Carbon 111 (2017) 322–333

Contents lists available at ScienceDirect

Carbon

journal homepage: www.elsevier.com/locate/carbon

Autophagy and lysosomal dysfunction: A new insight into mechanism of synergistic pulmonary toxicity of carbon black-metal ions co-exposure

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ARTICLE INFO

Article history:
Received 14 July 2016
Received in revised form
20 September 2016
Accepted 8 October 2016
Available online 11 October 2016

ABSTRACT

Fine particulate matter (PM 2.5) is the principal instigators of adverse health events, yet gaps still remain in understanding the mechanism mediating its toxic response. Similar to nanoparticles, PM 2.5, with large surface area to volume ratio, can absorb multipollutants in air, displaying toxicity profiles that are very different from those of coarse particles of the same composition. One particularly relevant interaction is that of PM 2.5 and the anthropogenic metals. In this study, we used carbon black nanoparticle (CBs) and metal ions as model materials to investigate the synergistic pulmonary toxicity and its mechanism. We demonstrated that excessive metal contaminants adsorbed on CBs contributed to the observed toxic effects both in vitro and in C57BL6 mice intratracheal instillation model. Significantly, we found that autophagy and lysosomal dysfunction accounted for the synergistic pulmonary toxic effect of co-exposure to CBs and metals. Our findings provide a new insight into understanding the toxicological and healthy effects of fine particles, which have potential to aid in mitigating their adverse health effect.

1. Introduction

A number of studies have investigated the toxicity of particulate matter (PM) [1–4], while gaps still remain in understanding the detailed mechanism of PM toxicity. As small PM go deeper into the lungs than the large PM that exist in the air, many scientific researches focus on evaluating the contribution of fine fraction such as PM 2.5 to health effects [5–7]. From another point of view, we consider that investigating the biological outcomes of nanomaterials with sub-micro nanometers is a good approach to understand the PM 2.5 toxicity mechanism.

Over the past five years or so, we have performed a series studies on nanotoxicology. In vitro cell experiments showed that different sized carbon blacks (CBs) were either nontoxic or slightly toxic, as vehicles of phenol red, they played an essential role in the cytotoxicity induced by phenol red [8]. Subsequently, we found that in serum-free medium, toxicity of nanodiamonds (NDs) to cells was related to the adsorption of sodium ions by NDs [9]. Recently, we further demonstrated that NDs could act as Trojan horse for intracellular delivery of metal ions to trigger cytotoxicity, while NDs alone showed good biocompatibility to cells [10]. As such, we consider that nanoparticles do not necessarily lead to toxicity due to their small size. In nanotoxicity assessments, we should investigate not the direct interaction between nanoparticles and living systems, but the complicated interactions including nanoparticles, multicomponents in environmental media and living systems [11].

Similar to nanoparticles, PM 2.5, with large specific surface area, can also absorb multipollutants in air, and then display toxicity profiles that are very different from those of coarse particles of the same composition [2]. One particularly relevant interaction is that

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http://dx.doi.org/10.1016/j.carbon.2016.10.015
0008-6223/

of PM 2.5 and the anthropogenic metals [12]. In this study, we used CBs and metal ions as model materials to investigate the synergistic toxic effects in vitro and in vivo. CBs have been used extensively in toxicology studies as a surrogate for elemental carbon in airborne PM [13,14]; while metals, including Ni, Cu, Cd and Cr presented in PM due to anthropogenic activities [15,16]. We demonstrate that CBs, acting as vehicles for metal delivery, promoted the metal ion-induced toxicity both in vitro and in C57BL6 mice intratracheal instillation model. More importantly, we indicate that autophagy and lysosomal dysfunction accounted for the synergistic toxic effect of co-exposure to CBs and metal ions (Fig. 1).

2. Materials and methods

2.1. Materials

CBs (Printex 90 and Printex G) were obtained from Degussa (Shanghai, China). The mean individual sizes are 14 and 51 nm for Printex 90 and Printex G, respectively. They were easily dispersed in aqueous solution by sonication. Resultant suspensions were stable for extended periods of time. Transmission electron microscopy (TEM) images and dynamic light scattering (DLS) analysis showed that the sizes of the majority of Printex 90 clusters in water, cell culture medium (containing 10% FBS) and normal saline (containing 0.05% Tween 80) were about 95, 102 and 106 nm, respectively (Table S1). The details for characterization have been described in our previous work [8].

Metal chlorides including $NiCl_2 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, $CdCl_2 \cdot 2.5H_2O$, and $CrCl_3 \cdot 6H_2O$ were obtained from Sigma-Aldrich, China. Stock solution of each metal ion was prepared with MilliQ water and was used to make serial dilutions. All other chemicals used were of analytical grade.

2.2. Cell lines and treatment

RAW264.7 macrophage-like cells were grown in the Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1.5 g/L $NaHCO_3$, 100 units/mL penicillin, 100 μg/mL streptomycin, 4.5 g/L glucose and 4 mM L-glutamine at 37 °C in a humidified 5% CO_2-containing atmosphere. Cells were seeded in 24-well plates at a density of 10^5 cells/well and incubated overnight to allow for cell adherence. After washing twice with phosphate buffered saline (PBS), cells were treated with Ni^{2+} (0–60 μg/mL), Cu^{2+} (0–40 μg/mL), Cd^{2+} (0–4 μg/mL), Cr^{3+} (0–300 μg/mL) with or without 50 μg/mL CBs (or LCBs) for 24 h. Cells incubated with the complete culture medium were used as controls. For CBs-metal ion mixture samples, each kind of metal ion solution was mixed thoroughly with the aqueous CB solution for 2 h prior to experiments.

BEAS-2B cells were grown in RPMI-1640 cell culture medium supplemented with 10% heat-inactivated FBS, 1.5 g/L $NaHCO_3$, 100 units/mL penicillin, 100 μg/mL streptomycin, 4.5 g/L glucose and 4 mM L-glutamine at 37 °C in a humidified 5% CO_2-containing atmosphere. A cell suspension (8×10^4 cells/mL) was dispensed into 24-well plates. After incubation overnight for cell adherence, they were treated with Ni^{2+} (0–40 μg/mL) with or without 50 μg/mL CBs (or LCBs) for 24 h.

The evaluation system was composed of the following: (1) cytotoxicity analysis, (2) in vitro distribution and (3) autophagy and lysosomal function assessment.

2.3. Animals and treatment

C57BL6 mice (male, 18–22 g) were purchased from Shanghai SLAC Laboratory Animal Co. Ltd., China and kept in conventional conditions. All animal experiments were conducted in accordance with the Institute's Guide for the Care and Use of Laboratory Animals and were approved by the ethical committee of Shanghai University of Traditional Chinese Medicine (Approval No. ACSHU-2014-200, approved in 16 July, 2014).

Groups of mice were instilled with 100 μL normal saline (NS), 20 μg CBs, 4 μg Ni^{2+} or CBs-Ni^{2+} mixture. All samples were suspended in NS containing 0.05% Tween 80. At 1 and 7 d post-instillation, animals in control and experimental groups were weighed and then sacrificed. The evaluation system was composed of the following: (1) the bronchoalveolar lavage (BAL) fluid biomedical index, (2) serum and BAL fluid (BALF) cytokine activity, (3) lung histopathological evaluation, (4) in vivo biodistribution and (5) autophagy and lysosomal function assessment.

2.4. Cytotoxicity analysis

After incubation, cells were washed with PBS to remove the uninternalized nanoparticles. Cell viability was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich, Shanghai, China) assay. The cell viabilities were expressed as a percentage of ODtest/ODcontrol. All of the viability assessment data was based on three independent measurements. The IC 50 value of each metal ion and CBs-ion mixture was determined by Origin Pro 7.5 software. The combination indexes of CBs and each metal ion were calculated by CompuSyn 1.0 software, and for each specific test, if the combination index is <1,

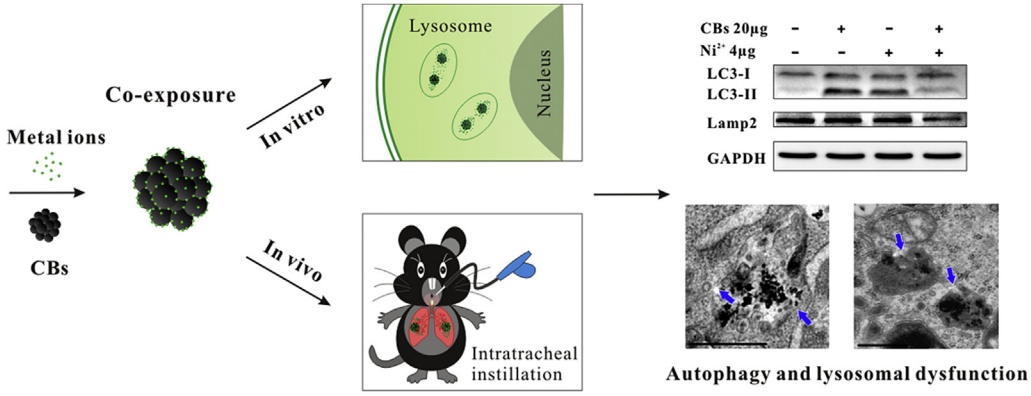

Fig. 1. Schematic showing of autophagy and lysosomal dysfunction accounted for the synergistic pulmonary toxic effect of co-exposure to CBs and metals. (A colour version of this figure can be viewed online.)