PS 603e Panel Biomarker Study in Workers Exposed to Multiwalled Carbon Nanotube Aerosol

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The aim of the study was to assess potential fibrogenic risk upon real occupational exposure to multi-walled carbon nanotubes (MWCNT) using biomarkers tested in preceding animal experiment. The study was conducted at 2 MWCNT-producing enterprises with the same reactor type. 11 workers who had more than 1 year contact with MWCNT aerosol composed the exposure group, the control group consisted of 14 people. Elemental carbon was evaluated in air samples and the CNT presence was confirmed by TEM-analysis. Blood and induced sputum samples were obtained from workers, TGF-β1, KL-6 and osteopontin levels evaluated. To assess the relationship between MWCNT exposure and biomarker levels (age, gender, smoking have been chosen as cofounders) generalized linear models including main effects and interactions in-pairs were created. The regression coefficients confidence intervals were refined by bootstrap analysis. TWA respirable MWCNT fraction was up to 6.11 mg/m3. TEM has shown the presence of MWCNT agglomerates sized 0,5-10 μm in all air samples. It was found that exposure to MWCNT aerosol at workplaces may alter the fibrosis biomarkers in blood serum and induced sputum. The levels of TGF-β1 in serum were dependent on exposure to MWCNTs (β=10.5, 95%BCa=1.2-51.8), the KL-6 levels in induced sputum were significantly higher in exposure group (β=235.9, 95%BCa=21.2-482). Osteopontin proved to be as an uninformative indicator. Data suggest that MWCNT exposure may lead to the changes in serum and induced sputum samples specific fibrogenic biomarkers content in workers. MWCNT-producing companies have to introduce control measures, as well as provide the adequate medical services organization.

PS 603f MWCNT Exposure Assessment in Occupational Settings

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The production of Multi-walled carbon nanotubes (MWCNTs) steadily increases all around the world. However, recent toxicological data raise concerns about their potential risk to human health, particularly upon occupational exposures. The aim of this study was to evaluate the occupational exposure to MWCNT at one of the manufacturing facilities in Russia. The sampling was performed at different sites during the entire technological process. Elemental carbon (EC) analysis was performed using modified NIOSH 5040 method. TEM analysis was performed using modified NMAM 7402 method. The highest MWCNT aerosol short-term concentration was registered during the reactor cleanout process, reaching 157 ug/m3. Time-weighted average (TWA) concentration was respectively 29.6 ug/m3 for the 8-hours shift. Respirable fraction TWA concentrations for EC at different workstations ranged from 0.53 to 6.11 ug/m3. TEM showed the presence of MWCNT-containing agglomerates at all technological stages, also detectable on non-working days. Agglomerates size ranged from 1 to 10 um; no individual MWCNTs were found. The highest EC values were found in samples obtained during reactor cleanout and harvesting, whereas laboratory handling produced the lowest MWCNT mass concentration values. In the absence of relevant OEL currently set in Russia we compared the maximum 8-hr TWA concentrations with the NIOSH recommended exposure level – 1 ug/m3. The 6.11 ug/m3 EC 8-hr TWA mass concentration value - determined during product harvesting - dictates the necessity of exposure control aimed at the reduction of potential adverse health effects in workers. Repeated exposure assessment is required after exposure controls installation. Direct online particle counters should be also used for mapping and identification of critical exposure points.

PS 603g Multiwalled Carbon Nanotubes Induce NLRP3 Inflammasome-Dependent Expression of Pro-Fibrotic Markers in Primary Human Bronchial Epithelial Cells

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Fibrogenic potentials of multi-walled carbon nanotubes (MWCNT) have been well documented; however mechanisms leading to lung fibrotic changes especially in primary human cells are not well understood. We aimed at deciphering the toxic effects of MWCNTs on primary human bronchial epithelial (HBE) cells and studied the effects of epithelial origin factors on human lung fibroblasts (MRC-5 cells). Well characterized MWCNTs and their suspensions were used to treat primary HBE cells (1.5-24 μg/mL). Fetal Lung fibroblasts (MRC-5 cells) were exposed to 1:4 dilated conditioned medium from MWCNT exposed BECs. MWCNT induce NLRP3 inflammasome dependent pyroptosis in HBE cells characterized by caspase-1 activation, cathepsin B release and inflammatory cell death. Cell death induction as well as inflammatory cytokine production can be significantly reduced by using siRNA to NLRP3, a caspase-1 inhibitor (z-WEHD-FMK) or a cathepsin B inhibitor (CA-074Me). Significant increase in mRNA expression of two well-known markers of fibrosis (TIMP-1 and Tenascin-C) in human lung fibroblasts was observed after treatment with conditioned media from MWCNT-treated HBE cells. Induction of these pro-fibrotic markers is significantly reduced when conditioned media from NLRP3 siRNA transfected HBE cells is used. In conclusion we demonstrate here that MWCNT induce inflammasome dependent pyroptosis in primary HBE cells which can initiate a profibrotic response. Thus we present a novel mechanism by which MWCNTs induce human airways epithelial damage.

PS 603h Mucociliary Differentiation May Protect Human Bronchial Epithelia from Multiwalled Carbon Nanotube-Induced Toxicity

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Multi-walled carbon nanotubes (MWCNTs) are a form of graphitic carbon engineered for a wide variety of applications in consumer and industrial products. Their unique physiochemical properties, including high tensile strength and conduction, have made carbon nanotubes one of the most widely-used nanomaterials today. However, the increasing public use of MWCNTs elevates the risk of human exposure, both to consumers and those involved in the manufacture of MWCNT-enabled products, particularly via inhalation. Previous studies have found elevated cytotoxicity, inflammation, and fibrosis in murine lungs and human cells exposed to MWCNTs. However, the effect of these materials on the normal differentiation of human bronchial epithelial cells (BECs) is not well studied. In this study, human primary BECs derived by bronchoscopy were seeded on Millicell CM transwell inserts and allowed to reach confluence in BEGM growth medium (Lonza). Cells were then exposed to MWCNT in suspension for 24 hours at 0.06, 0.25 or 1 μg/cm2. Treatment controls included an untreated control, vehicle (BEGM with 10μg/ml DPPC, 1% BSA) control, and a 1μg/cm2 nano graphitized mesoporous carbon (NG) shape control. BECs exposed during differentiation in either air-liquid interface (ALI) or submerged culture were compared. BECs grown in ALI fully differentiated into a mucociliary pseudostratified epithelium regardless of treatment. In submerged culture, 1 μg/cm2 MWCNTs were highly cytotoxic to BECs and 0.25 μg/cm2 MWCNTs prevented the BECs from prototypical squamous differentiation. The results of this study suggest that the process of mucociliary differentiation in BECs can provide resistance to the cytotoxic effects of MWNCTs even before the cells have fully matured.

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