4466 Lung Response to Crystalline Silica and Coal Dust Exposure in Rats

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Background and Purpose: The passage of the Federal Coal Mine Health and Safety Act (Coal Act) in 1969, along with the establishment of the Coal Workers Health Surveillance Program (CWHSP) managed by the National Institute for Occupational Safety and Health (NIOSH) in association with the Mine Safety and Health Administration (MSHA), significantly reduced the incidence of coal workers pneumoconiosis (CWP) in the U.S. Nevertheless, since the early 2000s, this trend has reversed with a notable increase in CWP cases, particularly in the central Appalachian states of Virginia, Kentucky, and West Virginia. However, the etiological factor(s) contributing to this resurgence remain unclear. While crystalline silica has been proposed as a potential contributor to the re-emergence of CWP cases, there is currently insufficient experimental evidence to confirm its definitive role in the resurgence. **Methods:** The potential role of crystalline silica in the re-emergence of CWP has been investigated using a rat whole-body inhalation-exposure and lung toxicity model. All experiments were done in an AAALAC International approved animal facility (NIOSH, Morgantown, WV) following a protocol approved by the CDC-Morgantown Animal Care and Use Committee. Aerosols containing crystalline silica (Min-U-Sil 5) or coal dust (Keystone Mineral Black 325BA) were generated using a custom-built automated system and rats exposed in a whole-body inhalation chamber. The particle size distribution in the aerosol samples was assessed with a micro-orifice uniform deposit impactor (MOUDI).Approximately 3-month-old male Fischer 344 rats (n=6/group) were divided into four exposure groups: 1. Filtered-air control (6 hours/day, 5 days/week for week 1 followed by filtered air again for 6 hours/day, 4 days/week for 3, 6, or 12 months); 2. Min-U-Sil 5 silica (15 mg/m^3, 6 hours/day, 5 days during week 1 followed by filtered air for 6 hours/day, 4 days/week for 3, 6, or 12 months); 3. Coal dust (filtered air for 6 hours/day, 5 days during week 1 followed by coal dust, 10 mg/m^3, 6 hours/day, 4 days/week for 3, 6, or 12 months); 4. Min-U-Sil 5 + coal dust (Min-U-Sil 15 mg/m^3, 6 hours/day, 5 days during week 1, followed by coal dust, 10 mg/m^3, 6 hours/day, 4 days/week for 3, 6, or 12 months). The silica content in the coal dust sample used in the study was 5.4%. Rats were euthanized at the end of the 3rd, 6th, or 12th month, after the first exposure, and bronchoalveolar lavage (BAL) was conducted in the right lung. The BAL samples collected were analyzed to determine the number of total cells, alveolar macrophages, and polymorphonuclear leukocytes. Simultaneously, the diaphragmatic and cardiac lobes of the left lung collected were fixed in formaldehyde, embedded in paraffin, sectioned at a thickness of 5 µm, stained with hematoxylin and eosin or Mason's trichrome stain, and examined for histological changes by a pathologist. **Results:** The mass median aerodynamic diameter of the crystalline silica and coal dust particles in the aerosol samples generated was 1.6 µm [geometric standard deviation (σ_g) 1.6] and 1.36 µm (σ_g 2.3), respectively. The body weights of the rats exposed to coal dust alone or a combination of coal dust and silica were lower compared to the corresponding controls in the 3- and 6-months groups, with reductions of approximately 5% for coal dust and about 10% for the combination of crystalline silica followed by coal dust. Exposure to crystalline silica alone did not result in any significant difference in body weight, compared with the control group at any of the time intervals.Results from bronchoalveolar lavage (BAL) analysis and lung histology indicated progression of lung toxicity in the aerosol-exposed rats, compared to the time-matched, air-only exposed control group. Notably, at the 3- and 6-month intervals, the lung response was more pronounced in rats exposed to both crystalline silica and coal dust aerosols than in those exposed to the individual agents. For instance, histological changes indicative of lung fibrosis were detectable as early as 3-months post-exposure in only the rats exposed to both types of aerosols. Additionally, hyperplasia of the bronchus-associated lymphoid tissue (BALT) was observed exclusively in the rats exposed to the combination of crystalline silica and coal dust aerosols. **Conclusions:** Taken together, the results suggest that exposure to a combination of crystalline silica and coal dust induces a more pronounced pulmonary response in rats, as indicated by the BAL parameters of pulmonary toxicity and lung histology, compared to exposure to either agent individually. These findings highlight the potential role of crystalline silica exposure in the re-emergence of CWP in the U.S.

4467 Gonadal sex hormones modulate the inflammatory response to house dust mites in female bronchial epithelial cells

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Background and Purpose: Asthma is a highly prevalent disease, affecting over 20 million adults in the US. This prevalence is not evenly distributed between genders; more adult women have asthma than adult men. While as children, asthma is more common in boys than girls; this sex disparity changes around the age of puberty, where adult women display higher asthma rates and severity than adult men. This fact prompted the current study investigating the role of sex hormones on allergic inflammatory responses using a common allergen, house dust mites (HDM). We hypothesize that in the presence of female sex hormones like estrogens, the female bronchial epithelium will produce a greater inflammatory response evidenced by increase expression of cytokines and chemokines, while in the presence of androgens the response to HDM will be attenuated. **Methods:** HBEC3-KT (female bronchial epithelial) cells were cultured in Airway Epithelial Cell Basal Medium (ATCC) with the supplemental growth kit and antibiotic/antimycotic and seeded with 50,000 cells per insert in 12 well Transwell inserts coated with collagen. The cells were cultured to confluency (72 hours) in the insert with the same media. Once confluent, the media in the basal compartment was replaced for media without supplements, and the apical surface was rinsed and aspirated to create an air-liquid interface (ALI). The cells were left at ALI for 24 hours before adding the exposure mixtures to the apical side. The exposure mixtures used for each insert totaled 100 µL, and included PBS, HDM (50 ng), 17β estradiol (1 nM, 10 nM), Progesterone (10 nM), and dihydrotestosterone (DHT, 1 µM). To assess the roles of hormone receptors, cells were co-cultured with fulvestrant, mifepristone, and flutamide as antagonists for estradiol, progesterone, and DHT, respectively. Each exposure group consisted of 3 individual inserts. 48 hours after the start of the exposure, cell basal media was collected, and cells were counted for viability via Trypan Blue exclusion using a cell counter. RNA was extracted using Trizol and retrotranscribed. To assess gene expression, TaqMan probes were used for GM-CSF, IL-8, IL-6, IL-1β, CCL2, CCL5, and 18S as an endogenous control. Gene expression changes were calculated using the -ΔΔCT method. Statistical analysis was performed using R/Rstudio with ANOVA and Tukey Post-Hoc analyses. **Results:** Exposure to HDM elicited some response in select pro-inflammatory genes: GM-CSF, IL-8, IL-6, IL-1β, CCL2, and CCL5. Co-exposure with estradiol increased the response to HDM for the following genes IL-8, (2.29 times), GM-CSF (2.54 times), CCL2 (2.90 times), IL-6 (1.79 times), IL-1β (1.47 times), and CCL5 (1.52 times). On the other hand, cells treated with DHT displayed the opposite response: IL-8 (1.83 times decrease), GM-CSF (3.36 times decrease), CCL2 (1.59 times decrease), IL-6 (1.94 times decrease), IL-1β (2.10 times decrease), and CCL5 (1.93 times decrease). **Conclusions:** Gonadal sex hormones modulated the response of the female cell line HBEC3-KT to HDM exposure. Female sex hormones enhanced the response of pro-inflammatory genes in a concentration-dependent manner, while male sex hormones inhibited the inflammatory response. These results have implications for environmental agents that induce injury through inflammation and our understanding of sex-specific responses in inflammatory lung disease.

4468 Drp1 Inhibition Attenuates Toxin-Induced Lipid Droplet Accumulation and Inflammation in Microglia

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Background and Purpose: Microglia are the resident immune cells of the central nervous system (CNS) and play a crucial role in brain homeostasis and function. In aging, neurodegenerative disorders, and inflammatory states, microglia lose their homeostatic molecular signatures and display a dysfunctional state characterized by elevated levels of proinflammatory cytokines, production of reactive oxygen species (ROS), and altered metabolic profile. This microglial state has recently been associated with lipid droplet accumulation. Lipid-accumulating microglia have reduced phagocytic activities and elevated release of inflammatory cytokines. Reducing such lipid accumulation, therefore, may be beneficial to the CNS. Our lab has been investigating the therapeutic potential of inhibiting dynamin-related protein 1 (Drp1), which we have shown to have other functions beyond its role in mitochondrial dynamics. In this study, we investigated the impacts of Drp1 knockout or Inhibition on lipid droplet accumulation and associated inflammation. **Methods:** Aging hippocampal metabolomics data (3-month- and 23-month-old) were accessed from the metabolomics workbench (ID PR001047). Three transcriptomic datasets were acquired from NCBI GEO. First, was microarray data (GSE139946) from substantia nigra of paraquat and maneb-treated mice. Second, RNA-Seq data (GSE89562) from the hippocampus of LPS-treated mice sorted into high and low lipid droplets. Lastly, Bulk hippocampal RNA-Seq data of LPS/Saline treated mice. In vitro studies, primary microglia were treated with lipopolysaccharides (LPS) at 100ng/mL for 24 hours with or without the small molecule peptide P110 (a Drp1 inhibitor). For in vivo studies, 3-month-old mice were intraperitoneally injected with lipopolysaccharide (LPS, 1mg/kg body weight) once daily for four consecutive days or paraquat (10 mg/kg body weight) every second day for five injections. At the end of each experiment, mice were saline perfused. One hemi-brain was post-fixed for immunohistochemistry and the other hemi-brain was micro-dissected for protein/RNA extraction. Lipid droplet staining was done using BODIPY (a dye that labels lipid droplets). **Results:** Lipid metabolism pathways (glycerophospholipid metabolism, sphingolipid metabolism, and synthesis of fatty acid) are significantly enriched in the aging hippocampus ($p<0.05$, n=8). Similar patterns have been reported in other brain regions of LPS and paraquat-treated mice. These pathways are associated with increased synthesis of fatty acids and cytokines mediators. Increased production of free fatty acids molecules leads to significant accumulation of lipid droplets in microglia than other brain cells. Using RNA-Seq data from NCBI GEO for LPS (Hippocampus) and paraquat (Substantia nigra), we determine the expression levels of lipid droplets-associated proteins. LPS and paraquat significantly upregulated hippocampal Plin2 mRNA transcripts ($p=0.03$, n=8) and nigral Plin4 mRNA transcripts ($p<0.0001$, n=3) respectively. To determine whether Drp1 levels correlated with the amount of lipid droplets in microglia, we used RNA-Seq data of microglia sorted into high and low lipid droplets. Sorting was confirmed by significantly higher Plin2 ($p =0.04$, n=3) and Plin3 mRNA ($p=0.03$, n=3) in the lipid high group compared to the lipid low group. Microglia with high lipid droplets had significantly higher *Dnm1l* mRNA transcripts than microglia with low lipid droplets ($p=0.007$, n=3), suggesting a dose-response correlation between Drp1 and lipid droplets. Using mouse primary microglia, we confirmed that more Drp1 protein was detected in microglia treated with LPS (100ng/mL, 24 hours) ($p<0.001$, n=3).

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