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Inorganic mercury exposure in drinking water alters essential metal homeostasis in pregnant rats without altering rat pup behavior

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ABSTRACT

The aim of this work was to investigate the effects of $HgCl_2$ exposure in the doses of 0, 10 and 50 μg Hg^{2+}/mL in drinking water during pregnancy on tissue essential metal homeostasis, as well as the effects of $HgCl_2$ exposure *in utero* and breast milk on behavioral tasks. Pregnant rats exposed to both inorganic mercury doses presented high renal Hg content and an increase in renal Cu and hepatic Zn levels. Mercury exposure increased fecal Hg and essential metal contents. Pups exposed to inorganic Hg presented no alterations in essential metal homeostasis or in behavioral task markers of motor function. In conclusion, this work showed that the physiologic pregnancy and lactation states protected the offspring from adverse effects of low doses of Hg^{2+}. This protection is likely to be related to the endogenous scavenger molecule, metallothionein, which may form an inert complex with Hg^{2+}.

1. Introduction

Essential metals such as zinc, iron, cupper, magnesium, and calcium are extremely important to mammals. They participate in a great number of cellular biochemical pathways, and some of the essential metals are structural components of macromolecules or enzymatic cofactors. The amount of essential metals required by the organism depends on age, gender, genetic factors, and physiologic state (pregnancy and lactation) [1,2]. During pregnancy and lactation periods, females have a high nutrient demand because they are responsible for providing nutrients such as vitamins, amino acids and essential metals to their offspring via uterus or breast milk [2,3]. For this, females present biological adaptations, such as increase of food consumption and gastrointestinal absorption efficiency, decrease on urinary and fecal excretion, and mobilization of tissue stores [4].

Due to the extreme importance of essential metals to the newborn development as well as to a healthy adult life, the World Health Organization (WHO) highlights the necessity to maintain essential metal homeostasis [2]. Studies have shown that essential metal deficiency (*e.g.* iron) [5], chelating agents [6–8], and heavy metal intoxication [9,10] may alter essential metal homeostasis. Symptoms from disturbances in essential metal homeostasis are variable and differ among the elements; however, organisms with essential metal deficiency generally present growth retardation, anemia, skin lesion, alopecia, osteoporosis [2], depression, and anxiety [11].

Among the toxic metals known for disturbing essential metal homeostasis, we can highlight cadmium, arsenic, lead, and mercury [9]. Hg is a non-essential metal ubiquitously distributed in the environment, which can be found in three chemical forms; namely organic, inorganic and elemental Hg. Indiscriminate and uncontrolled Hg use by humans may increase the release of this metal and consequently increase the risk of contamination. Mercury exposure may occur through pollution inhalation (elemental Hg) or ingestion of contaminated food (mainly organic Hg) or water (mainly inorganic Hg) [12,13]. In the last few decades, developing countries such as Brazil, China, India, and Mexico have been more susceptible to Hg and other environmental contaminant effects due to rapid industrialization and increased urbanization without adequate residue and waste control [14,15].

Mercury exposure during pregnancy and/or lactation periods is extremely dangerous to fetus and/or infants [16,17]. Contamination with Hg during pregnancy and lactation periods was first registered

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and studied at the decades of 60 and 70, in the case namely Minamata Disease [18,19]. Currently, we have seen some studies with populations that have in their diet basically seafood (main font of Hg) and the consequences of this diet to fetus, newborn and infant development [20–23].

Several studies of our research group have demonstrated the effects of subcutaneous inorganic Hg exposure in rats, such as inhibition of sulfhydryl enzymes, nephrotoxicity symptoms [24–28], and behavioral alterations [29–31]. Pups subcutaneously exposed to $HgCl_2$ presented alterations in the levels of Cu, Zn and Mg in liver, kidney [10] and brain [31]. Consequently, these alterations in essential metal homeostasis may contribute to the toxic effects of Hg. Interestingly, changes in renal and hepatic zinc homeostasis have not been observed when pups were indirectly (via breast milk) exposed to inorganic Hg [25,32]. In addition, our research team has also demonstrated a relation between the increase of metallothionein (an endogenous scavenger molecule) levels and the amelioration of the toxic effects of Hg [25,29,33,34].

Taking into consideration that cases of Hg contamination are still observed and that drinking water is the main exposure route to inorganic Hg, studies in this area are necessary. Recently, Oliveira et al. [35] showed that $HgCl_2$ exposure in drinking water during pregnancy causes a decrease in food intake and in body weight gain, without altering fetus weight. However, to our knowledge there are no studies relating $HgCl_2$ exposure in drinking water during gestational period with an alteration in essential metal homeostasis in fetuses. Thus, based on the importance of essential metals to the good development of offspring during gestational and lactation periods, the main objective of this work was to verify the effects of low doses of $HgCl_2$ exposure in drinking water on essential metal homeostasis in pregnant rats and their fetuses. Moreover, we verified whether pups exposed to $HgCl_2$ *in utero* and breast milk present behavioral alteration.

2. Material and methods

2.1. Chemicals

Mercuric chloride was purchased from Sigma Chemical Company (St. Louis, MO). Analytic standards (mercury, iron, copper, zinc), sodium chloride, potassium phosphate monobasic and dibasic, absolute ethanol, sodium hydroxide, nitric acid, ß-mercaptoethanol, sucrose, hydrochloric acid, phenylmethylsulphonylfluoride (PMSF), chloroform, calcium disodium ethylenediaminetetraacetate (EDTA), 5,5-dithio-bis(2-nitrobenzoic acid) (DTNB), and tris(hydroxymethyl) aminomethane (Tris) were purchased from Merck (Darmstadt, Germany).

2.2. Animals

Sixty-four Wistar rats (45 virgin female rats, 10–12 weeks old, 190–220 g; and 19 mature male rats, 14–15 weeks old, 250–300 g) obtained from the Animal House of the Federal University of Santa Maria were transferred to our breeding colony and maintained on a 12 h light/dark cycle and controlled temperature (22 ± 2 °C). Animals had free access to water and commercial food. After 2 weeks of adaptation, female and male rats (3:1) were placed in the same cage for mating. Studies were carried out in accordance with the national and institutional guidelines (University Ethics Committee Guidelines—Process number 096/2011) for experiments with animals.

2.3. Mating

Virgin female Wistar rats were placed in animal cages with mature male breeders as described in Oliveira et al. [35]. Briefly, three females and 1 male were placed in the same cage during the night (7 p.m.–9 a.m., 14 h). In the following morning, males were separated from females. Mating was confirmed by the presence of sperm in their vaginal smears and defined as day 0 of gestation (GD 0).

2.4. Treatment

Pregnant rats (GD0) were divided in two experimental protocols as described below:

- Protocol 1: Inorganic mercury exposure during the gestational period

Eighteen female rats (n = 6 per group) were randomly divided into three groups and were exposed to 0, 10 and 50 μg Hg^{2+}/mL during 20 days of gestation.

→ Control (n = 6): 0 μg Hg^{2+}/mL (distilled water)
→ Group10 (n = 6): 10 μg Hg^{2+}/mL
→ Group50 (n = 6): 50 μg Hg^{2+}/mL

- Protocol 2: Inorganic mercury exposure during the gestational and lactational periods

Twenty-seven female rats (n = 9 per group) were randomly divided into three groups and were exposed to 0, 10 and 50 μg Hg^{2+}/mL during the gestational (approximately 21 days) and lactational (approximately 21 days) periods. The experiment started with 27 female rats (n = 9 per group); however, 2 rats from the control group, 2 rats from Group10 and 1 rat from Group50 were not pregnant. Thus, the final experimental number used in this work is described below:

→ Control (n = 7): 0 μg Hg^{2+}/mL (distilled water)
→ Group10 (n = 8): 10 μg Hg^{2+}/mL
→ Group50 (n = 7): 50 μg Hg^{2+}/mL

Inorganic mercury doses (10 and 50 μg Hg^{2+}/mL) were chosen based on a previous study from our research team [35].

Animals from both experimental protocols were placed individually in polycarbonate cages and exposed to one of the two doses (10 or 50 μg Hg^{2+}/mL) of $HgCl_2$ for 20 days (gestational exposure: from day 0 until day 20 of gestation) or 41 days (gestational/lactational exposure: from day 0 of gestation until day 21 of lactation). $HgCl_2$ was dissolved in distilled water and supplied to females in the drinking water during the gestation and/or lactation. Drinking solutions containing Hg were replaced and prepared every 2 days from a stock solution (200 μg Hg^{2+}/mL) prepared weekly and stored (4 °C).

2.5. Sample preparation

On day 19 of gestation, pregnant rats from protocol 1 (n = 6 per group) were placed in individual metabolic cages to collect urine and feces for metal determinations. In the following day (GD 20), the animals were euthanized. At the time of euthanasia, rats were anesthetized with an intraperitoneal (i.p.) injection of ketamine and xylazine (70/30 mg/kg/2 mL saline) and after decapitated. Blood, liver, kidneys, placenta and fetuses were removed. In addition, liver and kidneys were removed from the fetuses. Due to the small amount of sample, a pool of fetal (males and females) liver and kidney was made (one pool by litter). Serum was obtained by total blood centrifugation at 3000*g* for 10 min and frozen until analyses (5 days).

For metallothionein level assay, tissues from the pregnant rats (liver, kidneys and placenta) were homogenized in 4 vol of cold 20 mM Tris–HCl buffer, pH 8.6, containing 0.5 mM PMSF as agent antiproteolytic and 0.01% β-mercaptoethanol as a reducing agent.