

## Response of a human alveolar cell line to urban fine particles

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Recently, particular attention has been paid to the toxicological properties of PM<sub>2.5</sub>, the fine fraction of air particulate matter, which penetrates deeply in respiratory tract and can easily reach the alveolar ducts (Billet *et al.*, 2007). PM<sub>2.5</sub> was collected in an urban area using a low volume gravimetric sampler. Samples were processed and the particles were detached from filters and used to test their cytotoxic effects on the human alveolar cell line A549.

A549 cells were exposed to urban particle suspensions at the concentrations of 0.1, 1, 10, 25 and 50 µg/cm<sup>2</sup>. Carbon Black particles (CB) of similar dimension were used at the doses of 1, 10, 25 µg/cm<sup>2</sup> as a reference inert particulate matter. Cell viability, intracellular reactive oxygen species (ROS) production, cytochrome P450 CYP1A1 and enzyme HMGB-1 expression, particle internalization with light and transmission electron microscopy were analysed.

A concentration-dependent decrease in cell viability was observed in A549 cells exposed to particle suspensions, showing a significant difference with respect to controls starting from the dose of 10 µg/cm<sup>2</sup>. Cell viability was not affected by CB until the concentration of 25 µg/cm<sup>2</sup>.

ROS production was investigated by flow cytometry using the fluorescent probe DCFH-DA. While control cells did not show ROS-linked fluorescence, a significant and concentration-dependent increase of ROS after exposure to 10 µg/cm<sup>2</sup> PM was observed. PM-induced ROS always resulted to be significantly higher than those induced by CB.

PM<sub>2.5</sub> cell exposure also induced a marked Cytochrome CYP1A1 expression, as revealed by the flow cytometry analyses.

HMGB1 is an enzyme passively released by damaged or necrotic cells and the correlation between the exposure to PM and the release of HMGB1 in the medium from A549 cells is under investigation.

Phagocytosis of particles by A549 cells was morphologically characterized and seemed to depend on both particle concentration and exposure time, with the majority of particles being engulfed in membrane-bound vacuoles after 24h of exposure. Cell-particle interaction is also under investigation for the molecular changes observed in the particle

carbonaceous structure (Zerbi *et al.*, 2008). Cytotoxic effects, mainly represented by necrotic changes, were visible at light microscope (Fig. 1). At ultrastructural level, particles clusters onto the outer layer of the cell membrane were observed with the phagocytic structures forming in correspondence of the points of cell-particles interaction. Cell membrane lyses and mitochondrial ultrastructural disruption appeared to be the main modifications induced by PM<sub>2.5</sub> on A549 cells (Gualtieri *et al.*, *in press*).

In conclusion urban PM<sub>2.5</sub> has a potentially high toxicological impact and can induce cell toxicity in a concentration-dependent manner being able to enter and damage the A549 cells, and even to provoke their death. CB, of an aerodynamic diameter similar to that of PM<sub>2.5</sub>, was consistently less effective. This finding suggests the leading role of the other components of PM such as transition metals and PAHs adsorbed onto the carbon core.

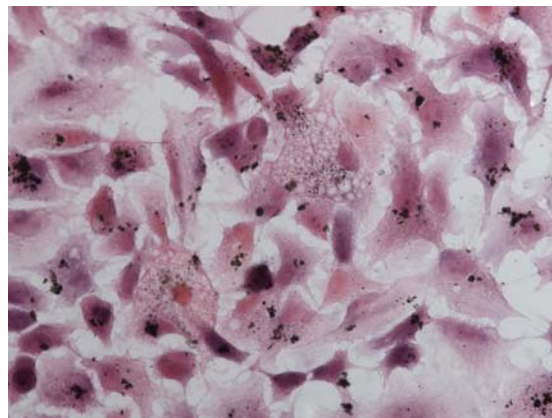


Figure 1. Light microscopy of HE stained A549 monolayers exposed to 25 µg/cm<sup>2</sup> PM<sub>2.5</sub> for 24h, showing extensive cytoplasmic vacuolations.

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