

**PS 2715 The Effect of Topically Applied Carbon Nanomaterial on Skin Inflammation in a Model of Allergic Contact Dermatitis**

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Carbon nanomaterials (CNM), both fullerenes and carbon nanotubes (CNT), are only available in a few consumer products, and the greatest skin exposure risk remains occupational in nature. However, CNM skin exposure could increase in the future, since present CNM research is focused on transdermal drug delivery, biomedical imaging systems, and textiles. There is limited research in the field of dermal nanotoxicity and most studies have only examined high dose exposures in healthy animal models. This study examines the effect of lower, more relevant CNM doses on the skin of both healthy mice and a dinitrofluorobenzene (DNFB) induced mouse model of allergic contact dermatitis (ACD). ACD is characterized by skin barrier disruption, swelling, and inflammation upon contact with a chemical hapten or allergen, and we hypothesize that carbon nanomaterial exposure to skin will be immunomodulatory and alter the progression of allergic contact dermatitis. Thus far, three doses (0.5, 5, 50  $\mu\text{g}/\text{cm}^2$ ) of both a long and short carboxylated multi-walled CNT have been tested in the ACD model. The skin thickness, and transepidermal water loss values are not altered by the CNT exposure, after a 24 hour exposure. However, IL-6 levels in the skin homogenate are decreased by the 5 and 50  $\mu\text{g}/\text{cm}^2$  doses, and macrophage migration from the skin is also reduced by the CNT exposure, as measured by flow cytometry. This data illustrates a possible immunosuppressive effect of CNT when applied to the skin of an ACD model, but this effect is insufficient to alter the skin morphology after a 24 hour exposure. Future work will examine sub-acute dosing of CNT and identify effects on long term dermatitis resolution. Identification of the possible hazards involved with CNM dermal application may help in exposure risk assessment and the design of safer materials. (Funding Sources: NIEHS Training Grant ES07026, NIH RO1 ES021492)

**PS 2716 Impaired Ciliogenesis in Differentiating Human Bronchial Epithelia Exposed to Nontoxic Doses of Multi-Walled Carbon Nanotubes**

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Multi-walled carbon nanotubes (MWCNTs) are nanoscale hollow fibers used for a variety of industrial and consumer products due to their unique properties. While MWCNT inhalation is known to cause inflammation and pulmonary fibrosis at high doses, the susceptibility of damaged and regenerating airways to MWCNT exposure remains unexplored. In this study, we investigate the effect of MWCNT exposure on cilia development in a differentiating air-liquid interface (ALI) model. Primary bronchial epithelial cells (BECs) were taken from healthy human donors via bronchoscopy and treated with non-cytotoxic doses (1  $\mu\text{g}/\text{cm}^2$  or less) of MWCNTs in submerged culture for 24 hours. Cultures were then allowed to differentiate in ALI for 28 days in the absence of further exposure and markers of mucociliary differentiation were compared. Whole-mount immunofluorescent microscopy of F-actin and  $\alpha$ -tubulin indicated significantly decreased cilia staining in cultures previously treated with MWCNTs. Analysis of cilia beating by high-speed motion capture also revealed significantly increased variability in beating frequency and amplitude in exposed BEC cultures after 28 days. Expression of mucociliary marker genes FOXJ1, MUC5AC, and MUC5B was unaffected by exposure. Ultrastructural imaging of ciliary axonemes showed a modest effect of MWCNT exposure on microtubule organization and ciliary architecture. Confocal imaging of actin and  $\gamma$ -tubulin staining during early ciliogenesis suggested a disruption of the cytoskeleton and impaired apical docking of basal bodies in MWCNT-exposed cells. Colocalization of CEP164 with  $\gamma$ -tubulin procentrioles was significantly reduced in days 1-3 of ciliogenesis following MWCNT exposure, suggesting that basal body synthesis may be disrupted prior to ciliary vesicle formation. The results of this study demonstrate that a brief exposure of undifferentiated or denuded epithelium to low, nontoxic doses of MWCNTs can impair normal ciliogenesis during cell differentiation, and that this effect appears to be driven by interactions with the cytoskeleton and basal body synthesis, rather than MWCNT effects on gene transcription.

**PS 2717 The Effects of Inhaled Multi-Walled Carbon Nanotube Exposure on Signaling Pathways Associated with Vascular Disease in Wild Type Mice**

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Cardiovascular disease (CVD), including coronary artery disease (CAD), contributes to the leading cause of death in the US, and is becoming a global risk as well. An emerging field, nanotechnology, has recently been implicated in contributing to CVD. The use of nanomaterials is becoming commonplace in its environmental, commercial, and therapeutic applications. While ongoing research has revealed that nanoparticle-exposure is associated with cardiovascular toxicity, the molecular signaling mechanisms involved have not yet been fully elucidated. When considering the etiology of CAD, it is likely that pathways that mediate expression of pro-atherosclerotic factors, such as oxidized low density lipoprotein (oxLDL) signaling, as well as those involved in vascular remodeling, such as matrix metalloproteinase (MMP)-9, are involved in nanoparticle-mediated vascular toxicity. As such, we investigated the hypothesis that inhalation exposure to multi-walled carbon nanotubes (MWCNTs) results in alterations in oxLDL signaling in the vasculature, via the oxLDL lectin-like receptor (LOX-1) and cholesterol efflux receptors (ABCA1), resulting in increased reactive oxygen species (ROS) and MMP-9 expression. To test this hypothesis, 2mo old male C57Bl/6 mice were exposed for 6 h/day for 14 consecutive days to atmospheres containing 1  $\text{mg}/\text{m}^3$  MWCNTs or filtered air (FA, n=7). Our results show increased ROS expression and MMP-2/-9 activity is within the systemic (aortic) vasculature of MWCNT-exposed mice using DHE staining and *in situ* zymography, respectively. Furthermore, immunofluorescence of coronary vascular endothelial expression of LOX-1 was found to be significantly upregulated (1.4-fold induction), while ABCA1 expression was decreased (0.75-fold reduction) in MWCNT-exposed hearts, compared to FA controls. This data suggests that LOX-1, ROS, and dysregulated MMP activity may be involved in MWCNT-mediated vascular toxicity. (Funding for this research was provided by R00 ES0126586 and UNT RIG Grant GA9306 to AKL)

**PS 2718 Osteopontin Modulates SWCNT-Induced Pulmonary Fibrosis Upstream of the TGF- $\beta$ 1 Signaling**

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It has been shown by a number of *in vivo* studies that pulmonary exposure to carbon nanotubes may lead to acute local inflammatory response, pulmonary fibrosis and granulomatous lesions. Among factors that play direct role in initiation and progression of fibrotic process are epithelial-mesenchymal transition and myofibroblasts recruitment/differentiation, mediated by the transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1). Yet, other contributors to the TGF- $\beta$ 1 associated signaling, such as osteopontin (OPN) has not been fully investigated. To explore this issue, OPN-knockout female mice (OPN-KO) along with their wild-type (WT) counterparts were exposed to single-walled carbon nanotubes (SWCNT) (40  $\mu\text{g}/\text{mouse}$ ) via pharyngeal aspiration and pulmonary fibrotic response was assessed 1, 7 and 28 days post-exposure. Simultaneously, RAW 264.7 and MLE-15 cells were treated with SWCNT (24 hours, 6  $\mu\text{g}/\text{cm}^2$  to 48  $\mu\text{g}/\text{cm}^2$ ) or bleomycin (0.1  $\mu\text{g}/\text{ml}$ ) in the presence of OPN-blocking antibody or isotype control and TGF- $\beta$ 1 was measured in supernatants. Diminished lactate dehydrogenase activity and protein levels at all time points, along with less pronounced neutrophil influx 24 h post-exposure, were registered in broncho-alveolar lavage fluid (BALF) of OPN-KO mice compared to WT mice. Pro-inflammatory cytokines release (IL-6, TNF- $\alpha$ , MCP-1) was also reduced. A significant two-fold increase in TGF- $\beta$ 1 was found in BALF of WT mice at 7 days, while TGF- $\beta$ 1 levels in OPN-KO animals remained unaltered. Histological examination revealed marked decrease in granulomas formation and less collagen deposition in the lungs of OPN-KO mice compared to WT. RAW 264.7 and MLE-15 cells exposed to SWCNT and bleomycin had significantly less TGF- $\beta$ 1 released in the presence of OPN-blocking antibody. Thus, we believe that OPN is important in initiating the cellular mechanisms that produce an overall pathological response to SWCNT and it acts upstream of TGF- $\beta$ 1. Further investigation to understand the mechanistic details of such interactions is critical to predict outcomes produced by carbon nanotubes pulmonary exposure.



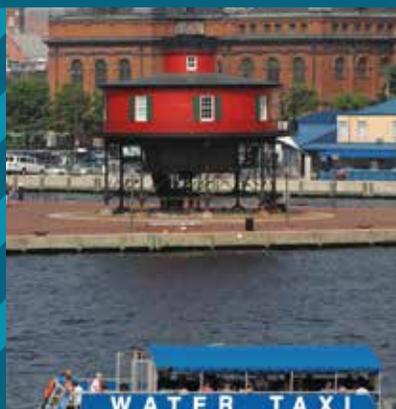
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