1081 Roles of TRPV3 and TRPA1 in Wood Smoke Pneumotoxicity

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Air pollution, particularly smoke produced from biomass combustion, is pneumotoxic. However, the mechanisms underlying the toxic effects of these materials on lung epithelial cells is not fully understood. Transient receptor potential (TRP) ion channels are a family of proteins that mediate some of the pro-inflammatory and cytotoxic effects of air pollutants, typically via disruption of intracellular calcium homeostasis. We hypothesized that TRP ion channels that are activated by wood smoke particles might regulate cytotoxicity in lung epithelial cells treated with wood smoke particles via mechanisms involving disruption of intracellular calcium homeostasis; specifically causing endoplasmic reticulum stress and EIF2aK3/PERK-dependent signaling. Using fluorescent calcium imaging and various human TRP channel over-expressing HEK-293 cells, we found that pine wood smoke preferentially activated transient receptor potential ankyrin-1 (TRPA1) and vanilloid-3 (TRPV3). Pine wood smoke particles were acutely cytotoxic to primary human lobar bronchial epithelial cells (LD_{50}~25ug/cm^2), and TRPV3 and TRPA1 antagonists both attenuated cytotoxicity. Using immunocytochemistry and functional assays, it was found that TRPV3 resides on the endoplasmic reticulum of human lobar bronchial epithelial cells. Consistent with the induction of endoplasmic reticulum stress and EIF2aK3/PERK activation in lobar cells, treatment with pine smoke particles promoted the time-dependent induction of pro-apoptotic DDIT3 and ATF3, biomarkers of EIF2aK3/PERK activation during ER stress. As above, both TRPV3 and TRPA1 antagonists substantially attenuated these responses. Collectively these data show that activation of TRPV3 and TRPA1 by wood smoke particles triggers ER stress in primary human lung epithelial cells, and that these events contribute to the acute cytotoxic effects of pine wood smoke particles on bronchial epithelial cells. These findings expand our understanding of how wood smoke adversely effects the lung. *Support: ES017431, ES027015, University of Utah Undergraduate Research Opportunities Program.*

1082 *In Vivo* and *In Vitro* Toxicity of PM2.5 from a Marcellus Shale Drilling Operation

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Marcellus Shale unconventional natural gas well development (UNGD) has reached an all-time high in recent years. Many rural communities and regions are experiencing increased industrial activities and possibly air pollutant exposures from shale gas extraction activities. Previously our laboratory has identified increased concentrations of particulate matter (PM) in the fine (<2.5 µm, $PM_{2.5}$) and ultrafine (<0.1 µm, $PM_{0.1}$) size ranges near UNGD areas. Epidemiological studies have associated emissions from these well pads with negative health consequences based on distance. In this study, we collected high volume $PM_{2.5}$ samples onto PTFE filters over 1 week during fracture stimulation at a Marcellus Shale gas well site. Additional samples were taken upwind, and 1, 2, and 7 km from the well pad downwind during the same timeframe. The particles were extracted from these filters in ultrapure water and dried via lyophilization. We exposed THP-1 cells *in vitro* for 24 hours. In the cell line, dose-dependent cytotoxicity was demonstrated with the on drill site sampling having the highest toxicity level at doses of 12.5 µg/mL of PM to 100 µg/mL (15.7%±1.0% and 34%±2.2%, respectively). LDH release demonstrated that upwind $PM_{2.5}$, at the same mass concentrations, had much lower cytotoxicity (100 µg/mL, 0.77%±0.90%). Proliferation, as measured by WST-1, was not different for any dose or particle type. In a separate study, young Sprague Dawley rats were exposed to 100 µg/rat $PM_{2.5}$ from the drill site via intratracheal instillation. Drill site $PM_{2.5}$ exposure significantly increased mean arterial blood pressure (sham: 88±4 mmHg, exposed: 103±6 mmHg) without significantly increasing heart rate or diastolic blood pressure. Additionally, arteriolar responses to phenylephrine and acetylcholine were not significantly different from sham. Taken together these data suggest that the on drill site $PM_{2.5}$ has a greater toxicity than downwind and upwind. Furthermore, exposure can significantly increase blood pressure, though the mechanisms are unknown.

1083 Neural Effects of Fracking Sand Dust Aerosols

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Hydraulic fracturing (fracking) is a process to recover oil and gas from shale rock formation. The technique involves fracturing the oil- and natural gas-laden rock with pressurized liquids. Water and fine mesh sand (proppant) generally make up 98 to 99.5 % of the fluid used during fracking. The transportation and handling of proppant at well sites generates aerosols, and, there is concern of worker exposure to such fracking sand dust (FSD) by inhalation. FSD are generally composed of silica in the respirable range, and other silicate/non-silicate minerals depending on the geological source of the proppant material. Field investigations by NIOSH suggest that the levels of respirable crystalline silica at well sites can exceed the permissible exposure limits. Thus, from an occupational safety perspective, it is of critical importance to evaluate the potential toxicological risks associated with FSD. To determine if FSD poses a neurological risk, male Sprague Dawley rats (8 weeks old) were exposed to FSD (10 or 30 mg/m^3; 6 h/d × 4 d) by whole-body inhalation. At 1, 7, 27 or 90 d post-exposure, neuroinflammatory mediators, blood-brain barrier (BBB), synaptic, and glial markers were evaluated as indices of neural injury. FSD elicited neuroinflammation (increased *Nos2, Il6* mRNAs) and altered the expression of BBB-related markers (increased or decreased *Mmp9, Cldn1* and *Cldn3* mRNAs) in the olfactory bulb, hippocampus and cerebellum. Increased glial fibrillary acidic protein and altered expression of myelin basic protein was also seen in these brain areas. An intriguing observation was the persistent reduction (25-42 % decrease at 7, 27, 90 d post-exposure; $P < 0.05$) of Synaptophysin 1 (SYP) and Synaptotagmin 1 (SYT) proteins in the cerebellum, suggestive of long-term synaptic changes. SYP and SYT are critical players in the exocytosis of synaptic vesicles, neurotransmitter release and synaptic plasticity. Synaptic loss and impaired synaptic plasticity are often associated with the functional decline of the nervous system. Whether such molecular aberrations will lead to neurodegeneration-like pathological changes remains unknown. Further, it needs to be determined if such effects follow direct FSD particulate translocation to the brain or an indirect neurogenic/systemic response. Thus, additional studies are warranted to investigate FSD translocation and evaluate the long-term neurological effects, including functional and behavioral outcomes, of FSD exposure.

1084 Air Quality: An Interface between Environment, Climate Change, and Public Health

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In recent years, Imperial County has consistently ranked as the top California County with the highest asthma rate in children. Community members are concerned their breathing problems are due to one or more of many sources of pollution that contaminate Imperial Valley (IV). Of particular interest is the potential toxicity of particles rising from the crusted lakebed of the Salton Sea. With polluted runoff as the only water source, the Salton Sea has become increasingly polluted and is rapidly shrinking. No one has differentiated the sources of particulate matter (PM) in IV or their connection to asthmatic symptoms. Our goal is to investigate the differences between, and potential harmfulness of airborne particles to which IV residents are exposed. A state-of-the-art mobile sampling unit has been designed to collect PM of various size fractions, from ultrafine (<0.10 µm) to PM10. The geography and meteorological conditions in IV expose the community to a unique combination of natural and man-made pollutants. These sources include agriculture, industrial plants, and large cities across the border of Mexico, as well as the Salton Sea. To account for seasonal changes and source variation, our sampler collects particles from different wind directions over the course of a year. Each PM sample is chemically characterized and screened in an *in vitro* system to before moving into an *in vivo* model of asthma. Due to the high incidence of asthma in youth in IV, we are specifically interested in how this PM may modulate sensitization of the immune system to house dust mite (HDM) allergen, and in turn, how this impacts subsequent encounters with the allergen. Our initial chemical characterization indicates organic species dominate IV particles (58%) followed by ammonium sulfate (37%). The chemical composition of the organic particles from IV is highly complex and likely composed of hundreds of carbon-containing compounds. An *in vitro* screening in a human macrophage cell line demonstrates a significant increase in gene expression of COX-2, CYP1a1, IL-8 and IL-1B cytokines, as well as a similarity between IV PM and other agricultural regions in California. The high average degree of oxidation and the high organic nitrogen contents suggest that ultrafine PM in IV is likely toxic and could be a major culprit for the health problems in the region. The increase of inflammatory cytokines when exposed to IV PM *in vitro* suggests the need for further testing in a murine model of asthma.

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