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Developmental effects of parental exposure to soil contaminated with urban metals

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HIGHLIGHTS

- We examine effects of exposure contaminated soil on offspring development.
- Decrease in pups locomotors and exploratory activity
- Negative influence of parental exposure to contaminated urban soil on development

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ABSTRACT

Soil is a highly complex material, and because of rapid population growth, intense industrial activity and petrochemical development, it has suffered from contamination with substances of various origins. These environmental contaminants may have detrimental effects on human health, particularly during development. Due to the ability to transmit contaminants to the fetus, evaluating the effects of exposure of pregnant women on the psychomotor development of their offspring is of particular interest. Therefore, this study aimed to investigate the effects of exposure of female rats to an urban soil influenced by the dispersion of air contaminants during periods of pre-pregnancy, pregnancy and lactation on offspring development. Using physiological, behavioral and hematological parameters, deleterious effects on offspring were assessed. In behavioral parameters, parental exposure during pregnancy and lactation resulted in no significant differences in the evaluated parameters when compared to the control group. In contrast, pups from the pre-pregnancy group displayed decreased locomotor and exploratory activity in addition to increased levels of anxiety. Furthermore, offspring of rats exposed to contaminated urban soil during pre-pregnancy demonstrated significant changes in weight gain and development length and a reduction in the number of platelets compared to controls. Significantly, pups born to mothers exposed to contaminated urban soil during the pregnancy displayed changes in birth weight, weight gain during the growth, development length, incisor eruption and opening of the ears in addition to a reduction in their physical performance and a change in the number of lymphocytes. These results clearly show the negative influence of parental exposure to contaminated urban soil on the general development of the rats during the periods studied. These data indicate that developing organisms are highly sensitive to external factors. Further, they demonstrate the utility of these various biomarkers for identifying and displaying toxic effects of exposure to contaminated soils.

1. Introduction

The study of the impact of contaminants on human health and the environment is critical for the prevention of poisoning in the population and the preservation of public health. These studies are increasingly important, given the higher risk of exposure associated with the industrial and technological growth. This is especially true for developing countries, where the risks are poorly assessed and controlled (Mattos et al., 2009).

Industrial processes release a variety of atmospheric contaminants that, along with fuel burning operations (especially firewood and oil), generate a large amount of pollutants. Moreover, environmental contamination is present in soils due to material from contaminated harbor

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sediments, landfills and contaminated industrial land. The resulting environmental matrix is a complex mixture of organic and inorganic compounds. The association between air pollutants from industrial activity and contaminants in the ground soil may contribute to the increased incidence of severe damage in exposed human populations. Toxicological studies addressing different routes of exposure to contaminants from the soil and the effects of this exposure on organisms has been the subject of several studies (Muccillo-Baisch et al., 2012; Da Silva-Júnior et al., 2012).

Several studies have demonstrated the important contribution of metals to environmental contamination in Rio Grande (Baisch et al., 2004; Mirlean and Conceição, 2006). However, studies involving chemical or biological approaches to evaluate contaminated soils in the state of Rio Grande do Sul as a whole are less numerous (Mirlean et al., 2003; Mirlean et al., 2005; Da Silva-Junior and Vargas, 2009).

Prenatal development can be altered by many factors, and environmental etiology is one of the main causes of adverse pregnancy outcomes. In children, a significant fraction of the congenital defects have an environmental component (Leite and Schüler-Faccini, 2001). Children are more vulnerable to exposure to contaminants than adults because they possess unique absorption and metabolisms (Soldin et al., 2003). This vulnerability varies with stage of development. There are critical periods of structural and functional development, both in the prenatal stage as well as postnatal, in which specific structures or functions may be more sensitive to damage. These effects may not be evident until a later stage of development (ATSDR, 2005).

Toxic effects in the offspring may result from in utero exposure to metals such as cadmium, because this and other xenobiotics can be transferred via the maternal–fetal circulation and through breast milk. The mobilization that begins during gestation remains during lactation (Barbosa et al., 2005). The effects of contaminants in the soil are closely related to human health, particularly concerning effects on reproduction, and are among the issues prioritized by environmental health programs. The use of controlled experimental models allows the association between parental exposure and abnormal offspring development to be evaluated. Thus, the use of physiological, behavioral and hematological markers is useful in identifying early and late effects of environmental exposure on health.

This study aimed to investigate the effects of exposure of female rats to a contaminated soil during 3 defined periods: before pregnancy (pre-pregnancy), pregnancy and lactation. Offspring from rats exposed during these critical windows were evaluated for deleterious effects on development using physiological, behavioral and hematological parameters.

In a previous study by Da Silva-Júnior et al. (2013), it was found that exposure of male Wistar rats to contaminated soil had deleterious effects on various parameters of health. This study demonstrated the potential harmful health effects of contaminated soils and raised the possibility that exposure may interfere with human development in its different stages. This highlights the importance of the current study and supports more in-depth investigation in the future.

2. Materials and methods

2.1. Animals

Adult Wistar rats of both sexes, aged between 3 and 4 months, were obtained from the Central Animal Laboratory of the Federal University of Rio Grande (FURG). The animals were kept in a vivarium at the Institute of Biological Sciences, under controlled temperature (21 ± 3 °C), humidity (51%) and photoperiod (12 h light/12 h dark) conditions. Animals were fed commercial pet food (Bio base, Bio-Tec, Cold Water, SC) and water ad libitum.

Rats were divided into three treatment groups: pre-pregnancy, pregnancy and lactation. Rats were exposed urban contaminated soil for 21 days either before mating, during gestation, or during breastfeeding for each group, respectively. For each treatment type, a corresponding control group of rats was exposed to uncontaminated soil. To determine pregnancy, rats were subjected to the test of vaginal swab 24 h after being placed in contact with the males and were considered pregnant after detection of sperm. The smear was performed by washing the vaginal canal with 50 μL of saline using a micropipette with the tip retracted. The lavage fluid was then smeared on a slide and imaged using light microscopy (200× magnification). The male rats used for mating were not exposed to any type of soil.

The soil was administered by oral gavage, and treatments were calculated according to the daily limits outlined by the American Environmental Protection Agency (U.S. Environmental Protection Agency, EPA, 2011). These guidelines permit the voluntary intake of 10 g of soil in a single geophagy event, based on a human being weighing 70 kg. Aimed to mimic the exposure of people living in the study region, crude extract (aqueous) of soil das diluted in distilled water in the ratio 1:2 and 1 mL was given daily. The project was approved by the Ethics Committee on Research in the Area of Health, process no. 59/2010-CEPAS/FURG.

2.2. Soil samples

Soil samples were collected in two areas: in the campus of the Federal University of Rio Grande (FURG), which is subject to minimal anthropic impact (control group) and a place of strong urban campus occupation (predominant population living in poverty) and industry located in Rio Grande, a municipality of Rio Grande do Sul near the mouth of the Patos lagoon connected to the Atlantic Ocean (contaminated soil groups). The sampling location of the contaminated soil was chosen because it has previously been characterized with high levels of contaminants (Da Silva Júnior et al., 2013). At each collection site, the soil samples were collected as described by Da Silva-Júnior et al. (2009).

2.3. Assessment of effects on offspring of exposure of females to contaminated soil

2.3.1. Reproductive outcomes

The number of pups born (alive and dead) and litter (alive) weight at birth was reported.

2.3.2. Postnatal growth

The weight and length of pups was monitored throughout development, from birth to 21 days of age. The first measurement of body weight was performed on the first postnatal day and pups were weighed on alternate days until the day of death. The individual monitoring of the weight started on the day of the detachment of the ears. Length was measured with the animal in the prone position on an anthropometric ruler, measuring from tip of nose to end of tail as described by Hughes and Tanner (1970).

2.3.3. Evolution of development

To evaluate early development, age for detachment of the ears (bilateral), appearance of fuzz, appearance of hair, eruption of incisors, opening ears (bilateral), opening eyes (bilateral) and descent of testes was recorded. The parameters were considered complete when observed in all pups in the litter. Morphological parameters were evaluated in the early morning and late afternoon.

To test swimming ability, two pups were selected from each litter (the selection was made by averaging the weights) and individually subjected to a swimming test, performed on days 7, 14 and 21 post birth. For the test, animals were placed individually in a tank with heated water (40 °C) whose depth was sufficient to prevent the animal's legs from touching the bottom. Each animal was placed in the tank to swim three times for a period of 10 s, with intervals of 10 min between each trial. Based on their performance, animals received one of two scores at each time point (postnatal days 7, 14 and 21): zero (0) when the