Toxicity of organic ultrafine particles in human bronchial epithelial BEAS-2B cells

Ana Teresa Juarez-Facio, J Yon, Cécile Corbière, Laurence Chevalier, Jean-Marie Vaugeois, Christelle Monteil

TOXICITÉ DE PARTICULES ULTRAFINES ORGANIQUES SUR LES CELLULES ÉPITHÉLIALES BRONCHIQUES HUMAINES BEAS-2B

A. T. Juarez Facio *[1], J. Yon [2], C. Corbière [1], L. Chevalier [3], J.-M. Vaugeois[1], C. Monteil [1]

[1]EA 4651 ABTE équipe TOXEMAC - Université de Rouen Normandie
[2] Normandie Univ, UNIROUEN, INSA Rouen, CNRS, CORIA, 76000 Rouen, France.
[3]GPM - UMR 6634 CNRS
*Courriel de l'orateur : anatere_jf@hotmail.com

TITLE

Toxicity of organic ultrafine particles in human bronchial epithelial BEAS-2B cells

RESUME

La pollution de l'air a des effets sanitaires importants et les particules en suspension y contribuent de façon majeure. Les particules ultrafines (PUFs) ont un diamètre aérodynamique égal ou inférieur à 0.1µm et peuvent pénétrer profondément dans l'arbre respiratoire et atteindre d'autres organes comme le foie, le cœur et le cerveau. Les PUFs seraient plus toxiques en raison de leur surface spécifique qui absorberait davantage des composés organiques. L'objectif de cette étude est de montrer les effets toxicologiques des PUFs avec une teneur importante en composés organiques sur le modèle BEAS-2B, en utilisant une exposition à l'interface air-liquide afin de maintenir leurs caractéristiques microphysiques.

ABSTRACT

Air pollution has significant health effects worldwide and airborne particles play a significant role in these effects. Ultrafine particles (UFPs) have an aerodynamic diameter of 0.1 µm or less and can penetrate deep into the respiratory tree and reach other organs such as the liver, the heart and the brain. UFPs would be more toxic due to their specific surface area which would absorb more organic compounds. The objective of this study is to show the toxicological effects of ultrafine particles with an important organic compounds content on Beas-2B model and by using an exposure at the air-liquid interface in order to keep their microphysic characteristics.

MOTS-CLÉS : BEAS-2B, Interface air-liquide, miniCAST, particules ultrafines, composés organiques / **KEYWORDS:** BEAS-2B, Air-Liquid Interface, miniCAST, ultrafine particles, organic compounds

1. INTRODUCTION

Air pollution by ambient air particulate matter is a serious issue due to its health impacts in many urban and industrialized zones. Due to its physical properties, airborne ultrafine particulate matter is having more attention. Ultrafine particles (UFPs) have an aerodynamic diameter of 100 nm or less. They enter the body through the lungs and can be translocated to extrapulmonary organs as liver, spleen, kidney, heart, and brain (Oberdörster et al. 2004; Kreyling et al. 2009; Schraufnagel 2020). Moreover, because of their higher specific area and surface reactivity, UFPs can absorb a significant amount of hazardous metals and organic compounds making them more threatening than larger PM (Kwon, Ryu, et Carlsten 2020).

The principal source of UFPs emissions in urban environments is on-road vehicles, more specifically motor vehicles driven by diesel engines. Particles from diesel exhaust comprise of polyaromatics hydrocarbons which can diffuse easily through cell membranes because of their hydrophobic properties. Once in the cell, as free radicals, they cause oxidative damage to biological macromolecules including DNA (Leikauf, Kim, et Jang 2020). Studies show finer particle size increases the mutagenic and tumorigenic potential (Piergiacomo Pagano et al. 1996). In addition, UFPs induce inflammatory cytokines release such as IL-6, IL8. Therefore, organic compounds presented in UFPs represent a significant contribution to the toxicity and health effects.

Particle exposure of lung cells at the air-liquid interface (ALI) allows to study toxicity of particles in conditions close to the physiological ones. In this approach, cells are cultured on semi permeable membranes of a transwell insert in a culture well where medium is supplied only from the basal pole, then cells are exposed to an aerosol at the apical pole (BéruBé et al. 2010; Bhowmick et Gappa-Fahlenkamp 2016). Concerning the aerosol generation, several devices exist to carry controlled and repeatable cell exposures at relevant airborne concentrations. MiniCAST is a soot reference generator where soot particles, produced from a co-flow propane diffusion flame, are quenched, and quickly diluted to stabilize the soot particle stream and prevent water condensation. Depending on the operating conditions, particles characteristics as organic content, number and size distribution can be modified while maintaining a stable and repeatable source (Moallemi et al. 2019; Moore et al. 2014; Jing 1999). Thus, MiniCAST soot generator depicts an adequate device for studying particles toxicity.

DOI : 10.25576/ASFERA-CFA2021-24802

This work aims to show the toxicological effects of ultrafine particles with an important organic compounds content. Beas-2B model was exposed at the air-liquid interface and cell response was linked to the microphysical, chemical and morphological properties of miniCAST particles.

2. MATERIALS AND METHODS

2.1. Cell culture

BEAS-2B cell line (ATCC – CRL9609™) derived from normal human bronchial epithelial cells were obtained from autopsy of non-cancerous individuals. BEAS-2B cell line was cultured in LHC-9 medium (GIBCO) supplemented with 100 U/ml penicillin and 100 mg/ml streptomycin in collagen pre-coated flasks to improve cell adherence until 75-80% of confluence. Then, they were seeded at a density of 90,000 cells per cm^2 on collagen-coated Transwell™ inserts. After 3 days, cells reached the confluence and medium is changed every 2 days. Culture is maintained in Liquid-Liquid interface for 7 days prior to exposure. Apical medium is removed, and Air-Liquid interface is carried during and after exposure. The cultures were maintained in 95% humidified air with 5% CO_2 at 37°C.

2.2. Exposure system and cell exposure conditions

The exposure system is comprised of several main components: soot generator miniCAST (5201c), the Vitrocell® exposure system, and several devices for physical characterization (PPS, SMPS). The operating condition CAST3 was chosen for its high contents on organic carbon and for assuring a size distribution lower than 100 nm (Bescond et al. 2016).

The Vitrocell® exposure system comprises two stainless steel modules (exposure module and air control module). Aerosols are delivered to the modules in parallel: the first module receiving the aerosol of study and the second module receiving filtered clean air as negative control. The modules are designed for simultaneous exposure of three independent replicates. Exposure module is equipped with electrodes for high voltage charging to increase particle deposition.

Prior to exposure, the exposure wells are filled with medium and heated at 37°C by a circulating water bath. During exposure, an electrical field (+1000 V) was used to increase the particle deposition efficiency. Aerosol is delivered to the system by the distribution/dilution column where is drawn by the trumpet pipe (isokinetic sampling) to each well. Here, aerosol passed over the apical pole of the cell layer cells at a flow rate of 10 ml/min/well covered by an external vacuum pump. Cells were exposed during 35 min and control samples were exposed for 35 min to ambient HEPA-filtered laboratory air through the Vitrocell System®. Each exposure condition was repeated on 2-3 separate days. We compared the response of soot-exposed cells with the response of control cells, 3 h and 24 h after exposure.

2.3. Characterization of PUFs

Number and particle size distributions were measured with Scanning Mobility Particle Sizer at a range of 12.2–552.3 nm immediately downstream of the Vitrocell® system after went through VKL dilutors. Mass emission was measured with the Tapered Element Oscillating Microbalance and controlled with the Pegasor Particle Sensor directly after VKL dilutors. Transmission electron microscopy (TEM) was used to examine the morphology of the miniCAST soot.

2.4. Toxicological endpoints

Cytotoxicity was measured by the MTT assay adapted to the exposures in transwell inserts. *Gene expression*. Total ARN was extracted from cells after exposure using TRI-REAGENT® (Sigma-Aldrich) and Direct-zol™ RNA MiniPrep (Ozyme). cDNA was prepared by reverse transcribing extracted RNA samples using Invitrogen™ Transcriptase inverse M-MLV. The expression of mRNA was determined by the quantitative real time polymerase chain reaction (qRT-PCR) using Brilliant III Ultra-fast SYBR Green QPCR Master Mix (Agilent Technologies). Expression profiles of Cyp1b1, Cyp1a1, NQO1, IL8 et IL6 were normalized to B2M.

3. RESULTS AND DISCUSSION

3.1. Aerosol characteristics

The size distribution by SMPS and transmission electron microscopy image are shown in Figure 1. The count median diameter (CMD) was 56.95 nm and total count concentration was 2.44E+08 N/cm^3. Mass concentration during exposure was 57.85 mg/m^3. Ratio of organic carbon (OC) and total carbon (TC) shows the organic

DOI : 10.25576/ASFERA-CFA2021-24802

content of particles and it was around 87±5 %. Estimated mass deposition during 35 min exposure was 1775 ng/cm^2. These data are presented in Table 1. According to these data, CAST3 particles can be classified as ultrafine particles with a high content in organic compounds.

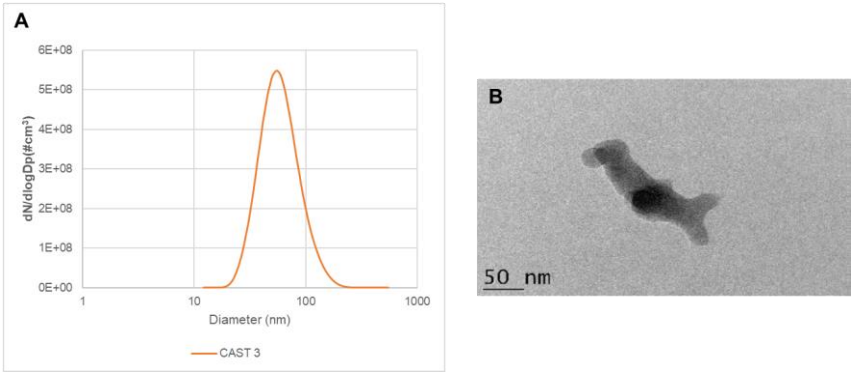

Figure 1. A: Size distribution by SMPS and B: Transmission electron microscopy images of CAST3.

Table 2. Count Median Diameter (CMD), Count and Mass Concentration, ratio of organic carbon (OC) and total carbon (TC) and estimated mass deposition during exposure to CAST3 particles.

3.1. Cellular response to CAST3 particles

CMD (nm)	56.95
Count concentration (N/cm^3)	2.44E+08
Mass concentration (mg/m^3)	57,85
OC/CT (%)	87±5

BEAS-2B were exposed to CAST3 particles during 35 min in Vitrocell® System. No cytotoxicity was observed at any condition (Figure 2.) nevertheless, gene expression, was modified. Expression of CYP1A1 and CYP1B1 were highly modified in both conditions and it was time dependent. Organic compounds as PAH's present in CAST3 can explain these results as they can induce cytochrome P450 (CYP) metabolic enzymes. Several studies have shown these enzymes play vital roles in oxidative stress and pro-inflammatory responses. IL8 and IL6 (inflammation markers) expression was reduced at 3 h and at 24 h was slightly increased. HO1, an antioxidant marker highly induced at 3 h but get back to the normal at 24 h. Finally, NQO1 another antioxidant marker was slightly induced in both conditions. The induction of NQO1 and IL8 after fine and ultrafine particles exposure has been shown in several studies (Hawley et al. 2014; Yuan et al. 2019; Steerenberg et al. 1998; Frias et al. 2020). Nrf2 has a protective role against atmospheric pollutants. Nrf2 induces the expression of NAD(P)H-quinone oxidoreductase (NQO1). This enzyme exerts cytoprotective, antioxidant and anti-inflammatory effects in lungs (Rubio et al. 2011).

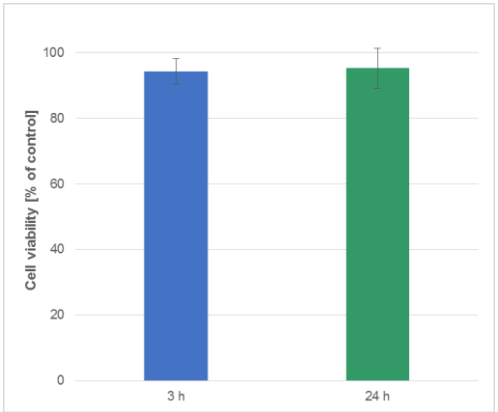

Figure 2. : *Cell viability* determined by MTT test after 3 h and 24h exposure of CAST3 (+1000V) particles. The data was expressed as mean ± SD of 2-3 independent experiments.

DOI : 10.25576/ASFERA-CFA2021-24802

4. CONCLUSION

CAST3 particles induce several modifications in gene expression. Physicochemical properties as organic content and surface could explain these results. However, in this work, complete aerosol exhaust was used during cell exposures so the biological response cannot be attribute only to particles themselves but also to the gas phase. Further investigations are needed to better understand the contributions of particles alone in the toxic response. UFPs are presented in urban ambient air and physicochemical properties depend on emission source. These particles can reach many other organs than lungs causing several health effects. Study of UFPs in the last years had got more attention and become a new area of toxicology research.

REFERENCES

BéruBé, Kelly, Zoë Prytherch, Claire Job, et Tracy Hughes. 2010. « Human Primary Bronchial Lung Cell Constructs: The New Respiratory Models ». *Toxicology*, Highlights of the 2010 Annual Congress of The British Toxicology Society. Includes the Abstracts of the British Toxicology Society, Spring 2010, 278 (3): 311-18. https://doi.org/10.1016/j.tox.2010.04.004.

Bescond, A., J. Yon, F. -X. Ouf, C. Rozé, A. Coppalle, P. Parent, D. Ferry, et C. Laffon. 2016. « Soot Optical Properties Determined by Analyzing Extinction Spectra in the Visible Near-UV: Toward an Optical Speciation According to Constituents and Structure ». *Journal of Aerosol Science* 101 (novembre): 118-32. https://doi.org/10.1016/j.jaerosci.2016.08.001.

Bhowmick, Rudra, et Heather Gappa-Fahlenkamp. 2016. « Cells and Culture Systems Used to Model the Small Airway Epithelium ». *Lung* 194 (3): 419-28. https://doi.org/10.1007/s00408-016-9875-2.

Frias, Daniela Perroni, Raquel Labiapari Nunes Gomes, Kelly Yoshizaki, Regiani Carvalho-Oliveira, Monique Matsuda, Mara de Souza Junqueira, Walcy Rosolia Teodoro, et al. 2020. « Nrf2 Positively Regulates Autophagy Antioxidant Response in Human Bronchial Epithelial Cells Exposed to Diesel Exhaust Particles ». *Scientific Reports* 10 (1): 3704. https://doi.org/10.1038/s41598-020-59930-3.

Hawley, Brie, Dave McKenna, Anthony Marchese, et John Volckens. 2014. « Time Course of Bronchial Cell Inflammation Following Exposure to Diesel Particulate Matter Using a Modified EAVES ». *Toxicology in Vitro* 28 (5): 829-37. https://doi.org/10.1016/j.tiv.2014.03.001.

Jing, Lianpeng. 1999. « Standard Combustion Aerosol Generator (SCAG) for Calibration Purposes », 9.

Kreyling, Wolfgang G., Manuela Semmler-Behnke, Jürgen Seitz, Wilfried Scymczak, Alexander Wenk, Paula Mayer, Shinji Takenaka, et Günter Oberdörster. 2009. « Size Dependence of the Translocation of Inhaled Iridium and Carbon Nanoparticle Aggregates from the Lung of Rats to the Blood and Secondary Target Organs ». *Inhalation Toxicology* 21 Suppl 1 (juillet): 55-60. https://doi.org/10.1080/08958370902942517.

Kwon, Hyouk-Soo, Min Hyung Ryu, et Christopher Carlsten. 2020. « Ultrafine Particles: Unique Physicochemical Properties Relevant to Health and Disease ». *Experimental & Molecular Medicine* 52 (3): 318-28. https://doi.org/10.1038/s12276-020-0405-1.

Leikauf, George D., Sang-Heon Kim, et An-Soo Jang. 2020. « Mechanisms of Ultrafine Particle-Induced Respiratory Health Effects ». *Experimental & Molecular Medicine* 52 (3): 329-37. https://doi.org/10.1038/s12276-020-0394-0.

Moallemi, Alireza, Mohsen Kazemimanesh, Joel C. Corbin, Kevin Thomson, Greg Smallwood, Jason S. Olfert, et Prem Lobo. 2019. « Characterization of Black Carbon Particles Generated by a Propane-Fueled Miniature Inverted Soot Generator ». *Journal of Aerosol Science* 135 (septembre): 46-57. https://doi.org/10.1016/j.jaerosci.2019.05.004.

Moore, Richard H., Luke D. Ziemba, Dabrina Dutcher, Andreas J. Beyersdorf, Kevin Chan, Suzanne Crumeyrolle, Timothy M. Raymond, Kenneth L. Thornhill, Edward L. Winstead, et Bruce E. Anderson. 2014. « Mapping the Operation of the Miniature Combustion Aerosol Standard (Mini-CAST) Soot Generator ». *Aerosol Science and Technology* 48 (5): 467-79. https://doi.org/10.1080/02786826.2014.890694.

Oberdörster, G., Z. Sharp, V. Atudorei, A. Elder, R. Gelein, W. Kreyling, et C. Cox. 2004. « Translocation of Inhaled Ultrafine Particles to the Brain ». *Inhalation Toxicology* 16 (6-7): 437-45. https://doi.org/10.1080/08958370490439597.

Piergiacomo Pagano, *, Tonino De Zaiacomo, Elena Scarcella, and Stefania Bruni, et Massimo Calamosca. 1996. « Mutagenic Activity of Total and Particle-Sized Fractions of Urban Particulate Matter ». Research-article. American Chemical Society. World. 27 novembre 1996. https://doi.org/10.1021/es960182q.

Rubio, Valentina, Jiawei Zhang, Mahara Valverde, Emilio Rojas, et Zheng-Zheng Shi. 2011. « Essential Role of Nrf2 in Protection against Hydroquinone- and Benzoquinone-Induced Cytotoxicity ». *Toxicology in Vitro* 25 (2): 521-29. https://doi.org/10.1016/j.tiv.2010.10.021.

Schraufnagel, Dean E. 2020. « The Health Effects of Ultrafine Particles ». *Experimental & Molecular Medicine* 52 (3): 311-17. https://doi.org/10.1038/s12276-020-0403-3.

Steerenberg, P. A., J. A. J. Zonnenberg, J. A. M. A. Dormans, P. N. T. Joon, I. M. Wouters, L. van Bree, P. T. J. Scheepers, et H. Van Loveren. 1998. « Diesel Exhaust Particles Induced Release of Interleukin 6 and 8 by (Primed) Human Bronchial Epithelial Cells (Beas 2b) In Vitro ». *Experimental Lung Research* 24 (1): 85-100. https://doi.org/10.3109/01902149809046056.

Yuan, Qi, Yaoyao Chen, Xiaobo Li, Zhengdong Zhang, et Haiyan Chu. 2019. « Ambient Fine Particulate Matter (PM2.5) Induces Oxidative Stress and pro-Inflammatory Response via up-Regulating the Expression of CYP1A1/1B1 in Human Bronchial Epithelial Cells in Vitro ». *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 839 (mars): 40-48. https://doi.org/10.1016/j.mrgentox.2018.12.005.

DOI : 10.25576/ASFERA-CFA2021-24802