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Lead accumulation and metallothionein content in female rats of different ages and generations after daily intake of Pb-contaminated food

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ABSTRACT

Female Wistar rats of different ages (45, 90 and 140 days) and generations (mothers and offspring) were fed a feed containing 2.0 mg of Pb kg^{-1} daily from weaning and the Pb accumulation was determined in different organs and in maternal milk, in addition metallothioneins (MTs) content was determined in the liver and kidneys. The results showed that Pb accumulation exhibited the following pattern: bone > liver > kidney > gut > blood cells > muscle > brain > ovary. Bones accumulated the most Pb in all animals, with its concentration increasing with age and prenatal exposure. Pb accumulation in the liver, kidney and blood cells, did not follow a consistent pattern with increasing age and our data did not indicate a relationship between the presence of MTs in liver and kidney and metal accumulation in these organs. However, in the offspring and with increasing age, Pb accumulated in more organs. Mothers fed with Pb produced contaminated milk, exposing their offspring to the metal via nursing Thus, increasing age and prenatal exposure increases susceptibility to Pb toxicity-induced damage.

1. Introduction

Lead (Pb) poisoning remains a major public health hazard, particularly in developing countries, by causing various deleterious effects on renal, hematopoietic, and reproductive functions as well as on the central nervous system (Flora et al., 2012; Beier et al., 2015). Pb is a metal that, mostly through anthropogenic action, can be found in soil, water, and the atmosphere; humans can be exposed via ingestion of contaminated water or food sources, among other routes. Nevertheless, the dietary intake of Pb is the main source of human exposure to this metal (EFSA, 2012).

Lead is one of the most studied metal elements, and its accumulation in organisms may cause harmful effects over time (Flora et al., 2012). Lead is absorbed into the bloodstream via the gastrointestinal tract and can be deposited in organs such as the liver, kidneys, lungs, brain, spleen, heart, muscles and bones; approximately 94% and 73% of the total Pb in the body is found in the bones in adults and children, respectively (ATSDR, 2007). Younger organisms tend to accumulate higher amounts of lead compared with adults, as their mucous membranes are more permeable and their defenses are not fully effective (Winiarska-Mieczan and Kwiecień, 2016).

Lead accumulated in bones may be gradually released into the bloodstream even after exposure has ceased, particularly during physiologic or pathologic bone demineralization periods, such as pregnancy, lactation and osteoporosis (EFSA, 2012). Thus, prenatal exposure to Pb may harm maternal health and fetal and infant development even if maternal exposure levels are low. Furthermore, as bones constitute the main Pb reservoir over decades, women and their offspring may be at risk of continuous exposure well after exposure has ceased (CDC, 2010).

Metallothioneins (MTs) are low-molecular-weight intracellular proteins that are rich in cysteine residues and have high affinity for essential and non-essential metals (Park et al., 2001). These proteins contribute to the homeostasis of some essential metals and act as a protective mechanism against metal toxicity (Klaassen et al., 1999). Although some studies have linked MTs to Pb detoxification, information on Pb-driven MTs induction mechanisms remains scarce (Dai et al., 2013).

Thus, the aim of the present study was to evaluate how age and maternal transfer influence lead accumulation in different organs. Furthermore, MTs content in kidney and liver Pb was also assessed. To accomplish these objectives, female Wistar rats of different ages

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and generations were fed a feed containing 2.0 mg of Pb kg^{-1} daily from weaning and were used to evaluate the accumulation of Pb in different organs and in maternal milk, in addition to the MTs content in the liver and kidneys.

2. Material and methods

2.1. Chemicals

All chemicals and reagents used were of analytical grade. Lead nitrate was purchased from Vetec (Brazil), grade > 99% pure. All other analytical laboratory chemicals and reagents were purchased from Merck (Germany), Sigma (USA) or Fmaia (Brazil), unless indicated otherwise.

2.2. Animals

Female Wistar rats (*Rattus norvegicus*) were provided by the central animal house of the State University of Londrina. Immediately after weaning (22 days), animals weighing 37 ± 5.58 g (n = 80) were transferred to the animal house of the Department of Physiological Sciences, where they were kept in individual cages at 23 ± 2 °C with a 12 h/12 h photoperiod. Female rats were fed commercial feed with and without added lead (Pb and control groups, respectively). Feed and water were available *ad libitum*. The animals were weighed, and feed consumption was assessed three times per week by comparing the weight of the feed provided with the weight of the feed remaining (including waste feed in the under-cage collecting tray). Two female rat groups 78 days of age were subjected to mating and kept under the same conditions described previously throughout pregnancy and nursing. For mating purposes, male Wistar rats (from the UEL central animal house) were placed in cages with females at a 1:3 or 1:4 (male:female) ratio. Assessment of weight and feed consumption was discontinued during the mating period. The ethics committee for animal experiments of the State University of Londrina, Brazil approved the present study (CEUA/UEL – Process: 34715.2011.16).

2.3. Feed composition

Nuvilab® commercial feed for rats was ground, and Pb was added in the form of lead nitrate ($Pb(NO_3)_2$) at a ratio of 2.0 mg Pb per kg feed (dissolved in 800 mL of water). New pellets were formed and dried at 60 °C for 24 h. The feed for the control group underwent the same process but without the addition of $Pb(NO_3)_2$. The Pb concentration of 2.0 mg kg^{-1} was defined based on the maximum Pb concentration for fish meat established by the Brazilian Health Surveillance Agency (ANVISA/685, 1998).

2.4. Experimental design

Female rats were divided into different treatment groups (n: 9 to 10 rats per treatment) as follows:

- Rats 45d: female rats fed commercial feed without lead (CTR 45d) or with added lead (Pb 45d) shortly after weaning at 22 days of age until they were 45 days of age;
- Rats 90d: female rats fed commercial feed without lead (CTR 90d) or with added lead (Pb 90d) shortly after weaning at 22 days of age until they were 90 days of age;
- Mothers 140d: female rats, which became pregnant and nursed, fed commercial feed without lead (CTR 140d) or with added lead (Pb 140d) shortly after weaning at 22 days of age until they were 140 days of age. These rats were also fed the manipulated feed (CTR or Pb) during pregnancy and lactation;
- Offspring 45d: female offspring from the Mothers 140d (CTR and Pb groups), fed commercial feed without lead (OffCTR 45d) or with added lead (OffPb 45d), respectively, shortly after weaning at 22 days of age until they were 45 days of age; and
- Offspring 90d: female offspring from the Mothers 140d (CTR and Pb groups), fed commercial feed without lead (OffCTR 90d) or with added lead (OffPb 90d), respectively, shortly after weaning at 22 days of age until they were 90 days of age.

In female rats, 45 days of age corresponds to puberty, while 90 and 140 days of age correspond to adulthood, when mating is possible (Anderson et al., 2004).

2.5. Sampling

After the experimental treatments, the rats were weighed, anesthetized with sodium thiopental (40 mg kg^{-1}), and euthanized by exsanguination without previous fasting. Blood was collected from the inferior vena cava and centrifuged (1870*g*, 15 min, Hsiangtai centrifuge, model MCD-2000, Taiwan); blood cells were used to evaluate Pb accumulation. One section of the small intestine (1 cm of the first duodenal portion), one small portion of the left lateral lobe of the liver, brain, ovaries, right kidney, left tibia, and one muscle from the left leg were also collected for an evaluation of Pb accumulation. Samples were stored in nitric acid-washed (for metal decontamination) cryogenic tubes. One small section from the right kidney and left lateral liver lobe were taken for a separate analysis of MTs.

One hour prior to milk sampling, the offspring were separated from their mothers. Then, the female rats were anesthetized intraperitoneally with ketamine (60 mg kg^{-1}) and xylazine 2% (10 mg kg^{-1}); following anesthesia, 0.125 mL of oxytocin (125 UI) was injected intraperitoneally. Milk was collected by manual milking with the help of a micropipette and stored in cryogenic tubes pre-washed with nitric acid, for Pb accumulation analysis.

2.6. Determination of lead in feed and biological samples

Feed, organs, blood cells, and milk samples were oven-dried at 60 °C and digested for 48 h at 60 °C in nitric acid (Suprapur, Merck) 5N (1:5, w:v). The digested material was analyzed for Pb using graphite furnace atomic absorption spectrometry (Perkin Elmer, AAnalyst 700, USA) against a reference Pb standard solution (Specsol, Brazil). The detection limit for Pb using this method is 0.05 μg L^{-1}. Two parallel determinations were performed for each sample and the differences in results were on average 3.6%.

2.6.1. Determination of MTs in the liver and kidney

The MTs content was determined according to the method described by Viarengo et al. (1997), with modifications. Liver and kidney samples were homogenized (1:3 and 1:2 w:v, respectively) in specific buffer (0.5 M sucrose, 26 mM Tris, 0.5 mM phenylmethylsulfonyl fluoride, 1.3 mM β-mercaptoethanol) and centrifuged 45 min, 21300*g*, 4 °C (Hettich, Univesal 320R, Germany). The supernatant was subjected to ethanol/chloroform fractionation to obtain a partially purified metalloprotein fraction. Sulfhydryl (-SH) groups in this fraction were quantified spectrophotometrically (Perkin Elmer, Victor^3 Multilabel Reader, USA) with Ellman's reagent (2 M NaCl, 0.43 mM DTNB buffered with 0.2 M Na-phosphate, pH 8) at 412 nm, using glutathione (GSH) as the standard. The MTs content was expressed as nmol MTs mg of protein^{-1}. The protein concentration in the homogenate was determined spectrophotometrically (BioTek Instruments, ELx 800 Absorbance Microplate Reader, USA) according to Bradford (1976) at 595 nm, using bovine serum albumin (BSA) as the standard.