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Characterization and genotoxicity evaluation of particulate matter collected from industrial atmosphere in Tamil Nadu State, India

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HIGHLIGHTS

- PM in the vicinity of 5 different industries were collected and characterized.
- Size of majority of the particles was less than 1 μm.
- Toxicity of PM samples depends on their metal and PAH content.
- PM-induced DNA damage was reduced by vitamin C or quercetin pretreatment.

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ABSTRACT

Ambient particulate matter (PM) collected in the vicinity of five industries (Cement, Chemical, Thermal power plant, Sponge-iron and Steel) in Tamil Nadu state, India was characterized for size distribution, metals and polycyclic aromatic hydrocarbons (PAHs) content. Genotoxicity of PM and organic matter (OM) extracted from PM was measured in human lung cancer cell-line, A549 and in human liver carcinoma cell-line, HepG2, respectively, using the comet assay. PM values varied from 57.0 μg/m^3 of air at Cement industry upstream to 561.0 μg/m^3 of air at Sponge iron industry downstream samples. Their metal content varied from 5.758 μg/m^3 of air at Chemical industry to 46.144 μg/m^3 of air at Sponge iron industry and PAH concentration varied from 0.5 ng/m^3 air in upstream Thermal power plant to 3302.4 ng/m^3 air in downstream Sponge iron industry samples. While all PM samples induced DNA strand breaks at higher dose levels, downstream samples of Steel and Sponge iron industries which contained relatively higher concentrations of PAHs and metals and exhibited higher levels of pro-oxidant activity as measured by DTT activity induced significantly higher levels of DNA damage in HepG2 and A549 cells. Pretreatment of A549 cells with vitamin C or quercetin significantly reduced PM induced DNA strand breaks.

1. Introduction

PM is a complex mixture of organic and inorganic substances of varying characteristics and has been associated with morbidity and mortality due to respiratory and cardiovascular disease [1,2]. PM is composed of both coarse and fine particles originating from a variety of sources, such as power plants, industrial processes, agricultural activities and traffic and is formed in the atmosphere by transformation of gaseous emissions. Their chemical and physical compositions vary depending on source, location, time of year, and weather [3,4]. Although the molecular mechanisms underlying association between PM exposure and cancer risks remain poorly understood, results of several experimental and epidemiologic studies suggest that PM mass and their metal and organic components are critical for inducing carcinogenesis related biological changes, including oxidative stress, immune deficiency and chronic inflammation [5]. However, to what extent such effects are influenced by physicochemical constituents of PM obtained from different sources or locations are still unclear.

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As toxicity of PM is influenced by its physical and chemical composition, many carcinogens and co-carcinogens such as PAHs, transition metals, and aldehydes that are present in PM and contribute to PM-related carcinogenesis [6]. The reactive metabolites of PAHs (e.g. quinones) are capable of interacting with cellular macromolecules, forming PAH-adducts, induce oxidative stress and DNA damage and mutations and initiate carcinogenesis [7]. Transition metals, although poor mutagens and carcinogens themselves, can enhance the mutagenicity and carcinogenicity of other potential carcinogens such as PAHs [8] and are considered to contribute to PM induced cytotoxicity and oxidative stress.

While air quality is slowly improving in the developed countries, it is rapidly deteriorating in developing countries due to rapid industrialization and increased vehicular traffic. Admittedly, the constituents of air pollution in different parts of the world are largely similar, but the magnitude of exposure, general health status of the people, nutritional and other disparities and level of health care facilities are different across the globe. These inherent differences make extrapolation of findings from developed to developing countries questionable. Therefore, in order to gain more insights on the adverse biological effects of PM originating from various industrial sources in India, this study was conducted to investigate the genotoxicity of the PM samples collected in the vicinity of five different industries (Cement, Chemical, Thermal power plant, Sponge iron and Steel) in Tamil Nadu state, India in relation to its composition. The PM samples were characterized for size distribution, metal and PAH content. Genotoxicity of PM samples was measured in human lung adenocarcinoma ephitelial cell line, A549 cells and that of OM extracted from PM was measured in human liver hepatocellular carcinoma cell line, HepG2 cells using the alkaline comet assay. Although, A549 cell line is often used for investigating the toxicity of air pollutants, activities of xenobiotic metabolizing enzymes, especially P450 activities, in this cell line are far below those present in human lungs [9]. Since many atmospheric organic chemicals need metabolic activation, genotoxicity of OM extracted from PM was measured in HepG2 cells as this p53 proficient cell line is retaining activities of several Phase 1 and Phase 2 enzymes and its expression profile of these genes are similar to those of the normal human cells and it does not need addition of extraneous metabolic activation system (e.g. S9 mix), it has been suggested as a good model system for evaluating the toxicity of environmental chemicals [10].

2. Materials and methods

2.1. Collection of PM

PM in ambient air at up- and down-stream locations of five industries: Chemical industry, Cement industry, Steel industry, Thermal power plant and Sponge iron industry, located in Salem area, Tamil Nadu, India were collected for 8 h using High Volume Air Sampler (Envirotech APM 415, India) and PM_{10} Wattman Quartz Filters (Cole-Parmer, USA). In all sampling days sky was clear with no rain fall; day time temperature ranged from 28.6 to 36.0 °C; mean wind velocity (predominantly north-west to south-east) varied from 3.4 to 8.5 km/h.

2.2. Determination of metals and PAH

Metals in PM were extracted using acid digestion and measured using AAS (Perkin Elmer Analyst 300, USA). PAHs from a portion of the filter were extracted ultrasonically with dichloromethane, concentrated using a rotary evaporator, purified with silica gel and then re-concentrated by rotary evaporation. The samples and a standard reference material (Aqua Standard, USA) were analyzed using HPLC-fluorescence (Shimatzu 10A, Japan). In this study the samples were analyzed for the presence of 16 PAHs including Napthalene (NaP), Acenapthylene (Acy), Acenapthene (Ace), Fluorene (Fl), Phenanthrene (Phe), Anthracene (Ant), Fluoranthene (Flu), Pyrene (Pyr), Benzo(a)anthracene (BaA), Chrysene (Chr), Benzo(b)fluoranthene (BbF), Benzo(k)fluoranthene (BkF), Benzo(a)pyrene (BaP), Dibenzo(a,h)anthracene (DBA), Benzo(ghi)pyrene (BghiP) and Indeno(123-cd)pyrene (IND).

2.3. Oxidative potential

The oxidative potential of PM samples was determined using DTT assay as described in De Vizcaya-Ruiz et al. [3]. Briefly, PM samples ultrasonically extracted in double distilled water and dried over a hot plate were incubated in triplicate (50 μg/ml) at 37 °C with 0.5 M phosphate buffered saline (PBS, pH 7.4), double deionized water and 1 mM DTT for 0–45 min. To an aliquot of the reaction mixture, 10% trichloroacetic acid was added and then mixed with Tris buffer (pH 8.9), 20 mM EDTA and 10 mM DTNB solution. The resultant 5-mercapto-2-nitrobenzoic acid was measured at 412 nm. The redox activity is expressed as the rate of DTT consumption (nM) per min per μg of sample minus the activity observed in the absence of PM.

2.4. Cell line and culture conditions

HepG2 and A549 cells were obtained from NCCS (National Centre for Cell Science, Pune, India) and cultured in DMEM (GIBCO, Invitrogen, USA), supplemented with 10% heat inactivated FBS, 1% L-glutamine and 100 U/ml penicillin–streptomycin.

2.5. Genotoxicity of OM

OM from PM samples were extracted from a portion of the filter with 100 ml of methanol by using a soxhlet apparatus for 30 min and concentrated in a rotary evaporator. The genotoxicity of the OM was evaluated using the comet assay by exposing the 24 h cultures of HepG2 cells (1×10^5 per well) cultured in 24-well plate to different concentrations (equivalent to 1 and 2 m^3 of air), in triplicates, of organics for a period of 4 h at 37 °C using MMS (100 μM, 30 min, 37 °C) as the positive control.

2.6. Toxicity of PM

The average hydrodynamic size and zeta potential of PM samples in water were determined by dynamic light scattering (Nano-ZetaSizer-HT, Malvern Instruments, Molvern, USA). PM from remaining portion of the filters were extracted ultrasonically using PBS (pH 7.4) and briefly centrifuged at 1000 rpm to remove large particles. Cytotoxicity of PM was estimated by treating the 24-h cultures of A549 cells (1×10^4 cells/well) cultured in 96 well plates with different concentrations of PM in PBS (equivalent to 1–10 m^3 of air) for 24 h at 37 °C followed by MTT assay. To measure genotoxicity of PM, the 24-h cultures of A549 cells (1×10^5 cells/well) cultured in 24 well plates were treated with different concentrations of PM in PBS (equivalent to 1–4 m^3 of air), in triplicates, for 4 h at 37 °C. Then the cells were trypsinized and subjected for the comet assay. Role of antioxidants on genotoxicity of PM was measured by treating the cells (1×10^5 cells/well in 24 well plates) with vitamin C, 50 μM or quercetin, 50 μM for 2 h at 37 °C followed by exposure to PM in PBS (equivalent to 4 m^3 of air sample) for 4 h 37 °C. Triplicate cultures and controls were maintained for all the samples. For the comet assay, H_2O_2 (100 μM, 5 min, 37 °C) was used as the positive control. The comet assay was performed under alkaline conditions following the procedure of Singh et al. [11] with