2889 Impact of Cholesterol on Engineered Nano-Material-Induced Membrane Permeability

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With an increase in the use of engineered nano-materials (ENM) in both consumer products and medical applications, there has come a greater risk of exposure to workers, consumers, and patients. Because of this increased risk of exposure, it is important to develop a better understanding of ENM and their potential for causing inflammation and disease. Some ENM have been reported to be bioactive and to trigger a pro-inflammatory response when inhaled. Alveolar macrophages have been demonstrated to be responsible for this inflammatory response due to their release of the cytokine, interleukin 1β (IL-1β). A key step preceding and linked to IL-1β release is phagolysosomal membrane permeability (LMP). Some ENM have been implicated in inducing LMP in alveolar macrophages, which in turn results in release of cathepsin B from the phagolysosome. Cathepsin B triggers the formation of the NLRP3 inflammasome, activating caspase-1, which in turn cleaves pro-IL-1β to its active IL-1β form. While, LMP has been implicated in this inflammatory pathway, the mechanisms of ENM-induced LMP remain unclear. It has been reported that ENM can have interactions with lipid membranes and so it is hypothesized that LMP is caused by a disturbance in lipid membrane fluidity and order. Therefore, lipid membrane fluidity was measured by fluorescence recovery after photobleaching (FRAP) using an Olympus FV-1000 confocal microscope. A model membrane system using intact human red blood cells (RBCs) was used as a surrogate for cellular organelle membranes. RBCs were exposed to several metal-oxide ENM for one hour at a 100 µg/ml dose, and the fluidity of localized regions of interest in the RBC membranes was measured by the rate of diffusion of the fluorescent probe, Di-4-ANEPPDHQ through the membrane. The more bioactive ENM, such as zinc oxide, produced a decrease in membrane fluidity, while less bioactive materials such as titanium dioxide produced little to no change in membrane fluidity. Addition of cholesterol increased RBC membrane fluidity. These results suggest that the more bioactive ENM act on LMP by inhibiting lipid mobility and thus potentially can induce membrane permeability triggering inflammation and disease. Additionally, membrane cholesterol may be able to minimize the effects of ENM on induced permeability. *(Funding provided by NIH R01ES023209, P30GM103338, and P20GM103546)*

2890 Toxicological Effects of Inhaled Multi-Walled Carbon Nanotubes on Reactivity of Isolated Rat Trachea

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Occupational exposure to multi-walled carbon nanotubes (MWCNT) can occur in many different settings such as research and product development, manufacturing, and waste disposal. Current studies of their health effects demonstrate that some MWCNT cause pulmonary inflammation and fibrosis, mesothelioma, and elicit changes in the cardiovascular and central nervous systems in animal models. However, whether changes in airways occur as a result of MWCNT inhalation has not been explored. To investigate the toxicological effects of inhaled MWCNT, rats were exposed to 1 or 5 mg/m^3 inhaled MWCNT (MWNT-7 provided by Mitsui & Co.) for 6 hours. Characterization of the MWCNT showed the expected crystalline structure, 20-50 walls, and little trace metal contamination. The MWCNT had a mass median aerodynamic diameter of 1.5 µm and a geometric standard deviation of 1.67. Tracheas from MWCNT and air-exposed animals were isolated from euthanized animals for *in vitro* examination using isolated, perfused trachea preparations at 18 hours and 7 days post-exposure. After exposure to 5 mg/m^3 MWCNT, no statistically significant changes in reactivity to intraluminally or extraluminally-applied methacholine (MCh) were observed at either 18 hours or 7 days post-exposure. However, after exposure to 1 mg/m^3 MWCNT for 6 hours, there was a significant increase in reactivity to intraluminal MCh as indicated by a decrease in the EC50 value in comparison to the air-breathing control animals. By day 7 post-exposure, the changes in reactivity to MCh had resolved. The increase in reactivity to intraluminally-applied but not extraluminally-applied MCh in the 1 mg/m^3 MWCNT-exposed animals indicates that the epithelial cells lining the trachea are affected by inhaled MWCNT, which could alter their regulatory effect on airway reactivity.

2891 Potentiation of Drug-Induced Phospholipidosis *In Vitro* through PEGlyated Graphene Oxide as the Nanocarrier

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Cationic amphiphilic drugs (CADs) are small molecules that can induce phospholipidosis (PLD), an adverse drug effect that causes an accumulation of phospholipid in the lamellar bodies of cells. Nanotechnology based drug delivery systems have been used widely, but it is unknown if drug induced phospholipidosis (DIP) can be potentiated through drug retention by undigestible nanocarriers. Due to the high drug loading capacity of graphene, we investigated if PEGylated graphene oxide (PEG-GO) loaded with CADs could potentiate DIP. Tamoxifen induced an accumulation of NBD-PE, a fluorescence labeled phospholipid in human hepatoma HepG2 cells, while PEG-GO loaded with tamoxifen (PEG-GO/tamoxifen) further potentiated the phospholipid accumulation. Additional alterations of PLD marker gene expression were found in PEG-GO/tamoxifen treated cells compared to tamoxifen treatment alone. Similar effects of PEG-GO enhanced DIP were observed for other CADs, indicating that nanocarrier potentiated DIP could be universal. Transmission electron microscopy confirmed the increased number of lamellar bodies within PEG-GO/tamoxifen treated cells compared to tamoxifen alone. PEG-GO/tamoxifen showed a delayed but potent PLD effect and a retarded PLD recovery, indicating the reversibility of DIP was interfered. Confocal microscopy revealed the increased number of lysosomes, greater expression of LAMP2 (a PLD marker), and an increase in the co-localization of lysosome/LAMP2 with NBD-PE by PEG-GO/tamoxifen rather than the drug alone. Mechanistically, there was no correlation of autophagy with PLD induced by PEG-GO/tamoxifen. This research suggests both pharmaceutical companies and regulatory agencies that adverse drug effects may be potentiated with a delayed recovery, if undigestible nanocarriers are used for drug delivery.

2892 Role of Specific Reactive Oxygen Species Scavengers of Fatty Acid Composites in the Nanotoxicity of TiO_2 Nanomaterials Used in Cosmetic Products

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The commercial use of nanomaterials is an emerging field of nanotechnology in the 21st century. Especially, Titanium dioxide (TiO_2) NPs have been used as UV-blocking filters for their enhanced protective effect in cosmetic industry. However, recent advances in molecular toxicology have revealed that NP exposure can promote cytotoxicity and oxidative damage, which has raised health concerns in the use of NPs in personal care products. Although many studies provide a mechanistic basis for the role of fatty acids in the beneficial effects of TiO_2 particles, the safety and toxicity of these composite particles have not been evaluated in cells or living systems. Also, the TiO_2 particles were not nanosized and thereby failed to represent the most commonly used forms of TiO_2 particles found in personal care products. Here we present the role of fatty acids composites in preventing oxidative stress and cytotoxicity that can be induced by TiO_2 NPs. Two types of cells, human fibroblast skin cells and adenocarcinoma lung cells, were exposed to either bare TiO_2 NPs or TiO_2 NPs containing fatty acid composites (palmitoleic acid, palmitic acid, stearic acid and oleic acid) commonly used in cosmetic products. NMR analysis confirmed that the fatty acid composites remained in the NPs after wash. The cytotoxicity of TiO_2 NPs was determined by cell viability measurement using quantitative confocal microscopy, and the localization of two different forms of TiO_2 NPs were assessed using electron spectroscopic imaging in transmission electron microscopy (ESI-TEM). TiO_2 NPs containing fatty acids posed significantly reduced cytotoxicity (80-88% decreases) than bare NPs in both cell types. Furthermore, there was decreased intracellular penetration of the NPs containing fatty acid composites compared with bare NPs. Our results also suggest that cosmetization reduces TiO_2 nanotoxicity by inhibiting the occurrence of reactive oxygen species (DPPH, superoxides, hydroxyl radicals, etc.). These results provide important insights into the role of fatty acids in protecting the cells from possible oxidative damage and toxicity caused by NPs used in the production of cosmetic products. Together, these efforts will increase our understanding about the nanotoxicity of metal oxide NPs, not only in the use of personal care products in everyday lives, but in other medical diagnostic and therapeutic applications.


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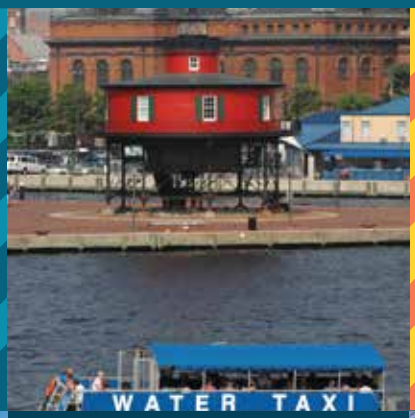

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