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Effects of methylmercury and retinol palmitate co-administration in rats during pregnancy and breastfeeding: Metabolic and redox parameters in dams and their offspring

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ABSTRACT

Ubiquitous low-dose methylmercury (MeHg) exposure through an increased fish consumption represents a global public health problem, especially among pregnant women. A plethora of micronutrients presented in fish affects MeHg uptake/distribution, but limited data is available. Vitamin A (VitA), another fish micronutrient is used in nutritional supplementation, especially during pregnancy. However, there is no information about the health effects arising from their combined exposure. Therefore, the present study aimed to examine the effects of both MeHg and retinyl palmitate administered on pregnant and lactating rats in metabolic and redox parameters from dams and their offspring. Thirty *Wistar* female rats were orally supplemented with MeHg (0,5 mg/kg/day) and retinyl palmitate (7500 μg RAE/kg/day) via gavage, either individually or in combination from the gestational day 0 to weaning. For dams (150 days old) and their offspring (31 days old), glycogen accumulation (hepatic and cardiac) and retinoid contents (plasma and liver) were analyzed. Hg deposition in liver tissue was quantified. Redox parameters (liver, kidney, and heart) were evaluated for both animals. Cytogenetic damage was analyzed with micronucleus test. Our results showed no general toxic or metabolic alterations in dams and their offspring by MeHg-VitA co-administration during pregnancy and lactation. However, increased lipoperoxidation in maternal liver and a disrupted pro-oxidant response in the heart of male pups was encountered, with apparently no particular effects in the antioxidant response in female offspring. GST activity in dam kidney was altered leading to possible redox disruption of this tissue with no alterations in offspring. Finally, the genomic damage was exacerbated in both male and female pups. In conclusion, low-dose MeHg exposure and retinyl palmitate supplementation during gestation and lactation produced a potentiated pro-oxidant effect, which was tissue-specific. Although this is a pre-clinical approach, we recommend precaution for pregnant women regarding food consumption, and we encourage more epidemiological studies to assess possible modulations effects of MeHg-VitA co-administration at safe or inadvertently used doses in humans, which may be related to specific pathologies in mothers and their children.

Abbreviations: RAE, retinol activity equivalents

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1. Introduction

Methylmercury (MeHg) is a well-documented ubiquitous contaminant (Sheehan et al., 2014; Sunderland et al., 2018). In the environment, MeHg bioaccumulates and biomagnifies through the food web, reaching concentrations many times higher than the levels in the surrounding water (Miklavčič et al., 2013) representing a serious risk to human health. As dietary fish intake is also a major MeHg source to fish consumers (Faial et al., 2015; USEPA, 1997), populations with a traditionally high dietary intake of fish are the most exposed to MeHg bioaccumulation and may consequently experience its deleterious effects on human health (Faial et al., 2015; USEPA, 2000). Once absorbed, MeHg is rapidly transported in red blood cells due to its increased membrane permeability, and widely distributed in the body, particularly in the central nervous system (CNS); however, MeHg conversion to inorganic Hg and posterior storage is mostly carried in the liver and kidney (Nabi, 2014). Like Hg, MeHg penetrates the placental and the blood-brain barrier of the fetus, which is not completely developed, increasing its exposure (Castoldi et al., 2001; Lepham et al., 1995). Effects observed following *in utero* exposure in poisoning events have included impaired motor development, spasticity, blindness, abnormal reflexes, deafness, seizures and deficiencies in memory, learning and psychological parameters, particularly in cases of low dose chronic exposure (Bellinger et al., 2016; Debes et al., 2016; Llop et al., 2017; Yorifuji et al., 2015). Therefore, as a particularly susceptible group, the effect of MeHg exposure on pregnant women remains an important issue for elucidation, especially in populations where fish is the main source of animal proteins (Stokes-Riner et al., 2011).

Maternal fish consumption during pregnancy exposes the fetus simultaneously to other substances and nutrients present in fish such as n-3 fatty acids, minerals, and vitamins, including vitamin A (VitA), a fat-soluble retinoid also present in eggs and dairy products (Reifen and Ghebremeskel, 2001). VitA may be obtained from both vegetal (provitamin A) and animal (preformed) diet (Napoli, 2012; Oliveira, 2015). High contents of preformed VitA can be encountered especially in liver and fish (Ross, 2010). Several important metabolic and physiologic processes in the organisms require VitA, such as vision, hematopoiesis, embryonic development, cell differentiation, immunocompetence and gene transcription (Chapman, 2012). In fact, in pregnant women, VitA contributes to early fetal development, especially in the CNS (Lane and Bailey, 2005; McCaffery et al., 2003, 2006; WHO, 2016) and VitA supplementation is recommended as safe in doses of 3000 or 7500 μg RAE/daily, independently of previous VitA consumption (WHO, 2009). Both MeHg and VitA may have pro-oxidant characteristics. Reactive oxygen species (ROS) generation has been linked to MeHg- induced toxicity both *in vivo* and *in vitro* systems. Evidence suggests that MeHg exposure causes production of ROS (Aschner et al., 2007; Ishihara et al., 2016), depletion of glutathione (GSH) (Yin et al., 2007), excessive accumulation of calcium ($Ca2+$) (Hare et al., 1993), apoptosis/necrosis (Shenker et al., 1998) and a decrease in mitochondrial membrane potential in nervous (Limke and Atchison, 2002) and immune (Shenker et al., 1998) systems. On the other hand, despite its importance in the healthy development of the fetus and the newborn, recent evidence supports that VitA is a redox-active molecule capable of inducing prooxidant effects in an animal model at safe/therapeutical doses (Behr et al., 2012; Gasparotto et al., 2015; Schnorr et al., 2015, 2011b, 2014). In rats, VitA supplementation at therapeutic doses resulted in impairment of liver and kidney redox balance in mothers and their offspring (Schnorr et al., 2011b). VitA also possesses teratogenic effects at higher doses (retinoic acid syndrome), originating a pattern of birth defects including craniofacial, cardiovascular and thymic affectations (Azais-Braesco and Pascal, 2000). Thus, during the first weeks of embryogenesis, supplementation with VitA must be carefully managed to avoid congenital malformations caused by either deficiency or excessive intake (Verma et al., 2017; West, 2002).

Individual effects of MeHg and VitA exposure are well documented. However, there is a lack of information about the impacts arising from their combined exposure. The particular nutritional requirements of women increases during pregnancy, especially for fish and shellfish, which are the primary source of long-chain polyunsaturated fatty acids (Razzaghi and Tinker, 2014). Despite recommendations from global committees in regard of MeHg exposure, only in the US, during the last years, fish consumption has increased among pregnant women (Cusack et al., 2017; Razzaghi and Tinker, 2014), creating a possible scenario of increasing MeHg and VitA bioavailability, imposing unknown risks to the expectant mother and her fetus. The present study aimed to examine the effects of both MeHg and VitA administered to pregnant and lactating rats. Thus, we assess redox state, mercury deposition, metabolic liver function and genomic damage in adult rats and their offspring, to evaluate possible additive/synergistic/antagonistic interactions between these compounds *in vivo*.

2. Materials and methods

Experimental procedures were conducted in accordance with the Principles of Laboratory Animal Care (National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals, revised 2011) (NIH, 2011), and were in compliance with recommendations of the Brazilian Society for Science in Laboratory Animals (SBCAL-COBEA). The Ethical Committee for Animal Experimentation (CONCEA) ascribed to Federal University of Rio Grande do Sul (UFRGS) approved the research protocol under authorization number 31672.

2.1. Animal and housing conditions

Male and female Wistar rats (*Rattus novergicus*) were obtained from our breeding colony and housed in groups of four animals under a 12 h light-dark cycle (7:00 – 19:00 h) at constant temperature (21 ± 1 °C). Standard commercial food (CR1 Lab Chow, Nuvilab, Curitiba, Brazil; 3600 μg RAE/Kg) and filtered water were provided *ad libitum*. Female nulliparous rats (200–250 g) with 120 days were checked daily for their estrous cycle using direct vaginal smear evaluation under light microscopy, as a previously described protocol (Marcondes et al., 2002). Estrous cycle monitoring was performed for two weeks, and through a later period of a week, to obtain a maximum number of rats in proestrous as possible in each reproductive cycle. Sexually receptive females (confirmed proestrous) were caged overnight with a single mature male (1F:1M). The next morning the presence of viable sperm in the vaginal smear was considered as successful mating and designated as gestation day 0 (GD0). Pregnant dams were caged individually and allowed to litter naturally. The date of delivery was defined as postnatal day 0 (PND0).

Restriction stress in late pregnancy (GD17–21) affects the dam and influences somatic growth and weight gain in offspring (Amugongo and Hlusko, 2014). Therefore, dams were minimally handled during a 4-day period (comprising GD20 and PND2) to avoid stress-related effects on pups, which also were only manipulated and registered on PND2. However, gavage continued during the restriction period performed by the same operant, to avoid additional stress in the dam. Pre-culled litter size possibly influences further development in pups from culled litters, due to maternal and offspring mutual adjustment to genetically defined litter sizes. The latter may increase variability in pup´s weight, as treatment-related mortality progresses in culled litters (Chahoud and Paumgartten, 2009). For that reason, we wanted to assure a maximum number of live pups. Consequently, full-sized litters were maintained with the dam until weaning (PND21) and immediately separated and sexed. Separated litters were co-housed in groups of the same sex to eliminate interferences on behavior related to sexual hormones (Dridi et al., 2014). No further treatment was applied to male and female pups, which continued in the same room conditions as their parents with standard food and filtered water *ad libitum*.