PS 443 Sonolytic Degradation of Pluronic® Surfactants to Toxic Byproducts: Implications for Nanotoxicity Testing.

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Poloxamers (known by the trade name Pluronic®) are triblock copolymer surfactants that contain two polyethylene glycol blocks and one polypropylene glycol block of various sizes. Poloxamers are widely used as nanoparticle dispersants for nanotoxicity studies wherein nanoparticles are sonicated with a dispersant to prepare suspensions. It is known that poloxamers can be degraded during sonication and that reactive oxygen species contribute to the degradation process. However, the possibility that poloxamer degradation products are toxic to mammalian cells has not been well studied. We report here that aqueous solutions of poloxamer 188 (Pluronic® F-68) and poloxamer 407 (Pluronic® F-127) sonicated in the presence or absence of multi-walled carbon nanotubes (MWNTs) can became highly toxic to cultured cells. Moreover, toxicity correlated with the sonolytic degradation of the polymers. These findings suggest that caution should be used in interpreting the results of nanotoxicity studies where the potential sonolytic degradation of dispersants was not controlled.

PS 444 Effects of Carbon Black Nanoparticles on Human Pulmonary Cell Lines and Precision Cut Lung Slices.

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Carbon black nanoparticles (CBNPs) are among the most abundantly used nanomaterials and have been reported to cause adverse health effects after inhalation exposure. The aim of this study was to compare the effects of Printex® 90 and acetylene soot particles in human pulmonary cell lines (16HBE14o-, Calu-3, A549) and precision cut lung slices (PCLS) of mice, rats and humans using a wide concentration range. Particle size distribution in the cell culture medium was determined by dynamic light scattering. Viability assays were LIVE/DEAD® staining and WST-1 assay for PCLS and WST-8 and neutral red assay for cell lines. CBNP-induced formation of reactive oxygen species (ROS) was assessed in A549 and 16HBE14o- cells by flow cytometry using the DCFH-DA assay. Furthermore, the effect of CBNP exposure on the transepithelial electrical resistance (TEER) was investigated in Calu-3 cells after 24, 48 and 120h treatment with 10 and 50 µg/ml CBNPs. With PCLS, the inflammatory response was assessed by measuring pro-inflammatory cytokines (i.e. IL-1α, TNF- α, IL-8). Both CBNPs tested were not toxic in physiologically relevant concentrations. Significant cytotoxicity was observed in the WST-8 assay for both CBNPs at 50 µg/ml after 48h, whereas no effects were found in the neutral red assay. Increased ROS formation was observed with both CBNPs after 24 and 48 h. Interestingly, acetylene soot particles cause significant TEER reduction at both dose levels and all time points tested whereas Printex® 90 reduced the TEER only after 120h at the high dose. Neither Printex® 90 nor acetylene soot particles induced the secretion of proinflammatory cytokines in mouse and rat PCLS. In conclusion, the combination of in vitro and ex vivo models provides a valuable tool to assess the acute irritation and inflammation effects of CBNPs on lung tissue.

PS 445 Rho-Kinases Are Involved in Caspase-1-Mediated IL-1β Secretion following *In Vitro* Exposure to Multiwalled Carbon Nanotubes and Asbestos in Human Monocyte.

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It has been reported that fibrous particles such as asbestos and carbon nanotubes (CNT) trigger interleukin (IL)-1β release through NLRP3 inflammasome in phagocytotic cells. GTPase effector Rho-kinases (ROCK1, and 2), are known to be associated with the organization of the actin cytoskeleton during phagocytosis. In this study we examined whether ROCKs are involved in asbestos- or multi-walled CNT (MWCNT)-induced IL-1β release in human monocytic THP-1 cells. THP-1 were differentiated to macrophages by PMA and were exposed to crocidolite, MWCNT or lipopolysaccharide (LPS) in the presence or absence of Y27632 (ROCKs inhibitor) or Z-YVAD (caspase-1 inhibitor). Concentrations of IL-1β in the culture medium were measured using ELISA. Cell-associated MWCNT or asbestos were assayed by turbidimetry. Protein levels of ROCK1 and ROCK2 were analyzed by western blotting. Treatment with PMA increased expression of ROCK1, whereas that of ROCK2 was not changed in THP-1 cells. Exposure of the cells to asbestos or MWCNT provoked IL-1β secretion the secretion was suppressed by either Y27632 or Z-YVAD, whereas LPS-induced IL-1β secretion was inhibited only by Z-YVAD, but not by Y27632. These results indicate that IL-1β secretion was increased by caspase-1 activation and ROCKs are involved in both asbestos- and MWCNT-induced IL-1β secretion. On the contrary, treatment with Y27632 did not change the amount of those fibrous particles associated with the cells. To further examine the effect of ROCK1 and ROCK2 on asbestos and CNT-induced IL-1β secretion, differentiated THP-1 were transfected with siRNA to knockdown ROCKs. siRNA designed for both ROCK1 and ROCK2 decreased asbestos- or MWCNT-induced IL-1β secretion and did not change LPS-induced IL-1β secretion, indicating that ROCKs are implicated in fiber-induced inflammasome responses.

PS 446 Multiwalled Carbon Nanotubes Damage the Mitochondria to Increase Reactive Oxygen Radical Production, Activate NF-κB Signaling to Induce Inflammatory Cytokines, and Stimulate Transforming Growth Factor β1 and Platelet-Derived Growth Factor Expression to Promote Fibroblast-to-Myofibroblast Transformation.

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Carbon nanotubes (CNTs) are novel material with unique electronic and mechanical properties. Here, we report that multi-walled carbon nanotubes (MWCNT) have potent, dose-dependent toxicity on cultured human cells. Molecular characterization revealed that MWCNT induced substantial ROS production and mitochondrial inner membrane depolarization at sub-toxic doses. MWCNT stimulated the secretion of a panel of inflammatory cytokines and chemokines (TNFα, IL-1β, IL-6, IL-10 and MCP1) from macrophages (Raw264.7) by activating the canonical NF-κB signaling pathway. Activation of NF-κB signaling involves rapid degradation of IκBα, nuclear accumulation of NF-κBp65, binding of NF-κB to specific DNA-binding sequences, and transactivation of target gene promoters. Finally, MWCNTs induced the production of fibrogenic growth factors TGFβ1 and PDGF that function as paracrine signals to promote the transformation of lung fibroblasts into myofibroblasts, a key molecular step in the development of lung fibrosis. These results demonstrated that MWCNT elicit multiple and intertwining molecular signaling events involving oxidative damage, inflammatory cytokine production, and myofibroblast transformation, which potentially underlie the toxicity and fibrosis in human lungs by MWCNTs.

PS 447 Functionalization-Associated Effects of Carboxylated Fullerenes on Cellular Aging.

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The systematic evaluation of critical cellular responses such as apoptosis, cellular proliferation, reproductive clonogenicity and cell cycle responses in lung cells by distinctly functionalized fullerenes demonstrated that fullerene-mediated responses are dependent on their ability to perturb cell division that ultimately impact cellular fate. Moreover, we postulate that the observed cellular responses were charge and functionalization specific, in that the positively charged fullerenes were cytotoxic as opposed to the negatively charged fullerene. Interestingly, the negatively charged fullerenes inhibited cellular apoptosis and necrosis. On further investigation we discovered that, depending on the functionalization, the negatively charged fullerenes could induce senescence in bronchial epithelial cells, a cellular response that we have previously reported as a potential toxicological endpoint of fullerenes in dermal cells. We demonstrate that the observed non-cytotoxic or cyto-protective effect of fullerenes may in fact be due to a more novel function of fullerenes to induce premature senescence in cells. In the present study we utilized immortalized but not tumorigenic human bronchial epithelial cells (Beas-2b) and normal human-derived bronchial epithelial cells (NHBE) to perform a systematic evaluation of the effect of a suite of positively and negatively charged engineered fullerenes on key biological

The Toxicologist

Supplement to *Toxicological Sciences*

52nd Annual Meeting and ToxExpo™

March 10–14, 2013 • San Antonio, Texas

An Official Journal of the Society of Toxicology

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www.toxicology.org

OXFORD UNIVERSITY PRESS

ISSN 1096-6080
Volume 132, Issue 1
March 2013

www.toxsci.oxfordjournals.org