2719 Thrombospondin-1 Mediates Multi-Walled Carbon Nanotube-Induced Impairment of Arteriolar Dilation

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Pulmonary exposure to multi-walled carbon nanotubes (MWCNT) and other nanomaterials has been shown to disrupt endothelium-dependent arteriolar dilation in the peripheral microcirculation. The molecular mechanisms behind these arteriolar disruptions have yet to be fully elucidated. The secreted matricellular matrix protein thrombospondin-1 (TSP-1) is capable of moderating arteriolar vasodilation by inhibiting soluble guanylate cyclase activity. We hypothesized that TSP-1 may be a link between nanomaterial exposure and observed peripheral microvascular dysfunction. To test this hypothesis, wild-type C57B6J (WT) and TSP-1 knockout (KO) mice were exposed via lung aspiration to MWCNT or a sham dispersion medium control. Following exposure (24hrs), arteriolar characteristics and reactivity were measured in the gluteus maximus muscle using intravital microscopy (IVM) coupled with microiontophoretic delivery of acetylcholine (ACh) or sodium nitroprusside (SNP). In WT mice exposed to MWCNT, skeletal muscle TSP-1 protein increased > 5-fold compared to sham exposed, and exhibited a 39% and 47% decrease in endothelium-dependent and independent vasodilation, respectively. In contrast, TSP-1 protein was not increased following MWCNT exposure in KO mice and exhibited no loss in dilatory capacity. Microvascular leukocyte activation was measured by assessing third order venular leukocyte adhesion and rolling activity. The WT + MWCNT group demonstrated 223% higher leukocyte rolling compared to WT + SHAM controls. TSP-1 KO animals exposed to MWCNT showed no differences from WT + SHAM control. These data provide evidence that TSP-1 mediates, in part, the systemic microvascular dysfunction in the periphery that follows pulmonary ENM exposure.

2720 Role of Matrix Metalloproteinases (MMPs) in Multi-Walled Carbon Nanotube (MWCNT) Exposure-Induced Inflammatory Responses

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The mechanism by which MWCNT inhalation exposure causes extrapulmonary health effects is currently poorly understood. We recently demonstrated that MWCNT could generate circulating bioactivity that was dependent on MMP-9 and interactions between circulating ligands and the scavenger receptor/immunomodulator CD36, which ultimately led to endothelial dysfunction and impaired vasodilation. In the present study, we interrogated endothelial cell inflammatory responses and the potential for a major endogenous CD36 ligand, thrombospondin-1 (TSP-1), to drive such systemic responses to pulmonary MWCNT exposure. We exposed C57BL/6 (WT) and MMP-9 (MMP-9$^{-/-}$) knockout mice to 0 (dispersion media; DM), 10 or 40μg MWCNT via pharyngeal aspiration in an acute or repeated, subchronic exposure design. Acutely, mice were exposed once to 0, 10, or 40μg of MWCNT and euthanized 4 or 24h post-aspiration. For the subchronic exposure, mice were subjected to either one dose of 40μg MWCNT or DM, followed by weekly doses of DM; or once weekly doses of 10μg MWCNT and euthanized 4 weeks post initial aspiration. Bronchoalveolar lavage (BAL) fluid and serum MMP levels, serum TSP-1 and 4-hydroxy-2-nonenal (4HNE) (oxidative stress marker), and markers of neuroinflammation were evaluated via immunoblotting. MMP activity was assessed via zymography and vascular and neuroinflammatory gene expression were evaluated via qPCR. Acute 10μg MWCNT exposure resulted in a 90% increase in serum TSP-1 in WT mice compared to DM controls. No significant increase in TSP-1 was observed in MMP-9$^{-/-}$ mice with 4h MWCNT exposure. TSP-1 was increased 136% in MMP-9$^{-/-}$ and 94% in WT serum with repeated exposure to 10μg MWCNT compared to 57% and 24% respectively following a single 40μg MWCNT exposure. Serum 4HNE levels were unchanged by MWCNT exposure. Serum from both WT and MMP9$^{-/-}$ mice exhibited inflammatory bioactivity, however, suggesting that MMP9-cleaved ligands may drive vasodilatory impairments, but not endothelial inflammatory responses. These preliminary results suggest that MWCNT exposure induces serum TSP-1 in an MMP-9 independent manner, but it is unlikely that TSP-1 drives the cumulative circulating inflammatory potential induced by MWCNT. *(Supported by NIOSH grant #OH010828)*

2721 Investigation of the Role of Osteopontin in Pulmonary Granuloma Formation and Fibrosis following Carbon Nanotube Exposure

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Pulmonary exposure to multi-walled carbon nanotubes (MWCNT) is known to induce granuloma formation with associated fibrosis. Granuloma formation following a pulmonary exposure is dependent on osteopontin (OPN) production by regulating macrophage accumulation. Levels of OPN are increased in silicosis, tuberculosis, and asbestos-related pathologies and circulating levels may serve to elucidate disease progression. In this study, we investigated the role of OPN in the fibrotic and granulomatous response following MWCNT exposure. Wild-type (WT) or OPN-deficient (OPN-/-) mice were exposed by oropharyngeal aspiration to vehicle (DM; 0.6 mg/ml mouse serum albumin and 0.01 mg/ml DPPC) or 40 μg of MWCNT, a dose known to induce granulomas, and sacrificed 1, 7, and 28 d post-exposure. Pulmonary OPN relative mRNA expression (peaked at 7 d) and protein levels were induced in wild-type mice following MWCNT exposure. No detectable levels of OPN were found in the OPN-/- mice, verifying the knockout model. With OPN deficiency, microgranuloma incidence (6/6 WT; 1/4 OPN-/-) and severity (1.50 ± 0.34 WT vs 0.25 ± 0.25 OPN-/-) was reduced 28 d post-exposure. Scoring of perivascular and peribronchial lymphoid infiltrates was also reduced (1.83 ± 0.17 WT vs 0.75 ± 0.25 OPN-/-). General fibrosis scored from Trichome-stained sections showed 100 % incidence in all exposed mice with no difference in severity (1.80 ± 0.20 WT vs 1.75 ± 0.25 OPN-/-). Alveolar fibrosis, measured as alveolar wall thickness (μm), from Picro-Sirius Red staining was increased due to exposure but not affected by deficiency of OPN (0.93 ± 0.05 WT/DM; 1.06 ± 0.05 OPN-/-/DM; 1.62 ± 0.07 WT/MWCNT; 1.50 ± 0.15 OPN-/-/MWCNT). Pulmonary cytotoxicity, inflammatory cell influx, and relative mRNA expression of genes related to macrophage function and recruitment (*Ccl2*, *Ccl22*, and *Arg1*) were increased as a result of MWCNT exposure but not affected by OPN deficiency. In conclusion, OPN deficiency prevented the development of granulomas but did not have a major influence on general inflammatory parameters or the development of fibrosis.

2722 Single-Walled Carbon Nanotubes Increase Influenza A Virus Infectivity through Oxidative Stress Mechanisms

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Background: Extensive application of nanomaterials has raised concerns regarding their potential health impacts. Our previous research had shown that pre-exposure of lung cells to single-walled carbon nanotubes (SWCNTs) modulated expression of several inflammatory and antiviral genes in concert with increased viral titers following subsequent exposure to influenza virus H1N1 (IAV). Evidence indicates that SWCNTs induce oxidative stress, which can impair cell function and impact on innate immune signaling pathways. To investigate possible mechanism of increased IAV infectivity by SWCNTs, we assessed the effect of oxidative stress induced by SWCNTs on innate antiviral responses in small airway epithelial cells (SAEC). Methods: Reactive oxygen species (ROS) were measured using a DCFDA method in SAEC exposed to SWCNTs (0.2-30 ug/ml) or IAV (MOI=0.5) singly and in combination for 2-6 hours. Before sequential exposures to SWCNTs (20 ug/mL) and IAV (MOI=0.5) for 24 hours respectively, SAEC were treated with the antioxidant (N-acetyl-l-cysteine (NAC)) as a means to block ROS production prior to measuring endpoints that include mRNA expression of inflammatory and antiviral genes as well as virus titers ($TCID_{50}$). Results: A dose-dependent increase of ROS production was observed in cells exposed to doses of SWCNTs ranging from 0.2 to 30 ug/mL with the lowest observed adverse effect level (LOAEL) determined to be 2.0 ug/mL. SWCNTs (20 ug/mL) synergistically produced ROS with IAV in SAEC and significantly inhibited expression of inflammatory and antiviral genes (RIG-I, MDA5, TLR3, IFNβ1, CCL5, IL8, IFIT2, IFIT3) while increasing IAV virus titers. With pre-treatment of NAC, the gene expression levels and virus titers in cells treated with SWCNTs+IAV showed no significant changes compared with those treated with IAV only. Conclusion: SWCNTs inhibited pulmonary immune responses and increased IAV infectivity in part through oxidative stress mechanisms.


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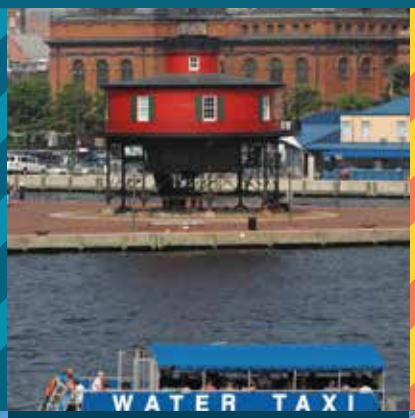

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