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Fibrosis biomarkers in workers exposed to MWCNTs

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ABSTRACT

Multi-walled carbon nanotubes (MWCNT) with their unique physico-chemical properties offer numerous technological advantages and are projected to drive the next generation of manufacturing growth. As MWCNT have already found utility in different industries including construction, engineering, energy production, space exploration and biomedicine, large quantities of MWCNT may reach the environment and inadvertently lead to human exposure. This necessitates the urgent assessment of their potential health effects in humans. The current study was carried out at NanotechCenter Ltd. Enterprise (Tambov, Russia) where large-scale manufacturing of MWCNT along with relatively high occupational exposure levels was reported. The goal of this small cross-sectional study was to evaluate potential biomarkers during occupational exposure to MWCNT. All air samples were collected at the workplaces from both specific areas and personal breathing zones using filter-based devices to quantitate elemental carbon and perform particle analysis by TEM. Biological fluids of nasal lavage, induced sputum and blood serum were obtained from MWCNT-exposed and non-exposed workers for assessment of inflammatory and fibrotic markers. It was found that exposure to MWCNTs caused significant increase in IL-1β, IL6, TNF-α, inflammatory cytokines and KL-6, a serological biomarker for interstitial lung disease in collected sputum samples. Moreover, the level of TGF-β1 was increased in serum obtained from young exposed workers. Overall, the results from this study revealed accumulation of inflammatory and fibrotic biomarkers in biofluids of workers manufacturing MWCNTs. Therefore, the biomarkers analyzed should be considered for the assessment of health effects of occupational exposure to MWCNT in cross-sectional epidemiological studies.

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1. Introduction

Engineered carbonaceous nanomaterials (CNMs) present tremendous opportunities for industrial growth and development and hold great promise through their applications in medicine, electronics, and numerous other areas. However, there are considerable gaps in our knowledge concerning the potential hazardous effects of CNMs on human health and the environment. The evidence for the potential adverse health effects associated with CNMs in humans comes from epidemiological studies of particulate matter (PM) arising from air pollution and other combustion processes (Delfino et al., 2005; Riley et al., 2005; Pope and Dockery, 2006; Brook, 2008a; Brook, 2008b; Craig et al., 2008; Simkhovich et al., 2008; Hamra et al., 2014). The U.S. EPA identified that PM represent serious public health problems in the United States causing premature mortality, aggravation of respiratory and cardiovascular disease, acute respiratory symptoms, chronic bronchitis and exacerbation of asthma. The International Agency for Research on Cancer (IARC) identified cancer concerns arising from core ultrafine particles with reactive chemicals adhered to their surfaces. The biological effects of CNMs can be comparable to or stronger than those of ambient PM. This emphasizes the urgent need for field studies focused on the assessment of exposure and health status in humans exposed to CNMs. Due to the lack of human data on CNMs exposure, only general non-specific precautionary measures can be taken. To establish effective regulatory standards, governments and regulating agencies around the world need precise and reliable data related to the potential adverse health effects associated with CNM exposures.

Multi-walled carbon nanotubes (MWCNTs) are the most promising CNMs that have been manufactured broadly during the last decade. MWCNTs are composed of layered graphene sheets, in the form of single-walled carbon nanotubes nested one inside the other. Their cylindrical nature, exceptional mechanical strength and intrinsic physico-chemical properties, render their feasibility for use in a number

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applications including electronics, optics, materials science, polymer chemistry and nanocomposites (Baughman et al., 2002; Wang et al., 2014). The large-scale manufacturing of MWCNTs in the past decade, and their expanded applications in new technologies, consumer products and biomedicine, raises the likelihood of human exposure to these materials, thus increasing concerns about their potential adverse health effects. In November 2014, the IARC classified MWCNT-7 (Mitsui ltd., Japan) to a Category 2B: as possibly carcinogenic to humans (Grosse et al., 2014).

The major health risks of exposure to MWCNT in workplaces are based on numerous in vivo animal studies that reported consistent non-cancerous adverse effects (Poland et al., 2008; Ma-Hock et al., 2009; Mercer et al., 2010; Pauluhn, 2010; Porter et al., 2010; Mercer et al., 2011; Wang et al., 2011; Murphy et al., 2012; Huizar et al., 2013; Dong et al., 2015; Khaliullin et al., 2015; Snyder-Talkington et al., 2015; Wang et al., 2015). Acute pharyngeal aspiration or inhalation exposure of rodents to CNT causes dose-dependent pulmonary damage accompanied by a robust inflammatory response and severe oxidative stress leading to fibrosis and formation of granulomatous lesions (Porter et al., 2010; Mercer et al., 2011; Huizar et al., 2013; Sargent et al., 2014). Mechanistically, the histological features of fibrosis in animal lungs were associated with a significant dose and time-dependent increase in TGF-β and osteopontin (OPN) measured in the serum, bronchial alveolar lavage (BAL) fluids or lung tissue (Pauluhn, 2010; Porter et al., 2010; Mercer et al., 2011; Huizar et al., 2013; Dong et al., 2015; Khaliullin et al., 2015). Additionally, it was shown that exposure to MWCNT activated TGF-b/Smad signaling pathway in fibroblasts and myofibroblasts thus facilitating pulmonary fibrosis (Wang et al., 2015). Moreover, inhalation exposure to MWCNT promotes the growth and neoplastic progression of initiated lung cells in mice (Sargent et al., 2014; Snyder-Talkington et al., 2016). These effects were observed at a dose of 31.2 μg/mouse – that is achievable in human exposures in occupational settings (Erdely et al., 2013; Khaliullin et al., 2015).

Overall, the health effects associated with CNT exposures in rodents have not yet been confirmed in humans. In spite of the insufficiency of data on health effects of CNTs of exposed individuals, there are several documented cases showing CNT deposits found in human lungs and other organs after various exposures. One of them is the tragic event on 9/11 – the attack on the financial district of New York. This incident has caused a number of devastating diseases among rescue and recovery workers. Some of the first responders were presented with respiratory illness and have been diagnosed with pulmonary fibrosis, chronic bronchiolitis and granulomatous formation. The tubular carbon nanostructures, similar to CNTs - produced as a result of high combustion temperatures - were detected in the biopsy specimens of first responders as well as air samples collected at the crash site (Wu et al., 2010).

There are also several epidemiological attempts to assess the effects of NM on exposed workers (Liao et al., 2014a; Liao et al., 2014b; Vermeulen et al., 2014; Lee et al., 2015; Liou et al., 2015). In these studies, the results of exposure to NM other than MWCNTs were analyzed; one common limitation of these studies was inconsistent assessments or low/short-interval exposures which has been discussed in detail in a recent review (Liou et al., 2015). Our recent pilot cross-sectional study (Shvedova et al., 2016) revealed changes in mRNA expression profiles of whole blood samples derived from workers exposed to MWCNT aerosol. Expression analysis revealed augmented levels of several fibrotic bio-markers including TGF-β, KL-6 in whole blood samples of workers exposed to MWCNTs.

In spite of all the concerning data, to date the unambiguous identification and characterization of accurate and reliable markers of pulmonary injury associated with CNT exposure in humans have not been developed. This is due, at least in part, to the complexity of studies evaluating the adverse effects of CNTs in humans including the essential requirement of non-invasiveness of methods involving human subjects. The goal of the current work was to identify potential biomarkers in nasal lavage, sputum and serum of workers as a prognostic tool for assessment of occupational exposures to MWCNT.

2. Materials and methods

2.1. Study details

The study was conducted at a company producing MWCNTs: Nanotechcenter Ltd. (Tambov, Russia). Carbon nanotubes are synthesized using the catalytic vapor deposition method (CVD). MWCNT characteristics were as follows: external diameter - 8–15 nm, internal diameter – 4–8 nm, length 2 μm and more, catalysts content (Ni and Co) – <5%.

Based on both workers' and supervisors' structured interviews and examination of workstations, potential workers in contact with the MWCNT aerosol for more than 1 year were selected and included as part of the exposure group ($n = 10$). These workers were represented by foremen, engineers, technical operators, and scientists. The personnel not exposed to the MWCNT aerosol, but who worked at the same enterprises as either engineers, scientists, or technical staff, were included as part of the control group ($n = 12$). In total, 22 workers of both genders (18 males, 4 females) aged 19–63 years participated in the study. Five of the exposed workers and 7 workers from the control group were under 30 years of age. None of them had a history of any diagnosed chronic respiratory diseases, including chronic obstructive pulmonary disease (COPD) and bronchial asthma, before they started working with CNTs. Six of 22 workers employed in the study were current smokers. Workers with acute respiratory symptoms, as a result of seasonal flu/cold, at the time of blood and sputum sampling were excluded from the study. The Institutional Review Board of Kazan State Medical University, Kazan, Russia, approved this study under the IRB protocol No. 14 dated 26.12.2011.

2.2. Exposure assessment

Exposure to MWCNT was monitored by transmission electron microscopy (TEM) and elemental carbon (EC) analyses. The EC analysis gave a quantitative measure of MWCNT exposure, while TEM confirmed the size and types of nanotube structures present. Air sampling was conducted in the employee's personal breathing zone (PBZ) simultaneously on mixed cellulose ester (MCE) filters (SKC Inc.) for TEM analysis and on ultraclean quartz-fiber filters (PallflexTissuquartz®) for EC analysis. At each sampling point, at least 400 liters (L), at a flow rate of 7–16 L/min, were sampled on the MCE filter; each set of samples was accompanied by at least 2 blank filters. For the EC analysis, sampling of both the inhalable and respirable aerosol fraction on quartz filters was conducted for 90 min in the breathing zone at a rate of 3 L/min (270 L total). TEM coupled with energy dispersive x-ray spectroscopy was performed using modified method NMAM 7402 (NIOSH, 2006). The MCE filters were analyzed with a JEOL 2100F transmission electron microscope. The EC content (quartz filters) was determined by thermal-optical analysis, using a modified method NIOSH 5040 (Birch et al., 2011; NIOSH, 2013). The 8-hour time-weighted average (8-h TWA) EC concentrations were calculated for each worker taking into account the person's duties and time spent at different workstations, as described in detail in Shvedova et al. (2016).

2.3. Collection of blood and induced sputum samples

Blood sampling was drawn by venipuncture by a qualified technician aseptically from the cubital vein using a BD Vacutainer® Safety-lok™ catheter (BD Diagnostic, Franklin Lakes, NJ; USA) into sterilized siliconed glass Vacu tubes (7ML LVDR 100EA/BX 10BX/CS; K3 EDTA 15%; BD Diagnostics, Franklin Lakes, NJ). Clotting was obtained at room temperature. Following the centrifugation (3000 rpm × 10 min) the serum was separated and stored at −20 °C until use.