3491 Toxicity of Carbon Nanotubes *In Vivo*: Biochemical and Histopathological Parameters

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Carbon nanotubes (CNT) are cylindrical nanoparticles with biomedical applications due to their ability to be functionalized with chemical substances. However, toxicity in *in vivo* models is not yet fully established and the results are contradictory. The objective of this project was to evaluate the toxic effect of different NTC *in vivo* model using biochemical and histopathological parameters. For this purpose, CNTs were synthesized by spray pyrolysis (UP-CNTs) and purified (P-CNTs) using sonication for 48 h. The P-CNTs were functionalized with fluorescein isothiocyanate (FITC-CNTs). Balb/c mice were exposed to a single intravenous dose of 60 µg/mouse: NP-NTC, P-NTC, FITC-NTC; or 0.2% pluronic F-127 as vehicle control. The toxicity testing was performed by determination of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), urea and creatinine metabolites in venous blood samples before exposure to the different CNTs, and 24 h, 14, 29 and 60 days post-exposure. The histopathological damage was assessed by dissecting and histological analysis of sections from liver, lung, kidney and spleen. The results showed high toxicity in animals exposed to UP-CNTs compared with other CNTs, showing extensive lesions in the lungs, kidneys and liver. Biochemical parameters in this group had a significant increase, related to hepatotoxicity and kidney damage. In the case of animals exposed to FITC-CNTs, these revealed toxic effects to a lesser degree, it was attributed to FITC. However, mice exposed to the P-NTC showed no significant minimal differences compared to control. These results suggest that the purification is a determining factor *in vivo* toxicity of CNTs, postulating the P-CNTs as potential candidates for biomedical applications.

3492 Multi-Walled Carbon Nanotube (MWCNT)-Induced Fibrogenic Signaling in a Human Lung Epithelial-Fibroblast Co-Culture System

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It has been established that MWCNT induce pulmonary fibrosis in *in vivo* models. *In vitro* studies of fibroblasts in monoculture have also investigated MWCNT toxicity. However, the role of other cell types and cellular communication in the mechanisms of MWCNT-induced fibrosis needs further study. Our lab previously elucidated inflammatory and vascular effects of MWCNT exposure in an epithelial-endothelial co-culture model; here, we employ a system to examine fibrogenic responses to MWCNT. In this study, we assessed the effects of pristine MWCNT at an occupationally relevant dose on fibroblasts grown in a transwell co-culture system of apical Beas-2B human bronchial epithelial cells and basolateral WI-38 normal human lung fibroblasts. To address the impact of cell-to-cell communication between the epithelium and underlying fibroblasts on pulmonary fibrosis, we first measured chemokine (C-C motif) ligand 2 (CCL2) concentrations by ELISA in monoculture and co-culture conditioned media. CCL2 levels were significantly elevated in the basolateral chamber of MWCNT-exposed cells in co-culture, without similar changes in WI-38 monoculture or unexposed WI-38 grown in the co-culture system. CCL2 has been shown to be increased and upregulated in patients with idiopathic pulmonary fibrosis, usual interstitial pneumonia, and bronchiolitis obliterans syndrome, and responsible for fibroblast production of TGF-β and procollagen. In further support, MWCNT-exposed WI-38 in co-culture exhibit increased collagen type I, a hallmark of fibrosis, as compared to unexposed WI-38 in co-culture. These results suggest that the epithelial-fibroblast co-culture system may be more reflective of the fibrotic disease state instead of monoculture systems, and may allow us to further elucidate the mechanism of MWCNT-induced fibrosis *in vitro*.

3493 Thirteen-Week Nose-Only Inhalation Study in Rats of Graphistrength© C100 Multiwalled Carbon Nanotubes (MWCNT) with 13- and 52-Week Recovery Periods

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Graphistrength© C100 provides superior electrical and mechanical properties for various applications. Graphistrength© C100 is formed of MWCNT (ca. 12 walls, outer mean diameter ca. 12 nm, length ca. 1 µm) agglomerated in particles with a granulometry centered on 400 µm. After investigating different parameters for the aerosol generation, male and female Wistar rats were exposed by nose-only inhalation (6h/day, 5d/week) to target concentrations of 0.05, 0.25 and 5.0 mg/m3 air of a respirable aerosol (MMAD < 3 µm) and sacrificed 24 hours after 4 weeks and 24 hours, 13 and 52 weeks after 13 weeks of exposure. Clinical, biological and histological evaluations were performed according to the OECD TG 413. Broncho-alveolar lavage fluid (BALF) was also collected and analyzed for cytokines and inflammatory parameters. Deposition of black particles (MWCNT) in lungs was observed at all test concentrations. An inflammatory lung reaction was observed in all rats exposed to 5.0 mg/m3. It was characterized by changes in the cytological, biochemical and cytokine parameters of BALF, an increase in the neutrophil blood cells count, an increase of the lung weight, an interstitial inflammation mainly around the alveolar ducts at the bronchiole-alveolar junction and a cell hypertrophy/hyperplasia in the terminal and respiratory bronchioles. The slight changes in BALF parameters observed at 0.25 mg/m3 recovered after a 13-week treatment-free period. Signs of lung clearance of the MWCNT were observed at 0.05 and 0.25 mg/m3. No pathological changes were observed on the pleura nor was there any brain translocation via the olfactory bulb. In conclusion, a lung inflammation characteristic of overload with insoluble particles was observed after a 90-day exposure to 5.0 mg/m3 of Graphistrength© C100, a No-Observed Adverse Effect Concentration (NOAEC) of 0.25 mg/m3 was established for the repeated-dose toxicity. Published in: Pothmann D et al. Lung inflammation and lack of genotoxicity in the comet and micronucleus assays of industrial multiwalled carbon nanotubes Graphistrength © C100 after a 90-day nose-only inhalation exposure of rats. Particle and Fibre Toxicology 2015, 12:21.

3494 Acute Phase Inflammatory Responses in Mice after Pulmonary Exposure to Carbon Nanotubes Functionalized by Zinc Oxide Thin Film Coating

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Introduction. Multi-walled carbon nanotubes (MWCNT) are used for a wide range of applications in material fabrication, electronics and medicine. Despite these benefits, MWCNTs cause lung inflammation and fibrosis in mice after inhalation exposure, suggesting a possible human health hazard. Atomic layer deposition (ALD) is a method for applying conformal nanoscale coatings to enhance/alter surface properties. We previously reported that ALD coating of MWCNTs with Al2O3 reduced cytokines in human monocytes *in vitro* and decreased lung fibrosis in mice compared to uncoated MWCNTs. Here, we investigated the lung inflammatory and fibrogenic responses of monocytes (THP-1 cells) and mice to MWCNTs coated by ALD with ZnO (Z-MWCNTs). Methods. MWCNTs were coated with ZnO to yield Z-MWCNTs. THP-1 cells were assayed for cytokine mRNA levels after treatment with uncoated MWCNT or Z-MWCNTs. Mice were exposed to MWCNT or Z-MWCNTs by oropharyngeal aspiration (4 mg/kg) and evaluated for lung inflammatory cell counts, histopathology and cytokines at day 1 and 28 post-exposure. Results. Z-MWCNTs or ZnO nanoparticles enhanced IL-6, IL-1β, CXCL10 and TNF-α mRNA levels in THP-1 cells *in vitro* relative to MWCNTs at 1 day. Mice exposed to Z-MWCNT had increased lung inflammation and bronchoalveolar lavage fluid (BALF) cells. IL-6, CXCL10 and TNF-α mRNAs were increased in lung tissue by Z-MWCNTs at 1 day. However, Z-MWCNTs increased only BALF protein levels of IL-6 and CXCL10. IL-6 mRNA was increased in the heart, liver and spleen of Z-MWCNT-treated mice. Lung fibrosis was not different between mice treated with uncoated or Z-MWCNTs 28 days post exposure. Conclusions. These findings indicate that ALD coating of MWCNTs with ZnO enhances acute lung inflammation in mice and therefore, induction of IL-6 in THP-1 cells *in vitro* predicts lung and systemic responses to Z-MWCNTs *in vivo*. Funding: Supported by NIEHS grant R01-ES020897

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Preface

This issue is devoted to the abstracts of the presentations for the Continuing Education courses and scientific sessions of the 55th Annual Meeting of the Society of Toxicology, held at the New Orleans Ernest N. Morial Convention Center, March 13–17, 2016.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 603.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 629.

The abstracts are reproduced as accepted by the Scientific Program Committee of the Society of Toxicology and appear in numerical sequence. Author names which are underlined in the author block indicate the author is a member of the Society of Toxicology. For example, J. Smith.

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