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Volcanogenic pollution and testicular damage in wild mice

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HIGHLIGHTS

- Volcanogenic pollutants cause histomorphometric alterations in seminiferous tubules.
- Apototosis in gametogenic cells is increased by volcanogenic pollution.
- Mice exposed to volcanically active environments have higher testicular injury.
- Testes of mice exposed to volcanic environments have higher levels of heavy metals.

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ABSTRACT

Many evidences have surfaced the adverse effects of environmental pollutants on male reproduction. Volcanogenic pollution is understudied, although it is a well-known source of hazardous contaminants. This study aims to assess the effects of chronic exposure to volcanogenic pollution on wild mice testes by studying: (i) diameter of seminiferous tubules; (ii) relative volumetric density of different spermatogenic cells and interstitial space; (iii) damage in the seminiferous tubules and (iv) apoptotic level in the germinal epithelium. The mice from the polluted site showed higher levels of the selected heavy metals than those from the reference site. The mean diameter of seminiferous tubules and the relative volume occupied by spermatozoa and lumen in exposed mice were significantly lower than in the unexposed group. Contrarily, exposed mice showed a significantly higher relative volume occupied by interstitium, as well as, a higher degree of damage and a significantly higher number of apoptotic cells in the germinal epithelium. Results show that secondary manifestations of volcanic activity can pose a serious risk of testicular injury and therefore for male reproduction.

1. Introduction

During eruptive and post-eruptive phases volcanoes release numerous hazardous contaminants, including toxic gases and heavy metals (Amaral and Rodrigues, 2011). Previous studies have reported that terrestrial invertebrates (Heikens et al., 2001; Lock and Janssen, 2001; Amaral and Rodrigues, 2005; Amaral et al., 2006a) and vertebrates (Wlostowski et al., 2000; Pereira et al., 2006; Amaral et al., 2007), including humans (Amaral et al., 2008), exposed to volcanically active environments accumulate heavy metals in different tissues and organs. Furthermore, several recent studies evidenced that chronic exposure to volcanogenic contaminants has adverse effects not only in animals like earthworms (Cunha et al., 2011) or mice (Camarinho et al., 2013), but also in humans (Rodrigues et al., 2012).

Present-day volcanic activity in the Island of S. Miguel (Azores, Portugal) is marked by several hydrothermal manifestations consisting of active fumarolic fields, thermal and cold CO_2 springs and soil diffuse degassing areas (Viveiros et al., 2008, 2009, 2010). At Furnas volcano (one of the three main active volcanoes in S. Miguel Island), CO_2 emanations ($\sim$968 t d^{-1} CO_2) by soil diffuse degassing is recognized as a permanent and hidden threat for public health; other volcanic main hazardous gaseous emissions from Furnas volcano activity include hydrogen sulfide (H_2S)

Abbreviations: DiamST, Diameter of seminiferous tubule; IntS, Interstitial space; L, Lumen of seminiferous tubule; Sc, Spermatocyte; Sg, Spermatogonia; St, Spermatids; Sz, Spermatozoa.

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and radon (^{222}Rn) (Baxter et al., 1999; Viveiros et al., 2009, 2010). Associated with volcanic gases and aerosols, heavy metals, such as arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), rubidium (Rb) and zinc (Zn) are also released (Delmelle and Stix, 2000; Durand et al., 2004; Amaral et al., 2008). According to Mathur et al. (2010) one of the major mechanisms behind metal toxicity has been attributed to oxidative stress (metals are capable of interacting with nuclear proteins and DNA and cause oxidative deterioration of biological macromolecules). Since metals are able to penetrate the blood testis barrier they can potentially affect spermatogenesis in different mammals (mouse, rat, rabbit, monkey and human). Moreover, several recent studies have reported the potential testicular toxicity of these metals in rats and mice, such as As (Mukherjee and Mukhopadhyay, 2009), Cd (El-Shahat et al., 2009; Oliveira et al., 2009; Predes et al., 2010; El-Refaiy and Eissa, 2012), mercury (Hg) (Massányi et al., 2007a; Mocevic et al., 2013) and nickel (Ni) (Murawska-Cialowicz et al., 2012; Toman et al., 2012).

Recent studies performed with Furnas volcano inhabitants show that chronic exposure to volcanogenic emissions is associated to a high incidence of chronic bronchitis and of some cancer types (e.g. breast, lip and oral cavity) (Amaral et al., 2006b; Amaral and Rodrigues, 2007) and, with a higher risk of DNA damage in buccal epithelial cells (Rodrigues et al., 2012). Last year, a study by Camarinho et al. (2013) showed that chronic exposure to volcanogenic air pollutants causes lung injury in wild mice caught in Furnas village.

Since volcanic environments are characterized by hypoxia, hypercapnia and hyperthermia, as well by the occurrence of elevated concentrations of toxic gases and some heavy metals, this study was designed to assess whether chronic exposure to volcanically active environments causes testicular damage, using the wild mice *Mus musculus* as model species. Testicular damage was assessed by studying the relative volumetric density of different spermatogenic stages and of interstitial space, the diameter of seminiferous tubules, the damage in the seminiferous tubules (percentage of luminal area occupied by spermatozoa and germinal epithelium structural organization), and by quantifying apoptotic spermatogenic cells in the seminiferous tubules.

2. Materials and methods

2.1. Mice sampling and preparation of samples for histology

Two separate sets of *M. musculus* were caught alive, during a period of 14 months, in the following sites of S. Miguel Island (Azores, Portugal):

- Furnas village (volcanically active environment) is a rural locality with 1500 inhabitants located inside the Furnas volcano caldera. Furnas village is considered a volcanically active environment, where hazardous gases and heavy metals are present in a daily basis (Viveiros et al., 2010, 2012). In this village, approximately 58% of the buildings are placed above anomalous soil CO_2 diffuse degassing of volcanic-hydrothermal origin (Viveiros et al., 2010, 2012); among these, 11.6% of the dwellings are also located in an area where the soil temperature is high (Sousa, 2003). In Furnas, temperatures at ground level are much higher when compared to the other areas of the Island, where secondary manifestations of volcanism do not occur. According to Cunha et al. (2011), the average temperature of Furnas soil at the surface is around 37 °C during the year, while in the areas without volcanism soil temperatures yearly range from 15 to 25 °C (circa 10 °C differential).
- Rabo de Peixe village (reference site), with 5000 inhabitants, is a rural location like Furnas village, but without any type of volcanic manifestations since the seventieth century (Carvalho, 1999). This village has no apparent sources of anthropogenic pollutants.

M. musculus is considered a good bioindicator species because it shares the habitat with humans (including the houses) and the mice population is usually large (Timm, 1994). Furthermore, since in the studied environment the major source of volcanogenic pollutants is soil diffuse degassing, mice, though preferentially nocturnal, are also exposed to volcanogenic pollutants during the day when hidden inside the holes.

Adult male mice were caught using live-catch mousetraps being housed no more time than the necessary to be euthanized. Only mice with a minimum weigh of 10 g were used, with a total of six mice caught for Furnas village and of twelve individuals for Rabo de Peixe village. The average (±SE) mice weight per site was similar (14.28 ± 3.12, volcanogenic; 15.83 ± 2.64 unexposed; $F = 1.224$; $df = 1$; $P = 0.285$; one-way ANOVA). Euthanization was made with chloroform, followed by a necropsy and extraction of the testicles. Both testicles were then fixed in 4% buffered formaldehyde for standard histology processing, and afterwards, they were dehydrated and embedded in paraffin wax. This study was carried out in accordance with the recommendations of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS 123) and 86/609/EEC Directive and Portuguese rules (DL 129/92).

A set of histological slides for the morphometric measurements and for the evaluation of the damage in the seminiferous tubules was prepared, consisting of several sections of 4 μm thickness, per slide, of each testicle for every mouse. The slides were then stained with hematoxylin and eosin according to Martoja and Martoja-Pierson (1970). Another set of histological slides was prepared for TUNEL assay to assess the number of apoptotic cells.

2.2. Metal contents in mice tails

The tail of each mouse was excised by cutting next to the sacrum bone. The tails were then dried at 130 °C. Mice tails from each site were randomly grouped in 2 pools per site, digested in concentrated nitric acid (HNO_3) and finally dissolved in 0.1 N HNO_3. The samples from Furnas and Rabo de Peixe were sent to Actlabs (Ontario, Canada) for metal analysis. Samples (tail tissues) were digested using nitric acid and hydrochloric acid in a hotblock. The completely digested solutions were then diluted and run on a sector field ICP-MS (Thermo Element 2) for metals analyses. Quality control was implemented and included reagent blanks and reference materials. NIST 1575a was utilized as the internal quality control, and the determined results were within the range of the certified values as listed in the certificate.

2.3. Histological morphometric parameters

Histological slides were observed to estimate the relative volumetric density of different spermatogenic stages [spermatogonia (Sg), spermatocyte (Sc), spermatids (St), spermatozoa (Sz)], lumen (L) and, interstitial space (IntS) in which the blood vessels were included, and consisted of 10 field analysis per testicle totalizing 20 field per individual, by using the M168 Weibel Multipurpose Test System (Weibel, 1979). Each field were randomly selected and observed at 200× magnification. Also, 60 seminiferous tubules per individual (30 per testis) were randomly chosen and photographed with a 200× magnification using an image analyzer (Image Pro-Plus 5.0 by MediaCybernetics®) coupled to a microscope (Leica® DM1000, Cambridge, UK). For each seminiferous