Biomonitoring of genotoxic risk of workers exposed to heavy air pollution

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Abstract

Epidemiological studies found an increased risk of cancers in occupations exposed to traffic air pollution. PM2.5 are toxic and can enter into the respiratory tract and circulatory system. PM2.5 can adsorb various substances, such as polycyclic aromatic hydrocarbons (PAHs) nitro-PAHs. The present study was carried out with 15 Rebouças tunnel (Rio de Janeiro, Brazil) workers (exposed group) and 11 healthy men (control group). The participants were informed about the study and asked to sign an informed consent form and to complete a standard questionnaire to obtain necessary data on their lifestyle. Samples of buccal mucosa cells and peripheral blood were evaluated using micronucleus (MN) assay. Urine samples were used to estimate the concentration of 1-hydroxyprene (1-HOP) and 2-naphthol (2-NAP). A significantly higher frequency (10.82) of MN in buccal cells and (4.42) binucleated lymphocytes were detected for the exposed workers. Higher concentrations of 1-HOP (16.47 μmol/mol creatinine) and 2-NAP (6.56 μmol/mol creatinine) were also detected in the exposure group. In conclusion, damage to the genetic material and the high concentrations of metabolites of PAHs detected in the biological samples taken from Rebouças tunnel workers can be related to daily exposure to pollutants in the tunnel.
Keywords: PAHs, nitro-PAHs, atmosphere, genotoxic, blood and urine.

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1 Introduction

Urban air pollution is a complex mixture of particles and gases derived from a variety of sources that is altered by the sun and temperature to produce a range of atmospheric transformation products [1]. Traffic is a major source of this air pollution, emitting carbon dioxide, carbon monoxide, various hydrocarbons, nitrogen oxides, particulate matter (PM), volatile organic compounds, heavy metals, and secondary reaction products such as ozone, nitrates and organic acids [1, 2]. The exhaust from gasoline and diesel vehicles is frequently a major source of the PM in urban air, [1, 3] especially PM2.5 (fine particulates with a median aerodynamic diameter less than 2.5 μm), which enters the respiratory tract and potentially the circulatory system [1, 4, 5].

The toxic effects of PM are mainly attributed to PM2.5. Because of their large specific surface, these particulates can adsorb various organic substances, such as polycyclic aromatic hydrocarbons (PAHs), nitro-PAHs, and oxygenated PAHs (oxy-PAHs) [6, 7]. Previous studies have demonstrated that increased concentrations of PAHs in the workplace environment could induce DNA damage in workers involved in road paving and bitumen [8]. In addition, an increased risk of oxidative damage has been found in PAH-exposed coke oven workers. According to Ulvestad *et al.* [9], tunnel workers showed loss forced expiratory volume and increased chronic obstructive pulmonary disease when exposed to pollutants. Another study showed a higher frequency of symptoms of chronic bronchitis in PAH-exposed foundry workers, [8] and an epidemiological study also found an increased risk of cancers in foundry workers, [8, 10] and in occupations with high exposure to traffic-related air pollution [11–13]. The exposure of local residents and certain occupational groups to heavy traffic, such as bus drivers, street policemen and street vendors, has been studied for its potential to assess the contribution of urban air pollution to DNA damage in urban residents [13–16].

Humans are exposed to genotoxic agents in the environment, at work, in medical treatments and through lifestyle choices. Biomarkers can be employed as endpoints for assessing human genotoxicant interactions from exposure to effects and individual host susceptibility [8, 17]. Two widely used biomarkers of carcinogen exposure are urinary metabolites that indicate the internal exposure dose and genetic biomarkers like micronucleus (MN) assays, which reflect the biologically effective dose [8].

In 2010, we conducted a PM2.5 monitoring study at three sites of the city of Rio de Janeiro: the campus of the Rio de Janeiro State University (moderate traffic 119,000 vehicles/day), Avenida Brasil express way (has heavy traffic, ~250,000 vehicles/day and is the city's biggest highway) and Rebouças tunnel (heavy traffic, ~190,000 vehicles/day) [7, 18, 19]. In these studies we detected PM2.5 values that exceeded the levels established by the World Health Organization, [20] mainly in Rebouças tunnel. Furthermore, this site showed highest concentrations of PAHs and nitro-PAHs, and the highest mutagenicity values for *Salmonella typhimurium* strain TA98 and its derivatives, YG1021 and YG1024, both sensitive to nitro-

compounds [7, 18, 19]. The aim of the present study was to investigate the genotoxic effects on Rebouças tunnel workers exposed to PM2.5.

2 Materials and methods

2.1 Subjects and sampling

The study was carried out on 15 Rebouças tunnel workers (exposed group), and 11 healthy men working on the campus of the Rio de Janeiro State University (control group), without signs of any occupational exposure to potential genotoxic substances. The participants were informed about the study and asked to sign an informed consent form and to complete a standard questionnaire to obtain necessary data on their lifestyle and personal factors (age, working hours, health, food consumption, medication, smoker and X-ray exposure). All procedures were submitted to and approved by the National Research Ethics Committee, CONEP (CAAE Nº. 27402014.6.0000.5259). The mean characteristics of the study group are presented in table 1.

Table 1: The mean characteristics of the study group.

	Control Group	Exposed Group
N	11	15
Age	44 ± 12	41 ± 10
Smoked	0	0
Exposure to X-ray	2	6
Use of medications	4	4
Alcohol intake	6	5
Consumption of smoked foods	7	10
Consumption of fried foods	9	15
Consumption of fruits	10	12
Consumption of vegetables	10	14

Exposure to X-ray at 12 months; Eventual alcohol intake; Regular consumption of smoked, fried foods, fruits and vegetables.

Buccal mucosa cells were obtained by scraping the inner cheek with a swab. The cells were rinsed in ice-cold physiological saline solution (0.9%) using individual coded centrifuge tubes. Samples of peripheral blood (2 mL) were collected in heparinized vacuum tubes by vanipuncture, and urine samples (30 mL) were collected by the workers. All the samples were stored on ice and protected from light until processed.

2.2 Micronucleus assay

Buccal mucosa cells were centrifuged three times in methanol: acetic acid (3:1) solution at 2000 g for 5 min, and then the pellet was dropped on duplicate slides. Slides were stained with Feulgen/fast green and cells were scored under 1000× magnification [21]. Two slides from each volunteer were blindly scored by two readers (1000 cells from each of the duplicate slides and for each reader).

2.3 Lymphocyte cultures, staining and binucleated cells with micronuclei (BNMN) scoring

Lymphocyte cultures were set up by adding 0.5 mL whole blood to 5 mL RPMI 1640 medium supplemented with 500 µL fetal bovine serum plus 100 µL phytohemagglutinin A, and incubated in CO_2 5% for 44 h at 37°C. Two cultures per subject were established. A final concentration of 6 µg/mL cytochalasin B (Cyt B) was added to the cultures 28 h later to arrest cytokinesis. At 72 h incubation, the cultures were harvested by centrifugation at 800 rpm for 8 min and treated with a hypotonic solution (0.075 mol/L KCl at 4°C). The cells were centrifuged and a methanol:acetic acid (3:1) solution was gradually added. This fixation step was repeated twice and the resulting cells were resuspended in a small volume of fixative and dropped onto clean slides. The slides of all the samples were stained with 5% Giemsa for 7 min [22]. Following the criteria proposed by Fenech [23] to determine the frequency of BNMN and the total number of MN in lymphocytes, a total of 1000 binucleated cells with well-preserved cytoplasm (500 per replicate) were scored per subject on coded slides [23, 24].

2.4 Urinary concentrations of 2-naphthol (2-NAP) and 1-hydroxyprene (1-HOP)

Urine samples (1.50 mL) were placed in a 2 mL vial and added to 100 mL buffer solution of sodium acetate to 0.2 mol/L to adjust the pH to 5.0. Then 10 µL β-glucoronidase/arylsulfatase (Merck) was added to promote enzymatic hydrolysis at 37°C for 18 h with shaking and 200 rpm. A SiO_2-C18 cartridge (Supelco Supelclean ship-18 SPE 100 mg) was prepared by passing 5 mL HPLC-grade methanol and 5 mL bidistilled water. The prepared sample was transferred slowly to the cartridge using a glass syringe to retain the organic molecules. 5 mL doubly distilled water was added to the cartridge to remove the soluble compounds in water. Then, 1.5 mL HPLC-grade acetonitrile was passed through the cartridge into a 2 mL vial. Chemical analyses were performed on a Perkin Elmer series 200 HPLC with fluorescence detector. An injection volume of 30 µL was used, with a mobile phase of 50% acetonitrile and 50% double distilled water. The flow rate employed during the analysis was 1.5 mL/min. The separation column was a SupelcosilLC-18 (column length: 250 mm; internal diameter: 4.6 mm; particle size: 5.0 µm), operating at 40°C. The fluorescence detector operated with an excitation wavelength of 240 nm and emission of 370 nm. Calibration curves were prepared with standard 2-NAP (Sigma) between 20 and 100 ng/mL and 1-HOP (Sigma) between 50 and 400 ng/mL. The determination coefficients were 0.99 for

both compounds. Calibration standards were prepared on a urine control and the same procedures were carried out as for the samples.

2.5 Statistical method

Student's t-test was used to assess the statistical significance of the results obtained in the different assays. Comparisons between the results of the micronucleus test and the data from the questionnaire were performed using the Pearson correlation test with a significance level of 0.05 using the SPSS/PC statistical program.

3 Results

The MN frequencies observed in the buccal mucosa cells and binucleated lymphocytes and the 1-HP and 2-NAP concentrations are given in table 2.

Table 2: The micronuclei frequencies in cells of the buccal mucosa, in binucleated lymphocytes and 1-HP and 2-NAP concentrations of control and exposed groups.

	Control Group (Means ± SD)	Exposed Group (Means ± SD)
MN Exfoliated Buccal Cells	1.19 ± 0.49	10.82 ± 4.90*
MN Binucleated Lymphocyte	1.71 ± 0.52	4.42 ± 2.78*
1-HP (μmol/mol creatinine)	5.54 ± 2.19	16.47 ± 6.05*
2-NAP (μmol/mol creatinine)	1.31 ± 0.40	6.56 ± 2.72*

*Student's t-test $p<0.05$. SD standard deviation. MN, micronuclei; 1-HP, 1-hydroxypyrene; 2-NAP, 2-naphthol.

The assessment of MN frequencies in exfoliated buccal cells revealed a significant difference between exposed workers and control subjects (table 2). The correlation test revealed a positive correlation (0.787) between alcohol intake and buccal MN frequency in the control group. None of the factors mentioned in the questionnaire (table 1) were found to correlate with the MN frequencies detected for the exposure group.

A significantly higher frequency of MN in binucleated lymphocytes was observed for the exposed workers than for the control group (table 2). No correlation was observed between lifestyle factors and frequencies of MN in binucleated lymphocytes.

Significantly higher concentrations of 1-HP and 2-NAP (μmol/mol creatinine) were detected in the exposure group (table 2).

4 Discussion

Human biomonitoring is becoming increasingly important in occupational and environmental health studies [25]. The use of biomarkers as integrated measures of exposure and/or effects is increasing as a result of difficulties in identifying exposure sources and demands for more integrated data for human exposure risk assessments [25]. The exposure of the general population to PAHs and substituted PAHs has gained great importance in environmental health [26]. The significance of this class of substances from an environmental medicine viewpoint is determined by their ubiquitous occurrence in the environment and their carcinogenic nature [26]. Heavy traffic is a major source of exposure to PAHs in urban areas. After metabolic activation, many PAHs have been shown to induce lung and skin tumors in animals by mechanisms that also operate in exposed humans [26]. In this study, we analyzed the effect of exposure to environmental pollutants in Rebouças tunnel workers using different exposure biomarkers. Rebouças tunnel has heavy traffic, and has high ambient air concentrations of PM 2.5 (141 $\mu g/m^3$), PAHs (5.41 ng/m^3) and nitro-PAHs (13.02 ng/m^3) [7, 18, 19]. Fifteen of the fifty Rebouças tunnel workers agreed to participate in this study, although N is small, it represents 30% of the population. Biological samples of these workers, including buccal mucosa cells and peripheral blood, were analyzed for MN frequency, and urine samples were used to estimate the concentration of hydroxyl metabolites of pyrene (1-HOP) and naphthalene (2-NAP).

The buccal mucosa cell samples taken from the Rebouças tunnel workers showed a significantly higher frequency of MN than the control group. Furthermore, this result showed no correlation with any of the data provided in the questionnaire. These results may be related to exposure to pollutants present in this tunnel. Other studies investigating human exposure to pollutants have found an increase in the frequency of MN in cells of the buccal mucosa in workers exposed to PAHs [8], heavy metals [27] and ozone [28]. Other workers also exposed to pollutants and found to have an increased frequency of MN in buccal mucosa cells are listed below: traffic police (China: 5.72 ± 2.57) [29], (Turkey: 0.10 ± 0.0) [30], (Philippines: 17.07) [31]; gas station attendants (Philippines: 18.90) [31], (India: 12.76) [32] and taxi drivers (Turkey: 0.12 ± 0.05) [30]. The correlation test revealed a positive correlation between alcohol intake and MN frequency in buccal mucosa cells for the control group. Several studies have indicated a relationship between the ingestion of alcohol and increased frequency of micronuclei [8, 33]. The same correlation was not observed in the exposure group, which reinforces the likelihood of the occurrence of MN being related to occupational exposure.

The binucleated lymphocyte samples from the Rebouças tunnel workers also showed a significantly higher frequency of MN than the control group. A similar increase was also observed in studies using a micronucleus assay in lymphocytes from tunnel workers (6.31 ± 0.61) in the Umbrian Apennine Mountains, Italy, compared with outdoor workers away from traffic (4.71 ± 0.28) [34]. An elevated frequency of MN in human lymphocytes compared with control groups has also been observed in individuals who have other occupations that expose them to

pollutants in different parts of the world: garage mechanics (Hungary: 23.5 ± 5.7) [35]; traffic police (Italy: 3.75 ± 1.65) [36], (China: 4.27 ± 0.68) [37] and diesel train attendants (China: 0.166) [38].

PAHs are a major group of carcinogenic compounds in ambient urban air, and most recent biomarker studies have focused on assessing PAH exposure. To assess internal PAH exposure the determination of 1-HOP and 2-NAP in urine has been successfully used in many studies in environmental and occupational medicine [25, 26]. Urinary excretions mainly contain metabolites of PAHs with a low molecular weight, such as naphthalene and pyrene. Assessments of humans exposed to naphthalene have attracted increasing interest in environmental health, since this most volatile PAHs has been classified as a possible human carcinogen by international agencies [26, 39, 40] and the general population's exposure to external naphthalene in the environment is reported to be higher than it is to other PAH compounds [26]. Our results showed a significant increase in the concentration of metabolites 1-HOP and 2-NAP in the urine of the exposure group. Other studies assessing occupational exposure to pollutants have also found higher concentrations of 1-HOP: coke oven and graphite electrode producing plant workers [41, 42]; coal liquefaction workers [43]; road pavers [44]; aluminum plant workers [35]; carbon black workers [45]. The same observation has been made in studies assessing 2-NAP concentrations in the urine of workers exposed to different classes of pollutants: charcoal workers [46, 47] and emission inspectors [47]. In general, exposure assessments and biomarkers have found differences between the control and exposed populations, suggesting a likely linkage between the class of agent measured by the exposure assessment and the damage detected by biomarker [1]. PAHs or PAH metabolites were the main class of chemical measured in the air and urine, respectively, and this class of compound is recognized as an important component of diesel and automobile exhaust and air pollution in general [1].

5 Conclusion

In conclusion, damage to the genetic material and the high concentrations of metabolites of PAHs detected in the biological samples taken from Rebouças tunnel workers (exposure group) can be related to daily exposure to pollutants in the tunnel.

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