Characterization of oxidative potential and toxicology in vitro study of fine and ultrafine PM generated by smoke grenades shots

V. Martin de Lagarde[1], T. Rogez[1], F. Cazier-Dennin[2], D. Dewaele[2], M. Janona[1], F. Cazier[2], C. Monteil[1], C. Corbière[1]

[1]University of Rouen Normandy, Health Department - ABTE EA4651 ToxEMAC, Rouen, France, [2] University of Littoral Côte D'Opale, Centre Commun de Mesures, Dunkerque, France

Pyrotechnic smokes are widely used for civilian and military applications: protection, security, festivity, obscuring or signaling. Particulate air pollution is a major public health concern and smokes combustion induces intense particulate matters pollution episodes. There are multiple types of smokes with various initial compositions, but there is a lack of data on chemicals produced after combustion and their toxic effects.

In this study, we evaluated the toxicity of particles of two different smokes, a red signaling smoke (RSS) and an hexachloroethane-based obscuring smoke (HC-OS) by measuring their oxidative potential (OP) and by exposing a 3D model of primary human pulmonary cells (NHBE) grown at the Air-Liquid-Interface to suspended particles. OP was examined with the dithiothreitol (DTT) and the antioxidant (acid ascorbic) depletion assays. Cytotoxicity (MTT, cell cycle, ATP production) as well as pro-inflammatory and antioxidant genes expression (RT-qPCR) were explored after 24h of exposure (RSS and HC-OS) and after 24h of recovery for RSS particles exposure.

Physico-chemical characterization of particles was previously studied and revealed that particles were smaller than 1 µm diameter; therefore particles are capable to penetrate deep into the airways after inhalation. Furthermore, RSS particles were more organic (quinones and polycyclic aromatic hydrocarbons) than HC-OS particles that were mainly metallic. Indeed, we noticed a higher metal content (133 282 µg/g) especially in Al (106 000 µg/g) and Fe (12 100 µg/g) compared to RSS particles (12 035 µg/g). Results showed (Mean +/- SD) that DTT was significantly depleted by RSS (73.7% +/- 1.1) and HC-OS (42.0% +/- 0.1) particles at the highest dose of exposure (50 µg/cm^2) but acid ascorbic was only depleted by HC-OS (42.3% +/-1.3). Both particles were not cytotoxic but genes expression was altered and was dependent on particles type. Particles from RSS (50 µg/cm^{2}l) but not from HC-OS significantly increased superoxide dismutase 1 (SOD1, 1.48 +/-0.10) and 2 (SOD2, 1.32 +/-0.06) and heme oxygenase-1 (HO-1, 8.32 +/-2.5) expression. Both particles significantly induced NADPH quinone oxidoreductase-1 (NQO-1, 6.35+/-0.6 with S1, 1.73+/-0.1 with S4) and IL-8 expressions (2.11+/-0.3 with RSS, 1.79+/-0.4 with HC-OS) whereas the catalase expression was unchanged. After 24h of recovery, genes expression were back to normal (SOD1, SOD2, HO-1), stayed significantly increased (NQO-1, IL-8) or remained unchanged (Catalase).

Because of their different chemical composition, smoke particles produced many different reactive species, which can be detectable by the two OP assays. Indeed, the reaction of DTT assay is mainly associated with organic components, which may explain the higher depletion by RSS particles. On the contrary, acid ascorbic depletion is generally attributed to metals corresponding to HC-OS depletion. Our study demonstrated that NHBE exposure to different military smoke particles triggered an adaptive antioxidant response that was reversible for RSS particles and lead to inflammatory response but without cytotoxicity. This study improves the knowledge of the toxicity of pyrotechnic mixtures like smoke particles to assess human health risk.

Keywords: smokes particles, oxidative potential, Air-Liquid Interface exposure, primary human pulmonary cells, antioxidant and inflammatory responses