This presentation reflects the views of the author and should not be construed to represent FDA's views or policies. ([1]CR grant LH12014)

PS 430 Carboxylated Multiwalled Carbon Nanotubes Induce mTOR Independent Autophagosome Accumulation in Endothelial Cells by Blockade of Autophagic Flux.

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Carbon nanotubes (CNTs) are attractive for various nanomedicine applications including their intravascular use. Therefore, the vascular biocompatibility of CNTs is a critical safety issue.
We have previously shown that in contrast to their pristine counterparts M60, carboxylated multiwalled carbon nanotubes M60COOH at 100μg/ml induced increase in the LC3B autophagosome protein marker in cultured human umbilical vein endothelial cells (HUVECs). Here we investigate a mechanism of this process. The autophagosome accumulation in M60COOH treated HUVECs was visualized by Laser Scanning Confocal Microscopy (LSCM) using immunodetection of the LC3B as well as in HUVECs transfected with Premo™ Autophagy Sensor LC3B-Green Fluorescent Protein using the baculovirus BacMam 2.0 technology. Moreover, western blotting (WB) analysis confirmed accumulation of LC3B in M60COOH treated HUVECs. The autophagosome accumulation can be caused either by induction of autophagy or by blockade of autophagic flux. The WB analysis of p62 (SQSTM1), a protein which is preferentially degraded by autophagy, showed that the induction of autophagy by serum starvation caused a significant decrease in p62 protein levels, while no change in p62 was observed in M60COOH treated HUVECs. The classical pathway of induction of autophagy involves inhibition of the mTOR kinase. The WB analysis of the mTOR substrate p-p70S6K showed no changes in levels of phosphorylation of this protein after M60COOH treatment. In addition, the LSCM kinetic study of HUVECs treated with Alexa555-conjugated M60COOH indicated the autophagic flux blockade.
Our results showed that the accumulation of autophagosomes in HUVECs induced by M60COOH likely resulted from the blockade of autophagic flux, rather than induction of autophagy. This presentation reflects the views of the author and should not be construed to represent FDA's views or policies. ([2]CR grant LH12014)

PS 431 Effects of the Protein Corona on the Interaction of Carbon Nanotubes with Blood Platelets.

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With the potential uses of carbon nanotubes (CNTs) in medicine and related CNT toxicity concerns, the importance of CNT–protein interaction studies cannot be stressed enough. We have shown that CNTs activate store operated calcium entry in blood platelets (De Paoli Lacerda SH et al, ACS Nano 2011). Here, we investigate how the PLT-activating effect of multiwalled carboxylated-CNTs (M60COOH) is influenced by interaction of M60COOH with different human proteins: albumin (HSA), fibrinogen (FBG), Gamma-globulins (HGG) and histone H1 (H1). Pymol molecular visualization was used to determine protein dimensions, electrostatic potential surfaces, and the number of aromatic residues available for binding to the M60COOH. Dissociation constants KD = 0.049, 0.053, 0.079 and 0.165 mg/mL for H1, FBG, HSA and HGG, respectively, were calculated for binding of these proteins to M60COOH. Circular dichroism revealed that the secondary structure of the studied proteins changed upon binding to M60COOH surface. Platelet (PLT) aggregometry showed that pre-incubation of M60COOH with HSA, HGG, and FBG reduced PTL-aggregating effect compared to bare M60COOH. In contrast, pre-incubation of M60COOH with H1 markedly increased their PLT-aggregating activity which was comparable to the effect of 20μM TRAP. In addition, the flow cytometry analysis of PTL membrane microparticles (CD41+CD62P+MP) showed that H1-M60COOH induced marked increase in CD41+CD62P+MP release compared to bare M60COOH. It is likely that positively charged H1 strongly interacts with the negatively charged plasma membrane of PTLs enhancing the nanopenatration of CNTs through the platelet plasma membrane. FBG leads M60COOH to self-assembly resulting in loss of the CNT surface area for interaction with PTLs which possibly explains the decrease in PLT-aggregating activity of FBG-treated M60COOH. In conclusion, binding of different proteins to CNTs greatly modulate their interactions with blood platelets. This presentation reflects the views of the author and should not be construed to represent FDA's views or policies.

PS 432 Genotoxicity of Multiwalled Carbon Nanotubes.

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Carbon nanotubes have many unique applications in industry and medicine. Although the low density and small size of carbon nanotubes makes respiratory exposures to workers likely during the production or use of commercial products, the genotoxicity is not fully investigated. We have previously shown mitotic spindle aberrations in cultured primary and immortalized human airway epithelial cells exposed to single-walled carbon nanotubes (SWCNT). In order to investigate whether mitotic spindle damage was unique to SWCNT, we examined mitotic spindle aberrations following dosing of cells to multi-walled carbon nanotubes (MWCNT) at concentrations anticipated in the workplace. MWCNT induced a dose responsive increase in disrupted centrosomes, abnormal mitotic spindles and aneuploid chromosome number. The data further showed that monopolar mitotic spindles comprised 95% of the disrupted mitoses. Cell cycle analysis demonstrated a greater number of cells in G1 and S-phase in MWCNT-treated compared to diluent control, indicating a G1/S block in the cell cycle. The monopolar phenotype of the disrupted mitotic spindles and the G1/S block in the cell cycle is in sharp contrast to the multi-polar spindle and the G2 block in the cell cycle observed in SWCNT-induced disruption. Three dimensional reconstructions showed carbon nanotubes integrated with the microtubules, the DNA and within the centrosome structure. The lower doses did not cause cytotoxicity or apoptosis 24 hours after exposure; however, after 72 hours, significant cytotoxicity was observed in the MWCNT-exposed cells. One month following exposure, MWCNT-treated cells had a dramatic increase in both size and number of colonies. Our results demonstrate significant disruption of the mitotic spindle by MWCNT at occupationally relevant doses.

PS 433 Loss of Epithelial Monolayer Integrity following Exposure of Primary Human Airway Cells to Multiwalled Carbon Nanotubes.

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Pulmonary health effects due to inhaled multi-walled carbon nanotubes (MWCNT) have been a growing concern, as MWCNT become more widely used due to their unique physical and chemical properties. Studies have implicated MWCNTs in the pathogenesis of pulmonary fibrosis and inflammation. Airway epithelia are crucial for the maintenance of airway homeostasis and epithelial injury leads to airway remodeling and inflammation. We therefore tested the effect of MWCNT on airway epithelial integrity. In vivo mouse exposure to MWCNTs induced loss of airway columnar and ciliated epithelium within 7 days, and changes consistent with airway epithelial metaplasia. We then explored the mechanism for these changes in vitro. Bronchial epithelial cells (BECs) were obtained from healthy human volunteers via bronchoscopy, grown on E10+ electrode arrays until confluent, and electrical resistance across monolayers was measured continuously for 7 days after treatment with either dispersion medium, nanographene shape control (12ug/ml), or MWCNT (3 or 12 ug/ml). MWCNT treatment induced a significant reduction in epithelial resistance over time, suggesting breakdown of monolayer integrity, as well as alterations in cell morphology, but cytotoxicity was observed only in the higher MWCNT dose. Epithelial-mesenchymal transition was ruled out by western blotting, RT-PCR and staining for relevant markers. Microarray analysis revealed that MWCNT induced significant downregulation of cornulin, cadherins, and keratins, as well as upregulation of genes involved in retinoid signaling. These results suggest that MWCNT disrupt airway epithelial integrity through cytotoxicity and metaplasia linked to by retinoid-related dedifferentiation.

PS 434 Human Pleural Mesothelial MeT-5A Cells Are a Limited *In Vitro* Model for Detection of Potential Asbestos-Like Genotoxic Effects of Multiwall Carbon Nanotubes.

C. Ziemann, S. Reamon-Büttner, A. Hackbarth, H. Brockmeyer, H. Rahmer and B. Bellmann. *Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany.* Sponsor: C. Dasenbrock.

Multiwall carbon nanotubes (MWCNT) are nanomaterials with important technological impact. But, depending on diameter and length some MWCNT may induce fiber-like toxicity/genotoxicity, similar to asbestos. Thus, a project funded by

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