Toxicology and Applied Pharmacology 294 (2016) 11–20

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

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

Mice housed on coal dust-contaminated sand: A model to evaluate the impacts of coal mining on health

Karina Caballero-Gallardo, Jesus Olivero-Verbel *

Environmental and Computational Chemistry Group, School of Pharmaceutical Sciences, Zaragocilla Campus, University of Cartagena, Cartagena, 130014, Colombia

ARTICLE INFO

Article history:
Received 4 November 2015
Revised 9 January 2016
Accepted 12 January 2016
Available online 13 January 2016

Keywords:
Total suspended particles
Toxicity
Mining
Liver
Colombia

ABSTRACT

Coal dust is the most important air pollutant in coal mining in regards to producing deleterious health effects. It permeates the surrounding environment threatening public health. The aim of this study was to evaluate the toxic effects associated with exposure to sand contaminated with coal dust particles below 38 μm in diameter, obtained from a mineral sample collected in the largest coal mine in South America, La Loma, Cesar, Colombia. Sterilized sand was spiked with coal dust to obtain concentrations ranging from zero to 4% coal dust. To model natural exposure, mice were housed for eight weeks in boxes containing this mixture as bedding after which, they were euthanized and blood and tissue samples were collected. Real time PCR analysis revealed an increase in *Cyp1A1* mRNA for living on sand with coal dust concentrations greater than 2% compared to mice living on sand without coal dust. Unexpectedly, for mice on coal dust-polluted sand, *Sod1*, *Scd1* and *Nqo1* hepatic mRNA were downregulated. The Comet assay in peripheral blood cells and the micronucleus test in blood smears, showed a significant potential genotoxic effect only at the highest coal dust concentration. Histopathological analysis revealed vascular congestion and peribronchial inflammation in the lungs. A dose–response relationship for the presence of hepatic steatosis, vacuolization and nuclei enlargements was observed in the exposed animals. The data suggest living on a soil polluted with coal dust induces molecular, cellular and histopathological changes in mice. Accordingly, the proposed model can be used to identify deleterious effects of exposure to coal dust deposited in soils that may pose health risks for surrounding wildlife populations.

1. Introduction

Coal mining is one of the most important economic activities in many countries. Coal mines are generally located far away from the final utilization location. Thus, throughout the entire coal mining, handling and transport process coal dust emissions are produced. Caballero-Gallardo et al. (2015) reported that marine sediments from coal ports, polluted by polycyclic aromatic hydrocarbons (PAHs) and metals, induced CYP1A1 and NQO1 in HepG2 cells. Coal dust is composed of micrometer-to-nanometer-sized particles, usually ranging between 0.1 and 30 μm (EPA, 2010), resulting from the collision, abrasion, crushing, and pulverization of coal. This fossil fuel is a complex mixture of various minerals, trace metals, and organic materials that vary depending on the nature of the extraction site (Dalal et al., 1995). Coal dust emissions depend upon different parameters related to the specific activity or to the characteristics of the material, such as particle size distribution, coal type, moisture content, pile configuration, dumping height, as well as weather related parameters (wind speed, and relative humidity among others).

Together with the concern in terms of pollutant content, coal dust emissions are capable of producing a considerable environmental impact in both coal workers and people in nearby residential areas (Petsonk et al., 2013). Inhalation of coal dust may cause a variety of lung diseases, including coal workers pneumoconiosis (CWP) (Mo et al., 2014), progressive massive fibrosis (PMF) (Cohen et al., 2008), lung function loss (Stansbury et al., 2013), chronic bronchitis and emphysema (Omland et al., 2014). Relationships between exposure to carbon particles and the occurrence of cardiovascular disease have also been identified (Nadadur et al., 2009). In humans and animal models of acute and chronic exposure to coal dust, inflammatory effects and oxidative damage to the lung parenchymal tissue are characterized by activation of antioxidant enzymes, such as superoxide dismutase (SOD); increased markers of lipid peroxidation and decreased antioxidant defenses (Pinho et al., 2004).

At the global level, several studies have documented that coal dust exposure in biota (Zocche et al., 2010, 2014) and humans (Rohr et al., 2013a) living near coal mining areas produces genotoxicity and diseases related to inhalation of coal dust (Petsonk et al., 2013). In Colombia, researchers have also found genotoxic damage as a possible effect of coal dust exposure (Leon et al., 2007; León-Mejía et al., 2011, 2014). Recently, Guerrero-Castilla et al. (2014) reported that hepatic concentrations of Cd, Cu and Zn in *Mus musculus* specimens were

* Corresponding author.
E-mail addresses: kcaballerog@unicartagena.edu.co (K. Caballero-Gallardo), joliverov@unicartagena.edu.co (J. Olivero-Verbel).

http://dx.doi.org/10.1016/j.taap.2016.01.009
0041-008X/

significantly higher in animals living near mining areas when compared to a reference site, as well as the mRNA expression of NAD(P)H dehydrogenase, quinone 1 (*Nqo1*), metallothionein 1 (*Mt1*), superoxide dismutase 1 (*Sod1*), metallothionein 2 (*Mt2*), and DNA-damage-inducible transcript 3 (*Ddit3*). However, there is no direct evidence that coal dust is contributing to these effects.

The expansion of the coal mining industry has negative effects on the ecosystem. This is reflected in erosion, destruction of water resources, land subsidence, air pollution, declining biodiversity, landscape fragmentation, release of contaminated water, generation of solid waste and the loss of agricultural land (Keating, 2001; Mamurekli, 2010), among other problems. However, there is little information on how the health effects on animals living near areas of coal mining activity. The goal of this study was to expose mice to soil-containing coal dust, aiming to replicate the conditions under which mice interact with this pollutant in areas that receive permanent atmospheric depositions from coal mining activities. This allows the comparison between biochemical and cellular effects published for field-collected animals and those housed with coal dust under laboratory conditions.

2. Materials and methods

2.1. Coal dust preparation

Bituminous gross coal obtained from a mine, located in La Loma, Department of Cesar (Colombia), was used to prepare the coal dust sample. The coal was ground and passed through an Rx-812 sieve shaker to obtain particle sizes of less than 38 μm in diameter (Mesh No. 400, Serial No. 15917). This particle size was employed in the experiments since it roughly corresponds to the fraction of total suspended particles (TSP) (Cyrys et al., 2005; EPA, 2010) that could be formed during opencast mining (Chaulya, 2004), a practice that allows coal particles to reach neighboring mining areas. The sample was split and stored in sterile glass vials under laboratory conditions (26 ± 2 °C and relative humidity 70–85%).

2.2. Coal dust characterization

The coal dust sample used in the experiment was tested for trace elements content, analyzing forty-six trace elements (Li, Be, Sc, V, Cr, Co, Ni, Cu, Zn, Ga, Ge, As, Se, Rb, Sr, Y, Zr, Nb, Mo, Cd, Sn, Sb, Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Hf, Ta, W, Tl, Pb, Bi, Th, and U). Briefly, 0.1 g coal dust sample was digested with a HF + HNO_3 (8 mL:3 mL) solution, drying, and a second dissolution in HNO_3 (3 mL) and HCl (3 mL). All the acids used were Suprapur® High Purity Acid, Merck. Three multi-elemental solutions Spec® 1 (rare earth elements, REE), Spec® 2 (alkalis, earth alkalis, and metals) and Spec® 4 (Nb) were employed to construct an external calibration curve read on an AGILENT 7700 ICP/MS at the Central Laboratory of the University of Huelva, Spain. The average precision and accuracy for most of these elements fall in the range of 5–10%, and were controlled by repeated analysis of the SARM-1 (granite) and SARM-4 (norite) international rock standard of the South African Bureau of Standards. The lower detection limit (LDL) for most elements in the solution was 0.01 ppb. Total Hg in coal dust was analyzed employing a direct mercury analyzer (Tri cell DMA-80) from Milestone, Italy. Operational conditions were established according to EPA method 7473 (EPA 7473, 2007). The sensitivity and accuracy of the DMA were monitored by analyzing certified reference material. The detection limit (DL) was 0.003 μg/g.

2.3. Preparation of coal dust in sand mixture used for bedding

Sand was purchased from a local store and passed through a sieve to obtain a particle size less than 1 mm. Subsequently, the sand was washed eight times with tap water, three with distilled water and two with ultrapure water (Milli-Q), followed by sterilization in an autoclave at 120 °C × 1 h and 15 min, and finally dried in an HDF-120 oven at 70 °C for 24 h. The sand was stored in sterile glass bottles and maintained at room temperature.

2.4. Animals

Female ICR (Institute of Cancer Research) mice, 6 weeks old were purchased from the National Institute of Health, Bogota (Colombia). Animals weighing between 19 and 20 g each were housed six per polycarbonate cage (32 cm long × 20 cm wide × 21 cm high) and maintained under standard laboratory conditions, 26 ± 2 °C, 70–85% relative humidity, and dark/light cycle 12/12 h. Animals were acclimated to the lab for one week prior to the experiment. Standard diet and water were provided *ad libitum*. Animal care and experimental procedures were approved by the Institutional Ethics Committee of the University of Cartagena.

2.5. Experimental protocol

Thirty-six mice were randomly and equally divided into six groups. The non-exposure group was caged on sand only as bedding. Animals in treatment groups were housed during eight weeks on coal dust-contaminated sand at different concentrations (0.25, 0.5, 1, 2, and 4% *w*/w) (see Fig. S1 in Supplementary material), varying from a level where sand looks completely black in color (4%) to that where the black color from dust particles is not distinguished from the sand alone (0.25%). Moreover, as seen in Table S1 (Supplementary material), metal concentrations reported for several coal mining sites (da Silva et al., 2000a; Niu et al., 2015; Reza et al., 2015; Pandey et al., 2016), are greater than those obtained after incorporating coal dust at the highest concentration (4%) to the sand.

This approach was carried out based on the following criteria: first, rodents living near or within coal mine areas are in permanent contact with sand, material where dust particles are deposited from the air. Therefore, we simulated that specific environment. Second, several authors have shown that wildlife from coal mining areas, including mice (Leon et al., 2007), rats (Leon et al., 2007), fish (Holm et al., 2003), and collared tuco-tuco (da Silva et al., 2000a), experience different types of toxic stress, effect that we have monitored at the gene expression level in mice (Guerrero-Castilla et al., 2014). Taken together, the basis for these experiments was to replicate what occurs in the wild but under laboratory conditions, allowing the mice to be in contact with dust particles present in the sand, through dermal, inhalation and ingestion exposure.

Bedding was changed weekly. Food remains that fell onto the sand and excreted stools were removed daily. Mice were weighed every three days over the course of the study. The experiment was repeated once. In selecting the concentrations of coal dust that the mice would be exposed to, we took into account several studies that reported levels of metals in soil from areas of coal mining (see Table S1 in Supplementary Material) (da Silva et al., 2000a; Niu et al., 2015; Reza et al., 2015; Pandey et al., 2016). These data showed that the concentrations used in this study were lower than those found in the environmental dust levels near coal mines.

2.6. Tissue and blood collection

At the end of the exposure period, mice were anesthetized with sodium pentobarbital given ip at a dose of 60 mg/kg. Once anesthesia was induced, the animals were dissected and the blood was collected *via* vena cava flow, stored in tubes with sodium citrate to a final concentration of 0.76%, and maintained under refrigeration for a maximum of 24 h prior to analysis. An aliquot of the blood was used within the next 8 h for genotoxicity assays and another for enzyme activity measurements. During necropsy, tissue samples from lung, liver, kidney, and spleen