Effect of airborne particulate matter on oxidative stress, pro-inflammatory response and intracellular calcium signaling in pulmonary artery endothelial cells

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Introduction

Many epidemiological studies have revealed the involvement of airborne particulate matter (PM) in increasing of respiratory and cardiovascular mortality and morbidity. It has been shown in humans, a correlation between exposure to particles and an increase in pulmonary arterial pressure. The pulmonary circulation could be one of the primary targets of inhaled particles and people with pulmonary hypertension (PH) could be a population at risk. Despite the main role of calcium signaling and oxidative stress in the pathogenesis of this disease, the effect of particulate pollution on these cellular targets is poorly described. In this context, the objectives of this study are to evaluate, on pulmonary vascular cells, under no stretch or cyclic mechanical stretch that mimic wall pressure found in PH, the effects of particles (particulate Matter-$PM_{2.5}$ and nanoparticles-NP) on the biological responses such as calcium signaling, oxidative stress and pro-inflammatory response.

Methods

Human Pulmonary Artery Endothelial Cells (HPAEC) are cultured in physiological (0% to 5% stretching and normoxia: 21% O_2) and pathological conditions that mimic the PH (stretch system: 30 cycles/min and 15% stretching and hypoxia: 1% O_2), using STREX®, B-bridge International:system. HPAEC cells are exposed for 4h or 24h to $PM_{2.5}$ or NP (carbon black-FW2) from 5 to 20 µg/cm². Different endpoints are studied (i) production of reactive oxygen species by a fluorescent probe (H_2DCF-DA) or electronic paramagnetic resonance, (ii) calcium signaling using the fluorescent indicator dye Fluo-4 and confocal microscopy analysis, (iii) pro-inflammatory response by measuring the release of various cytokines (IL-6, IL-8) by ELISA.

Results

In HPAEC, a 4h-exposure to both nanoparticle (FW2) and $PM_{2.5}$ (5 - 20 µg.cm^2) induced a concentration-dependent increase of intracellular ROS levels. $PM_{2.5}$ and FW2 NP induced also, after 24h exposure, a pro-inflammatory response characterized by an IL-8 and IL-6 secretion. In addition, after a 24h-exposure to HPAEC, FW2 attenuated, in a concentration-dependent manner, the ATP (10^{-5}M)-induced increase in intracellular calcium ion level ([Ca^{2+}]i,) (Figure 1).

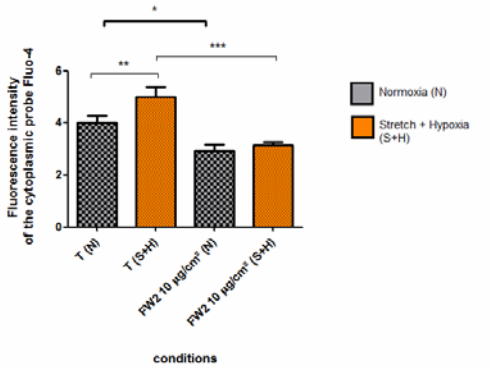

Figure 1: Intracellular [Ca^{2+}] (ATP response) in normal and pathological conditions.

A 24h-exposure to FW2 NP (10 µg/cm²), induced a modification of the intracellular Ca^{2+} level. These modifications appear to be more significant in pathological condition. Indeed, there is a significantly decrease for the ATP response: 28% $p<0.05$ (*) in normoxia condition, against a very significantly decrease in stretch and hypoxia condition 37.2% $p<0.001$ (***). T means control.

When cells are treated with both FW2 NP and thapsigargin (known to deplete endoplasmic reticulum Ca^{2+} stores), we show a significant decrease of the calcium response as compared to control cells suggesting an effect of these particles on the intracellular calcium release from the endoplasmic reticulum.

A 1h-pretreatment with polyethylene glycol (PEG)-superoxide dismutase (300 U/ml) and polyethylene glycol (PEG)-catalase (600 U/ml), significantly decreases the modifications of intracellular Ca^{2+} level induced by FW2 NP. Thus, the perturbation of intracellular calcium homeostasis induced by particles seems also to be correlated to oxidative stress.

Conclusion

In conclusion, the present study shows that, in HPAEC, particulate pollution (i) produces reactive oxygen species, (ii) induces a pro-inflammatory response characterized by an IL-8 and IL-6 secretion and (iii) impairs calcium homeostasis. In conditions that mimic PH, the perturbation of intracellular calcium homeostasis induced by particles could be correlated to a deterioration of the endoplasmic reticulum stocks and to oxidative stress.

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