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Effects of physical characteristics of carbon black on metabolic regulation in mice☆

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ABSTRACT

Potential adverse effects of human exposure to carbon black (CB) have been reported, but limited knowledge regarding CB-regulated metabolism is currently available. To evaluate how physical parameters of CB influence metabolism, we investigated CB and diesel exhaust particles (DEPs) and attempted to relate various physical parameters, including the hydrodynamic diameter, zeta potential, and particle number concentrations, to lung energy metabolism in female BALB/c mice. A body weight increase was arrested by 3 months of exposure to CB of smaller-size fractions, which was negatively correlated with pyruvate in plasma. There were no significant differences in cytotoxic lactate dehydrogenase (LDH) or total protein in bronchoalveolar lavage fluid (BALF) after 3 months of CB exposure. However, we observed alterations in acetyl CoA and the NADP/NADPH ratio in lung tissues with CB exposure. Additionally, the NADP/NADPH ratio was associated with the zeta potential of CB. Mild peribronchiovascular and interstitial inflammation and multinucleated giant cells (macrophages) with a transparent and rhomboid appearance and containing foreign bodies were observed in lung sections. We suggest that physical characteristics of CB, such as the zeta potential, may disrupt metabolism after pulmonary exposure. These results, therefore, provide the first evidence of a link between pulmonary exposure to CB and metabolism.

1. Introduction

Carbon black (CB) is produced by the thermal decomposition of hydrocarbons and is composed of primary particles with chloride and sulfur on its surface (Zielinski et al., 1999). Although CB is commonly used, it has been classified as a possible carcinogen to humans by the International Agency for Research on Cancer (IARC) (IARC, 1996). The bioreactivity of CB has been extensively studied because of its low solubility with no considerable harm compared to organics or metals (Wilson et al., 2002). However, previous reports showed that CB is able to reduce lung function and increase lung inflammation and histopathological injury (Barlow et al., 2005; Schreiber et al., 2013; Zhang et al., 2014). Long-term exposure to CB in rats was found to induce DNA damage, which was highly affected by CB in the lungs (Gallagher et al., 2003). Recently, CB was related to modification of energy metabolism *in vitro* (Pink et al., 2014). Mitochondrial proteins and DNA are susceptible to

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attack by reactive oxygen species (ROS), which can disrupt the regulation of energy metabolism (Maher et al., 2012; Meyer et al., 2013). Risom and colleagues indicated that free radicals and oxidants on CB surfaces can traverse to nuclei or initiate free radical chain reactions which damage DNA (Risom et al., 2005). However, the association between CB exposure and energy metabolism has not been well established.

Characteristics of pulmonary deposition and the toxicokinetic fate of nano-sized primary particles are mainly attributed to their agglomeration status. Physicochemical characteristics of particles, such as the size, surface area, and surface charges, play important roles in determining particle toxicity (Oberdörster et al., 2005) and may affect inhalation into the pulmonary environment (Oberdörster et al., 2005). The physicochemistry of CB regulates its oxidative-inflammatory potency (Schreiber et al., 2013). For example, our previous study showed that the surface area of CB may be an important dose metric for oxidative stress and DNA damage in rats (Chuang et al., 2015). Recently, the particle surface charge was linked to oxidative-inflammatory responses (Cho et al., 2012; Pan et al., 2014). The zeta potential, for example, is the electric potential produced between charged groups, which is associated with the surface of a particle and the suspension medium. The zeta potential is used to derive information concerning electrostatic repulsive forces and the surface charge of a particle (Cho et al., 2012). The lungs with their high inner surface represent a first-line target tissue for CB exposure; therefore, it is important to determine the physicochemistry of CB in response to pulmonary toxicity.

Interactions of the environment with mitochondria and DNA have been defined as key gaps in research (Shaughnessy et al., 2014). In addition, increasing numbers of studies have observed an association between particulate matter and metabolism (Lee et al., 2016; Magnani et al., 2016; Yan et al., 2014). Therefore, gene-environment interactions are critical in these events, leading to oxidative imbalances that result from mitochondrial dysfunction. The objective of this study was to evaluate how physical parameters of CB influence metabolism. Relatively low toxic potencies of CB of various size fractions were intratracheally installed into female BALB/c mice at various concentrations, and inflammation- and metabolic-related indicators were evaluated at various time points between 1 and 3 months.

2. Materials and methods

2.1. Particles and reagent sources

Three size fractions of CB were obtained from Degussa (Incheon, Korea), including CB of 14 (CB14), 95 (CB95), and 280 nm (CB280). A Standard Reference Material (SRM) of diesel exhaust particle (DEP) 2975 was obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). All other reagents were obtained from Sigma (St. Louis, MO, USA), unless explicitly stated otherwise.

2.2. Field emission-scanning electron microscopy (FE-SEM) and energy-dispersive x-ray (EDX) microanalysis

The physicochemistry of particles was investigated using an FE-SEM (JEOL JSM-6500F, Japan) and an EDX microanalysis instrument, according to our previous report (Chuang et al., 2014). To prepare FE-SEM samples, particles were adhered onto 12-mm carbon adhesive tabs on 13-mm aluminum SEM stubs. The stubs were subsequently coated with platinum (Pt) to an average thickness of 10 nm using a sputter coater. FE-SEM images were taken at an accelerating voltage of 20 kV and a spot size of 5. The EDX Genesis Microanalysis System was used to determine elements of the particles.

2.3. Specific surface area and endotoxin

The specific surface area was measured by nitrogen adsorption at −196 °C using a Tristar 3020 gas adsorption analyzer (Micromeritics; Norcross, GA, USA), according to the Brunauer-Emmett-Teller (BET) method. The amount of endotoxin in the particles was determined using a Pierce LAL Chromogenic Endotoxin Quantitation Kit (Rockford, IL, USA), according to the manufacturers' instructions.

2.4. Hydrodynamic diameter, zeta potential, and particle number concentration

To determine the physical characteristics of particles when suspended in solution, their hydrodynamic diameter, zeta potential, and particle number concentration were determined. Particles were thoroughly dispersed in phosphate-buffered saline (PBS) supplemented with 5% bovine serum albumin (BSA) at final concentrations of 5, 140, 290, and 450 μg/ml after 15 min of sonication. Hydrodynamic diameters and the zeta potential were determined using a Malvern Zetasizer Nano-ZS (Malvern Instruments, Worcestershire, UK). Particle number concentrations were determined using a NanoSight (LM-10; Amesbury, UK), the principle of which was previously reported (Filipe et al., 2010). To prepare particle samples, particles were prepared at 20 μg/ml in PBS (with 5% BSA) for the Nanosight analysis. Nanoparticle Tracking Analysis software vers. 2.3 was set to the automatic/basic mode using the same settings for the camera shutter/gain, detection threshold brightness, and gain prior to 1 min of sonication. To estimate the equivalent number concentrations of particles at the four mass concentrations, the particle number counted at 20 μg/ml was timed by the mass concentration to obtain the estimated particle number concentration.

2.5. Animals

Six-week-old female BALB/c mice were obtained from BIOLASCO (Taipei, Taiwan). Mice were housed in plastic cages under a 22 ± 2 °C temperature and 55% ± 10% relative humidity in a 12/12-h light/dark cycle. Lab Diet 5001 (PMI Nutrition International, USA) and water *ad libitum* were provided during acclimatization, pre-exposure, and post-exposure. Animal experiments were performed in compliance with the Animal and Ethics Review Committee of the Laboratory Animal Center at Taipei Medical University (Taipei, Taiwan).

2.6. Experimental design

The experimental design is shown in Fig. 1. To investigate subacute effects of particle exposure, mice were randomly divided into 4 groups for exposure to CB14, CB95, CB280, and DEPs. On day −1, facial vein blood was collected for plasma. On day 0, the mice received intratracheal (IT) instillation of 0, 5, 140, 290, and 450 μg of particles in 50 μl PBS (with 5% fetal bovine serum (FBS)) per mouse under light anesthesia induced by 2% isoflurane vapor (2 ml/min) using a rodent anesthesia machine (Northern Vaporiser; Skipton, UK). On day 28, facial vein blood was collected for plasma following euthanasia ($n = 6$ per subgroup). On days 56 and 84, mice were euthanized ($n = 6$ per subgroup). The lungs were lavaged with 1 ml of PBS followed by centrifugation at 1500 × *g* for 5 min at 4 °C. Lung tissues were snap-frozen in liquid nitrogen or fixed in 4% (m/v) paraformaldehyde in PBS for histological analyses. Mass

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