Usefulness of normal or diseased human bronchial epithelial cell models differentiated at air-liquid interface to study the effects of air pollution-derived PM_4

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Keywords: Air pollution-derived PM_4; human bronchial epithelial cell models; air-liquid interface; repeated exposure; underlying mechanisms of toxicity.
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Air pollution-derived particulate matter (PM) is a well-recognized human health risk factor (Beelen *et al* 2013). Its health effects, observed from indoor and outdoor environments, have been of great concern due to the high exposure risk even at low concentrations (Kim *et al* 2015). Many scientists, policy analysts, and governmental agencies worldwide also believe that current levels of air pollution-derived PM are deadly causing thousands of premature deaths annually. However, PM is generally a heterogeneous and complex mixture of particles, originating from a myriad of natural and anthropogenic sources, whose chemical composition varies over space and time (Loomis *et al* 2013). The knowledge of the underlying mechanisms by which PM exerts its health effects is still incomplete. Consequently, detailed studies realized in more relevant *in vitro* models are highly needed. Hence, we evaluated the usefulness of normal human bronchial epithelial (NHBE) or chronic obstructive pulmonary disease (COPD) cells differentiated at air-liquid interface (ALI) to better study the toxicological effects of repeated exposure to air pollution-derived PM_4

ALI differentiated primary cultures of NHBE or COPD cells were exposed either one or three times to PM_4 (NIST-SRM 2786) at 5µg PM/cm² for 4h, with 20h-time intervals. Cytotoxicity (i.e. glucose 6-phosphate dehydrogenase, G6PD), oxidative endpoints (i.e. malondialdehyde, MDA; protein carbonyl, CO; 8-hydroxy-2'-deoxyguanosine, 8-OHdG; total antioxidant status, TAS; glutathione, GSSG/GSH; superoxide dismutase, SOD), inflammatory mediators (i.e. tumor necrosis factor-alpha, TNF-α; interleukine-1 beta, IL-1 β; interleukine-6, IL-6; interleukine-8, IL-8; transforming growth factor-alpha, TGF-α), and gene expression of some xenobiotic-metabolizing enzymes were studied 24h after the last exposure.

No cytotoxicity was noted in PM_4-exposed NHBE cells whereas a low cytotoxicity was seen in PM_4-exposed COPD cells ($p<0.05$), thereby supporting their expected higher sensitivity. Dose and time-dependent oxidative damage were reported in PM_4-exposed NHBE and particularly COPD cells ($p<0.05$). Indeed, early protein-CO and 8-OHdG formations, on the one hand, and TAS, GSSG/GSH, and SOD alterations, on the other hand, occurred in PM_4-exposed NHBE and particularly COPD cells ($p<0.05$). Only a late MDA production appeared in PM_4-exposed NHBE and particularly COPD cell models ($p<0.05$). Highest concentrations of TNF-α, IL-1 β, IL-8 and TGF-α were observed in non-exposed COPD *versus* NHBE cells ($p<0.05$). In contrast, lower concentrations of IL-6 were detected in non-exposed COPD *versus* NHBE cells ($p<0.05$). Dose and time-dependent increases of inflammatory mediators, except interleukin-6, were reported in PM_4-exposed NHBE and particularly COPD cells ($p<0.05$). In addition, the transcriptomic profiles of some xenobiotic-metabolizing enzymes were differently modified in non-exposed and PM_4-exposed COPD cells as compared to non-exposed and PM_4-exposed NHBE cells, respectively.

In conclusion, our results supported the usefulness of primary cultures of NHBE and COPD cells differentiated at ALI and repeatedly exposed to air pollution-derived PM_4 to better study its health effects. The use of COPD cells also allows to better take into account specific pathological sensitivity.

This work was financially supported by the "Institut National de Recherche contre le CAncer" (INCA) and "Institut National de la Santé et de la Recherche Médicale" (INSERM), the Nord/Pas-de-Calais Regional Council, and Mines Douai.

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