 16% of the administered dose was detected in the total renal mass of dams exposed to CH_3HgCl and treated subsequently with saline. Treatment of rats with 200 mg/kg DMPS reduced in the renal burden of mercury by approximately 70%. When rats were treated with the same dose of DMSA, the renal burden of mercury was reduced by about 50%.

The disposition of mercuric ions in each of the four renal zones is also shown in Fig. 1. The greatest amount of mercury was detected in the cortex, followed by the outer stripe of the outer medulla. The content of mercury in the inner stripe of the outer medulla and in the inner medulla was significantly lower than that in the other two zones. Treatment of dams with DMPS reduced significantly the amount of mercury in the cortex and the outer stripe of the outer medulla. Similarly, DMSA also reduced the amount of mercury in the cortex and outer stripe of the outer medulla. The renal disposition of mercuric ions in pregnant rats exposed to CH_3HgCl on day 17 of pregnancy (Fig. 1) was not