PS 667 ACTIVATION OF THE NLRP3 INFLAMMASOME CORRELATES WITH THE PULMONARY BIOACTIVITY OF MULTIWALLED CARBON NANOTUBES.

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Nanotechnology is one of the world's most promising new technologies. In turn, carbon nanotube production is estimated to reach into the millions of tons within the decade. In addition, surface modification of carbon nanotubes alters their charge, functionality, and reactivity; therefore extending their already broad applications. Utilizing two MWCNT samples, two hypotheses were tested in this study. First, we investigated whether MWCNTs with different surface chemistries exhibit different bioactivities in vivo. Second, we investigated if differences in bioactivity were related to activation of the NLRP3 inflammasome. To test these hypotheses, mice (C57BL/6J) were given 0-40 μg/mouse either bare (BMWCNT) or COOH-coated (FMWCNT) multi-walled carbon nanotubes via pharyngeal aspiration. The results show the BMWCNT were more bioactive in the lung, causing a more robust inflammatory and fibrotic response than FMWCNT. The BMWCT also caused significantly higher levels of the cellular mediators associated with NLRP3 inflammasome activation. Specifically, cathepsin-B activity, IL-1β, IL-18, and IL-33 levels were significantly higher in BMWCNT than FMWCNT treated mice. In conclusion, this study provides evidence that the NLRP3 inflammasome is activated in vivo, after pulmonary exposure to MWCNTs, and the severity of the activation differs from BMWCNT to FMWCNT. The results confirm that modification of the surface of the MWCNT with COOH-groups decreased the bioactivity of the MWCNT. This difference in bioactivity correlated with the activation of the NLRP3 inflammasome. Taken together, the results demonstrate that coating the MWCNT surface, without affecting their intrinsic structure, may constitute a useful strategy for decreasing MWCNT toxicity. This work was supported by NIH grant RC2-ES01872.

PS 668 CARBOXYLATED CARBON NANOTUBES INDUCE AUTOPHAGY AND APOPTOSIS IN ENDOTHELIAL CELLS.

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Carbon nanotubes (CNTs) have profound impact on the development of medical devices for intravascular use. Therefore, the vascular toxicity of CNTs is a critical safety issue. Here we investigate effects of carbon nanomaterials on cultured human umbilical vein endothelial cells (HUVEC). We prepared carboxylated multi-walled CNTs (M60(COOH)) by refluxing pristine multi-walled CNTs with a 60 nm diameter (M60) in a H2SO4/HNO3 mixture. Electron microscopy revealed that both M60 and M60(COOH) self-assemble in plasma forming rope-like structures. The CCK-8 toxicity assay in HUVEC showed that M60(COOH) at 20 μg/mL decreased cell viability to 66±5% in 24 hrs. M60 and fullerenol (C60(OH)24) induced a slight decrease of cell viability to 91±1% and 84±6%, respectively; fullerene nC60 had no effect. The annexin V/PI flow cytometry showed that M60(COOH) induced apoptosis in HUVEC. We further investigated HUVEC autophagy using Premo™ Autophagy Sensor. Laser Scanning Confocal Microscopy visualized authophagosomes in both M60(COOH) and C60(OH)24 treated cells (20 and 100 μg/mL for 24 hrs). In contrast, nC60 and M60 induced only a slight or no increase of autophagy, respectively. The results were confirmed by direct visualization of autophagosomes in the nanomaterial treated cells and using western blotting with densitometry of LC3B. Finally, we showed that autophagy inhibitor 1 mM 3-methyladenine (3-MA), which does not affect HUVEC viability alone, significantly enhanced the cytotoxic effect of M60(COOH). In conclusion, M60(COOH), but not their pristine counterparts induce autophagy in HUVEC. M60(COOH) are also more potent than M60 to induce HUVEC apoptosis. Autophagy in M60(COOH) treated HUVEC initially plays a cytoprotective role, however, consequent dysregulation of this process may contribute to the endothelial cell toxicity of CNTs. The findings and conclusions in this study have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Agency determination or policy.

PS 669 PULMONARY EXPOSURE TO GRAPHENE OXIDE AND FULLERENES CAUSES INFLAMMATION AND MODIFIES THE IMMUNE RESPONSE.

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Carbonaceous nanoparticles (CNP) have distinctive physical and chemical properties that make them useful in a wide range of applications. As the production of these particles increases, there is a growing need to explore their potentially harmful effects due to environmental and occupational exposure. Mounting evidence indicates that toxicological outcomes of CNP exposure may vastly depend on surface properties, size, shape and functionalization. In the current study, we evaluated pulmonary inflammation and systemic immune responses in mice after pulmonary exposure to structurally different CNP: pristine C60 fullerene; TRIS-functionalized C60 (C60-TRIS) and graphene oxide (GO). The inflammagenic potential of the tested CNP was found to be as follows: GO>C60-TRIS>C60, as evidenced by accumulation of PMNs, macrophages and lymphocytes as well as changes in lung permeability and inflammatory cytokine profiles in the lungs on days 1 and 7 post exposure. Further, GO and fullerenes were found to induce reactive oxygen species production by RAW 264.7 macrophages in vitro. To investigate if pulmonary exposure to CNP altered systemic immune reactivity, we tested the proliferative response of spleen T cells of exposed animals. In mice exposed to GO, T cell proliferation was decreased; however, it was increased in fullerene-exposed animals. Co-incubation of OVA-specific B3Z hybridoma T cells with OVA-loaded dendritic cells (DC) exposed to GO or fullerenes resulted in altered IL-2 production by B3Z cells, suggesting that modified T cells responses seen in vivo can be partially attributed to a direct modulation of DC functions by GO. Overall, our study shows the potential of fullerenes and GO to induce pulmonary inflammation.

PS 670 INCREASE IN LUNG NEUTROPHIL AND MACROPHAGE LEVELS IN RATS EXPOSED TO INHALED AGGLOMERATED TIO2 NANOPARTICLES.

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Recruitment of activated immune cells, such as neutrophils and macrophages is involved in the host's mechanisms of pulmonary defense against particles, whether nanometric or micrometric. Agglomeration of metal oxide nanoparticles (NP) is an important phenomenon occurring in aerosol generation, resulting most often in exposures to NP agglomerates exceeding the nanoscale (>100 nm). Thus, consequent biological responses may not represent the toxicity of NP (<100 nm) but rather that of micrometric NP agglomerates. The objective of this study was to compare the pulmonary immune cell response of rats exposed by inhalation to agglomerated TiO2 NP of different initial size. Three groups of Fisher 344 rats were nose-only exposed for 6 h to an aerosol made of either 5, 10–30 or 50 nm TiO2 NP and sacrificed 16 h after exposure. The control group was exposed to clean air only. A fluidized bed (Model 3400A, TSI Inc.) was used to aerosolize the NP powders. The average exposure concentrations in the inhalation chamber measured gravimetrically were 19.30, 21.99 and 21.94 mg/m3 and respective median number aerodynamic diameters measured by an electrical low pressure impactor (Dekati) were 369, 255 and 321 nm. Total and differential cell counts were measured in bronchoalveolar lavage fluids. All exposed rats showed a significant ($p<0.05$) increase in neutrophils. The total cell count and number of macrophages were also significantly increased ($p<0.05$) for rats exposed to aerosols made of 10-30 and 50 nm NP. Exposures to 20 mg/m3 agglomerated (>100 nm) TiO2 NP induced a significant increase in the number of neutrophils and macrophages in rat lungs in a similar manner for all three different NP initial size. Overall, this data show that increases in lung inflammatory cells is associated to the size of NP agglomerates rather than that of the initial NP.

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