PS 1253 Enhanced Influenza Virus Infectivity through Suppression of Toll-Like Receptor Activity by Single-Walled Carbon Nanotubes

X. Zheng[1], J. Loeb[1], J. Lednicky[1], N. B. Saleh[2] and T. Sabo-Attwood[1]. *[1]University of Florida, Gainesville, FL and [2]University of Texas at Austin, Austin, TX.*

Despite recent advancement in manipulating nanomaterials and their growing application in a wide variety of fields, sound understanding regarding toxicity associated with potential exposures is still urgently needed. Single-walled carbon nanotubes (SWCNTs), allotropes of carbon with a cylindrical structure that share a resemblance to asbestos, raise concerns regarding long-term adverse health effects associated with inhalation. This underscores the critical need to comprehend how SWCNTs impact the respiratory system. While numerous toxicological studies have focused on fibrosis, cancer, and exacerbation of asthma, the ability of SWCNTs to modulate infectivity of pathogens has been minimally explored. Our recent work has indicated that SWCNTs increase influenza virus infectivity in small airway epithelial cells (SAECs) and suppress anti-inflammatory and -viral genes. To decipher the molecular mechanisms driving viral infectivity, we investigated whether SWCNTs mediate TLR3, a receptor that recognizes viral dsRNA as the first line of defense. Our hypothesis was that SWCNTs reduce TLR activity, resulting in inhibition of downstream anti-inflammatory and -viral genes mediated by transcription factors NF-kB and/or IRFs. For these studies, SAECs were exposed to Poly (I:C), a TLR3 agonist, singly and following pre-treatment with SWCNTs. TLR3 activity and gene expression were measured by luciferase reporter assays and qRT-PCR, respectively. The results demonstrated that SWCNTs did not alter TLR3 activation alone, but suppressed TLR3 activity by Poly (I:C) via NF-kB and IRFs in a dose-specific manner. SWCNTs also repressed genes induced by Poly (I:C), including IFIT2/3, CCL5 while further stimulating IL8. Collectively, these data suggest that SWCNTs suppress the innate immune response to viruses in lung cells, rendering them more susceptible to infections. Our study highlights a novel mechanism of SWCNT toxicity.

PS 1254 Intratracheal Exposure of Multiwalled Carbon Nanotubes Induces a Nonalcoholic Steatohepatitis in C57BL/6J Mice

J. Kim[1], Y. Jang[1] and M. Cho[1, 2, 3]. *[1]Laboratory of Toxicology, BK21 PLUS Program for Creative Veterinary Science Research, Research Institute for Veterinary Science and College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea, [2]Graduate School of Convergence Science and Technology, Seoul National University, Suwon, Republic of Korea and [3]Graduate Group of Tumor Biology, Seoul National University, Seoul, Republic of Korea.*

The effects of multi-walled carbon nanotubes (MWCNTs) exposure have been received attention from the field of public health, due to the high aspect ratio of MWCNTs. Because obesity prevalence increases all over the world, nonalcoholic fatty liver disease (NAFLD) is currently the most common prevalent liver disease and is regarded to be a component of metabolic syndrome, which is a cluster of disorders that also includes dyslipidemia, diabetes mellitus, arteriosclerosis, and hypertension. Exposure to MWCNTs is known to be a risk factor for respiratory and cardiovascular diseases, but its effect on NAFLD is unknown. In this study, we investigated the effects of intratracheal exposure of two different types of MWCNTs, namely, pristine multi-walled carbon nanotubes (PMWCNTs) and acid-treated multi-walled carbon nanotubes (TMWCNTs), on liver pathogenesis. Direct instillation of the test material into the lungs has been applied as a quantitatively reliable alternative method of inhalation exposure. The 10% weight loss dose was assessed in 3 months of subchronic study and is defined here as the maximum tolerated dose (MTD) of PMWCNTs and TMWCNTs; by this metric, MTD for a 1-year exposure of MWCNTs was determined to be 0.1 mg/mouse. Mice exposed to PMWCNTs and TMWCNTs for 1 year, developed a nonalcoholic steatohepatitis (NASH)-like phenotype, characterized by inflammation, hepatic steatosis, and fibrosis. In addition, PMWCNTs induced a more severe NASH-like phenotype than TMWCNTs, which was shown as consistent up-regulation of interleukin (IL)-6 and plasminogen activator inhibitor (PAI)-1. Impaired cholesterol homeostasis, overexpression of NF-κBp65, and suppression of peroxisome proliferator-activated receptor gamma (PPARγ) in the liver were also identified.

PS 1255 Single-Walled Carbon Nanotubes (SWCNT) Mediate Cytotoxicity via Apoptosis and Initiate Collagen Production in Rat Pleural Mesothelial Cells

S. B. Kallakuri and J. M. Cerreta. *PHS, St. John's University, Queens, NY.*

Biological systems are more frequently encountering nanoparticles such as single-walled carbon nanotubes as their production and use increase. SWCNT can become airborne due to their size and can easily be inhaled. Although a number of studies have been carried out to date, little is known about the SWCNT toxicity to pleural mesothelial cells. In the present study, the toxicity caused by SWCNT in rat pleural mesothelial cells (RPMC) and the possible cellular pathway involved was investigated. Confluent cultures were exposed to increasing concentrations (1.56, 3.125, 6.25, 12.5, 25, 50, 100, 200, 400 and 800μg/ml) of SWCNT for 24hrs. A MTT assay demonstrated that at all SWCNT concentrations there was a significant decrease in cell viability (10.4%, 13.3%, 21.1%, 31.3%, 34.9%, 35.9%, 39.2%, 41%, 48.8% and 62.1% decrease respectively) with the calculated LC_{50} value being 476μg/ml. Phase-contrast microscopy of SWCNT-treated groups revealed cell shrinkage, rounding and detachment of RPMC. At concentrations of 25, 50 and 100μg/ml of SWCNT, loss of microvilli and attachment of nanotubes to RPMC were observed by SEM. Cultures exposed to a concentration of 400μg/ml SWCNT had increase in the activity of caspase 8 (111%, $p<0.0001$) and caspase 9 (113.7%, $p<0.001$). Caspase 3 activity was determined in cultures exposed to 400μg/ml SWCNT and indicated an increase (59.9%, $p<0.0001$) compared to control cultures. 10μM of caspase 3 specific inhibitor, Z-DEVD-*fmk*, had significant protective effect on RPMC co-treated with 400μg/ml SWCNT (41.3% increase, $p<0.05$). The hydroxyproline levels were increased at a treatment concentration 6.25μg/ml SWCNT (72 ± 9.9μg/ml, $p<0.001$) compared to control cultures (34.4 ± 9.1μg/ml). Cytochrome C levels in the cytosolic fraction were increased from 0.6 ± 0.1ng/ml in the controls to 3.8 ± 0.3ng/ml ($p<0.0001$) in 400μg/ml SWCNT treated cultures. These data indicate that SWCNT initiate apoptosis via both the extrinsic and mitochondrial-mediated pathway and increase the production of hydroxyproline, a marker for collagen production in RPMC.

PS 1256 Pathology-Directed Mass Spectrometry Determines Proteomic Differences Associated with Carbon Nanotube Exposure in the Lung

E. H. Seeley[1], G. Boyce[1], L. Bishop[2], R. Salmen[2], L. A. Battelli[2], P. C. Zeidler-Erdely[2], J. M. Antonini[2], G. W. Kilby[1] and A. Erdely[2]. *[1]Protea Biosciences Inc, Morgantown, WV and [2]NIOSH, Morgantown, WV.*

Multi-walled carbon nanotubes (MWCNT) have tremendous industry application however, their unique physicochemical properties may pose health risks after inhalation. For insight into the adverse mechanisms associated with MWCNT deposition in the lung, pathology-directed mass spectrometry profiling was used; a method to identify regionally-specific alterations in proteins, lipids, and metabolites. Mice were exposed by oropharyngeal aspiration to vehicle or MWCNT (10 or 40 ug). Lungs were perfused 24 h post-exposure, inflated with 50% OCT, snap frozen and sections collected on MALDI targets with a serial section stained with H&E. Areas of interest (alveolar and terminal bronchial areas, regions with MWCNT deposition, and regions in treated mice without MWCNT deposition; 50 um in diameter; n=15-20 per section per area) were annotated on H&E stained sections and matched digitally to the corresponding serial section for mass spectra collection to determine proteomic differences. Principal component analyses showed separation of sham from MWCNT-exposed animals and in treated mice in areas with and without deposition. Genetic algorithm classification models indicated spectral classification accuracies of 85% for control, 80% for MWCNT deposition, and 80% for treatment with no deposition. Spectral data (2-40 kDa) indicated numerous peaks separating areas of interest. For example, m/z 10165 and 12973 were increased in areas of MWCNT deposition whereas m/z 3954 was decreased as a result of exposure irrespective of deposition. Segregation was evident in the exposed group as areas without obvious deposition, which either showed no alteration compared to sham (m/z 9450) or the response was blunted compared to areas of obvious deposition (m/z 17097). This methodological approach not only reveals a dynamic range of alterations due to exposure but provides context regionally with respect to MWCNT deposition, thus providing insight into specific mechanisms of MWCNT-induced lung pathology.

The Toxicologist

Supplement to *Toxicological Sciences*

54th Annual Meeting and ToxExpo™

March 22–26, 2015 • San Diego, California

The Official Journal of the Society of Toxicology

OXFORD UNIVERSITY PRESS

ISSN 1096-6080
Volume 144, Issue 1
March 2015

www.toxsci.oxfordjournals.org

SOT | Society of Toxicology

Creating a Safer and Healthier World by Advancing the Science of Toxicology

www.toxicology.org