junctional protein expression changes. In combination with IL-4 pretreatment, DA challenge synergistically decreases barrier function as determined by decreases in TEER and increases in permeability. Since IL-4 is produced during allergic inflammation, these results support the hypothesis that allergic conditions such as asthma might exacerbate DA-mediated epithelial damage *in vivo*.

4270 Effect of age on crystalline silica-induced pulmonary toxicity in rats

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Background and Purpose: Millions of workers are exposed to crystalline silica in the United States. Silica exposure at levels above the OSHA PEL (50 µg/m^3) is known to contribute to potentially fatal pulmonary diseases such as silicosis, cancer, and chronic obstructive pulmonary disease. The age composition of workers is changing in the U.S. and worldwide and it is anticipated that many workers will continue to work at older ages. It is currently unknown what relationship, if any, the age of the worker has on his/her response to occupational exposure to silica. Furthermore, it is critical to determine the mechanisms underlying the potential modification of the silica-induced lung toxicity by age. The objective of the proposed study, therefore, was to determine the effect of age on the pulmonary toxicity induced by crystalline silica exposure by employing a rat lung toxicity model. **Methods:** All experiments in this study 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. To conduct these studies, two age groups of male F344 rats were used. The first group of animals (6 months old at the time of exposure) was designated as the young age group. This age group of rats simulate workers who are approximately 18 to 20 years old. The second group of rats (18 months old at the time of exposure) was designated as the old age group. This age group of rats simulate workers who are between the ages of 45 to 50 years. The young and old age groups of rats were exposed to either air (control) or Min-U-Sil 5 crystalline silica (15 mg/m^3, 6 hours/day, 5 days) in a whole-body inhalation system. At two post-exposure time periods (1-day and 6-months) the animals were euthanized with an intraperitoneal injection of ≥100 mg sodium pentobarbital/kg b.w. followed by exsanguination by severing the descending abdominal aorta. Immediately following euthanasia, a tracheal cannula was inserted, the right lobes were tied off and the left lung lobe underwent bronchoalveolar lavage (BAL). The BAL fluid and the cells collected were employed to assess the pulmonary response to silica exposure. Lactate dehydrogenase (LDH) activity was determined in the BAL fluid to assess lung injury. Using the BAL cells from the lavage samples, cell counts and differentials were conducted to assess pulmonary inflammation. To determine the generation of reactive oxygen species (ROS) by the BAL phagocytes, a luminol-dependent chemiluminescence assay was performed. **Results:** silica exposure resulted in an increase in the BAL fluid LDH activity, suggesting the induction of lung injury. In the young-aged animals, LDH activity was higher in silica treated animals compared to controls, at both 1-day and 6-month post-exposure. However, this elevation in LDH activity was not significantly different ($p>0.05$) from control at both time points. The increase in LDH activity was significantly ($p<0.05$) higher in the silica exposed groups compared to air exposed groups in the old-aged animals at both 1-day and 6-month post-exposure. At 6-months post-exposure in the old-aged animals LDH activity was 2.6-fold higher in the silica exposed animals compared to age-matched controls. Furthermore, the results also show increases in BAL cell ROS generation between animals treated with silica and controls. Specifically, in both the young-aged and old-aged animals, ROS generation was significantly ($p<0.05$) higher in silica treated animals compared to controls, at both 1-day and 6-month post-exposure. However, at 6-months post-exposure ROS generation was 2.4-fold higher in the silica exposed old-aged animals compared to the silica exposed young-aged animals. Additionally, silica inhalation resulted in a significant increase in neutrophil infiltration at both 1-day and 6-months post-exposure for both the young-aged and old-aged rats. At 1-day and 6-months post-exposure, in the young animals, silica exposure caused a 150-fold and 124-fold increase in neutrophil infiltration respectively, compared to the controls. On the other hand, at 1-day post-exposure in the old animals, silica caused a 271-fold increase in neutrophil infiltration while at 6- months post-exposure in the old animals, silica caused a 243-fold increase in the infiltration of neutrophils compared to the corresponding controls. Thus, the neutrophil infiltration in the silica exposed older rats was approximately 2-fold higher, compared to their younger counterparts. **Conclusions:** Inhalation exposure to crystalline silica results in pulmonary toxicity in the rats, regardless of their age. However, our results reflect that advanced exposure age leads to an enhanced pulmonary response to crystalline silica exposure in the rats. In the lungs of the older animals, silica exposure facilitates significantly higher neutrophil infiltration, LDH activity, and oxidant generation compared to young animals exposed to crystallin silica. In conclusion, our results support the hypothesis that enhanced age exacerbates the pulmonary response to crystalline silica inhalation exposure.

4271 Silica-induced lung and systemic inflammation was prevented by pretreatment with a water-soluble, organosilane-based coating in an animal model

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Background and Purpose: Inhalation exposure of respirable crystalline silica puts thousands of workers from multiple industries at risk for pulmonary disease. Inhalation of silica dust can cause pulmonary fibrosis and inflammation, chronic obstructive lung disease, and lung cancer. One of the primary causes of silica-induced lung disease is the highly reactive surface of fractured silica particles which generates cell-damaging reactive oxygen species. Coating silica particles with specific organosilanes has been shown to reduce silica-induced toxicity in lung macrophages *in vitro*. The objective of the current study was to use an *in vivo* model to evaluate acute and sub-chronic toxicity and the resolution kinetics in lungs induced after silica exposure with and without a specific water-soluble, organosilane-based coating (SIVO160). **Methods:** Male Sprague-Dawley rats were intratracheally instilled with silica (1 mg/rat), silica coated with SIVO160, SIVO160 alone, or saline (vehicle control). At 3, 10, 45, and 90 d after exposure, bronchoalveolar lavage (BAL) was performed to assess lung inflammation and injury. Whole blood was collected at each of the time points post-exposure to evaluate systemic inflammation by differentiating circulating white blood cells. In addition, samples of uncoated and coated silica were analyzed by different methods [(1) RapiFlex MALDI-ToF/ToF mass spectrometry; (2) digestion in phagolysosomal simulant fluid (PSF; pH 4.5; model of lung macrophage phagolysosome) and serum ultrafiltrate (SUF; pH 7.3; model of extracellular lung lining fluid)] to confirm the SIVO160 coating on the surface of the silica particles after an extended incubation period in biological media. **Results:** At each time point after exposure, silica significantly increased BAL fluid 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 completely blocked at each time point when silica was coated with SIVO160 before exposure. Pulmonary exposure to SIVO160 alone produced no increase in lung injury and inflammation. Changes in peripheral blood phenotypes were not observed until 45 d after silica exposure as total white blood cells, neutrophils, and lymphocytes were significantly elevated in the blood compared to the other groups. Coating the silica with SIVO160 before exposure prevented the observed changes in blood cell profiles. As assessed by mass spectrometry, multiple unique spectral peaks were detected on the surface of the silica+SIVO160 particle samples after an overnight incubation in saline. The peaks were absent in the uncoated silica sample spectra, confirming the coating's presence on the particles. The removal of the SIVO160 coating on the silica had a delay of approximately 3 d after incubation in both PSF and SUF, suggesting some protection could be conveyed to lung cells by the coating during phagocytosis and particle deposition on lung tissue structures. Microscopic evaluation of tissue slices showed that particles were still visible at 90 d within lung cells of the animals treated with uncoated silica but were not present in the lungs of the animals treated with silica coated with SIVO160. This indicated the coated silica was likely not toxic to phagocytes and was cleared from the lungs, whereas the uncoated silica persisted in the lungs throughout the course of the study. **Conclusions:** SIVO160 coating of highly toxic silica particles: (1) prevented lung and systemic toxicity at both acute and sub-chronic time points after exposure; (2) increased the rate of clearance of the deposited particles compared to uncoated particles from the lungs; and (3) persisted on the surface of the particles in different simulated biological fluids. Organosilane materials may be used as a possible mitigation strategy to potentially protect large numbers of workers exposed to crystalline silica in multiple industries.

4272 Investigating the Toxic Effects of Lunar and Martian Dust Simulants

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Background and Purpose: The NASA Thrive in Deep Space (TIDES) program is dedicated to the advancement of space exploration, with a specific focus on lunar and Martian missions. An area of concern within these missions is the potential toxicity and inflammatory effects associated with respirable-sized lunar and Martian dusts. Prior studies have shown that lunar and Martian regolith simulants, characterized by their high silica content, can induce toxic reactions, including lung inflammation, which may lead to lung fibrosis and silicosis. These adverse health outcomes can be attributed to nearly free silanols (NFS) found on the silica surface. Once these particles are phagocytosed by alveolar macrophages they are internalized into lysosomes. NFS on the surface of silica have a disruptive effect on lipid membranes, particularly within lysosomes leading to cellular toxicity and inflammation. **Methods:** In this study, we sought to investigate the relative toxicity and underlying mechanisms of lunar regolith simulant JSC-1 and Martian regolith simulant JSC-MAR-1 in inducing inflammation. To expedite data collection while minimizing the need for vertebrate testing, a novel model using murine *ex vivo* alveolar macrophages (mexAM) derived from primary alveolar macrophages (AM) of C57Bl/6 mice was employed. Cytotoxicity assays were conducted to establish dose-response curves for the regolith simulants, with quartz silica and TiO2 used

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