2122 Silver Nanoparticle-Mediated Mast Cell Degranulation Metabolic Pathway Shifts Are Distinct from IgE-Mediated Degranulation

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Silver nanoparticles (AgNPs) are incorporated into a variety of consumer and medical products, primarily due to their antimicrobial properties, thereby leading to increased exposure in the general population. We have previously demonstrated that 20 nm AgNPs induce robust mast cell degranulation through a novel non-IgE mechanism. Mast cells are important effector cells in the immune system and are essential to an allergic response. To better understand this novel mechanism of mast cell degranulation, we characterized and compared cellular metabolic shifts across several mechanisms of degranulation (AgNP, IgE, compound 48/80 [non-IgE]) in murine bone marrow-derived mast cells (BMMCs). To explore these metabolic changes, we utilized functional assays to measure glycolysis and aerobic mitochondrial respiration in response to treatments. We observed an increase in ATP production which correlated to degranulation, however, differing levels of mitochondrial dysfunction and glucose uptake were observed between AgNP and other treatments. In addition, we utilized Seahorse XFp technology to measure: 1) preferential metabolic pathway phenotypes, 2) glycolytic metabolism, and 3) mitochondrial respiration metabolism. The cell phenotype test revealed that BMMCs shift away from glycolysis when degranulated via AgNP whereas IgE mediated degranulation preferentially utilized glycolysis. The Cell Mito stress test revealed a decrease in respiration for all mechanisms of degranulation, compared to control, with consistently lower levels for non-IgE degranulation. Compound 48/80, but not AgNP, also caused complete inhibition of glycolytic mitochondrial respiration reserve. We next observed a decrease in glycolysis that was most prominent in non-IgE degranulation. There was a complete depletion of glycolytic reserve, similar to mitochondrial respiration, with non-IgE degranulation (AgNPs and compound 48/80) that did not occur with IgE mediated degranulation. In conclusion, mast cell metabolism varies significantly between AgNP degranulation and IgE mediated degranulation suggesting novel cell regulatory mechanisms are potentially driving AgNP mediated mast cell degranulation.

2123 Potential Mitochondrial-Targeted Toxicity of Silver Nanoparticles in Mouse Hepatocytes

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Silver nanoparticles (AgNPs) are a well-proven antimicrobial nanomaterial, which hold great promise for a wide range of biological applications. Despite the promising advantages, the significant human exposure to AgNPs has given rise to concerns over potential human health and environmental hazards. While multiple studies have investigated the toxic effects of various AgNPs at different levels, the underlying mechanism is still largely elusive. In this work, we explore the possible relationship between mitochondrial activity and the cytotoxicity of AgNPs with different coating and sizes, compared to silver ions, in cells cultured in glucose and galactose-based media. Since cells cultured in galactose rely mostly on oxidative phosphorylation (OXPHOS) to produce their ATP, they become more sensitive to mitochondrial toxicants than cells grown in glucose medium. Analysis of bioenergetic function with the XF Seahorse extracellular flux analyzer further confirmed that oxygen consumption rate (OCR) was significantly increased whereas extracellular acidification rate (ECAR), a measure of glycolysis, was decreased in cells grown in galactose. Cytotoxicity assays, mitochondrial stress analyses, and fluorescence and darkfield microscopy were used to investigate the effects of AgNPs on cell proliferation and metabolism. Our data suggest the sensitivity of mitochondria to AgNPs, which could in turn have an impact on cell viability and proliferation.

2124 *In Vitro* Pulmonary Toxicity of Copper Carbonate ($CuCO_3$) Particles Used as Outdoor Wood Preservatives

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Micronized $CuCO_3$ containing nanoparticles (NPs) are employed as outdoor wood preservatives. This application raises a risk of human exposure to $CuCO_3$ NPs or their products with unknown health effects. Research examined the *in vitro* pulmonary toxicity of milled $CuCO_3$ particles and saw dust extracts (SDE) obtained from $CuCO_3$ treated and untreated wood. A thiobarbituric acid reactive substance (TBARS) assay measured the oxidative reactivity of all samples with and without the copper chelator triethylenetetraamine dihydrochloride (TETA). Analysis of milled $CuCO_3$ samples demonstrated samples containing the smallest size distribution displayed the greatest TBARS reactivity. SDE from only $CuCO_3$ treated wood displayed TBARS reactivity similar to milled $CuCO_3$ samples. TBARS reactivity of milled $CuCO_3$ and SDE from treated wood was eliminated after filtration through a 3kDa filter. TETA significantly attenuated TBARS reactivity of milled $CuCO_3$ samples but not SDE from treated wood indicating reactivity was not due to ionic Cu in the $CuCO_3$ treated wood. BEAS2B cells were exposed to either milled $CuCO_3$ samples (25 - 200 µg/ml) or SDEs (2.75 - 88 mg/ml) and cytotoxicity assessed using the WST1 assay at 22h post-exposure. Milled $CuCO_3$ samples with the smallest size distribution were most cytotoxic to BEAS2B cells. SDE from treated wood was more cytotoxic when compared to SDE from untreated wood. TETA attenuated BEAS2B cytotoxicity for both milled $CuCO_3$ particles and SDE from treated wood. On a molar dose metric, SDE from treated wood was more cytotoxic to BEAS2B cells compared to milled $CuCO_3$ samples. BEAS2B IL-6, IL-8, and HO-1 gene expression was examined by qRT-PCR after 3, 6, 12, 24, and 48h exposure to milled $CuCO_3$ samples or SDEs. Milled $CuCO_3$ and SDE samples induced proinflammatory and stress gene expression to varying extents in a time dependent manner and did not correlate with TBARS reactivity. Milled $CuCO_3$ particles elicited a completely different gene expression profile when compared to SDE from treated wood. These findings highlight the challenges in assessing the *in vitro* pulmonary toxicity of $CuCO_3$ treated outdoor wood samples using milled $CuCO_3$ with similar size distribution. The results indicate *in vitro* pulmonary toxicity should be assessed using the nano-enabled product. *This abstract does not represent US EPA policy.*

2125 Biological Effects of Long-Term Exposure of Human BEAS-2B and Met-5A Cells to Riebeckite/Tremolite Asbestos and Their Respective Cleavage Fragments

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Asbestos is a commercial term that refers to 6 different fibrous minerals including riebeckite (RF) and tremolite (TF) amphibole asbestos. Inhalation of respirable forms of amphibole fibers, can cause asbestosis, lung cancer and both pleural and peritoneal mesothelioma. Amphiboles can also occur in a non-fibrous habit that can be mechanically broken into cleavage fragments (CF) which can meet the mineralogical/regulatory criteria for fibers. While the effects of RF and TF on health are well documented, there is uncertainty regarding the toxicity of riebeckite and tremolite CF. In this study, human epithelial (BEAS-2B) and mesothelial (MET-5A) cells were evaluated for the presence of several cancer hallmarks indicating the neoplastic-like transformation following continuous long-term (5 weeks) exposure to sub-toxic (2.5µg/cm^2) concentrations of RF, TF and their CF. TF- and RF-exposed cells, both BEAS-2B and MET-5A, revealed a neoplastic-like transformation phenotype characterized by significant increase in invasion/migration, anchorage-independent growth, proliferation, and morphological transformation, compared to controls. No anchorage-independent growth and invasion was observed in both cell types treated with riebeckite CF although an increase in DNA damage, migration, proliferation, and morphological changes were detected in BEAS-2B cells. In the case of tremolite CF, although a significant increase in proliferation, transformation and DNA damage was observed in both cell types, an increase in invasion/migration and anchorage-independent growth was detected only in BEAS-2B cells. Similarly, analysis of inflammatory responses suggested cell-type specific effects as well as treatment related differences. Overall, our data are compatible with the findings that amphibole asbestos fibers demonstrate higher neoplastic transformation potential compared to the respective CF (at the same mass dose) in both bronchial epithelial and mesothelial cells.

2126 Advanced *In Vitro* Models for the Assessment of Nanomaterial-Induced Lung Fibrosis

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Nanotechnology promises significant scientific, economic and societal benefits, but commercialization and growth are threatened by safety uncertainties. Existing *in vitro* hazard testing strategies to define the human health impact of nanomaterials (NM) commonly apply unrealistic acute, high-doses to cellular models that poorly reflect the *in vivo* environment. The H2020 project "*Physiologically Anchored Tools for Realistic nanOmateriaL hazard aSsessment*" (PATROLS) aims to establish innovative hazard assessment tools to predict adverse effects caused by long-term NM exposure, and support regulatory risk

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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 59th Annual Meeting of the Society of Toxicology, held at the Anaheim Convention Center, Anaheim, California, March 15–19, 2020.

An alphabetical Author Index, cross-referencing the corresponding abstract number(s), begins on page 542.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 580.

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