Environment International 60 (2013) 163–170

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Genotoxic effect of exposure to metal(loid)s. A molecular epidemiology survey of populations living and working in Panasqueira mine area, Portugal

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ARTICLE INFO

Article history:
Received 13 April 2013
Accepted 18 August 2013
Available online 13 September 2013

Keywords:
Environmental contamination
Genetic polymorphisms
Genotoxicity biomarkers
Metal(loid)s
Mining activities

ABSTRACT

Previous studies investigating the exposure to metal(loid)s of populations living in the Panasqueira mine area of central Portugal found a higher internal dose of elements such as arsenic, chromium, lead, manganese, molybdenum and zinc in exposed individuals. The aims of the present study were to evaluate the extent of genotoxic damage caused by environmental and occupational exposure in individuals previously tested for metal(loid) levels in different biological matrices, and the possible modulating role of genetic polymorphisms involved in metabolism and DNA repair. T-cell receptor mutation assay, comet assay, micronucleus (MN) test and chromosomal aberrations (CA) were performed in a group of 122 subjects working in the Panasqueira mine or living in the same region. The modifying effect of polymorphisms in *GSTA2*, *GSTM1*, *GSTP1*, *GSTT1*, *XRCC1*, *APEX1*, *MPG*, *MUTYH*, *OGG1*, *PARP1*, *PARP4*, *ERCC1*, *ERCC4*, and *ERCC5* genes was investigated. Significant increases in the frequency of all biomarkers investigated were found in exposed groups, however those environmentally exposed were generally higher. Significant influences of polymorphisms were observed for *GSTM1* deletion and *OGG1* rs1052133 on CA frequencies, *APEX1* rs1130409 on DNA damage, *ERCC1* rs3212986 on DNA damage and CA frequency, and *ERCC4* rs1800067 on MN and CA frequencies. Our results show that the metal(loid) contamination in the Panasqueira mine area induced genotoxic damage both in individuals working in the mine or living in the area. The observed effects are closely associated to the internal exposure dose, and are more evident in susceptible genotypes. The urgent intervention of authorities is required to protect exposed populations.

1. Introduction

Mining industry is a major economic resource for many countries, although it is one of the most hazardous activities, both for workers and for the surrounding environment. Panasqueira mine area is one of the most important Portuguese mining sites, and the environmental effect of this activity is a source of great concern. Environmental studies performed in this area identified an anomalous concentration of several metals and metalloids [= metal(loid)s] in stream sediments, superficial and ground waters from local courses, road dust, soils, and plants for human consumption from nearby villages (Ávila et al., 2008; Ferreira da Silva et al., 2013; Grangeia et al., 2011; Salgueiro et al., 2008). Indeed, populations living in the small villages around the mine site are strongly dependent on agriculture and farming, and moreover the local river flowing nearby — Zêzere river — feeds the Castelo do Bode dam (located 90 Km downstream from the mine), the principal water supply for the Lisbon metropolitan area. Any significant spillages into the river can cause serious environmental contamination and potential health consequences to populations living downstream.

Most metal(loid)s are very toxic to living organisms and when present in excess may become an important threat for the human health. This is also true for even those elements that are considered as essential (Murray et al., 2009). Major health effects include developmental retardation, endocrine disruption, kidney damage, immunological and neurologic effects, and several types of cancer (Mudgal et al., 2010).

A number of studies have been published on the genotoxic effects of metal(loid)s, demonstrating that elements like arsenic (As), cadmium (Cd), chromium (Cr), iron (Fe), mercury (Hg), manganese

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http://dx.doi.org/10.1016/j.envint.2013.08.014

(Mn), nickel (Ni), lead (Pb), and their compounds increase the frequency of micronuclei (MN), chromosomal aberrations (CA), sister chromatid exchanges (SCE), and also chromosomal loss (reviewed in Jadhav et al., 2006). Hartwig (1994) postulated a mechanism of genotoxicity based on the interference of some metal(loid)s with the fidelity of DNA repair. Subsequent studies confirmed that metal(loid)s such as As, Cd, cobalt (Co), and Ni interfere with both base and nucleotide excision repair (BER and NER) pathways (HERAG05, 2007).

Individuals working in the Panasqueira mine and populations living nearby were previously tested for metal(loid) contents in different biological matrices (Coelho et al., in press). The results revealed that the populations are exposed to significantly higher concentrations of As, Cr, Mn, Mo, Pb, and Zn when compared to matched controls. The group environmentally exposed showed higher levels of metal(loid)s than the one occupationally exposed, with females presenting significantly higher concentrations of toxic substances than males. Preliminary studies performed in a smaller group of individuals living and working in the area revealed increased levels of MN and T-cell receptor mutations in exposed populations (Coelho et al., 2011, 2012).

The current study was designed to investigate the presence of genotoxic damage associated with both occupational and environmental exposure to metal(loid)s. TCR mutation assay, MN test, CA, and comet assay were analysed as biomarkers of genotoxic effect.

A number of polymorphisms of genes involved in the metabolism (*GSTA2*, *GSTM1*, *GSTP1*, and *GSTT1*) and in DNA repair (*XRCC1*, *APEX1*, *MPG*, *MUTYH*, *OGG1*, *PARP1*, *PARP4*, *ERCC1*, *ERCC4*, and *ERCC5*) were selected according to the literature and investigated as possible effect modifiers (Hartwig, 1994; HERAG05, 2007; Mateuca et al., 2008; Skjelbred et al., 2011; Teixeira et al., 2002).

2. Methods

2.1. Study population

The study population consisted of a total of 122 subjects living in the area of the Panasqueira mine as detailed in Coelho et al. (in press). Forty-one individuals living in villages located in the vicinity of the mine and downstream of Zêzere river (*S. Francisco de Assis* and *Barroca do Zêzere*) were classified as environmentally exposed (16 males and 25 females). The 41 male miners and ex-miners from the Panasqueira mine represented the group of occupationally exposed. Whereas 40 additional subjects without environmental and/or occupational exposure to mining activities, or other known toxic exposure, were the controls. The control group included individuals living in non contaminated areas upstream the river and on the western side of the mine (*Casegas* and *Unhais-o-Velho*). The individuals in the control group worked mainly in administrative offices and were matched with the environmentally exposed group by age, gender, lifestyle, and smoking habits (17 males and 23 females). The criteria used to select the individuals for the study were age over 18 years and living in the same village for at least 5 years before the study. A questionnaire was used to assess the personal health status, medical history, medication, diagnostic tests (X-rays, etc.), and lifestyle factors. All subjects were fully informed about the procedures and objectives of this study, and signed an informed consent form. Approval for this study was obtained from the Institutional Ethical Board of the Portuguese National Institute of Health.

2.2. Sample collection

Blood samples were collected by venipuncture in BD Vacutainer® CPT™ tubes with sodium heparin for mononuclear leukocytes isolation prior to TCR mutation and comet assays, and in tubes containing sodium heparin for cytokinesis block MN test, CA, and for genetic polymorphism analysis.

Samples were transported under refrigeration and stored at approximately +4 °C until mononuclear leukocyte isolation and analysis for MN test and CA, and at- −20 °C for DNA extraction and genotyping. All samples were coded and analysed under blind conditions.

2.3. TCR mutation assay

Isolation of mononuclear leukocytes was performed using BD Vacutainer® CPT™ tubes, following manufacturer's instructions. The mononuclear leukocyte layer (buffy coat) was removed and washed three times with ice-cold phosphate buffer solution (PBS) of pH 7.4, at 1000 rpm (~270 ×g) for 10 min. TCR mutation assay was carried out with flow cytometry according to García-Lestón et al. (2011).

2.4. Comet assay

Mononuclear leukocytes were isolated as described in the TCR mutation assay protocol. Cells were suspended in freezing medium (50% foetal calf serum, 40% RPMI 1640, 10% DMSO) to obtain 10^7 cells/mL, and stored at −80 °C until the time of analysis. The mononuclear leukocytes were quickly thawed at 37 °C. Cell viability was assessed by trypan blue exclusion technique where in all cases it was found to be higher than 85%. The alkaline version of the comet assay was performed as described by Costa et al. (2008) with a number of modifications. Briefly, cells were suspended in 100 μL low melting point agarose and dropped (5 μL drops — 4 drops per individual, 12 drops per slide) onto a frosted slide precoated with normal melting point agarose. After lysis, unwinding and electrophoresis the slides were washed with PBS 7.4 and ice-cold bi-distilled water. They were then further dehydrated in ethanol solutions (70% and 96%). Prior to analysis, gels were stained with SYBR Green solution, washed twice with ice-cold bi-distilled water and left to dry for 30–60 min. Before each slide was scored a drop of water and a cover slip were placed on top of them. Twenty-five randomly selected cells from each gel (100 cells/donor) were examined.

2.5. Cytokinesis-block MN test

Aliquots of 0.5 mL heparinised whole blood were used to establish duplicate lymphocyte cultures for cytokinesis-block MN test, as described in Costa et al. (2006). To determine the total number of MN in binucleated cells, a total of 1000 binucleated cells with well-preserved cytoplasm (500 per replicate) were scored for each subject. MN was scored by the same reader and identified according to the criteria defined by Fenech (2007).

2.6. CA assay, aneuploidies and gaps

Duplicate lymphocyte cultures for CA were established using 0.5 mL of heparinised whole blood as described in Roma-Torres et al. (2006). One hundred metaphases were analysed for each individual, and fifty from each duplicate culture, according to the criteria of Therman (1980).

Metaphases with 46 chromosomes were scored. The structural aberrations were classified as follows: Total CA frequency (CA-total) was defined as the number of aberrations, excluding gaps; Chromosome-type aberrations (CA-chromosome) included chromosome-type breaks, ring chromosomes, and dicentrics; Chromatid type aberrations (CA-chromatid) included chromatid-type breaks. Aneuploidies (cells with 45 and 47 chromosomes) and gaps (single and double) were also scored.