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Single-walled carbon nanotubes disturbed the immune and metabolic regulation function 13-weeks after a single intratracheal instillation

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ABSTRACT

Due to their unique physicochemical properties, the potential health effects of single-walled carbon nanotubes (SWCNTs) have attracted continuous attention together with their extensive application. In this study, we aimed to identify local and systemic health effects following pulmonary persistence of SWCNTs. As expected, SWCNTs remained in the lung for 13 weeks after a single intratracheal instillation (50, 100, and 200 μg/kg). In the lung, the total number of cells and the percentages of lymphocytes and neutrophils significantly increased at 200 μg/kg compared to the control, and the Th1-polarized immune response was induced accompanying enhanced expression of tissue damage-related genes and increased release of chemokines. Additionally, SWCNTs enhanced the expression of antigen presentation-related proteins on the surface of antigen-presenting cells, however, maturation of dendritic cells was inhibited by their persistence. As compared to the control, a significant increase in the percentage of neutrophils and a remarkable decrease of BUN and potassium level were observed in the blood of mice treated with the highest dose. This was accompanied by the down-regulation of the expression of antigen presentation-related proteins on splenocytes. Moreover, protein and glucose metabolism were disturbed with an up-regulation of fatty acid β-oxidation. Taken together, we conclude that SWCNTs may induce adverse health effects by disturbing immune and metabolic regulation functions in the body. Therefore, careful application of SWCNTs is necessary for the enforcement of safety in nano-industries.

1. Introduction

Carbon nanotubes (CNTs) can be naturally produced by burning organic compounds either indoor or outdoor (Lam et al., 2006; Murr et al., 2004; Yuan et al., 2001). CNTs are also uniformly manufactured by various synthetic methods, including arc discharge, laser ablation, high-pressure carbon monoxide disproportionation, and chemical vapor deposition. Owing to their unique electrical, mechanical, thermal, and physical (extremely light and strong) properties, CNTs have been widely applied as additives in various consumer products such as baseball bats, tennis rackets, swimsuits, car parts, and golf clubs, as well have biomedical, agricultural, and industrial applications (Husen and Siddiqi, 2014). Particularly, single-walled CNTs (SWCNTs), which are one-dimensional structured CNTs, have been extensively evaluated for applications in industrial area, such as production of composite materials, nanoelectronics, field effect emitters, and energy research over the last decade (Baughman et al., 2002). SWCNTs have also attracted tremendous attention in the field of biomedicine, such as biological sensing, bio-imaging, drug delivery, and cancer therapy (Liu et al., 2009; Gomez-Gualdrón et al., 2011; Gong et al., 2013; Porter et al., 2007). Thus, exposure to CNTs can occur intentionally or accidentally not only during innovative and manufacturing processes, but also during usage, disposal, recovery, and recycling processes of CNTs and their commercial products (Dong and Ma, 2015), and the possibility of exposure rapidly increases with extensive application of CNTs. Moreover, accumulating reports suggest that CNTs can cause harmful health effects themselves and that their adverse health effects are enhanced in the presence of foreign bodies such as respiratory particulate materials, parasites, viruses, and bacteria (Fonseca et al., 2015; Kuijpers et al., 2015; Lam et al., 2006; Nowack et al., 2013; Shvedova et al., 2008; Sanpui et al., 2014; Swedin et al., 2012).

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When foreign substances enter the human body, the immune system cleans them up by activating an orchestrated defense mechanism (Kindt et al., 2006; Belardelli and Ferrantini, 2002). Phagocytic cells, including macrophages, neutrophils, and dendritic cells, recruit other immune cells to inflammation sites by releasing cytokines and chemokines against foreign substances in the early phases of the immune response (Linton and Fazio, 2003). Meanwhile, professional antigen-presenting cells (APCs), including dendritic cells, macrophages, and B cells, engulf and digest foreign substances, then provide the information on foreign substances with MHC class II molecules on their surface in the late phase of the immune response (Murtaugh and Foss, 2002). Additionally, T cells can distinguish between self and non-self by recognizing information provided by APCs, but not by the foreign substances themselves. Recently, some researchers have studied the effect of nanoparticles on the function of APCs (Jiménez-Periáñez et al., 2013; Koike et al., 2008; Tkach et al., 2011; Villa et al., 2011). For example, SWCNTs suppressed T cell responsiveness in the spleen with a direct effect on dendritic cells (Hogg and van Eeden, 2009). Meanwhile, pulmonary carbon black caused antigen-related airway inflammation and immunoglobulin production by increasing the expression of MHC class II and co-stimulatory molecules, as well as the number of APCs in the lung (Koike et al., 2008).

Although nanoparticles generally enter the body via the respiratory system, they can translocate into many tissues and organs through the circulatory, lymphatic, and nervous systems, thus can result in subsequent systemic adverse health effects as well as local effects in the exposure site, disrupting their normal function (Hogg and van Eeden, 2009; Ali-Boucetta and Kostarelos, 2013; Ong et al., 2016). However, very limited information is currently available regarding these simultaneous effects (Dong and Ma, 2015). In our previous study, a single intratracheal instillation of SWCNTs (100 μg/kg) induced early lung fibrosis and subchronic tissue damage (Park et al., 2011a). Herein, we first identified a local immune response within the lung 13 weeks after a single intratracheal instillation, an alternative exposure technique for the evaluation of respiratory tract toxicity (Driscoll et al., 2000, Fig. S1). In addition, we investigated the systemic health effects following the pulmonary persistence of SWCNTs using blood and splenocytes from mice.

2. Materials and methods

2.1. Preparation and characterization of SWCNTs

Pristine SWCNTs (ASP-100F, purity of 60–70 wt%, Hanwha nanotech.com, Incheon, Korea), which were produced by conventional arc-discharge process using Fe-based metal catalysts, were dispersed in phosphate-buffered saline (PBS) containing 0.5% Pluronic F127 (Fig. 1, Park et al., 2014). The final concentration of dispersed SWCNTs was 56.9 μg/mL. Dispersed SWCNTs were coated on one side of a copper grid, dried in vacuum oven for 1 h, and then imaged with a transmission electron microscope (TEM, JEM-2010, Tokyo, Japan) at an accelerating voltage of 200 Kv. Additionally, the surface charge and hydrodynamic diameter (HDD) of dispersed SWCNTs were characterized using a Zeta Potential and Particle Size Analyzer (ELSZ-2, Otsuka Electronics Korea Co. Ltd, Seongnam-si, Gyeonggi-do, Korea). Moreover, dispersed SWCNTs were digested in a mixed solution of HNO_3 (70%) and H_2O_2 (30%) by using a microwave digestion system (Milestone, Sorisole, Italy) under high temperature and high pressure (120 °C, 8 min; 50 °C, 2 min; 180 °C, 10 min), and the metal concentrations in lysates were determined using inductively coupled plasma-mass spectrometry (ICP-MS, 7700, Agilent Technologies, Hachioji-shi, Tokyo, Japan). Table 1 shows characterization of dispersed SWCNTs.

2.2. Animal care and SWCNTs treatment

Specific-pathogen free male ICR mice (Five-weeks-old, 25–27 g, 80 mice, OrientBio, Seongnam, Korea) were acclimatized for 1 week before the start of the study at a constant temperature (23 ± 3 °C), relative humidity (50 ± 10%), a 12 h light/dark cycle with a light of intensity 150–300 lx and ventilation of 10-20 times/h. Considering that the upper limit dose volume of 5 mL/kg is recommended for intratracheal instillation in terms of animal welfare, SWCNTs (50, 100 and 200 μg/kg, 20 mice/group) were treated using a 24-gauge catheter under light tiletamine anesthesia. The control group was treated with a vehicle used for suspension of SWCNTs, and mice were euthanized on 13-weeks after a single instillation (Fig. S1). Body weight was measured weekly, and significant decrease in body weight gain following persistence of SWCNTs was observed at the maximum dose (200 μg/kg, Fig. S2). The experiments were assessed by the Institutional Animal Care and Use Committee (IACUC) of Ajou University (Suwon, Korea, IACUC No. 2013-0069) and were performed in accordance with the "Guide for the Care and Use of Laboratory Animals," an Institution of Laboratory Animal Research (ILAR) publication.

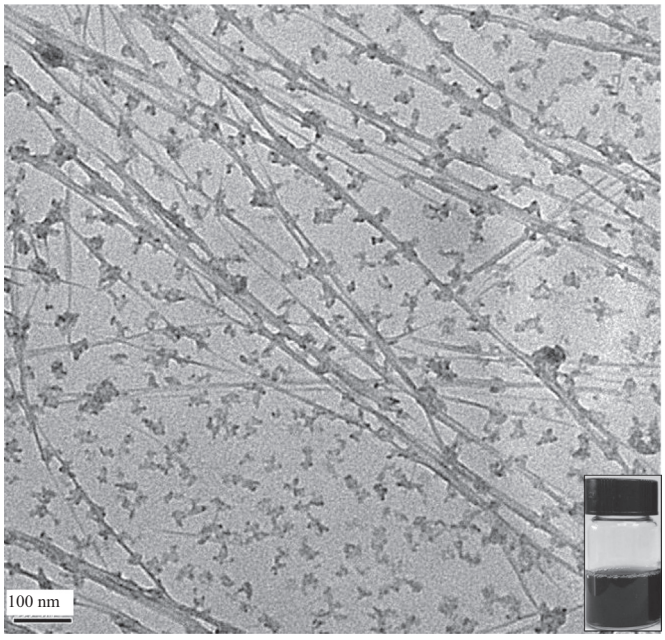

Fig. 1. TEM image of SWCNTs suspended in vehicle. Box shows a picture of SWCNTs suspended in vehicle.

2.3. Blood analysis

Whole blood was collected from the caudal vena cava, and the part was centrifuged at 3000 rpm for 10 min to obtain serum for the immunoglobulin and NMR analysis. Hematological and biochemical analysis were performed in Neodin Veterinary Science Institute (Seoul, Korea) using a blood autoanalyzer (HemaVet850, CDC Technologies, Inc., Dayton, Ohio, USA) and chemistry analyzer (BS-400, Mindray, Shenzhen, China), respectively.

2.4. BAL cell analysis

As described previously, bronchial alveolar lavage (BAL) fluids were obtained by cannulating the trachea and lavaging the lungs twice with cold sterile PBS (1 mL) on 13-weeks after a single instillation (Park et al., 2015). After centrifuging for 5 min at 1,500 rpm, cell pellets were used for BAL cell, cell cycle, and immunophenotypic analysis, and the supernatants were used for cytokine assay. The total cell number was recorded using an automatic cell counter (ViCell XR, Beckman Coulter, CA, USA). In addition, cells were spin-downed on slides, fixed in methanol, stained with Diff-Quick (Sysmex Corporation, Tokyo, Japan), and then differentiated by morphological characterization. A part of BAL cells were also fixed with 70% ethanol and stained with propidium iodide and RNase (Sigma-Aldrich, St. Louis, MO, USA). Then, changes in cell cycle were analyzed by measuring the DNA content using the FACSCalibur system and CellQuest software (BD Biosciences, Franklin Lakes, NJ, USA).

2.5. Cytokine assay

The concentration of pro-inflammatory cytokines (Interleukin (IL)-1β, tumor necrosis factor (TNF)-α, IL-6), Th0-type cytokine (IL-2), Th1-type cytokines (IFN-γ and IL-12/IL-23), Th2-type cytokines (IL-4, IL-5, IL-10, and IL-13), chemokines (macrophage inflammatory protein (MIP)-1α, macrophage chemoattractant protein (MCP)-1, and granulocyte-macrophage colony-stimulating factor (GM-CSF)), and transforming growth factor (TGF)-β in BAL fluid and immunoglobulins (IgE, IgG, and IgM) in serum were determined with enzyme-linked immunosorbent assay