Genotoxic and oxidative damage related to $PM_{2.5}$ chemical fraction

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Abstract

Many studies have pointed out a correlation between airborne PM quantitative exposure and health effects. The aim of this research is the investigation of the role of the $PM_{2.5}$ chemical fraction in the DNA damage induction in human cells (A549). Air samples ($PM_{2.5}$) were collected in different sites (urban, industrial and highway) using a high–volume sampler. Organic and water-soluble extracts of $PM_{2.5}$ were tested on A549 cells to evaluate genotoxic and oxidative damage using the Comet assay without and with formamido-pyrimidine-glycosylase (Fpg). Organic and water extracts were analysed for determination of PAHs by GC-MS methods and metals by the ICP-MS technique respectively. The $PM_{2.5}$ organic extract of all the samples caused a significant dose-dependent increase of the A549 DNA damage. The genotoxic effect was related to IPA $PM_{2.5}$ content and the highest effect was observed for the motorway site sample (65.03 $CL/10m^3$) while the oxidative damage was observed in $PM_{2.5}$ water extract of the industrial and motorway sites. The extent of the oxidative damage seems to be related to the type and concentration of metals present in these samples. The results of this study emphasize the importance of evaluating the PM chemical composition for the biological effect determination. This concern highlights the need for considering its qualitative composition in addition to its size and air concentration for PM health effect evaluation and exposure management.

Keywords: PM, genotoxicity, oxidative damage, Comet assay, PAH, metals.

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1 Introduction

Particulate matter (PM) is an important environmental health risk factor for many diseases. Different studies show that long-term exposure to high concentrations of PM increases the risk of lung cancer, respiratory and cardiovascular diseases [1, 2]. Although epidemiological studies have consistently demonstrated adverse effects of PM exposure on human health, the physical and chemical properties of PM responsible for toxicity as well as the mechanisms underlying particle-induced carcinogenesis are still not fully known [3]. It is probable that different characteristics of PM are responsible for its adverse health effects. The particles size influences the capacity of PM fractions to reach the deepest sites of the respiratory system. Moreover, because of their large specific surface, PM can contain various organic substances that are known human mutagens and carcinogens, such as polycyclic aromatic hydrocarbons (PAHs) and nitroaromatic hydrocarbons (nitro-PAHs), as well as metals [4]. In particular, transition metal ions (Fe, Cu, Zn etc) are abundantly present in PM and have been shown to be potent inducers of oxidative DNA damage through the generation of reactive oxygen species (ROS) [5]. The health effects are mainly attributed to the small size particles with an aerodynamic diameter below 2.5 μm ($PM_{2.5}$) that penetrate deeply into the alveoli. Moreover the large and irregular surface areas favour the better adsorption of pollutants. The aim of this research is the investigation of the role of the $PM_{2.5}$ chemical fraction on the oxidative and genotoxic effects in human cells. With this scope the biological effects caused by $PM_{2.5}$ samples collected in sites with different emission sources were compared.

2 Materials and methods

2.1 Airborne particulate sampling

$PM_{2.5}$ samples were collected in different sites near the city of Alessandria (Piemonte): urban site (UR) (medium vehicular traffic density and home heating); highway site (HW) (high vehicular traffic density); industrial site (IN) (located near a foundry). The 24h sampling was performed in February 2007 using a high-volume air sampler (TISH Environmental, INC.) and glass fibre filters [6].

2.2 Extraction of PM components

The glass fibre filters were fractionated and one fraction (1/4) was added with 30mL of Milli-Q ultrapure water for 30min in an ultrasonic bath to extract water-soluble compounds and with 300 mL of dichloromethane to extract organic-extractable compounds. Soluble components were separated from the insoluble ones by centrifugation at 5000rpm for 10min. The supernatant was filtered with polypropylene membrane filters (0.45μm). PM extracts were separated into two aliquots (one for the chemical analysis and the other for Comet assay). Organic extracts were concentrated by rotary evaporation.

2.3 Chemical analysis

The PAHs level in the organic extracts was evaluated using a GC-MS Finnigan Trace GC Ultra-Trace DSQ (Thermo Scientific, San Jose, CA, USA) instrument with quadrupole mass analyzer. The column was a Thermo TR-5MS (60m x 0.25mm i.d.) coated with a 0.25μm film of 5% phenyl polysilphenylene-siloxane. The inlet temperature was 250°C and the splitless time was 1.00min. The column temperature was: 70°C (4min), then at 10°C min^{-1} from 70 to 120°C and then at 2°C min^{-1} from 120 to 300°C (21min). Helium was the carrier gas at a constant flow (1.0mL min^{-1}). Electron impact (EI+) mass spectra were acquired with an ionization energy of 70.0eV in full scan mode between 30-300amu (scan rate 500.0amu s^{-1}). The ion source and transfer line temperatures were at 250°C. The compounds identification was based on the comparison of their retention times and mass spectra with those of reference standards. The metal content in water extracts were measured by ICP-MS by ThermoFisher XSeries1 ICP-MS (Winsford UK), software PlasmaLab V2.5.4.289, equipped with an Apex-Q fully-integrated inlet system. The instrumental conditions were as follows: main run was: peak jumping, sweeps at 60ms, dwell time at 10000ms, channels per mass was 1, acquisition duration was 33750ms and channel spacing is 0.02amu. Resolution was standard.

2.4 Comet assay

The organic extracts were dissolved in DMSO for the biological test. A549 (human alveolar carcinoma) cells were maintained in nutrient mixture F-12 Ham supplemented with L-glutamine (200mM), 10% heat-inactivated newborn calf serum, 100U/mL penicillin, 100μg/mL streptomycin and 25mM Hepes. The cultures were incubated in a humidified incubator at 37°C with 5% CO_2 in air. The A549 cells were cultured for 24h before exposure to PM extracts. The proportion of living cells was determined by the trypan blue staining (overall > 90%). Cells were exposed (24h at 37°C) to serial dilutions of the PM extracts. After exposure, cell viability was checked again. The Comet assay was performed under alkaline conditions (pH>13) according to the method of Moretti *et al.* [7]. The slides were stained with ethidium bromide (20μl/mL) and examined with a fluorescent microscope (Axiovert 100M, Zeiss). One hundred cells per sample (two slides), randomly selected, were analysed using an image analysis system (CometScore). The Comet Length (CL) was selected as the parameter to estimate DNA damage. The data obtained were statistically evaluated by the Student's t-test (SYSTAT statistical package) to assess the significant differences ($P \leq 0.05$) between control and exposed cells.

2.5 Fpg-modified Comet assay

The Fpg-modified Comet assay was carried out as described above with the exception that, after lysis, the slides were washed 3 X 5 min with Fpg Buffer (40mM Hepes, 0.1M KCl, 0.5mM EDTA, 0.2mg/mL bovine serum albumin, pH 8). Then the slides were incubated with 1 unit of Fpg enzyme at 37°C for 1h.

Control slides were incubated with the buffer only. For each experimental point the mean comet length for enzyme untreated cells (CL) (direct DNA damage) and the mean comet length for Fpg-enzyme treated cells (CLenz) (direct and indirect DNA damage) were calculated.

3 Results and discussion

3.1 Gravimetric and chemical analysis

The obtained $PM_{2.5}$ air concentration and the abundance of different PAHs in PM extracts of the three sites investigated are summarized in table 1.

Table 1: $PM_{2.5}$ air concentration and $PM_{2.5}$ organic extract PAH concentration.

Parameter	UR	HW	IN
$PM_{2.5}$ (μg/m^3)	27.5	75.3	56.6
Naphthalene (ng/m^3)	n.d.	n.d.	0.1
Acenaphthylene (ng/m^3)	n.d.	n.d.	n.d.
Acenaphthene (ng/m^3)	n.d.	n.d.	n.d.
Fluorene (ng/m^3)	n.d.	n.d.	n.d.
Phenanthrene (ng/m^3)	0.5	1.5	0.8
Anthracene (ng/m^3)	n.d.	n.d.	n.d.
Fluoranthene (ng/m^3)	0.8	4.6	2.3
Pyrene (ng/m^3)	0.9	n.d.	2.5
Benzo(a)anthracene (ng/m^3)	1.4	n.d.	8.5
Benzo(b)anthracene (ng/m^3)	1.2	n.d.	2.7
Benzo(b)fluoranthene (ng/m^3)	1.1	11.0	8.2
Benzo(k)fluoranthene (ng/m^3)	1.1	5.0	n.d.
Benzo(a)pyrene (ng/m^3)	n.d.	10.4	4.3
Benzo(e)pyrene (ng/m^3)	2.2	5.8	2.5
Indeno(1.2.3cd)pyrene (ng/m^3)	n.d.	17.9	9.7
Dibenz(ah)anthracene (ng/m^3)	n.d.	n.d.	n.d.
Benzo(ghi)perylene (ng/m^3)	5.6	28.4	8.3
Benzo(ghi)fluoranthene (ng/m^3)	0.3	n.d.	0.5
Total mutagenic PAHs (ng/m^3)	10.9	77.3	43.8
Total PAHs (ng/m^3)	15.1	84.6	50.4
Total PAHs (ng/100μg $PM_{2.5}$)	54.4	112.5	89.0

The results of the gravimetric analysis showed that in all the sites the PM level exceeded the WHO guideline (10 μg/m^3), the future limit value proposed by the new European Directive (25 μg/m^3 mean annual level) and the standard value recently approved by the US EPA (15 μg/m^3 standard value for the annual level) [8]. The highest level of PM was observed in the HW site followed by the IN and UR sites. The great content of atmospheric $PM_{2.5}$ in the HW site is due to

the high traffic density of the highway [9]. The chemical analysis of the PM organic extracts showed a variability of PAH composition in the three different sites. In the HW site the highest concentration of total and mutagenic PAHs was measured and the highest PAH concentration per μg of PM was found. The type and density of vehicular traffic likely affects PAH concentration. The chemical analysis (Table 2) of the PM water extracts points out the presence of 14 metals in all the PM samples investigated being the more abundant Fe, Cu, Zn, Sb and Ba.

Table 2: Metal concentrations in the $PM_{2.5}$ water extracts.

Parameter	UR	HW	IN
V (ng/m^3)	158	299	118
Cr (ng/m^3)	46	154	99
Mn (ng/m^3)	296	781	730
Fe (ng/m^3)	2127	7275	2747
Co (ng/m^3)	41	32	22
Cu (ng/m^3)	324	1236	3588
Zn (ng/m^3)	58906	72893	594011
As (ng/m^3)	21	137	74
Cd (ng/m^3)	21	64	644
Sn (ng/m^3)	n.d.	17,933	6321
Sb (ng/m^3)	92403	284229	233376
Ba (ng/m^3)	11160	10513	10805
Pt (ng/m^3)	25	69	21
Pb (ng/m^3)	213	460	839
Transition metals (ng/m^3)	61898	82671	601,314
Total metals (ng/m^3)	165741	396076	853394
Total metals (ng/μg $PM_{2.5}$)	6027	5260	15078

The highest concentration of total and transition metals was observed in the industrial site. Calculating the total load of soluble metals per unit mass of PM (μg) the IN site showed also the highest concentration of metals confirming the data expressed per m^3. The result is probably related to the presence in this area of a foundry that typically releases metals (Zn, Cu, Pb, Fe etc.). The presence of metals seems to be more affected by industrial emissions than by vehicular traffic.

3.2 Genotoxic damage and oxidative stress of PM extracts

The alkaline version of the Comet assay (sensitive to DNA strand breaks, direct oxidative DNA lesions and alkali-labile sites) was used to evaluate the genotoxic effect of organic extracts. The exposure of the A549 cells to $PM_{2.5}$ organic extracts showed a statistically significant (t-test) dose-dependent increase of the genotoxic effect with respect to the control cells in all the samples investigated (Figure 1).

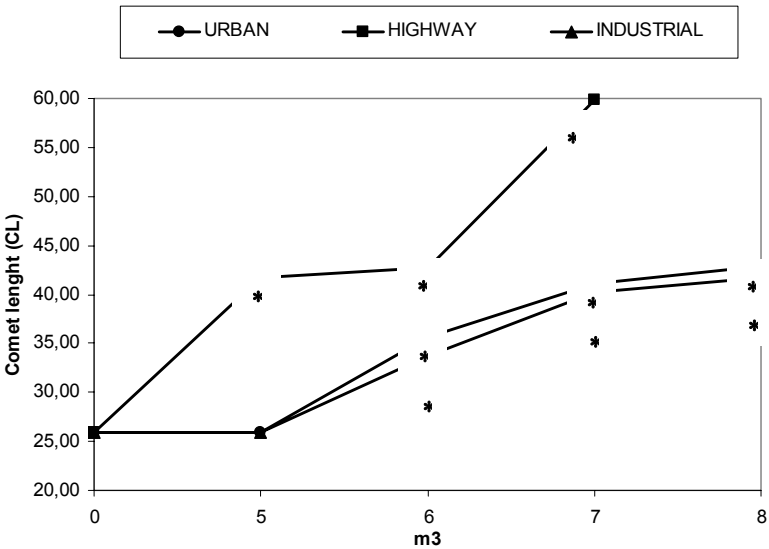

Figure 1: Effect of A549 cells exposure to organic extracts evaluated by alkaline version of the Comet assay (*: P<0.001).

In order to compare the results of the genotoxic effect the data obtained with $PM_{2.5}$ extracts sampled in the different sites were analysed as genotoxic parameter (CL) referred to 10 m^3 of air calculated from the dose-response curves by linear regression analysis (r^2> 0.7). The results obtained showed a variable degree of genotoxic damage in the monitored sites. The highest genotoxic activity was evidenced in the HW site (65.03 CL/10m^3) and the lower effect in the UR site (44.54 CL/10m^3). The biological effect seems to be related to PAH concentration observed in the sites investigated. In fact the HW site showed higher levels of total and mutagenic PAHs with respect to the IN and UR sites. Other studies showed the relationship between PM PAH concentration and the biological effects [10]. To evaluate the direct and oxidative DNA damage of water extracts the Fpg-modified Comet assay was used. The results obtained in the IN site showed the presence of a genotoxic effect both in enzyme untreated cells (CL) (direct DNA damage) and in enzyme treated cells (CLenz) (direct and indirect DNA damage) (Figure 2).

On the other hand, for the HW site a biological effect only using Fpg enzyme was observed. No genotoxic effect was showed in water extract of the UR site. For the IN site the DNA damage observed in enzyme untreated cells underlines the presence of pollutants with direct genotoxic effect. The subtraction of the mean CL from the relative CLenz value of the exposed cells (Clenz-CL) compared with unexposed cells at each experimental point provides the intensity of the oxidative damage. A significative oxidative damage was observed only in the IN site (39.40 CL/m^3) and a lower oxidative effect was revealed in the HW site (31.33 CL/m^3). The presence of the oxidative genotoxic damage could be related to the composition of metal in these sites. In fact in the UR site the lower

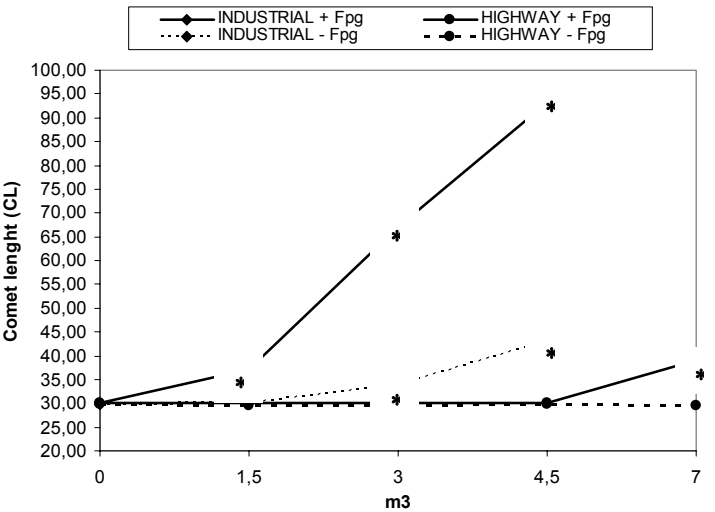

Figure 2: Effect of A549 cells exposure to water extracts evaluated by Comet assay and Fpg-modified Comet assay (*: P<0.001).

concentration of total and transition metals was observed while in the IN and HW sites a higher levels of these compounds was revealed. In particular the high level of oxidative damage in the IN site with respect to the HW site could be ascribed to the concentration of Cu, Zn e Cd that is 3-10 fold higher then in the PM sampled in the HW site. This finding supports the results obtained in other studies that showed the role of transition metals in reactive oxygen specie production and in oxidative stress induction [11, 12]. In different studies the genotoxic and oxidative activity of Cu and Zn are reported [13].

4 Conclusion

The results of this study highlight that emission sources characterized by different prevalent pollution (PAHs, metals) can significantly affect the intensity and type of the biological effect observed, emphasizing the importance of the PM chemical composition for the biological effect. This concern showed the need of considering the qualitative composition of the PM in addition to its size and air concentration for the PM health effect evaluation and the exposure management.

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