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Short note - Nota breve

Biological effects induced by chemical characteristics of $PM_{2.5}$

Sara BONETTA[1*], Valentina GIANOTTI[1], Silvia BONETTA[1] Dario SCOZIA[2], Fabio GOSETTI[1], Matteo ODDONE[1], Maria Carla GENNARO[1] & Elisabetta CARRARO[1]

[1] Dipartimento di Scienze dell'Ambiente e della Vita, Università del Piemonte Orientale "A. Avogadro", Viale Teresa Michel 11, 15121 Alessandria, Italy
[2] AMC, Azienda Multiservizi Casalese S.p.A., Via Orti 2, 15033 Casale Monferrato, Italy
[*] Corresponding author e-mail: *sara.bonetta@mfn.unipmn.it*

RIASSUNTO - *Studio degli effetti biologici indotti dalle caratteristiche chimiche del particolato atmosferico* - Obiettivo dello studio è determinare la relazione tra la composizione chimica del $PM_{2.5}$ e la capacità di provocare danni al DNA. I campioni di $PM_{2.5}$ sono stati prelevati in 3 siti (urbano, autostradale, industriale). Gli estratti organici ed acquosi del $PM_{2.5}$ sono stati testati su cellule A549 per valutare gli effetti biologici (Saggio Comet) e analizzati per quantificare IPA e metalli. Negli estratti organici il danno al DNA è risultato associato alla quantità di IPA. La presenza di stress ossidativo è stata evidenziata solo nel sito industriale ed in quello autostradale e potrebbe essere legata alla quantità/tipologia di metalli rilevati.

Key words: PM, genotoxicity, oxidative damage, Comet assay, PAHs, metals
Parole chiave: PM, genotossicità, stress ossidativo, saggio Comet, IPA, metalli

1. INTRODUCTION

Epidemiological studies have consistently demonstrated that long-term exposure to high concentration of PM increases the risk of lung cancer, respiratory and cardiovascular diseases but the biological mechanisms behind these associations are not yet fully understood (Pope *et al*. 2002; Knaapen *et al*. 2004). It is probable that different characteristics of PM are responsible of 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 surface, PM can contain various organic substances that are known as human mutagens and carcinogens (e.g. PAHs, Nitro-PAHs) (Claxton *et al*. 2004). Transition metal ions are also abundantly present in PM and have been shown to be potent inducers of oxidative DNA damage (Knaapen *et al*. 2002). The health effects are mainly attributed to the small size particles ($PM_{2.5}$) 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.

2. MATERIAL AND METHODS

Airborne particulate sampling

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

Extraction of PM components

One fraction of each filter was extracted with Milli-Q water in a ultrasonic bath to extract water-soluble compounds and with dichloromethane to extract organic-extractable compounds. PM extracts were separated into 2 aliquots (one for the chemical analysis and the other for Comet assay).

Chemical analysis

The PAH level level was evaluated using a GC-MS Finnigan Trace GC Ultra-Trace DSQ instrument with quadrupole mass analyzer. The metal content was measured by ThermoFisher XSeries1 ICP-MS, software PlasmaLab V2.5.4.289, equipped with an Apex-Q fully-integrated inlet system.

Comet assay

The A549 (human alveolar carcinoma) were exposed (24h) to serial dilutions of the PM extracts. The Comet assay was performed under alkaline conditions (Moretti *et al*. 2002). The slides were stained with ethidium bromide and examined with a fluorescent microscope. The cells were analysed using the CometScore image system. The comet length (CL) was used to estimate DNA damage. The data were statistically evaluated by the Student's t-test.

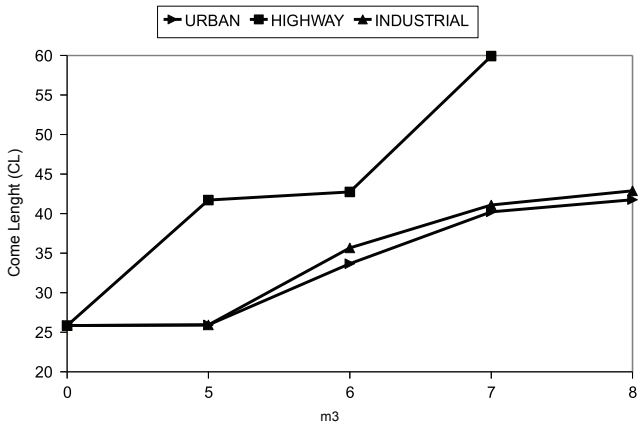

Fig. 1 - Effetti dell'esposizione delle cellule A549 agli estratti organici (Saggio Comet).
Fig. 1 - Effect of A549 cells exposure to organic extracts (Comet assay).

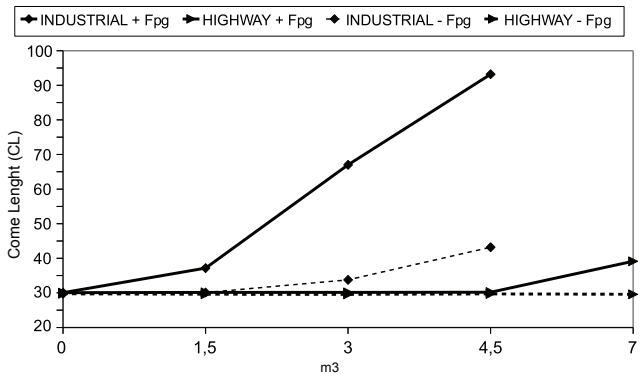

Fig. 2 - Effetti dell'esposizione delle cellule A549 agli estratti acquosi (Saggio Comet e Saggio Comet modificato con l'enzima Fpg).
Fig. 2 - Effect of A549 cells exposure to water extracts (Comet assay and Fpg-modified Comet assay).

Fpg-Modified Comet assay

The Fpg-modified Comet assay was carried out as Comet assay with the exception that, after lysis, the slides were incubated with Fpg enzyme (1h). For each experimental point the mean comet length from enzyme untreated cells and the mean comet length for Fpg-enzyme treated cells were calculated. The subtraction of these parameters compared with unexposed cells at each experimental point provides the intensity of the oxidative damage.

3. RESULTS AND DISCUSSION

The results of the gravimetric analysis showed that in all the sites the PM level exceeded the limit values proposed by WHO, the new European Directive and US-EPA (Ballester *et al*. 2008). The highest level of PM was observed in the HW site probably due to the high traffic density of the highway. 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.

The type and density of vehicular traffic likely affects PAH concentration. The chemical analysis of the PM water extracts pointed out the presence of 14 metals in all the PM samples investigated being the more abundant Fe, Cu, Zn, Sb and Ba. The highest concentration of total and transition metals was observed in the IN site. The result is probably related to the presence in this area of a foundry that typically releases metals. The presence of metals seems to be more affected by industrial emissions than by vehicular traffic. The alkaline version of the Comet assay 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 dose-dependent increase of the genotoxic effect in all the samples investigated (Fig. 1). In order to compare the results of the genotoxic effect the data were analysed as genotoxic parameter (CL) referred to 10 m^3 of air calculated from the dose-response curves. The results 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).

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. To evaluate the 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 and in enzyme treated cells (Fig. 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. A significant oxidative damage was observed only in the IN and HW sites.

The presence of the oxidative genotoxic damage could be related to the composition of metal in these sites. In fact in these sites a higher levels of total and transition metals was revealed. This finding supports the results obtained in other studies that showed the role of transition metals in oxidative stress induction (Gutierrez-Castillo *et al*. 2006).

4. CONCLUSIONS

The results of this study highlight that emission sources characterized by different pollution can significantly affect the intensity and type of the biological effect observed. This concern showed the need of considering the qualitative composition of the PM in addition to its size and air level for the PM health effect evaluation and exposure management.

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