SEM analyses suggested the attachment of LPO on the carboxylated side-walls of the bundles of SWCNTs. We also confirmed the ex vivo biodegradation of SWCNTs in murine bronchoalveolar lavage (mBALF) shown to express peroxidase activity. Lastly, we addressed whether the pre-occupancy of LPO with SWCNTs would interfere with its antibacterial properties. To this end, experiments with Pseudomonas aeruginosa, an opportunistic human pathogen, were conducted and we noted that the pre-occupancy of LPO with SWCNTs severely impaired the antibacterial activity of LPO. Our study provides evidence for the degradation of carboxylated SWCNTs by LPO, a secreted peroxidase that is present in the airways. However, the antibacterial activity of LPO is impaired as a result of the pre-occupancy of LPO with SWCNTs. This latter finding implies that airway defenses may be compromised in individuals exposed to SWCNTs, possibly leading to more persistent infections.

PS 587 Effects of MWCNT and Nitrogen-Doped MWCNT in Lung Epithelial Cells

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The growing use of multi-walled carbon nanotubes (MWCNT) and their derivatives in academic and industrial settings has raised the need to efficiently and accurately determine their potential toxicity in the workplace. Nitrogen-doped multi-walled carbon nanotubes (ND-MWCNT) are modified MWCNT that have enhanced electrical conductivity and are increasingly used in a variety of aerospace and fuel cell applications. Although similar in structure to MWCNT, limited toxicological data is available, and the biocompatibility and mechanism of action of ND-MWCNT have yet to be elucidated. Recent in vivo data showed that ND-MWCNT induced inflammation and fibrosis in mouse lungs. In this study, we assess uptake of ND-MWCNT into small airway epithelial cells (SAEC), which may induce molecular toxicological effects. We showed that ND-MWCNT induced higher levels of reactive oxygen species (ROS) in SAEC when compared to their parent MWCNT in short-term in vitro exposure. Treatment of SAEC with low doses (1.2μg/mL) of ND-MWCNT and MWCNT indicated that both induced a significant increase in cell proliferation in a time-dependent manner. Furthermore, significant alterations to the cell cycle were observed in cells treated with both ND-MWCNT and MWCNT in a time- and dose-dependent manner, as shown by an increased percentage of cells in the S and G2 phases of the cell cycle. Confocal images showed alterations in acetylated and total tubulin levels in both ND-MWCNT and MWCNT treated cells as well. Since increases in ROS production and cellular proliferation are associated with multiple pathological conditions, ND-MWCNT exposure may play a role in the initiation of these diseases.

PS 588 Autophagy and Extracellular HMGB1 Are Mediators of Inflammasome Activity in Response to MWCNT Exposure

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RATIONALE: Pulmonary exposure to Multi-walled Carbon Nanotubes (MWCNT) has been shown to induce chronic inflammation in animal models, and therefore may pose a health risk to humans. Pathology induced by MWCNT exposure is dependent upon NLRP3 Inflammasome activity, specifically IL-1β signaling. Mechanisms regulating Inflammasome activity are not clear. In this study, we establish critical roles for High Mobility Group Box 1 (HMGB1) and autophagy in regulating Inflammasome activity. METHODS: Secretion of HMGB1 was assessed in vitro from primary Alveolar Macrophages (AM) and in vivo in C57Bl/6 mice (24 and 72 hours) after MWCNT exposure. The contribution of extracellular HMGB1 to Inflammasome activity was delineated in vitro via HMGB1 elimination by immunoprecipitation techniques and in vivo using neutralizing antibodies. To assess the contribution of autophagy in mediating inflammasome activity, primary AM exposed to MWCNT were treated with molecular inhibitors (LY294002, Bafilomycin, Imipramine) targeting different sites along the autophagic flux, and IL-1β production assessed after 24 hours. RESULTS: HMGB1 secretion and autophagic flux are increased following MWCNT exposure. HMGB1 removal by immunoprecipitation techniques or neutralization reduces IL-1β production in primary AM in vitro and in vivo, respectively. Additionally, targeting autophagy by stabilizing the lysosome also decreases IL-1β production after MWCNT exposure. However, inhibition of PI3 kinase activity, responsible for autophagosome formation, enhances IL-1β secretion. CONCLUSIONS: HMGB1 signaling and autophagy play critical roles in the acute Inflammasome response to MWCNT exposure, and may be potential therapeutic targets in particle induced inflammatory diseases.

PS 589 Effects of SWCNT Fiber Length and Functionalization on ROS and Collagen Production

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Single-walled carbon nanotubes (SWCNTs) present a significantly higher chance of occupational and environmental exposure owing to their widespread use in technology and commercial products. Currently, there is a lack of consensus on the various CNT characteristics that induce pulmonary toxicity. Therefore, our aim was to study the effect of SWCNT length and chemical functionalization on ROS production and collagen synthesis in human lung fibroblasts. SWCNTs with varying functionalization including carboxylic acid, amine and hydroxyl, and varying lengths, i.e. long 5-30 nm and short 1-2 nm, obtained from Cheap Tubes Inc. were used. SWCNT dimension and elemental composition characterization was conducted using atomic force microscopy and energy dispersive X-ray spectroscopy respectively. SWCNT toxicity in normal human lung fibroblast were first determined using WST-1 assay and sub-toxic concentrations of SWCNTs were selected for further experiments. Cellular ROS production was determined fluorometrically using DCF-DA as fluorescent probes for peroxide measurement. Type I Collagen expression was determined by western blotting in the presence and absence of ROS inhibitors. Cellular collagen content was determined by Sircol® assay. Our results showed that: 1) Pristine > COOH > NH_2 > OH SWCNT in ROS and collagen production in a dose- and time-dependent manner; 2) longer length SWCNTs induced higher ROS generation and collagen I expression than those of short length; and 3) SWCNT-induced collagen expression was significantly blocked by various ROS inhibitors revealing a role for oxidative stress in SWCNT-induced fibrogenesis. These results suggest that SWCNT length and functionalization impact lung fibroblast ROS and collagen synthesis, which contribute to SWCNT-induced pulmonary fibrosis.
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Research findings and conclusions are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

PS 590 Differential Gene Expression in SAEC and HMVEC Grown in Monoculture or Coculture and Exposed to MWCNT: Correlation with *In Vivo* Studies

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In vitro coculture systems are at the forefront of molecular research due to their increased ability for cell-cell communication. In this study, small airway epithelial cells (SAEC) and human microvascular endothelial cells (HMVEC) were grown separately in monoculture or together in an alveolar-capillary coculture and exposed to either dispersion media control or multi-walled carbon nanotubes (MWCNT) for 6 or 24 h. Global mRNA profiling determined genes that were differentially expressed in coculture as compared to monoculture, and Ingenuity Pathway Analysis determined the biological functions and related pathways of these genes. A total of 1505 SAEC and 54 HMVEC genes, commonly involved in the cell cycle and cell proliferation, were differentially expressed in control coculture experiments as compared to monoculture (SAM 1%, FC >1.5). A total of 1601 SAEC and 2016 HMVEC genes, commonly involved in cell movement and survival, were differentially expressed in coculture as compared to monoculture following MWCNT exposure (SAM 1%, FC >1.5). A correlation study of gene expression between monoculture, coculture, and in vivo gene expression from mice lungs exposed to MWCNT determined that coculture gene expression had a better correlation with in vivo gene expression than monoculture. In this study, we determined that gene expression in cells from coculture models is different from expression in the corresponding cells in monoculture. As coculture gene expression better correlates with gene expression seen in vivo, we hypothesize that coculture may offer an enhanced in vitro model for nanoparticle risk assessment.

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