2717 Cellular Uptake and Toxicity of Ultrasmall Superparamagnetic Iron Oxide Nanoparticles (USPION) in a Vascular Cell Model

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USPION exhibit physicochemical properties that are advantageous for theranostic biomedical products, such as intravenously administered medical imaging contrast agents; however, the toxicity mechanisms are not completely elucidated. Therefore, the goal of this study was to evaluate cellular interactions of USPION (PVP-coated) on human coronary artery endothelial cells (HCAECs) as a vascular cell model. USPION had an average size of ~20 nm, were negatively charged, and of spherical shape as assessed by transmission electron microscopy (TEM), dynamic light scattering, and zeta potential analyses, respectively. Using the alamar blue (AB) assay, cells treated with 25 or 200 µg/mL exhibited decreased cell viability to ~80% and 50% of controls, respectively, after 6 h of USPION exposure. Cellular uptake was evaluated by TEM after 3 h exposure of HCAEC to 25 µg/mL USPION. To evaluate the role of reactive oxygen species (ROS) production on cytotoxicity, cells were incubated with DCFDA dye, exposed to 25, 50, 100 or 200 µg/mL of USPION for 6 and 16 h, and fluorescence emission was evaluated by fluorescence spectrophotometry and microscopy. To further determine the role of ROS, HCAEC were pretreated with the antioxidant N-acetyl-cysteine (0.25, 0.5 or 1 mM) for 1 h prior to USPION (100 µg/mL) treatment, and cell viability was assessed by AB. Internalization of USPION in secondary lysosomes and perinuclear localization was observed as early as 3 h of exposure. No ROS production was apparent using spectrophotometric DCFDA assay and fluorescence was detected in only a few cells exposed to 200 µg/mL USPION, with signal interference caused by particle overload in the cytoplasm. Our results indicate that there is no evidence of increased ROS production after USPION exposure nor increased cell viability by pretreating HCAEC with NAC prior to USPION exposure; thus, the toxicity mechanism appears unrelated to ROS production under the experimental conditions used.

2718 Single-Walled Carbon Nanotubes Inhibit RIG-I/MAVS Innate Immune Pathway through Oxidative Stress *In Vitro*

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Despite of extensive application, nanomaterials has also raised concerns regarding their potential health impacts. Previous research showed that pre-exposure of lung epithelial cells to single-walled carbon nanotubes (SWCNT) modulated expression of several inflammatory and anti-viral genes in concert with increased viral titers following subsequent exposure to influenza A virus H1N1 (IAV). But the mechanisms of increased IAV infectivity by SWCNT remained unclear. Evidences indicated that SWCNT can induce oxidative stress which would have an impact on innate immune signaling pathways. Thus, in the present study, we assessed the effect of oxidative stress induced by SWCNTs on retinoic acide-induced gene I (RIG-I)/mitochondrial antiviral signaling (MAVS) signaling pathway in small airway epithelial cells (SAEC). Reactive oxygen species (ROS) were measured using a DCFDA method in SAEC exposed to SWCNT (0.2-30 ug/mL) or IAV (MOI=0.1-5.0) singly and in combination. Mitochondria respiration of SAEC was measured using a Seahorse assay following exposure to SWCNT for 24 hours. Antioxidant N-acetyl-l-cysteine (NAC) was applied alongside the sequential exposures to SWCNT and IAV. ROS production, RNA expression of inflammatory and antiviral genes, virus titers ($TCID_{50}$), and immunofluorescence of mitochondria, RIG-I and MAVS protein was measured. A dose-dependent increase of ROS production was observed at 0.2-50 ug/mL of SWCNT while the co-treatment of NAC blocked ROS from SWCNT. Mitochondrial respiration by Seahorse assay showed cell energetics was not affected by SWCNT at the tested dose range. SWCNT significantly inhibited expression of inflammatory and antiviral genes (RIG-I, MDA5, TLR3, IFNβ1, CCL5, IL8, IFIT2, IFIT3) and repressed the signalosome formation of MAVS while increasing IAV virus titers. With co-treatment of NAC, the gene expression levels, MAVS signalosome formation and virus titers in cells treated with SWCNT+IAV showed no significant changes compared with those treated with IAV only. SWCNT inhibited RIG-I/MAVS signaling and increased IAV infectivity in part through oxidative stress mechanisms.

2719 Time-Course Relationships between Airway Inflammation, Circulating Exosome Formation, and Systemic Vascular Responses following Pulmonary Multiwall Carbon Nanotube Exposures

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We previously reported that short-term MWCNT exposure produces serum bioactivity that impairs endothelial function leading to vasodilatory insufficiencies, as well as induction of blood-brain barrier (BBB) impairments. The mechanisms underlying observed systemic effects are incompletely understood and the contribution of extracellular vesicles, or exosomes, to the systemic delivery of lung-derived inflammatory mediators was explored in this study. We hypothesized that the systemic effects of MWCNT were mediated via exosomal delivery of pulmonary-derived bioactivity in serum. To test this hypothesis, male wild-type C57BL/6 mice (6-8 weeks) exposed to 0 (dispersion media; DM), 3 or 10 µg MWCNT via oropharyngeal aspiration were euthanized at 1, 3 or 7 days post-exposure. Bronchoalveolar lavage fluid (BALF), serum and tissues were collected. Inflammatory cytokine/chemokine expression in BALF was determined by electrochemiluminescence. Serum bioactivity (with and without exosomal fractions) was assessed via 1) serum cumulative inflammatory potential (SCIP) assay on mouse brain endothelial cells (MBEC) and 2) myography using naïve thoracic aorta from male C57BL6 mice incubated with 1% serum from exposed mice to evaluate vasodilatory changes. Analyses of BALF inflammatory cytokines revealed dose- and time-dependent increases in IL-1β, IL-4, IL-5, TNF-α and KC/GRO. Maximal neutrophil influx was observed 1d following the 3 µg dose, but at 3d following the 10 µg dose. A significant increase in TNF-α gene expression was observed with serum containing the exosomal fraction when compared with exosome depleted fractions and DM controls. No significant BBB changes were observed using sodium fluorescein cerebral uptake assay. Further evaluations of BBB integrity will be done via immunohistochemistry. Evaluation of other systemic parameters are ongoing. These preliminary findings suggest that MWCNT pulmonary exposure induces inflammatory activation in the lungs of C57BL6 mice in a dose- and time-dependent manner and may produce serum bioactivity via exosomal delivery.

2720 DHA-Supplemented Diet Reduces Nanoparticles-Induced Lung Inflammation with Methylation Changes

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Nanoparticles such as multi-walled carbon nanotubes (MWCNT) have many benefits and are used for many different purposes, such as medical and personal products. Exposure to MWCNT have been found to cause inflammation and lung disease, but the mechanism is still unclear. It is becoming increasingly evident that dietary and environmental influences can result in physiological changes through epigenetics. Epigenetic alterations are known to hold substantial potential as biomarkers for environmental exposures; this, in turn, may provide insight into mechanisms of environmentally related diseases and allow for a better understanding of disease etiology. Therefore, we utilized a murine model to determine the effect of docosahexaenoic acid-supplemented diet (DHA-SD) on MWCNT-induced inflammation, lung disease, and epigenetic changes. Balb/c mice were fed with normal diet or 50uM DHA-SD for 4 weeks, then both normal and DHA diet-mice were exposed to either dispersion media, 50 ug FA-21 (high Ni-MWCNT), or silica (SiO_2) as a positive control via oropharyngeal instillation. One week post-exposure to particles, lung and blood were harvested for analyses of pathology and epigenetic alterations. Laser Scanning Cytometry and pyrosequencing assay were used to measure airway thickness in lung tissue and methylation changes in inflammation and fibrosis-related genes, respectively. SiO_2 and FA-21 induced significant airway thickness, and these thicknesses decreased in the groups with the DHA-SD. Both SiO_2 and FA-21 induced hypomethylation in the promoter of *INFγ*, but there was no significant effect of DHA-SD on the methylation changes of *INFγ*. *Thy-1* promoter was hypermethylated in the lung after exposure to SiO_2 or FA-21, and DHA-SD reduced the methylation levels of *Thy-1* in both particle groups. There were no significant methylation changes in blood DNA of mice exposed to particles and/or DHA-SD. Obtained results suggest that DHA-SD can have anti-inflammatory effects with methylation changes.

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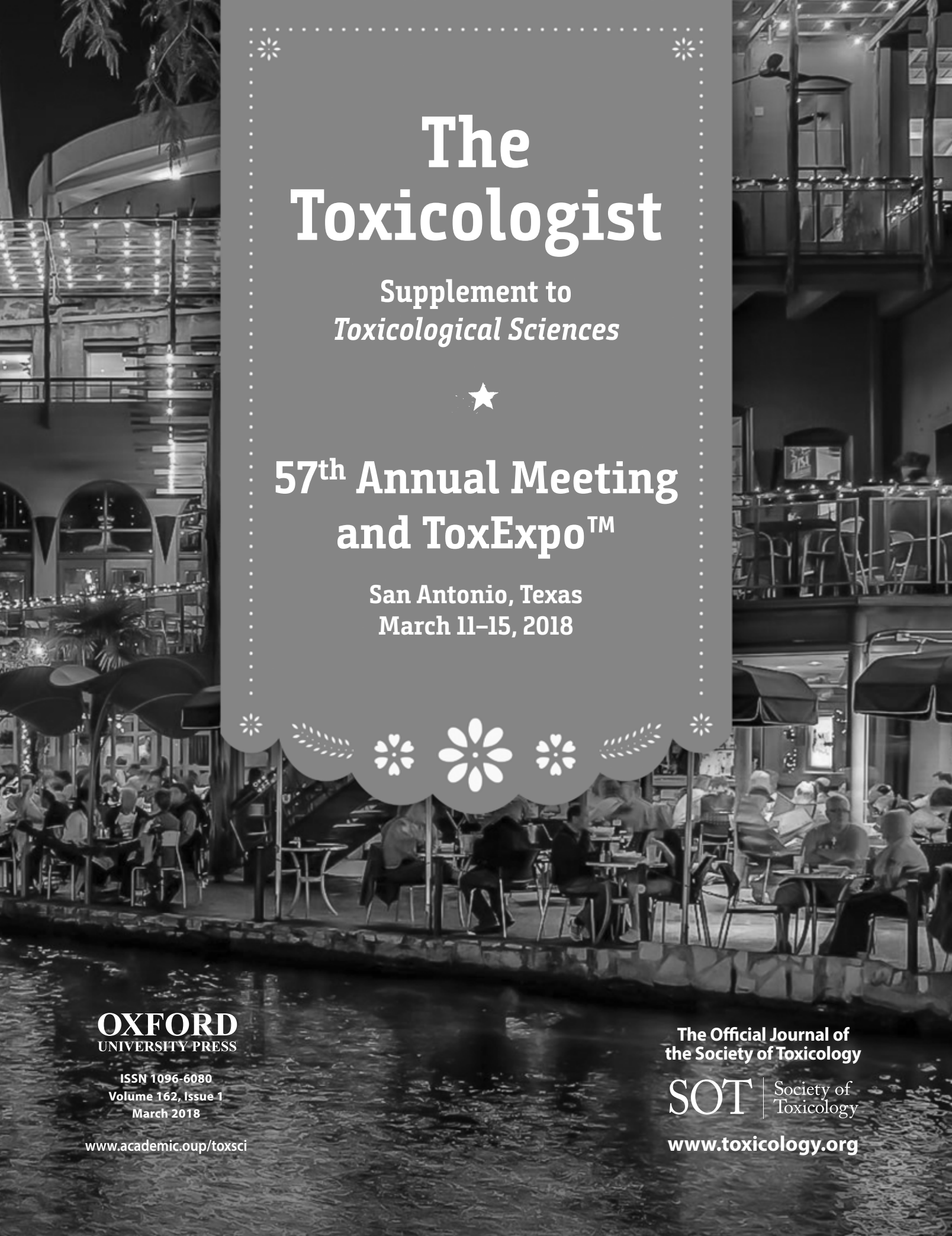

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