

Study of the toxicological effect of winter and summer PM₁ on BEAS-2B

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Particulate matter (PM) is a complex and heterogeneous mixture of particles and has been recognized as a threat for human health. Hazardousness of PM is not only dependent on its dimensions, but also and in particular on its chemical composition. Initially, PM deposition in human alveoli induces oxidative stress and production of pro-inflammatory mediators. Later, the onset of DNA damages and their possible misrepair can lead to mutations.

This project is part of the PRIN 2017 project RHAPS (Redox-activity and Health-effects of Atmospheric Primary and Secondary aerosol). The aim of this research is to evaluate the effect of PM₁ on a bronchial epithelial cell line (BEAS-2B) considering oxidative stress, genotoxicity and inflammation as main endpoints. RHAPS campaign allowed the collection of filter in winter and in summer 2021. Every week a clean filter was kept in the PM-sampler and not subjected at air flow, in order to have a negative control for the samples. Winter PM was collected in Bologna (urban site) and San Pietro Capofiume (BO, rural site), while summer only in the site of Bologna. PM₁ was extracted in water with mechanic agitation and cells were treated with the extract diluted 1:10 in complete medium. All the analysis were performed at 24 hours, with the only exception of reactive oxygen species (ROS) detection that was analysed at 30 minutes and 1 hours.

Cell viability was assessed with the MTT assay and results were considered in order to avoid the use of cytotoxic concentration of further tests. ROS were detected with DCFH-DA assay. Moreover, comet assay modified with endonucleases ENDOIII and FPG allowed the detection of oxidative damage on the DNA bases. The alkaline version of the comet assay, the immunofluorescence for the phosphorylated histone H2AX (γ -H2AX), and the micronuclei test were used to highlight DNA damage at single and double strands as well as at chromosomal level. Inflammation was evaluated through the analysis of the release of interleukin-8 (IL-8) and CXCL-8 mRNA expression. Preliminary data were obtained also on the gene expression of other genes involved in the DNA repair pathway (ATM and GADD45 α) and oxidative stress (HMOX and NQO1).

Differences were detected in all the assays' results among the days analysed and in the two sites of sampling. Some samples showed increase level of ROS and oxidised DNA bases. Moreover, cells resulted to be very sensitive to DNA damage as analysed by the presence of micronuclei and γ -H2AX. IL-8 secretion is stimulated in particular in some of the samples analysed, and its gene expression does not differ much from control level. mRNA expression analysis of ATM, GADD45 α , HMOX, and NQO1 highlighted the activation of these genes in some of the days considered. These results could be explained with the chemical composition of the PM which is under analysis and seems to really drive the toxicological effect highlighted by these results.

Keywords: particulate matter, oxidative stress, genotoxicity, inflammation, BEAS-2B.

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