Alteration in oxidative stress and F-actin assembly by incense particles

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Research has indicated that the smoke from incense combustion contains toxic pollutants, such as particulate matter with an aerodynamic diameter less than 2.5 μm ($PM_{2.5}$), metals and organic components, which have been associated with adverse human health effects.

The physicochemistry of incense $PM_{2.5}$ emitted from the three types of incense joss sticks (A-C) have been outlined in our previous study.[1] The physicochemical characterization included particulate and gaseous emissions, as well as the determination of the inorganic compounds. The collected incense $PM_{2.5}$ consisted of spherical singlets, chains and irregular-shaped aggregates (Figure 1).

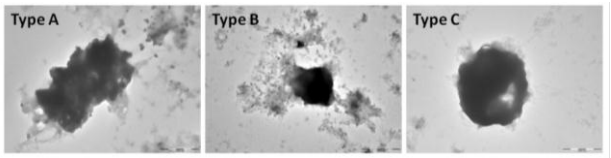

Figure 1. Transmission electron micrographs of incense $PM_{2.5}$ (a-c) collected in distilled-water.

To understand the effects of oxidative stress *in vitro* caused by the incense $PM_{2.5}$, A549 cells were exposed to the $PM_{2.5}$ (± N-acetyl-L-cysteine, NAC).[2] The cells significantly exhibited incense $PM_{2.5}$ induced intracellular reactive oxygen species (ROS) formation ($p<0.05$; compared to background (BG) levels in a dose-dependent manner (Figure 2a), and a quadratic time response (Figure 2b). The increased levels of ROS production caused by incense $PM_{2.5}$ were significantly reduced by the addition of NAC ($p<0.05$; Figures 2a, 2b), but the levels still persisted, especially at the higher concentrations of the incense $PM_{2.5}$, when compared to the BG levels ($p<0.05$).

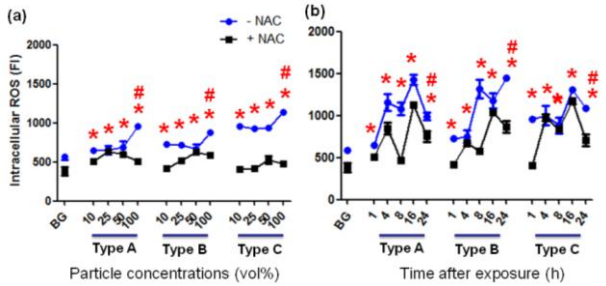

Figure 2. Dose-dependent (a) and 24-h (b) induction of oxidative stress stimulated by incense $PM_{2.5}$ with (+) and without (−) antioxidant NAC (mean ± S.D. n = 4). * Significant difference in comparison of BG at the same concentration ($p<0.05$); # Significant difference in comparison of NAC.

Control epithelial cells incubated with or without NAC exhibited elongated cell morphology with pronounced aggregation of actin filaments, seen as bundles at the cell periphery or cortical cytoskeleton (Figures 3a, 3b). Significant cell shrinkage and the formation of actin stress fibers was observed with increasing incense $PM_{2.5}$ concentrations (Figure 3c) and incubation time (Figure 3e). Antioxidant pre-treatments significantly reduced the formation of stress fibers in cells under the same exposure conditions (Figures 3d, 3f). The elongated cytoskeleton became polygonal following incense $PM_{2.5}$ exposure (+NAC), especially after 24 hours incubation (Figure 3f). Microscopic observations confirmed that externally-derived ROS could alter the actin cytoskeletal dynamics towards an apoptotic-like morphological organization in A549 cells.

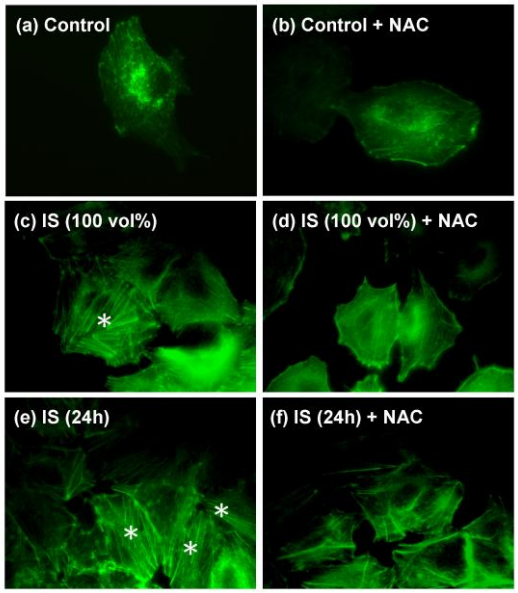

Figure 3. F-actin cytoskeleton remodeling during ROS-induced apoptosis (stain FITC-phalloidin). (* denote stress fibers; 20x).

This study demonstrates that incense $PM_{2.5}$ contained ROS induced cytoskeletal changes, suggesting that incense burning pose an environmental risk with regard to respiratory cell dysfunction. These results show that changes in the actin oxidation state activated an oxidative stress response. This response was also suppressed by the clinically important antioxidant NAC. Therefore ROS, generated via combustion derived processes such as incense burning, is a probable risk

[1] Chuang, H.-C., Jones, T., Lung, S.-C., and BéruBé, K. (2011). Soot-driven reactive oxygen species formation from incense burning. Sci Total Environ 409, 4781-4787.
[2] Chuang, H.-C., Jones, T., Chen T-T. and BéruBé, K. (2013). Cytotoxic effects of incense particles in relation to oxidative stress, the cell cycle and F-actin assembly. Toxicol Lett, in press.