Ecotoxicology and Environmental Safety 107 (2014) 133–139

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Genetic damage in coal miners evaluated by buccal micronucleus cytome assay

Grethel León-Mejía [a,*], Milton Quintana [b], Rafaela Debastiani [c], Johnny Dias [c], Lyda Espitia-Pérez [d], Andreas Hartmann [e], João Antônio Pêgas Henriques [a,f,*], Juliana Da Silva [g]

[a] Departamento de Biofísica, Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brasil
[b] Unidad de Investigación, Desarrollo e Innovación en Genética y Biología Molecular, Universidad Simón Bolívar, Barranquilla, Colombia
[c] Laboratório de Implantação Iônica, Instituto de Física, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brasil
[d] Laboratorio de Investigación Biomédica y Biología Molecular, Universidad del Sinú, Montería, Colombia
[e] Novartis Pharma AG, Basel, Switzerland
[f] Instituto de Biotecnologia, Universidade de Caxias do Sul (UCS), Caxias do Sul, RS, Brasil
[g] Laboratório de Genética Toxicológica, Universidade Luterana do Brasil (ULBRA), Canoas-RS, Brasil

ARTICLE INFO

Article history:
Received 18 March 2014
Received in revised form
30 April 2014
Accepted 19 May 2014
Available online 11 June 2014

Keywords:
Coal
Coal miners
Buccal micronucleus cytome assay
Inorganic elements
Occupational exposure

ABSTRACT

During coal mining activities, large quantities of coal dust, ashes, polycyclic aromatic hydrocarbons and metals are released into the environment. This complex mixture presents one of the most important occupational hazards for health of workers. The aim of the present study was to evaluate the genetic damage together with the presence of inorganic elements, in an exposed workers population to coal mining residues of Guajira-Colombia. Thus, 100 exposed workers and 100 non-exposed control individuals were included in this study. To determine genetic damage we assessed the micronucleus (MN) frequencies and nuclear buds in buccal mucosa samples (BMCyt) assay, which were significantly higher in the exposed group than non-exposed control group. In addition, karyorrhectic and karyolytic cells were also significantly higher in the exposed group (cell death). No significant difference was observed between the exposed groups engaged in different mining activities. No correlation between age, alcohol consumption, time of service and MN assay data were found in this study. However, the content of inorganic elements in blood samples analyzed by a Particle-induced X-ray emission technique (PIXE) showed higher values of silicon (Si) and aluminum (Al) in the exposed group. In this study we discuss the possibility of DNA damage observed in the mine workers cells be a consequence of oxidative damage.

1. Introduction

It is known that coal mining activities are a major source of environmental contamination. Mining activities release large amounts of substances that can form complex mixtures containing CO_x, NO_x, SO_x, aluminum silicon crystals, quartz, metals (arsenic, boron, cadmium, chromium, lead, copper, selenium, iron, and zinc), and polycyclic aromatic hydrocarbons (PAH) into the environment (Zhou et al., 2005).

The main route of coal mining exposure to these potentially hazardous residues is by inhalation of coal dust particles from the extraction and manipulation activities. Currently, it is known that chronic inhalation of coal dust particles can result in lung disorders including simple pneumoconiosis, progressive massive fibrosis, bronchitis, lung function loss, emphysema and cancer. Studies were able to establish that some of these disorders could have their origin in genetic damage generated by the inhalation of mineral particles. In particular interaction of particles with macrophages, epithelial cells and other cells could lead to generation of reactive oxygen species (ROS) (Schins and Borm, 1999; Cooke et al., 2003).

The effects of coal exposure have been studied using bacteria (Nakajima et al., 2008), bats (Zocche et al., 2010), rodents (Da Silva et al., 2000, León et al., 2007) and human cells (Celik et al., 2007; Rohr et al., 2013a, 2013b). Some studies in workers exposed to coal mining residues assessed by chromosomal aberrations (Santa Maria et al., 2007), sister chromatid exchange, and micronuclei

* Corresponding authors at: Departamento de Biofísica, Prédio 43422, Laboratório 210, Campus do Vale, Universidade Federal do Rio Grande do Sul, Avenida Bento Gonçalves 9500, Bairro Agronomia, CEP 91501-970, Porto Alegre, RS, Brasil. Fax: +55 5133167003.
E-mail addresses: grethelleon@gmail.com, grethy40@hotmail.com (G. León-Mejía), pegas@cbiot.ufrgs.br (J.A.P. Henriques).

http://dx.doi.org/10.1016/j.ecoenv.2014.05.023
0147-6513/

(MN) in peripheral blood lymphocytes (Donbak et al., 2005; León-Mejía et al., 2011) demonstrated that occupational exposure to coal dust can lead to a significant induction of cytogenetic damage. In a previous study, we found elevated DNA damage in coal mining workers from Guajira-Colombia, assessed by the Comet assay and MN test in lymphocytes (León-Mejía et al., 2011). Despite these findings, coal dust remains classified as "not classifiable as to its carcinogenicity to humans" (Group 3) by the International Agency for Research on Cancer (IARC, 1997).

The fact that a very high percentage of cancers have an epithelial origin suggests that micronuclei in epithelial cells are an important biomarker that can be used for epidemiological studies. Micronuclei that are detected in exfoliated buccal cells reflect genotoxic events that occurred in basal cells, and these events can be observed in exfoliated cells over an approximately three week period (Holland et al., 2008). The buccal micronucleus cytome assay (BMCyt assay) is considered a fast and simple method for *in situ* biomonitoring of human populations exposed to environmental genotoxicans (Majer et al., 2001; Bonassi et al., 2011).

The aim of the present study was to evaluate the genotoxic effects in exfoliated buccal cells and concentrations of inorganic elements in a population exposed to coal residues in the open-cast mine "El Cerrejón" in Guajira-Colombia using the buccal micronucleus cytome assay (exfoliated buccal cells; BMCyt assay) and the particle-induced X-ray emission (PIXE) in blood samples. The MN data in buccal were compared to MN data in lymphocytes from our previous study (León-Mejía et al., 2011) to assess whether buccal cells can be used as a non-invasive source to investigate biomarkers of genetic damage in exposed individuals.

2. Materials and methods

2.1. Individuals and sampling

This study was approved by the Committee on Research Ethics at University of Sinú Ethic and details of the study through the informed consent were obtained from each individual before the research began.

This study involved a total of 200 individuals, who live in the same region in order to ensure a comparable genetic background and life habits. The exposed group were 100 workers occupationally exposed to coal with a minimum time of service of 5 years in "El Cerrejón" open-cast coal mine, in the Guajira Department in the north coast of Colombia, South America. The non-exposed control group consisted of 100 individuals with no known exposure to genotoxic agents including coal, radiation, chemicals or cigarettes. Both study populations (exposed and non-exposed groups) lived in the same region; it was considered that the two populations should have presented the same genetic background and the same life habits.

The workers were involved in different activities in the mine: (i) *transport* of *extracted coal* ($n=50$), in which the workers are involved in coal transport up to arrival in the storing centers; (ii) *equipment field maintenance* ($n=18$), these workers drive trucks to spread water onto the roads where large quantities of coal dust are generated, and also maintain the coal extraction equipment; (iii) *coal stripping* ($n=17$), these workers are engaged in coal stripping activities and the accumulation of the material for the transport in trucks, they also extinguish fires generated by spontaneous combustion of coal; (iv) *coal embarking* ($n=15$), these workers are involved in shipping of coal in containers to be exported to other countries. All workers were exposed to large quantity of coal dust, but was perceived that the coal stripping group was the most exposed to coal mining residues.

All individuals in the study were required to answer a questionnaire and participate in a face-to-face interview, which included determination of standard demographic data and questions concerning medical issues (exposure to X-rays, vaccinations, medication, *etc.*), life style (smoking, alcohol consumption, diet, *etc.*), cancer history, other chronic diseases and occupation (number of working hours per day, protective measures adopted). All individuals included in the study were non-smokers and have time of service ≥ 5 years. Buccal cell and blood samples were obtained from all individuals.

2.2. Buccal micronucleus cytome assay (BMCyt assay)

After informed consent was obtained from each individual, buccal mucosa samples from all 200 individuals were collected. The subjects were asked to rinse their mouth with water before sampling. The exfoliated buccal mucosa cells were collected using a cytobrush to gently scrape the mucosa of the inner lining of both cheeks. All buccal sample tubes were coded and kept in upright position at room temperature.

The cells were washed three times in 0.9 percent phosphate saline buffer, the smears were made from the pellet and fixed in methanol:acetic acid (3:1). For microscopic analysis, the slides were incubated at 37 °C overnight and then stained with Giemsa (Stich and Rosin, 1984; Acar et al., 2001). The frequency of MN was determined in 2000 cells for each person following recommendations of Thomas et al. (2009). All slides were scored by one reader blinded to the exposure status of the individuals.

MN and other nuclear abnormalities were classified according to Tolbert et al. (1992) and Thomas et al. (2009). Nuclear anomalies, such as karyorrhectic and karyolytic cells (different forms of cell death), and nuclear buds (indicative of gene amplification) were assessed in 2000 cells/individual and recorded separately.

2.3. Particle-induced X-ray emission (PIXE)

Peripheral blood samples from all 200 individuals were collected by venipuncture. Thus, 5 mL of blood were drawn into heparin tubes (Becton Dickenson, vacutainer) for the particle-induced X-ray emission (PIXE) analysis. All blood samples tubes were coded and kept at room temperature. Blood samples were analyzed for the total content of metals by the particle induced X-ray emission (PIXE) technique (He et al., 1993; Johansson et al., 1995). This technique has been successfully employed to detect trace elements in plants and animals because of its multielemental character, high sensitivity, simplicity and high sample throughput (Mireles et al., 2004).

For the analyses, the blood samples were dried at 40 °C for 72 h, then macerated using a mortar, and finally pressed into pellets which were positioned on the target of the reaction chamber. A 3 MV Tandetron accelerator provided 2.0 MeV proton beams with an average current of 5 nA at the target. The X-rays induced by the beam in the samples were detected by a Si(Li) detector with an energy resolution of about 155 eV at 5.9 keV. The spectra were analyzed with the GUPIXWIN software package (Maxwell et al., 1995; Campbell, 2000) and the final results are expressed in parts per million (ug g^{-1}). The chemical elements analyzed in the samples by the PIXE method were: sodium (Na), magnesium (Mg), aluminum (Al), silicon (Si), phosphorus (P), sulfur (S), chlorine (Cl), potassium (K), calcium (Ca), iron (Fe), copper (Cu), zinc (Zn), bromine (Br) and rubidium (Rb). The organic matrix of the blood (the organic composition of the sample) was determined by the Rutherford Backscattering Spectrometry (RBS) technique.

2.4. Statistical analysis

The normality of the variables was evaluated using the Kolmogorov–Smirnov test; χ^2 and *t*-tests were used to compare the demographic characteristics of study populations and chemical elements analyzed by PIXE. The statistical analysis of differences in MN frequency between the exposed and control group were carried out using the non-parametric Mann–Whitney *U*-test, and statistical differences between the five groups (non-exposed control, extracted coal transport, equipment field maintenance, coal stripping, and coal embarking) were analyzed using the non-parametric two-tailed Kruskal–Wallis test with the Dunn correction. Correlations between MN frequency in lymphocytes obtained in our previous study (León-Mejía et al., 2011) and MN frequencies in buccal cells of the present study in control and exposed individuals were determined by Spearman rank correlation test. The critical level for rejection of the null hypothesis was considered to be $P<0.05$. All analyses were performed with the PRISMA 5.0 statistical software package.

3. Results

The mean age and standard deviation of exposed group was 44.0 ± 7.5 years (range, 24–60 years), and non-exposed control group was 43.7 ± 7.8 years (range, 27–60 years). The mean time of service of the exposed group was 17.7 ± 6.9 years (range, 5–30 years). The percentage of alcohol consumption for non-exposed group was 45 percent and for exposed group was 55 percent, considering as alcohol consumer to drink alcohol in excess of once/week.

Table 1 summarizes the values of the MN frequencies for both study groups, exposed and control groups, with exposed group differentiated by the mining area activities. There was no statistically significant difference between the different mining area activities ($P>0.05$; Kruskal–Wallis test), however the micronuclei frequencies observed to each individual subgroup exposed to coal mining were significantly increased compared to control group values ($P<0.05$; Kruskal–Wallis test).