(MCh). Transepithelial potential difference, short-circuit current and transepithelial resistance were measured using tracheas mounted in Ussing chambers and treated with the ion transport inhibitors amiloride (Na^+ channel blocker), NPPB (Cl^- channel blocker) and ouabain (Na^+, K^+ -pump blocker). There was no effect of COV treatment on basal or inhibitor-mediated bioelectric responses, indicating that ion transport and tight junction integrity in the airway epithelium were unaffected by COV. In isolated, perfused tracheas COV had no effect on reactivity to methacholine applied extraluminally or intraluminally indicating that epithelial integrity was intact and airway smooth muscle contractility was unchanged. Measurement of isometric contractions using isolated tracheal strips stimulated with electric field stimulation indicated that neural innervation of tracheal smooth muscle was not affected by COV. In conclusion, a 6 h exposure to COV did not alter function in the systems investigated in this study.

2381 Silica Inhalation-Altered Expression of Telomere Maintenance Genes in Lung Tissue of Rats

J. M. Antonini, P. Joseph, V. Kodali, C. Umbright, J. R. Roberts, A. Erdely, and M. Shoeb. *NIOSH, Morgantown, WV.*

Occupational exposure to silica causes severe health effects, such as pulmonary fibrosis and lung carcinoma. Identification of molecular targets and mechanisms of silica-induced pulmonary toxicity is important for intervention and prevention of lung disease. Telomeres consist of tandem repeats of TTAGGG DNA sequences and are located at the end of chromosomes, preventing chromosomal fusion and degradation. Telomeres shorten with cell division leading to genomic instability and cellular senescence. Shelterin (e.g., POT1) and other proteins (e.g., TTI2, RTEL1) involved in telomere maintenance play an important role in maintaining telomere length and integrity. The goal was to assess the effect of silica exposure on the regulation of different genes involved in telomere maintenance in an animal model. Male Fischer 344 rats were exposed by inhalation to silica using two regimens: (1) 15 mg/m^3 for 6 hr/d x 3, 6, and 12 wk, assessed 1 d post-exposure; (2) 15 mg/m^3 for 6 hr/d x 1 wk, assessed 44 wk post-exposure. After exposure, portions of right lungs were homogenized, total RNA was isolated, cDNA was obtained, and expression of telomere maintenance genes was assessed. At all time points post-exposure, mRNA expression of POT1, RTEL1, and TTTI2 was significantly decreased in lung tissue of silica-exposed animals compared to air controls. Reduced expression of these genes causes disruption of assembly of the telomere and induces DNA damage. Analysis of a focused array for genes associated with telomere function and regulation indicated a reduced expression ($p<0.01$) of 49 genes after 3 wk post-exposure. However, by 44 wk after a 1 wk exposure, 10 of these genes were overexpressed, whereas 29 of these genes remained downregulated. Array findings indicated acute and subchronic effects on telomere-associated genes after silica exposure. This study indicates that measurement of genes involved in telomere maintenance may serve as a potential biomarker related to silica exposure and also may offer insight into the mechanism of silica-induced lung disease and tumorigeneses.

2382 Quantification of Lung Injury with Phase Contrast Analysis in a Rodent Model of Postnatal Hyperoxia-Induced Pulmonary Dysplasia

L. Ooi, Q. Zhang, A. Ebrahimnejad, F. Paniagua, R. Sukhu, C. Meschter, and D. Warren. *Comparative Biosciences, Sunnyvale, CA.*

Hyperoxia-induced lung injury is well characterized in neonatal rats; hallmarked by alveolar wall fibrosis, alveolar space enlargement and inflammation. The fibrotic and alveolar changes provide a model for bronchopulmonary dysplasia in human neonates, smoke-inhalation injury, and other conditions involving alveolar wall fibrosis. In non-clinical phases of pharmaceutical development, histopathology provides a robust assessment of pulmonary changes following hyperoxia. To supplement this, quantitative assessments of alveolar changes facilitate evaluation of potential pharmaceutical therapies. Traditionally, Mean Linear Intercept (MLI) measurements of lung sections are used to assess alveolar airspace, by measuring linear distance between septal walls on a structured grid. Such MLI measures are laborious (~30 min/section) and do not portray the complexity of pulmonary changes following hyperoxia. We describe an improved method of quantitative alveolar assessment involving Phase Contrast Image Analysis (PCA) that is efficient (~5 min/section), robust, and broadly captures the profile of alveolar changes than MLI measurements. For induction, newborn pups were exposed to 95% oxygen conditions for 10 days followed by normoxic air for 14 days. Lungs were evaluated by histopathology and PCA. Induced pulmonary changes are characterized by inflammation with multifocal to diffuse distribution of lesions, fibrotic thickening of alveolar walls (10-60 µm, normal 5-10 µm) and expansion of the alveolar diameter. PCA assesses the area of alveolar airspace as percentage of total tissue area: first, RGB images of lung sections are converted to binary images using pixel thresholds to differentiate airspace and adjacent tissue. Pixel areas are presented as % alveolar space and % septum respectively. In hyperoxic lungs, alveolar space is significantly decreased (-7.4%) while septum area is significantly increased (+8.0%) relative to normoxic controls, consistent with reported changes. Anti-inflammatory therapeutics are under evaluation for demonstration of PCA as a primary endpoint. PCA provides a robust and efficient quantitative measure of pulmonary response dynamics with improved descriptions of alveolar landscapes.

2383 Persistent and Progressive Lung Injury Is Linked to Recurring DNA Damage, Cellular Senescence, and a Pro-Fibrotic Epithelial Response

T. Beach, C. Johnston, A. Groves, J. Williams, and J. Finkelstein. *University of Rochester, Rochester, NY.*

Radiation induced lung injury (RILI) is a common outcome in patients requiring radiation treatment for lung cancer. Approximately 43% of patients receiving radiation treatment at a dose of 15 Gy or greater will experience some type of RILI, either in the form of acute pneumonitis and/or late onset pulmonary fibrosis. Known effects of radiation injury include DNA damage, inflammation, and a cellular senescence among the exposed cell population, events associated with onset and progression of fibrosis in lung tissue. Resident Club cells and Type 2 Alveolar Epithelial cells (AEC2) play an active role in tissue repair through their ability to proliferate in response to injury. Therefore, we sought to determine the role of pulmonary epithelia in the development and progression of radiation induced pulmonary fibrosis (RIPF). We have hypothesized that following radiation induced immediate and recurrent DNA damage, there is continuous cellular senescence, and both a pro-fibrotic and inflammatory phenotype in the surviving epithelial cells. Whole lung tissue and isolated pulmonary (CD326+) epithelia were collected from fibrosis prone C57BL/6J female mice exposed to12.5Gy thorax only γ-radiation and examined at 24 hrs, 1,4,12, and 32 weeks post radiation treatment by RNA sequencing and histological analysis. Following radiation exposure we observed a loss of both Club cells and Type 2 epithelia. DNA damage as evidenced by γH2A.X foci was persistently increased in whole lung tissue compared to non-irradiated controls, and this data was further supported by an increased abundance of DNA Damage Response (DDR) associated transcripts such as p53bp1 and xrcc1. Senescence associated β-galactosidase and transcripts indicating cell cycle arrest such as cyclin dependent kinase inhibitors Cdkn1a, and Cdkn2b were similarly upregulated in both whole lung tissue and pulmonary epithelia following RT. Epithelial transcripts associated with mediating the immune/injury response appear to diminish over time. In contrast growth factors Ctgf, Vegf, Fgf, and Pgdf are persistently increased in transcript abundance following radiation, and are potentially important contributors of fibrosis. These initial results have revealed ROS production and activity, as well as stimulation or suppression of immune responses as possible targets for mitigation of fibrotic outcomes following radiation exposure.

2384 Effects of Inhaled Aerosolized Carfentanil on Real-Time Physiological Responses in Mice

B. Wong, M. W. Perkins, J. Tressler, A. Rodriguez, J. Devorak, and A. M. Sciuto. *USAMRICD, Gunpowder, MD.*

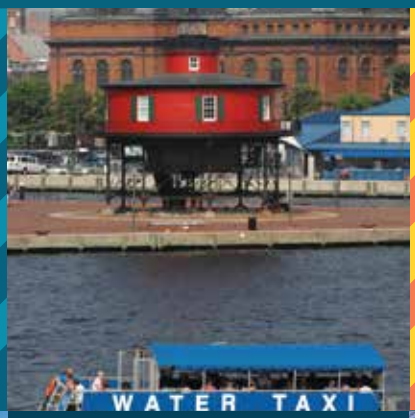
This study examined the real-time exposure-response effects of aerosolized carfentanil (CRF) on opioid-induced toxicity, respiratory dynamics, and cardiac function in mice. Unrestrained, conscious male CD-1 mice (25-30 g) were exposed to 0.4 or 4.0 mg/m^3 of aerosolized CRF for 15 min (Ct = 6 or 60 mg×min/m^3) in a whole-body plethysmograph chamber, in which minute volume (MV) was recorded in real-time. Various clinical observations of classical opioid-induced toxicity were recorded during exposure and up to 24 hr post-exposure. Core body temperature (T_c), mean arterial blood pressure (MAP), and heart rate (HR) were evaluated in telemeter-implanted animals exposed to CRF or sterile H_2O. Loss of consciousness and Straub tail were observed in < 1 min following exposure initiation to 6 or 60 mg×min/m^3 of CRF. Clinical signs of opioid-induced toxicity were observed in a dose-dependent manner during exposure and at 24 hr post-exposure to CRF. Exposure to 6 or 60 mg×min/m^3 of CRF resulted in decreases in MV, MAP, HR, and T_c, as compared to controls. Post-exposure administration of naloxone (NX, 0.05 mg/kg, *i.m.*) did not increase the MV of animals exposed to CRF to control levels within 24 hr but decreased the intensity and total number of clinical signs of opioid-induced toxicity as well as total time of respiratory depression. This is the first study to evaluate real-time respiratory


The Toxicologist

Supplement to *Toxicological Sciences*

56th Annual Meeting and ToxExpo™

Baltimore, Maryland | March 12–16, 2017

OXFORD
UNIVERSITY PRESS

ISSN 1096-6080
Volume 156, Issue 1
March 2017

www.toxsci.oxfordjournals.org

The Official Journal of the Society of Toxicology

SOT | Society of Toxicology

www.toxicology.org