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ORIGINAL RESEARCH

Pulmonary toxicities from a 90-day chronic inhalation study with carbon black nanoparticles in rats related to the systemical immune effects

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Background: Recent years, there occurs heavy haze pollution in northern China during wintertime. The potential influence of airborne particulate matter (PM) on human health attracts great concern. The fuel-derived PM in the inhalable size range is dominated by aggregates of nanoparticles of Carbon black (CB). However, there are still lack of evidences especially regarding long-term exposure to explain the chronic effects of nanoscaled CB and the relative mechanism.

Purpose: The objective of this study was to identify the potential mechanism of chronic effects of nanoscale CB. The systemic toxicity, immune suppression or activity and local toxicity were evaluated.

Methods: 32 rats were divided into 2 groups: 30 mg/m3 CB exposure (nose only, 90 d, 6h/d) and control (clean air). Half of rats were scarified after exposure and another half of rats recovered for 14 days. Eight rats in each group were executed the lung function tests using a ventilated bias flow whole body plethysmograph (WBP). SDS-PAGE protocol was used to detect the deposition and retention of CB in lung of rats. HE staining was used to observe the changes of histopathology. Cell apoptosis was examined by TUNEL assay or flow cytometry. The levels of IL-6, IL-8, IL-17 and TNF-α in serum and lung tissue were evaluated with commercially available ELISA kit. The peripheral blood cell counts were detected by Auto 5-diff hematology analyzer.

Results: The lung burden of CB was 16 mg in lung of rats after a 90-day exposure by MPPD. Fourteen percentages of the amount of CB accumulated at the end of the exposure period was cleared from the lung during the 14 dys recovery period. The lung function was significantly decreased and could not recover after a short time recovery. The fibroblasts and granuloma formation were found in lung. The levels of apoptosis and DNA damages were significantly increased in lung cells after CB inhalation. The cytokines levels in lung but not in serum were significantly increased in CB exposure group. The cell counts of WBC, monocytes and neutrophils had 1.72, 3.13, and 2.73-fold increases after CB exposure, respectively. The percentages of CD4+ lymphocytes and the rates of CD4+/CD8+ were statistically increased after CB exposure. The stimulation indexes of the peripheral blood lymphocytes were significantly decreased after CB exposure. In the CB exposure group, the disrupted histomorphology of thymus and spleen were found as well as the early apoptotic thymocytes had a 2.36-fold increase.

Conclusion: CB induced the localized or direct toxicity and systemic immune toxicity. The direct and systemic immune responses had a combined effect on the lung damages caused by CB.

Keywords: nanoscale CB particles, pulmonary toxicology, immune toxicity, chronic toxicity, inhalation

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Introduction

Recently, the potential influence of airborne particulate matter (PM) on the environment and human health attracts great concern.[1] Combustion-derived carbonaceous aerosols have traditionally been associated with pollution in urban areas.[2] Emissions of carbonaceous matter in China have increased by approximately 20% from 1996 to 2010.[3] In the north of China, 46% contribution of the monthly average PM2.5 concentration was residential coal burning.[4] The recent studies suggested that the formation of haze in northern China during wintertime was attributed to the primary emissions from residential coal burning.[5,6] Because it is difficult to determine the weighting coefficient of each component to the overall toxicity under different settings because of the complicated composition of airborne particles,[7] the study on the characteristics of primary particles from residential coal burning has been necessary. Black carbon (originates from incomplete combustion of fossil and biomass fuels and represents an important constituent of atmospheric aerosols, with an estimated global emission rate of 4.3–22 $Tg \cdot year^{-1}$.[8] In occupational environment, the concentration of carbon could reach 15 mg/m^3.[9]

The fuel-derived PM in the inhalable size range is dominated by aggregates of nanoparticles of Carbon black (CB).[10] Nanoscale CB (Printex 90) at the concentration of 11.7 mg/m^3 caused lung tumors in rats after 2 years of inhalation.[11] However no information of detailed deposition, clearance, and retention was provided. In spite of plenty of evidence of adverse effects induced by CB particles, according to the report of overall reevaluation to CB by IARC in 2010, the mechanistic information of the diseases induced by CB is still does not clear enough based on current evidence.[12]

Two main kinds of toxicities may exist due to CB exposure: Localized and systemic toxicities.[13] In fact, systemic toxicity involves an immune response that causes damage to the lungs.[14–16] The immunotoxic potential and ability of nanoparticles to alter immune responses has been documented.[17–19] Frampton et al, reported that CB exposure increased neutrophils and decreased monocytes and basophils among healthy adults after exposure to 25 $\mu g/m^3$ of ultrafine carbon particles for 2 hrs.[20] Intratracheal instillation of 14 and 95 nm CB in mice caused pulmonary inflammation. It triggered the induction of proinflammatory cytokine and chemokine release in the lung as well as translocated to the mediastinal lymph nodes.[21] Previous research has shown that exposure to 0.2 mg/mouse CB nanoparticles by intranasal route can contribute to immunological responses, including allergy.[22] Airway exposure to CB nanoparticles induces pulmonary inflammation, affects allergy associated T helper (Th2) cytokine bias[23] and induces genotoxic damage.[24] In animal models, acute respiratory, subcutaneous and direct immune cell exposure to CB nanoparticles enhances allergy to chicken egg ovalbumin protein.[25,26] However, because of the short-term exposure, it is uncertain whether the observed chronic changes in blood cell counts constitute adverse health effects or reflect a normal immune response to these environmental stimuli in healthy individuals. In our previous study, the elevated levels of pro-inflammatory cytokines in CB packing workers after 15 mg/m^3 CB exposure were found. We observed statistically significant increases of cytokines interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-17 (IL-17), tumor necrosis factor alpha (TNF-α), and Macrophage inflammatory protein-1.[9] Eosinophils were reported to be increased in workers exposed to nanoscaled CB particles for an average of 11.5 years but we did not observe the statistically significant differences in lymphocytes and monocytes.[27]

Therefore, there is still a lack of evidence especially regarding long-term exposure to explain the chronic effects of nanoscaled CB and the relative mechanism. The localized and systemic toxicities involved immune response after short-time CB exposure contributed to the damages of lung.[13–16] Though there were significantly different responses and mechanisms between short term and chronic toxicity of particles, the inflammation, and immune responses were found in long-term CB exposure workers.[9,27] Therefore, our hypothesis is chronic inflammation triggered by immune response could contribute to the toxicity of CB. In the present study, we established a 90-day nose-only inhalation CB model in rats to explore the possible mechanisms. The systemic toxicity, immune suppression or activity, and local toxicity were evaluated. Our results suggested that the direct and systemic immune responses had a combined effect on the lung damages caused by CB. Data collected from the present study will serve to explore health effects related to PM components and might provide strategies that reduce diseases induced by particle materials.

Material and methods

Animals and CB exposure study design

Male, pathogen-free Sprague–Dawley rats at 6 weeks of age were acclimated for a week before the commencement of inhalation exposure. During the acclimation and inhalation

exposure, the rats were housed in polycarbonate cages and provided food and tap water ad libitum in a controlled humidity and temperature environment with a 12 hrs light/dark cycle. We divided 32 rats into two groups: control and exposure. Rats in the exposure group were exposed to CB in the nose-only inhalation chamber at 30 mg/m^3 for 6 hrs/day for 90 days. The control animals were exposed to filter air for 24 hrs/day. After exposure for 90 days, eight rats were selected randomly and scarified in each group, respectively. The rest eight rats in each group were recovered for 14 days after exposure in the chamber with HEPA-filters. Rats were anesthetized by pentobarbital sodium and sacrificed at the endpoint of treatment. Blood samples were collected from abdominal aorta. Organs were separated to further detect. The present protocol was approved by the Committee of the Ethics Animal Experiments of Hebei Medical University (IACUC-Hebmu-20160047) and carried out under the Hebei Medical University institutional guidelines for ethical animal use.

Characterization of CB, aerosol generation, and conditions in the inhalation chamber

CB (≥99.8%) was purchased from the CB manufacturing factory (Jiaozuo Chemical Industry Limited Company, Henan, China) and its characteristics were the same as our previous studies.[9,27] Briefly, Tecnai G220 transmission electron microscope (TEM, FEI, USA) and Sirion 200 scanning electron microscope (SEM, FEI, Holland) were used to evaluate the size and morphology of CB particles. Nitrogen adsorption capacity was calculated to evaluate the specific surface characteristics of CB by Brunauer–Emmett–Teller method. The size and zeta potential of CB particles were performed on DelsaTM Nanoparticle analysis instrument (Beckman coulter, CA, USA).

CB was baked at 80°C for 12 hrs before exposure and packed in an aerosol generator column (Beijing Huironghe Technology Co., Ltd. Beijing, China). The aerosol generator system was employed to reliably disperse non-cohesive powders such as mineral dusts, medical compounds, pollen, and so forth in the size range <100 μm. In the present study, the CB powder was filled into the powder reservoir and uniformly compressed along a 20 cm filling height by a tamper. Then, the CB powder was transported onto a rotating brush at 13 L/min feed rate. The powder that came from the reservoir and had been loosened from the rotating brush was dispersed into practically all individual particles at 23 L/min speed in the dispersion head by dispersion air at 10 L/min flow rate. Dosing was performed using a precisely controlled feed rate of the feed piston (Figure 1). The concentration of CB aerosol in the exposed chamber was 22.50–42.50 mg/m^3 by filter weighing method, and the average was 30.06 ±4.42 mg/m^3 (Supplemental Figure S1A and S1B). During exposure, each rat was placed in

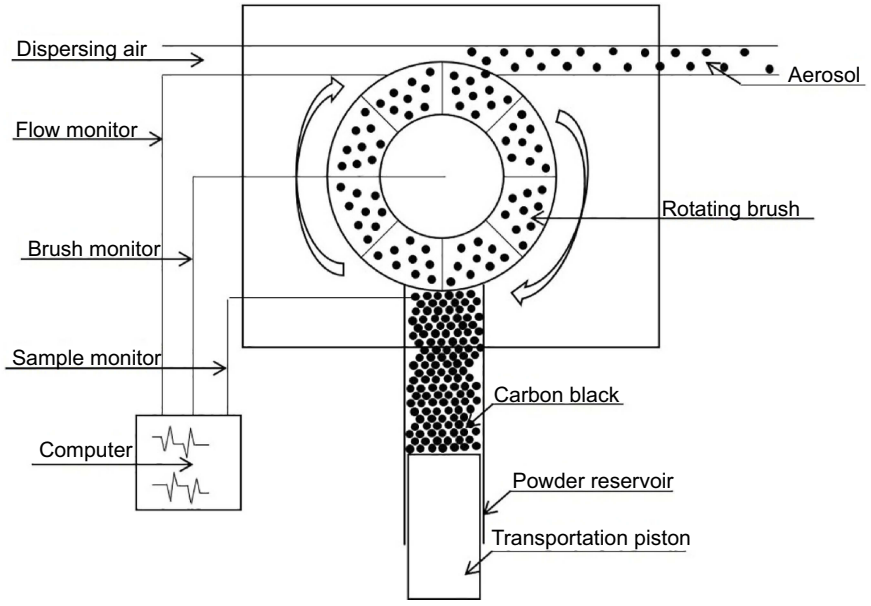


Figure 1 The diagram of the aerosol generator structure.