3709 Pulmonary Lipid Alteration Patterns and Inflammation Following Silver Nanoparticle Exposure

N. Medel, C. Ferreira, S. Alqahtani, *L. Xia*, and *J. Shannahan*. Purdue University, West Lafayette, IN.

Background and Purpose: Silver nanoparticles (AgNPs) are commonly used in manufacturing processes and consumer/biomedical products. Inhalation is a primary route of nanoparticle exposure and AgNPs have demonstrated lung toxicity including oxidative stress, inflammation, and pulmonary injury. Pulmonary inflammation is associated with the development of diseases including fibrosis, asthma, and cancer. Bioactive lipids govern the initiation and resolution of inflammation. Currently, there is little understanding regarding pulmonary lipid-mediated mechanisms of inflammation following nanoparticle inhalation. This knowledge gap impedes our ability to treat exposures and diseases where inflammation is a primary component. Within this study, we hypothesize AgNP exposure will induce a pulmonary inflammatory response via the dysregulation of lipid mediators. **Methods:** To test this hypothesis, mice were exposed to 50µg of AgNPs or vehicle (control) via oropharyngeal aspiration. Three days following exposure, bronchioalveolar lavage fluid (BALF) and the right lung lobes were collected while the left lung lobe was fixed in carboxymethyl cellulose. Collected samples were analyzed for endpoints of pulmonary lung injury and inflammation, lipid dysregulation, histological alterations, and AgNP deposition. **Results:** BALF analysis demonstrated increased total protein levels and neutrophils following AgNP exposure compared to controls demonstrating pulmonary inflammation and injury. AgNP exposure increased gene expression of inflammatory genes including interleukin-1β (IL-1β), macrophage chemoattractant protein-1 (MCP-1), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and C-X-X motif chemokine ligand 1 (CXCL1) while no alterations were observed for genes associated with resolution of inflammation interleukin-4 (IL-4) or interleukin-10 (IL-10). Assessment of BALF cytokines demonstrated elevations of the pro-inflammatory mediators macrophage inflammatory protein-2 (MIP-2) and macrophage chemoattractant protein-1 (MCP-1) and no alterations in IL-10 in mice exposed to AgNPs. Fixed lung lobes were sectioned and evaluated via a variety of imaging techniques. Hyperspectral darkfield imaging was utilized to determine AgNP localization while staining with hematoxylin and eosin histologically evaluated inflammation within the lung. Desorption electrospray ionization mass spectrometry (DESI-MS) was employed to assess spatial alterations in lipid mediators and demonstrated AgNP-induced alterations in lipid mediators. Hematoxylin and eosin staining and hyperspectral darkfield microscopy allowed for cross referencing of areas of inflammation and AgNP deposition with MassLynx data from DESI imaging to select regions of interest. **Conclusions:** A workflow was developed for processing, analyzing, and attributing the mass spectra data to compare the various metabolites between the AgNP exposed and control samples. Overall, our study demonstrates lipid dysregulation may contribute to AgNP-induced inflammation following particulate inhalation. This information can be utilized to identify disruptions of bioactive lipid mediators to better inform therapeutic strategies regarding inflammatory-mediated diseases resulting from exposures.

3710 Toxicity evaluation of silver nanoparticle in the kidneys of Wistar Rats

A. Patlolla[1], A. Kumari[2], and Z. Lusk[1]. [1]Jackson State University, Jackson, MS; and [2]Nizam College, Hyderabad, India.

Background and Purpose: Silver nanoparticles (Ag-NPs) also known as nano-silver possess unique physico-chemical properties, regarded as the best known nanoproducts and have been used in several applications. With the increasing use of Ag-NPs, the public has a higher risk of exposure in daily life, through occupational environments and consumer products. Additionally, the adverse effects of Ag-NPs on human health and the environment are of increasing concern. This study aimed to evaluate nephrotoxic effects of silver nanoparticles (AgNPs) in the Wistar rats using biochemical, oxidative stress and histopathological changes. **Methods:** Three groups of six rats were orally administered AgNPs once a day for 28 days with doses of 100, 500, 1000 mg/kg bodyweight. A control group was administered with deionized water. Blood and kidneys were collected 24 h after the last treatment following standard protocols. The activities of creatinine and blood urea nitrogen against AgNP-induced toxicity was determined in the serum. Various activity levels of oxidative stress including, Catalase (CAT), Superoxide dismutase (SOD), Glutathione peroxidase (GPx) and Lipid hydroperoxides (LPO) were evaluated in the kidney tissue. Scanning (SEM) and transmission electron microscopy (TEM) was used to determine the histopathological evaluation of the kidneys. **Results:** A significant increase in the levels of serum creatinine, blood urea nitrogen, CAT and LPO, were noted in AgNPs exposed rats compared to that in control rats. In contrast, decreased activities of SOD and GPx in a dose- dependent manner was observed in AgNPs exposed rats relative to control rats. SEM and TEM study showed significant morphological alterations in kidneys of AgNPs exposed rats in accordance with the biochemical markers. **Conclusions:** The results of the study demonstrate that AgNPs might be nephrotoxic, and its toxicity is mediated through oxidative stress mechanism.

3711 Local and systemic immune responses following aspiration of nickel oxide nanoparticles in a humanized Toll-like receptor-4 mouse model

K. A. Roach, J. L. Aldinger, A. B. Stefaniak, C. Waggy, and *J. R. Roberts*. CDC/NIOSH, Morgantown, WV.

Background and Purpose: It has been estimated that 20% of the global population exhibits contact sensitivity to nickel. Despite such prevalence in humans, recreating nickel allergy in laboratory rodents has proven challenging historically, ultimately limiting our understanding of many underlying immunological mechanisms responsible for the disorder. In 2010, it was discovered that species-specific differences in Toll-like receptor-4 (TLR-4) structure contribute to the discrepancies in susceptibility between mice and humans; subsequent findings in a humanized (h)TLR-4 mouse model demonstrated that the model more accurately depicts human immune responses to nickel in the skin, but the role of hTLR-4 in nickel's biological effects in other tissues remains unclear. Consequently, the primary goal of this study was to characterize alterations in various immune parameters in both sexes and genotypes of mice from the hTLR-4 colony following lung exposure to nickel. **Methods:** On 0d, a group of hTLR-4 negative and positive mice of both sexes (n=6) were exposed to vehicle control (dispersion media, DM) or nickel oxide nanoparticles (NiONP, 48 nm, one of three doses: 2.5, 5, or 20 µg) once by oropharyngeal aspiration. A set of mice from each sex/genotype combination was euthanized 1, 7, 14, or 28 d post-exposure. Bronchoalveolar lavage (BAL) was performed to evaluate cellular constituents and biochemical markers of inflammation within the airways. Blood was collected, circulating leukocyte profiles were characterized, and serum cytokine levels were evaluated. Finally, the lung-associated lymph nodes, thymus, and spleen were harvested, weighed, and phenotyped. **Results:** NiONP exposure resulted in dose-dependent increases in the total number of immune cells present in the lungs of all animals. Higher innate immune cell (e.g., neutrophils) influx was observed in all groups, but increases in the number and proportionality of lymphocytes in the BAL were only detected in hTLR-4 positive animals. Lymphocytes comprised 3.25% of the BAL cell pool in females at 14d (compared to 1.12% in DM, 2.13% in hTLR-4 negative) and 2.30% in males at 7 d (compared to 0.88% DM, 1.28% in hTLR-4 negative). Exposure to the 20 ug NiONP dose also induced significant increases in total cellularity of the lung-associated lymph nodes in all groups. In females, lymph node cell number peaked at 14 d, increasing 1.5-fold over DM control values in the hTLR-4 negative group ($5.01x10^6$) and 2.5-fold in hTLR-4 positive females ($6.46x10^6$). In males, lymph node cellularity increases were evident by 7 d, though responses were not as pronounced as those observed in females. Total cell number reached 1.2x control values ($5.21x10^6$) in the hTLR-4 negative group and 1.7x ($5.62x10^6$) in hTLR-4 positive males. NiONP exposure induced several notable changes in lymph node cellular composition as well—most of which were seen exclusively in hTLR-4 positive mice and were overall more pronounced in females. Increases in lymph node CD4+ T-cell and B-cell activation were observed in both females and males but increases in the proportionality of these two lymphocyte populations were only noted in hTLR-4 positive females. Cellular composition in the spleen exhibited similar alterations as those seen in the lymph nodes, except for one notable difference—in the lymph nodes, NiONP was associated with an increase in the CD4:8 T-cell ratio in hTLR-4 positive females and males, but in the spleen, this ratio was significantly decreased in hTLR-4 positive males. No clear relationship could be discerned between changes in the circulating leukocyte profile and any other parameter of the study; however, the few statistically significant changes observed occurred in the hTLR-4 positive groups. **Conclusions:** Overall, mice expressing hTLR-4 exhibited significantly enhanced immunological responsivity to NiONP compared to non-carriers. In general, female mice were more susceptible to nickel-induced immune alterations in the lung and associated lymphoid tissues, as well as in the spleen and blood. These findings, in combination with our previous findings in the skin, suggest that the hTLR-4 mouse model exhibits a heighted degree of reactivity to nickel that more closely resembles human responses to the metal, and thus, represents an improved approach for studying the general toxicity and allergenicity of nickel and its various formulations (e.g., nanoparticulates, salts, etc.) *in vivo*.

3712 Pulmonary toxicity of nickel oxide nanoparticles in a transgenic mouse model expressing humanized Toll-like receptor-4 (TLR-4)

J. R. Roberts, J. L. Aldinger, A. B. Stefaniak, and K. A. Roach. CDC-NIOSH, Morgantown, WV.

Background and Purpose: We have previously demonstrated that murine expression of humanized Toll-like receptor-4 (hTLR-4) confers enhanced immunological responsivity to nickel in the skin and increased susceptibility to contact sensitization *in vivo*; however, it remains unclear if hTLR-4 expression is similarly implicated in inflammatory reactions elicited by nickel in other relevant tissues. Consequently, the primary goal of this study was to characterize the effects of nickel nanoparticles on the lung in both sexes and genotypes of mice from the hTLR-4 colony to help elucidate its potential utility for future studies of nickel's health effects. **Methods:** hTLR-4 negative and positive mice of both sexes (n=6 per group) were exposed to vehicle control (dispersion media, DM) or nickel

SOT 63RD ANNUAL MEETING & TOXEXPO
SALT LAKE CITY, UTAH • MARCH 10–14, 2024

The Toxicologist

Supplement to *Toxicological Sciences*

SOT | Society of Toxicology

Toxicological Sciences

The Official Journal of the Society of Toxicology

OXFORD UNIVERSITY PRESS

ISSN 1096-6080 Volume 198, Issue S1 (March 2024)
www.academic.oup.com/toxsci

Publication Date: March 5, 2024