

Lonati Giovanni / Politecnico di Milano

In vitro assessment of proinflammatory and genotoxicological effects of wood combustion-generated ultrafine particles

Background

The TOBICUP (TOxicity of Blomass COmbustion generated Ultrafine Particles) project was designed to gain deeper insight on the possible health effects of ultrafine particles (UFP, $dp < 100$ nm) by assessing the potential toxicological responses of UFP samples collected either directly from the Residential Wood Combustion (RWC) emissions or where biomass burning for residential heating is widely used.

In this work, pro-inflammatory and genotoxicological responses of THP-1 and A549 cell lines, used as surrogates of alveolar macrophages and lung epithelial cells, treated with UFPs generated by the combustion of wood pellets and logs are presented.

Methods

Ultrafine particulate matter generated by wood (beech and fir) combustion in a 11 kW pellet stove and in a 8 kW wood stove was sampled with three parallel multistage impactors; a fourth impactor was used for the measurement of the number concentration and size distribution.

Combustion cycles were intended to simulate the behaviour of a real-world user.

UFP samples were analyzed for metals (ICP-AES), for the main inorganic ions (IC), for anhydrosugars (HPLC coupled to pulsed amperometric detection), for total organic carbon by thermal-optical approach, and for PAH (GC-MS).

Cell viability was assessed by lactate dehydrogenase leakage, and IL-8 was measured to evaluate pro-inflammatory effects; for genotoxicological assessment cells were treated with UFP samples at 50-100 $\mu\text{g/ml}$ concentrations for 24h. Genotoxicological effects have been evaluated in terms of DNA damage (Single SB and double breaks DSB in alkaline comet assay and DSB evaluation with immunostaining of γH2AX histone proteins), and of intracellular ROS (oxygen reactive species) and RNS (nitrogen reactive species) formation time course. The UFP intracellular disposition was evaluated by transmission electron microscopy analysis (TEM). Observed pro-inflammatory and genotoxicological effects were compared to those in cells treated with Diesel exhaust particles (DEP, dp 2.5 μm).

Results

Both A549 and THP-1 cells responded to UFP producing IL-8, with logwoods UFPs more active compared to pellet UFPs. With the exception of the higher effect of beech logwood UFPs only in THP-1 cells, the induced release of IL-8 was not influenced by the kind of wood; in addition, on a weight base, IL-8 release was similar or even lower compared to DEP, arguing against a higher biological activity of UFP compared with larger particles. The release of IL-8 induced by UFP could be significantly reduced by SB203580, indicating a role of p38 MAPK activation in IL-8 production. A qualitatively different protein adsorption profile was observed, with less proteins bound to beech UFPs compared to fir UFPs or DEP, which may provide higher intracellular availability of bioactive components, e.g. levoglucosan and galactosan.

From the genotoxicological standpoint, statistically significant increase of all DNA damage markers was highlighted in all samples, more evident for wood combustion than for pellet samples but without differences between the two types of wood. Cells treated with DEP showed more damage than those treated with UFPs from both logwood and pellet combustion. ROS/ RNS production was evident only after 30-60 minutes of treatment with pellet UFP.

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