1187 A Model for Evaluating Synergistic Opioid-Induced Respiratory Depression

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Drug overdose is one of the leading cause of injury-related death in the United States with almost 68% of reported cases in 2017 involving a prescription or illicit opioid. Opioid overdose or combining opioids with other sedative medication causes respiratory depression leading to mortality. With the recent emergence of the opioid abuse crisis, it has become increasingly important to understand the potential interactive effects on respiratory parameters of new chemical entities (NCEs) that may be taken with opioids. For such assessments, positive and negative assay controls are useful to demonstrate proper evaluation. In this regard, respiratory function of male Sprague Dawley rats was assessed by plethysmography following administration of an intravenous (IV) morphine challenge (3 mg/kg) and/or oral baclofen (20 mg/kg). The conditions tested included oral vehicle (negative control), oral vehicle with IV saline (negative control), oral vehicle with IV morphine (positive control), oral baclofen alone (positive control), and oral baclofen concomitant with IV morphine (double positive control). In this model we demonstrate that administering morphine together with baclofen resulted in synergistic decreases in respiratory frequency (RF) and substantial decreases in minute volume (V) of 66% and 62% from baseline, compared to administering morphine (RF:17%; V:55%) or baclofen (RF: 38%; V: 48%) independently. Hence, baclofen with a morphine challenge model can be effectively used as a comparator to assess potential interactive effects of NCEs with opioids.

1188 Development of a Thermal Spray Coating Generator and Exposure System for Toxicology Studies

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Thermal spray coating is a surface treatment process that enables different types of materials to be deposited on various substrates- metal, metal alloys, ceramics, and plastics. The process involves spraying a metal coating product which is melted by high temperatures and then sprayed under pressure onto a surface. Applications for thermal spray processes have a broad range across all industrial sectors. This technique is widely used in repairing bridges, water towers, wind turbines, pipelines, and ship tankers as well as in the automobile and aircrafts industries. During the process, large quantities of aerosols composed of fine and ultrafine metal particles are generated. Little is known about the physical (e.g., particle size and morphology) and chemical (e.g., metal composition, solubility, surface chemistry, metallurgy) properties of the particles formed during the process. The goals of this research were to construct a thermal spray coating generator and exposure system for toxicology studies and to characterize the properties of the formed aerosols during different spray coating processes in a laboratory setting. Initial studies have evaluated twin-wire arc thermal spray coating using P-MET 730 stainless steel consumable wire. In twin-wire arc thermal spraying coating, two consumable metal wires are fed independently into the spray gun. The wires are charged, forming an arc between them. The heat from the arc melts the wires generating metal particles which are entrained in an air jet from a spray gun. The entrained molten metal is then deposited onto a rotary substrate via compressed air. Count median electric mobility diameter was measured to be 196 nm with a geometric standard deviation of 1.5, using a scanning mobility particle sizer (SMPS). Importantly, a significant portion of the primary particles were observed via scanning electron microscopy (SEM) to be in the ultrafine size range with diameters <100 nm. The particles were primarily composed of iron, chromium, nickel, and manganese. With the development of this system, it is possible to investigate the pulmonary and systemic health effects associated with the inhalation of aerosols generated from different thermal spray coating processes in an animal model.

1189 Effect of Polyhexamethylene Guanidine Phosphate on Epithelial Barrier Function in Human Bronchial Epithelial Cells

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Polyhexamethylene guanidine phosphate (PHMG-p) has been used as a disinfectant and biocide, and was reported to involve in lung diseases, such as pulmonary fibrosis. However, the effect of PHMG-p on the barrier function of the bronchial epithelium is unknown yet. Tight junctions (TJs), which comprise the interacting proteins, such as occludin, zona occludens-1 and claudins maintain epithelial barrier function. The damage of TJ is the major cause of epithelial barrier breakdown during lung inflammation. In this study, we investigated whether PHMG-p modulates the protein expression of TJ in human bronchial epithelial BEAS-2B cells. PHMG-p decreased the TJs and the other proteins including E-cadherin and β-catenin, which also maintain in barrier function in the epithelium. In addition, PHMG-p impaired the cellular F-actin architectures, involved in the maintenance of TJ structure and barrier function. Furthermore, PHMG-p increased the active calpain1, calcium-dependent protease, which is known to breakdown TJs. In addition, PHMG-p increased in the intracellular calcium levels via extracellular space. Interestingly, addition of calpain1 inhibitor, ALLN, and removal of calcium source in extracellular space significantly reversed the impairment of TJs and F-actin architectures induced by PHMG-p. These results suggest that epithelial barrier dysfunction is one of the symptoms to lead pulmonary diseases by PHMG-p via calpain1 activation.

1190 Early De-Risking Approaches for Identification of Anti-Fibrotics for Idiopathic Pulmonary Fibrosis (IPF) Treatment

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Idiopathic Pulmonary Fibrosis (IPF) is a chronic progressing lung disease with a progressive and irreversible decline in lung function. It has a poor prognosis (3-5 yr survival from diagnosis) and increasing global prevalence (20 people per 100,000 population). To date, only Nintedanib (NINT) and Pirfenidone have been licensed to treat IPF, therefore there is increased effort to discover new therapies. NINT, a tyrosine kinase inhibitor was profiled in a fibroblast to myofibroblast human IPF lung assay to determine both efficacy and cytotoxicity using high content imaging. Myofibroblast formation is strongly associated with fibrotic lesions. NINT inhibited the generation of α smooth muscle actin formation (a marker of the transition to a myofibroblast phenotype) at doses of 30 nM and above, with an IC50 of 370 nM. Cytotoxicity (measured as changes in nuclei number, >50% reduction) was observed at >10 uM concentrations. In a bleomycin-induced lung fibrosis rat model, NINT dosed orally at 60 mg/kg BID inhibited lung fibrosis at lung concentrations of ca 100 nM (Median Modified Ashcroft Score reduced from 3 to 2, P<0.001, and hydroxyproline (collagen) levels in the lung were reduced by 30%, P<0.001). At these exposures mild side effects were observed, including reduced food consumption, lessened activity and body weight loss. These effects increased in severity with increasing dose and were seen in mouse as well as rat models. Hence, it may be possible to link early *in vitro* assessments of efficacy and cytotoxicity with *in vivo* pharmacokinetics and exposure estimates to help optimize the testing of compounds *in vivo* and de-risk compounds.

1191 The Role of Matrix Metalloproteinases in Multiwalled Carbon Nanotube (MWCNT)-Induced Inflammation in C57BL/6 Mice

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Matrix metalloproteinases (MMPs) are ubiquitously expressed extracellular matrix (ECM) proteases that are activated in inflammation and injury and play varied roles in cellular homeostasis, adaptation, tissue remodeling and immunity. We previously demonstrated that acute MWCNT exposure led to impaired vascular reactivity that was dependent on MMP-9 activation and CD36 signaling. We hypothesized that MWCNT inhalation exposure alters broad spectrum MMP activity, leading to serum-borne factors that enhance vascular permeability and activate inflammatory pathways. To test this hypothesis, male C57BL/6 mice (6-8 weeks) were given 10 mg/kg of the broad-spectrum MMP inhibitor, Marimastat, and then dosed with 0 (dispersion media; DM), 10 or 40 µg MWCNT via oropharyngeal aspiration 1 hour after Marimastat dosing. Pulmonary inflammation was evaluated 24 hrs following MWCNT exposure, in terms of cell and protein quantification of bronchoalveolar lavage fluid (BALF). Serum bioactivity was determined in mouse brain endothelial cells (MBEC) via serum cumulative inflammatory potential (SCIP) assay. Vascular integrity was evaluated via electric cell-substrate impedance sensing (ECIS) assay, and confocal assessment of intracellular gap formation. Neutrophil infiltration and total BALF protein significantly increased in 40 µg-dosed mice (n=6/group; p<0.01) and was not altered by MMP blockade. MBECs treated with serum from MWCNT-treated mice exhibited a ~75% reduction in barrier integrity compared to DM controls. A ~50% of DM recovery in barrier integrity was observed with MMP blockade. Additionally, MMP blockade diminished

MWCNT serum-enhanced thrombin-mediated loss of barrier integrity and enhanced sphingosine 1-phosphate-induced barrier stability in MBEC. Actin immunostaining of MBEC confirmed that MWCNT serum exposure disrupted cell-cell junctions in a MMP-dependent manner. A thrombospondin type-1 domain-containing peptide reduced regrowth of MBECs in a dose-dependent manner ($p<0.0001$). Thus, while MMP blockade did not alter lung inflammation resulting from MWCNT exposure, subsequent pathologic serum bioactivity arising from MWCNT treatment was dependent on pulmonary MMP activity.

1192 Use of Cell Media Nicotine Concentration as a Marker to Predict Cell Surface Deposited Nicotine in Transwells after Fresh Smoke/ Aerosol Exposure

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Exposure of organotypic 3D lung models at the air liquid interface (ALI) to fresh whole smoke/aerosol provides a more human relevant exposure assessment of combustible cigarettes and e-vapour products. The aim of this study is to present a method for determination of nicotine deposition at the 3D MucilAir™ tissue surface, in transwells of 24 MWP after repeated exposure to 30, 60 or 90 puffs of tobacco cigarette (3R4F, 1:17 dilution) or undiluted *my*blu™ e-vapour aerosol over 4 weeks. Exact measurement of nicotine deposited at the cell surface is difficult due to absorption of nicotine in to the cells. The nicotine serves as a general marker of exposure. We wanted to determine if cell media nicotine concentration was correlated with cell surface deposition. To measure this, the deposition efficiency onto a glass plate inserted directly into the transwell was determined and was compared to glass discs with cells grown on the surface (BEAS-2B, V79). During the four weeks of repeated exposure of MucilAir™ tissues at ALI to 3R4F smoke and *my*blu™ aerosol, basal culture media were collected for nicotine quantification. Additionally nicotine deposition on to glass discs representing the cell surface area was measured. Toxicological effects observed over the exposure time in comparison to the puff numbers were compared on nicotine basis. With regard to increasing puff numbers, dilution factors, and the surface area of glass plates nicotine deposition on glass discs in the transwells correlated well with the deposited nicotine in the cell media (measured using LC MS/MS). However the correlation coefficients obtained with the different products regarding nicotine deposition and nicotine concentration in the cell media deviated from each other due to the different physical characteristics of 3R4F smoke and myblu™ aerosol.

1193 DNA Damage from Regional Metal-Enriched Particulate Matter in a549 Lung Epithelial Cells

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Inhalation of particulate matter (PM) promotes the development of pulmonary diseases and cancer. Exposure to heavy metal-containing PM induces genotoxicity as mediated through the initiation of an inflammatory and oxidative response. However, the initial biochemical and physical interaction between PM and pulmonary epithelial cells that drives DNA damage is incompletely understood. This study therefore sought to develop an *in vitro* model to examine the genotoxic effect of PM on lung epithelial cells. PM was derived from 1) sediments from the Jackpile and St. Anthony uranium mines in NM; 2) from a church attic in Paguate, NM; and 3) tungsten carbide PM. All samples were resuspended in air and collected in a next-generation cascade impactor to ensure PM was respirable. Cells were labeled with a viability stain and a monoclonal antibody specific to the pH2AX histone variant, then imaged through the Cellinsight High-Content-Screening platform. H2AX fluorescence appeared greater in cells exposed to PM from the Jackpile mine, and dusts from the nearby church sample. Findings suggest that physical/chemical interaction with PM produce genotoxic effects on lung endothelium in the absence of inflammation. Further research is needed to elucidate underlying cellular mechanisms and putative physicochemical drivers of PM-induced genotoxicity.

1194 Effect of PHMG-p on Increasing of TRAIL-Related Death Receptors in Human Bronchial Epithelial Cells and Animal Model

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PHMG-p was used as the main component of the humidifier disinfectant to clean the humidifier and eliminate the growth of microorganisms. Epidemiological and toxicological studies have indicated that PHMG-p induces pulmonary inflammation and fibrosis caused by PHMG-p inhalation, but the toxicological mechanism of PHMG-p is still unknown. This study investigated whether PHMG-p-mediated upregulation of TNF-related apoptosis-inducing ligands (TRAIL) death receptors was associated with increased cell death. PHMG-p enhanced TRAIL-associated apoptosis, which correlated with upregulation of both TRAIL and TRAIL death receptor (DR) 4/5 expression through the induction of CHOP. PHMG-p enhanced the apoptosis-associated with extrinsic target protein (Fas and FADD) and intrinsic target protein (caspase-3, capase-8, and PARP). Also, PHMG-p promoted the pro-apoptotic (Bax) and reduced the anti-apoptotic (Bcl-2 and Bcl-xL) protein expression via upregulation of DNA damage associated proteins (H2AX and p53). Finally, PHMG-p increased Bid-activated cytochrome c release. Furthermore, the same symptoms as cells were seen in the PHMG-p-inhaled animal model. PHMG-p enhanced the apoptosis-associated with extrinsic and intrinsic protein target in mice. These results demonstrated the view that PHMG-p induces TRAIL-associated the cell death via the assembly of a death-induced signal complex (DISC) by TARIL-mediated DR4/5, FADD and caspase-8 activation in the early stages.

1195 The SILIFE Project: Production, Toxicity Screening, and Industrial Application of Quartz Species with Reduced Lung Toxicity

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In 1997 the IARC classified respirable crystalline silicas (RCS) as human carcinogens (category 1), strongly affecting work places in silica-dependent industries. IARC acknowledged differences among RCS species, based on source, chemical, thermal and mechanical history. As abundance, density and heterogeneity of surface silanol groups/radicals seem to be involved in RCS-mediated lung toxicity, adverse RCS lung effects might be reduced by coatings, covalently blocking these groups. Persistent problems with silicosis and the diversity of quartz applications stimulated issuance of the EU-project SILIFE, aiming at developing a dry surface-coating technology to abate RCS toxicity on industrial scale. The development process included choice of feasible, cost-effective coating additives (i.e. organosilanes and catalysts), definition of treatment parameters (technology, reaction time, dosage, application), proof of coating effectiveness and toxicity-reducing functionality by physico-chemical (e.g. ζ potential) and predictive *in vitro* and *in vivo* toxicity tests, with final implementation of coated RCS into industrial processes. Primary rat alveolar macrophages (4 h of incubation, 75 µg/cm^2 of pristine/coated quartzes) served as sensitive *in vitro* toxicity screening model with membrane (LDH-release) and DNA damage (Comet assay) as relevant endpoints. The very promising *in vitro* results were validated in a 28-/90-days intratracheal instillation study in male Wistar rats (pristine v. coated industrial quartz species; 2 mg/lung; positive control: quartz DQ12, 1 mg/lung). In bronchoalveolar lavage fluid both latency and variable, quartz species-dependent adverse reactivity of three industrial quartz samples was noted, using classical inflammatory parameters (differential cell count, LDH, ß-glucuronidase, total protein) and pro-inflammatory mediators (CINC-1, TXB_2) as meaningful readouts. But, more importantly, the study clearly demonstrated that some covalent RCS-coatings were indeed able to effectively block RCS lung toxicity in the rat for up to 90 days, without markedly compromising the technical process quality. Thus, covalent surface-coating of biologically reactive RCS species represents a promising strategy to render RCS handling safer. *Funding: LIFE 2014: project no. LIFE14 ENV/ES/00238.*

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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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