3063 Establishing an *In Vitro* Aerosol Exposure Method for Evaluating the Respiratory Toxicity of Ortho-phthalaldehyde

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Ortho-phthalaldehyde (OPA) is a high-level disinfectant for sterilizing reusable, heat-sensitive medical devices. Even though its use in this context has been cleared by the FDA, inhalation exposure limits have not been set due to the lack of comprehensive mammalian data. To fill this data gap, we previously assessed the respiratory toxicity of OPA aerosols in a human *in vitro* air-liquid-interface (ALI) airway tissue model. As a proof-of-concept study, the VITROCELL® Cloud System was employed for generating a bolus concentration of OPA aerosols within a short duration (i.e., less than 5 min). To mimic better the occupational exposure experienced by hospital workers while sterilizing medical instruments, we expanded our previous study by increasing the daily exposure duration to 4 h. For this purpose, a combination of a VITROCELL® BioAerosol Nebulizing Generator and a 24/48 Exposure Module was used for creating and delivering a continuous flow of OPA aerosols to the apical surface of the ALI cultures. Deposited concentrations of OPA aerosols were quantified using UPLC-ES/MS/MS. Cultures were exposed to OPA aerosols at concentrations ranging from 0.52 to 2.7 µg/cm^2 4 h per day for 5 days. Biological responses, including tissue integrity, cilia beating frequency (CBF), mucus secretion, and expression of key protein markers were assessed throughout the 5-day treatment phase. OPA aerosols significantly increased tissue permeability, as indicated by the concentration-dependent release of lactate dehydrogenase. The metabolic activity of the cultures, however, was not altered by the exposure at all concentrations tested. Consistent with our previous study, OPA is a potent ciliostatic agent that significantly suppressed CBF, as early as after 2 daily exposures and inhibited the expression of ciliary biomarkers. Together with a decrease in mucus secretion, these data suggest that the mucociliary clearance mechanism may have been compromised by OPA aerosol exposures. Besides its effects on tissue functions, OPA also induced squamous differentiation as indicated by the upregulation of involucrin expression and increase in transepithelial electrical resistance. Work is underway to establish a method for deriving a toxicological point-of-departure from the *in vitro* concentration-response data.

3064 A Hydrophilic Organosilane-Based Coating Blocked Acute and Subchronic Silica-Induced Lung Toxicity in an Animal Model

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Fracking is a process in which natural gas-laden rock is fractured to improve removal of underground reserves of gas. Sand is present in the fracking fluid pumped in the well to stabilize the fissures. The handling and use of sand at well sites generate respirable dust by which workers in the oil and gas industry are exposed. Respirable crystalline silica has been identified as a significant exposure hazard. Inhalation of silica dust can cause silicosis (pulmonary fibrosis and inflammation), chronic obstructive lung disease, and lung cancer. The surface of fractured silica particles generates cell-damaging reactive oxygen species. Coating silica particles with specific organosilanes has been shown to reduce *in vitro* silica-induced toxicity of lung macrophages. The objective was to assess whether a hydrophilic organosilane-based coating (SIVO-160) would reduce or prevent acute and sub-chronic silica-induced lung toxicity in an *in vivo* model. Male Sprague-Dawley rats were intratracheally instilled with silica (1.0 mg/rat), silica coated with SIVO-160, SIVO-160 coating alone, or saline (vehicle control). Bronchoalveolar lavage (BAL) was performed at 3, 10, 45, and 90 d after exposure, and markers of lung inflammation and injury were assessed. Also, suspensions of uncoated and coated silica were analyzed using RapiFlex MALDI-ToF/ToF mass spectrometry to confirm the presence of SIVO-160 on the surface of the silica particles. At acute and sub-chronic time points, silica exposure significantly increased BAL lactate dehydrogenase (lung injury) and the number of recovered lung macrophages and neutrophils (lung inflammation) compared to the saline-treated controls. These silica-induced elevations in lung toxicity were reduced to control levels at each time point when silica was coated with SIVO-160 before exposure. Pulmonary exposure to SIVO-160 alone produced no lung toxicity. Importantly, multiple unique spectral peaks were detected on the surface of the silica + SIVO-160 sample after incubation in saline that were absent in the uncoated silica sample spectra. During fracking operations, sand coated with organosilane materials may be used as a possible mitigation strategy and potentially protect large numbers of workers exposed to dusts generated at oil and gas extraction sites.

3065 Immune Cell Profiling of LPS-Induced Acute Lung Injury in Mice Treated with I3C Reveals Downregulation of CCR and CXCR Chemokine Receptors Associated with Trafficking of Myeloid Cell Populations

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Immune cell trafficking during inflammation is a result of chemotaxis mediated by CXC and CC chemokines and their receptors. The severity of acute lung injury and acute respiratory distress syndrome (ALI/ARDS) due to endotoxins or environmental toxins stems from trafficking of peripheral immune cells to the lungs. The pulmonary infiltration of innate immune cells leads to destruction of lung epithelial cell barriers, which cause systemic oxygen deprivation. However, indole-3-carbinol (I3C), a naturally occurring AhR ligand found in cruciferous vegetables, has been shown by our lab to suppress inflammatory mediators through the regulation of immune cell transcriptome. In the current study, we determined if I3C treatment prevents ALI by altering the expression of genes associated with cellular movement, infiltration, and antigen presentation in effector cells such as neutrophils and macrophages, which can cause damage to epithelial tissue, leading to development of ALI. Towards this, mice were intranasally administered lipopolysaccharide (LPS; 5mg/kg) to induce ALI. Three hours later, mice were treated with I3C (80mg/kg) or vehicle. After 48 hours, CCL2 (MCP-1) protein levels were increased in lung cell supernatant, bronchoalveolar lavage fluid, and serum in LPS+Veh vs. LPS+I3C mice. In addition, flow cytometric analysis revealed that ALI mice treated with I3C decreased expression CXCR2 in neutrophils and CCR2 in macrophages compared to mice treated with vehicle. Interestingly, scRNASeq showed that in myeloid cell tSNE clusters of LPS+I3C treated mice vs. LPS+Veh, I3C reduced expression of *CCL2, CCL5, CXCL3*, *CXCL10, FN1*, *MALT1*, and *HLA-A*, genes involved in movement of neutrophils, lymphocyte migration, metastasis, leukopoiesis, and immune cell adhesion. However, the mRNA expression of *CXCL2* and *IL-10*, genes involved in MDSC trafficking and anti-inflammatory responses, was increased in diseased mice treated with I3C compared to LPS+Veh mice. Furthermore, antigen presentation genes, *H2-K1 and H2-AB1* in AM/DC/M2 clusters and interferon-associated genes in Neutrophil 2 and M1/Mφ clusters were upregulated. These findings suggest that I3C induces distinct transcriptional profiles of neutrophils and macrophages, and alters immune cell trafficking and proinflammatory signatures of innate immune cells in ALI. *Supported by NIH grants P01AT003961, P20GM103641, R01ES030144, R01AI129788, R01AI123947, R01AI160896 and R01AI123947-S2.*

3066 Exploring the Toxicity of Azobenzene Disperse Dyes to Human Lung Cells

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Azobenzene disperse dyes (ADDs) are a class of dyes used to color synthetic fabrics. ADDs make up 60% of all dyes in the world and account for 70% of the 9.9 million tons of industrial dye colorants used annually. Select azo dyes are known to have carcinogenic and mutagenic properties. Research has primarily focused on the effects of azo dye reduction in the human intestine, liver, and skin; meanwhile, the effects on the pulmonary system remain less explored despite more recent epidemiology reports finding occupational asthma to be associated with azo dye exposure. To begin to elucidate the toxicity of ADDs on the respiratory system, small airway epithelial cells (SAECs) and A549 cells were exposed to three azo dyes that constitute an abundant black dye used in textiles: disperse blue 373 (DB373), disperse violet 93 (DV93), and disperse orange 61 (DO61). Because ADDs have been associated with allergic dermal responses, it was hypothesized that structurally different ADDs would illicit varying levels of cytotoxicity and changes in genes known to be associated with allergic airway disease (i.e. asthma). For the approach, SAECs and A549 cells were exposed to a range of ADD doses (0.01ug/mL to 4ug/mL) and cell viability was quantified after 48 hours using a CellTiter-Glo assay. To identify elevated mRNA expression of genes associated with an asthmatic response *in vitro*, RT-qPCR was performed on A549 cells exposed to 1ug/mL of DB373 and 1ug/mL of DV93 for 8, 24 and 48 hours. Results showed that exposure of SAECs and A549 cells to DV93 and DB373 led to a statistically a significant decrease in cell viability in a dose-dependent manner, whereas exposure to DO61 did not. Additionally, DV93 and DB373 resulted in a significant upregulation of lung injury and asthmatic genes (TGF-β1, CXCR4, IL-8, etc,.) compared to control cells. These observations demonstrate for the first time that commonly used ADDs can alter cytotoxicity and gene expression in human lung cells in a dye-dependent manner. These results also stress the need to further investigate pulmonary health implications of azo dyes that are abundantly used in consumer goods. Future studies will examine the ability of ADDs to act as respiratory sensitizers as the molecular mechanism driving toxicity.

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