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Mutagenic effect of contaminated soil on the offspring of exposed rats

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ABSTRACT. This study aimed to measure irreversible DNA damage in the offspring of rats exposed to contaminated soil. Bone marrow cells were removed from the femurs of the offspring of rats that were exposed to contaminated soil via gavage during three different periods: 21 days prior to detection of pregnancy, during pregnancy and 21 days after birth, during the lactation period. For each group, we also had a control group under the same experimental conditions but exposed to uncontaminated soil. The results showed that pups borne of rats exposed to contaminated soil had an increase in the number of micronuclei in the different exposure groups compared with their respective control groups. This suggests a potential risk of mutagenic damage to the offspring of pregnant women who live at the sampling site.

Keywords: soil contamination, reproduction, wistar rats, metals, micronucleus.

Efeito mutagênico da exposição a solos contaminados sobre a prole

RESUMO. Este estudo objetivou medir os danos irreversíveis de DNA na prole de ratas expostas a um solo contaminado, através do teste do micronúcleo. O mesmo foi realizado na medula óssea retirada do fêmur de filhotes de ratas expostas, via gavagem, em três diferentes períodos, ratas expostas durante 21 dias antes da detecção da prenhez, durante a prenhez e 21 dias após o nascimento, durante o período de aleitamento. Para cada grupo foi utilizado um grupo controle, mantido nas mesmas condições experimentais e expostos a um solo não contaminado. Os resultados obtidos nos filhotes expostos ao solo contaminado mostraram um aumento no número de micronúcleos nos três diferentes grupos expostos quando comparados aos seus respectivos grupos controle. Assim, os dados sugerem um potencial risco de dano mutagênico aos descendentes das gestantes residentes no local de amostragem.

Palavras-chave: contaminação terrestre. reprodução. ratas wistar. elementos metálicos. micronúcleo.

Introduction

To optimally thrive, humans should live in a quality environment to not compromise their physical and mental well-being. For this purpose, environmental science aims to improve public health by prevent damage that may be caused by increased chemical contaminant levels in the environment (Amorim, 2003). Among these contaminants are metallic elements present in the soil, which are of interest in this study.

Soil has the characteristic of being static; thus, contaminants that are deposited therein accumulate and remain for an extended period of time, unlike other environmental compartments that are continuously displaced such as air and water (Siqueira, Moreira, Grisi, Hungria, & Araújo, 1994; Stenberg, 1999).

The toxic compounds in the soil can be absorbed by humans through direct skin contact, inhalation, ingestion of percolated water or geophagy (Watanabe & Hirayama, 2001; Van De Wiele, Verstraete, & Siciliano, 2004). Within this context, soil research has become more prominent in studying environmental contamination.

Pregnant women exposed to these contaminants may develop mutations in their reproductive organs because there are critical periods of structural and functional development in the reproduction process in which a specific function or structure may be more vulnerable to damage during both the prenatal and postnatal stages (Agency for Toxic Substances and Disease Registry [ATSDR], 2005). Moreover, due to the frequent habits of geophagy and taking objects and hands to their mouth, children are more susceptible to the intake of particulate material containing contaminants and are more severely affected because they have a different distribution and metabolism (Soldin, Hanak, & Soldin, 2003).

Evaluating the consequences of toxic compounds on genetic content is important for predicting their effects on the health of exposed populations, which are represented here by a model animal system. Three different exposure periods in rats were used in this study to simulate the exposure situations that the population of interest in this study (children and women at reproductive age) encounter.

The mutagenicity in the offspring of mothers exposed to soil contaminants was evaluated with the use of biomarkers. In this study, the micronucleus test was performed to measure irreversible damage to the chromosome and establish an association between exposure to contaminated soils and possible mutagenic damage in bone marrow erythrocytes (White & Claxton, 2004).

Thus, the aim of this study was to investigate the effects of exposure to contaminated soil on the health of Wistar rats' offspring for periods of 21 days prior to pregnancy, during pregnancy and during lactation.

Material and methods

Soil samples were collected in the following two areas, previously analyzed by Silva-Junior et al. (2013): an area with minimum human activity on the campus at the Federal University of Rio Grande (FURG) (control soil), and near the mouth of the Patos Lagoon in the Atlantic Ocean in the region known as *Coroa do Boi* located in the city of Rio Grande, Rio Grande do Sul State, southern Brazil (contaminated soil). There are various chemical industries in this estuarine region, which is considered to be contaminated by liquid and atmospheric effluents and primarily characterized by its proximity to the industrial complex of the city and the strong urban occupation and human activity (Mirlean, Baraj, Niencheski, Baisch, & Robinson, 2001). This region was described as containing a high concentration of metallic elements (Garcia, Mirlean, & Baisch, 2010). Soil collection and analysis were performed as described by Silva-Junior & Vargas (2009).

Adult Wistar rats of both sexes were obtained from the Central Animal Laboratory at the Federal University of Rio Grande. Female rats were divided into the following three groups based on the period of exposure to contaminated soil: 21 days prior to the detection of pregnancy ('pre-pregnancy' group), during the 21 day gestation period ('pregnancy' group), and 21 days after birth during the period of lactation ('lactation' group). For each exposure group, we used a control group under the same experimental conditions but exposed to uncontaminated soil.

Female rats were subjected to a vaginal swab 24 hours after contact with males and considered pregnant if sperm was detected. The smear was prepared by vaginal washing with 50 μL of physiological saline applied to the vaginal canal and collected with the tip of a micropipette. The content was smeared fresh on a slide for light microscopy and observed at 200× magnification (Olympus CX 41). Male rats used for mating were not exposed to any type of soil.

The soil was administered by gavage based on the daily limits considered by the US Environmental Protection Agency [USEPA] (1996), which admits the voluntary ingestion of 10 g of soil in a single geophagy event, considering a human weighing 70 kg. To simulate natural conditions of human exposure in the study area, 1 mL of crude soil extract diluted in distilled water in the proportion 1:2 was administered daily according to the animal's weight. After exposure, the pups were euthanized by decapitation for bone marrow extraction. Micronuclei induction was measured using the cell smear test with the bone marrow extracted from the femurs of rats as described by Iarmarcovai, Ceppi, Botta, Orsière, & Bonassi (2008) and Ribeiro, Salvadori, & Marques (2003). Using a microscope at 1000× magnification (Olympus CX 41), 1,000 polychromatic erythrocytes were counted in each slide, and the number of micronuclei observed was registered. Smears were made in duplicate for each animal, and the average of the two results was considered.

The results are expressed as the mean ± standard deviation. Data were statistically analyzed with the software STATA 10. An analysis of variance (Kruskal-Wallis) test was performed to compare the means, followed by the Mann-Whitney test to compare between groups. The data was considered to be statistically significant when $p < 0.05$.

Results and discussion

The statistical analysis of the data showed that all of the groups exposed to contaminated soil were significantly different compared with the control groups ($p < 0.05$). Figure 1 shows the results of micronuclei counts per 1,000 polychromatic erythrocytes in each animal of different groups.

The results indicating mutagenicity showed a chromosome instability capable of generating very significant chromosomal alterations in the process of carcinogenesis contributing to the onset and/or progression of tumors (Nowak et al., 2002).

During pregnancy and after birth, the cells in the sex organs and the central nervous system that are still differentiating can be influenced by the substances present in maternal blood during pregnancy via the placenta and during lactation via breast milk. Individuals exposed during critical periods of development are more vulnerable to the action of chemical substances, as they have a lower metabolic and excretory capacity and absence of many feedback mechanisms of the endocrine system (Zenick & Klegg, 1989).

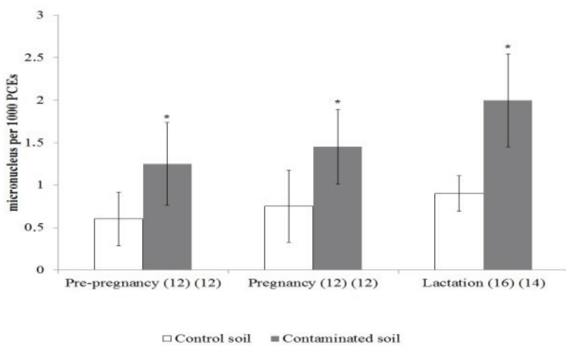

Figure 1. Number of micronuclei in polychromatic erythrocytes (PCEs) in the bone marrow from the offspring of rats exposed to either contaminated or control soils during the three exposure periods (pre-pregnancy, pregnancy and lactation). The values are expressed as the mean ± standard deviation. *$p < 0.05$ compared with the respective control group. The numbers in parentheses represent the n of the control group and the exposed group, respectively.

Thus, exposure of rats to leachate containing contaminated soil before pregnancy, during pregnancy and during lactation (post-natal) showed that the contaminated soil of the study area was capable of generating mutagenic damage in the bone marrow erythrocytes of their descendants throughout the periods analyzed, considering the reproductive period of rats.

The exposure in different reproductive periods showed that the negative effects of soil contaminants can extend for long periods even after exposure is terminated. This finding may be evidenced by pre-pregnancy group exposure where their offspring had mutagenic damage, although they were not exposed during any period of direct way. Our results also suggest that the metals circulating in the organism are able to cross the placental barrier to reach the fetus as well as pass into breast milk.

In a study that evaluated endocrine disruptors, heavy metals are cited as substances capable of crossing the placental barrier and accumulating in breast milk (Bila & Dezotti, 2007).

The metallic elements found in the soils used in this study, a higher concentration of all the metals analyzed was found in soils influenced by the presence of the industrial complex compared with the control soil (As, Cd, Cr, Cu, Ni, Pb and Zn), highlighting the values of As, Ni and Pb as greater than the standards of *Conselho Nacional do Meio Ambiente* [CONAMA] (Brasil, 2009) for groundwater.

Lead (Jagetia & Aruna, 1998) and nickel (Dhir, Agarwal, Sharma, & Talukder, 1991) are clastogenic agents that induce micronucleus formation. However, these are not the only metals that generate mutagenic effects because the studied soil presents a variety of metallic elements in varying degrees of contamination that mix in complex ways (Silva-Junior, Rocha, & Vargas, 2009).

The US Environmental Protection Agency [USEPA] (2001) labeled arsenic as carcinogenic to man and determined an allowable daily intake dose with minimal risk to health. This dose proved to be sufficient to induce micronuclei formation in the animals evaluated by Khan, Kesari, & Kumar (2013).

Both voluntary and involuntary soil ingestion can cause health damage when the soil contains toxic molecules, including metallic elements. In soil, metals are the most relevant compounds of probable anthropogenic origin that can cause toxicity (e.g., mutagenesis, carcinogenicity and teratogenicity) in mass populations (Monarca et al., 2002; Silva-Júnior et al., 2009; Silva-Júnior et al., 2013).

In this study, the micronucleus test, a common biomarker of mutagenicity, was effective in assessing the damage caused by soil contaminants on the health of the animals studied. This work supports the hypothesis that the contaminants in the soil can cause damage to the health of the population living at the sampling site.

Conclusion

The maternal exposure to metallic elements from a contaminated soil caused mutagenic damage in bone marrow cells of their offspring. This scenarios showed irreversible cytogenetic damage and offers an alert to mothers living in sites contaminated by metals.

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Ethical Approval

All procedures performed in studies involving animals were in accordance with the ethical standards of the Brazilian general rules as determined by the Brazilian College of Animal Experimentation

(Colégio Brasileiro de Experimentação Animal [COBEA], 1991). The project was approved by the Ethics Committee for Research in the Health Area (Comitê de Ética em Pesquisa na Área da Saúde - CEPAS/FURG) number 59/2010.

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