3709 Pulmonary Lipid Alteration Patterns and Inflammation Following Silver Nanoparticle Exposure

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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

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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

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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)

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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

oxide nanoparticles (NiONP, 48 nm) in one of three doses (2.5, 5, or 20 µg) once by oropharyngeal aspiration (0 d). Mice were euthanized 1, 7, 14, or 28 d post-exposure and bronchoalveolar lavage (BAL) was performed to evaluate cellular constituents and biochemical indices of inflammation. **Results:** The highest dose of NiONP induced significant increases in BAL lactate dehydrogenase (LDH) activity in all groups, the magnitude of which, was dependent on sex and genotype. Overall, females were more sensitive to LDH responses than males, while hTLR-4 expression was associated with greater maximal LDH values in both sexes (374 vs 239 U/L in females, 251 vs 189 U/L in males). A similar trend was observed with respect to total BAL cell number, which increased to the greatest degree in female hTLR-4 positive mice at 7 d (4.1x increase over DM). BAL cell count increased 2.3-fold over DM control values in hTLR-4 negative females, 2.1x in hTLR-4 negative males, and 2.6x in hTLR-4 positive males. BAL neutrophil number also peaked in NiONP-exposed females at 7 d, constituting 36.5% of the total BAL cell pool in hTLR-4 negative animals and 59.9% in the hTLR-4 positive group. Neutrophil counts peaked between 7 and 14 d in males and comprised 38.5% (hTLR-4 negative) and 37.8% (hTLR-4 positive) of all BAL cells. BAL macrophage activation status was differentially impacted by hTLR-4 genotype in female and male mice. Interestingly, the greatest increase in macrophage activation was seen in hTLR-4 negative females at 14 d (36.5%), despite a lack of significance at 1 d (4.9%) and 7 d (5.2%). In hTLR-4 positive females, activation status was significantly increased at all time points of the study but peaked on 7 d at 32.4%. Unlike in females, the time course of macrophage activation in male mice was consistent between groups, reaching peak values at 14 d (17.7% in hTLR-4 negative males, 22.3% in hTLR-4 positive). **Conclusions:** Overall, hTLR-4 positive mice exhibited a significantly greater degree of biological responsivity to NiONP than same-sex hTLR-4 negative controls. Female mice were generally more susceptible to nickel-induced airway inflammation than males, and the greatest maximal responses for most markers of interest in the study were observed in hTLR-4 positive females. These findings are consistent with information obtained previously from our hTLR-4 skin studies, collectively suggesting that the hTLR-4 mouse strain is able to more accurately emulate acute inflammatory responses to nickel in humans.

PS 3713 Role of dopaminergic REST in manganese-induced behavioral deficits and dysregulation of neurotransmitter levels in mice

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Background and Purpose: Chronic overexposure to elevated levels of manganese (Mn) induces manganism, a neurological disorder sharing similar symptoms of Parkinson's disease (PD). Despite evidence linking Mn neurotoxicity to dopaminergic (DAergic) dysfunction, the detailed molecular mechanisms involved remain to be established. Studies have shown that RE1-silencing transcription factor (REST) is protective against many neurodegenerative diseases, including PD and Mn toxicity. In the present study, we investigated if DAergic REST played a role in Mn-induced toxicity, such as behavioral deficits and alteration of several neurotransmitters, including dopamine, using DAergic-specific REST-deleted (REST cKO) mice. **Methods:** Behavioral tests such as locomotor activity, motor coordination, and cognitive function were tested, and neurotransmitter levels using a high-performance liquid chromatography-electrochemical detector (HPLC-ECD) system and microdialysis were measured. REST cKO mice were generated using *Cre-loxP* technology by crossing DAT-Cre mice with REST loxP mice to delete DAergic REST. Mice were exposed to Mn (330 µg Mn as $MnCl_2$ 30 mg/kg, daily, intranasally) for 3 weeks, followed by endpoint assessment of various experiments. **Results:** Results showed that DAergic REST deletion exacerbated Mn-induced impairment of locomotor activities and motor coordination in wild-type (WT) mice. Mn-induced cognitive dysfunction in WT was also further exacerbated in REST cKO mice. In line with these behavioral deficits, Mn decreased dopamine levels in the striatum and midbrain of WT, which were further reduced in REST cKO mice. The metabolites of dopamine, DOPAC, and HVA were also disrupted. Other brain regions, such as cortex, hippocampus, and cerebellum, did not show similar effects of Mn-induced dysregulation of dopamine and its metabolites in the nigrostriatal regions. Moreover, Mn decreased serotonin levels in the striatum and midbrain of WT mice, which were further exacerbated in REST cKO mice. Interestingly, these Mn effects were similarly shown in other regions, including cortex, hippocampus, and cerebellum. Mn also dysregulated glutamate and γ-aminobutyric acid (GABA), but not as consistent as dopamine or serotonin levels. In addition, using microdialysis experiments, Mn decreased synaptic dopamine release in the striatum of WT mice. Mn increased synaptic glutamate release while reducing synaptic GABA release in the striatum of WT mice by single acute Mn treatment (50 mg/kg, i.p.). **Conclusions:** Taken together, our findings demonstrate that DAergic REST deletion exacerbates Mn-induced motor and cognitive deficits and disrupts not only DAergic but also other neurotransmitters including serotonergic, glutamatergic and GABAergic systems. Our results suggest that DAergic REST may be a critical molecular target for developing therapeutics to treat Mn-induced neurotoxicity. Supported by R01 ES024756, R01 ES10563, and R01 ES020852.

PS 3714 Combined *in utero* and early-life inhalation exposure to CuO nanoparticles exacerbates asthmatic outcomes in adult ICR CD-1 outbred mice

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Background and Purpose: CuO nanoparticles (CuO NPs) are utilized in numerous industrial processes and consumer products. It has been shown that inhalation exposure to CuO NPs causes significant pulmonary inflammation and immunomodulation and that inflammation resolves once the exposure stops. However, their immunomodulatory consequences may persist even after exposure ceases. Exposure to these ultrafine materials during gestation or early life in offspring has been less explored. **Methods:** ICR CD-1 outbred dams and non-pregnant mice were exposed to filtered air (controls) or CuO NP aerosol (3.5 mg/m^3) prior to (7 days before breeding) and during gestation (from gestation date 6.5-15.5) *via* a whole-body inhalation. Delivered pups (males and females) were exposed to CuO NPs or filtered air (shams), using the same whole-body exposure from post-natal day (PND) 3–23. After weaning, pups (females only) from both experimental groups were enrolled in house dust mite (HDM) asthma model (PND 24). Remaining female pups from both exposure groups received sensitizations and challenges using normal saline. Pulmonary mechanics (with methacholine challenge) and inflammation by quantifying pro-inflammatory cytokines and number of macrophages, neutrophils and lymphocytes in bronchoalveolar lavage (BAL) fluid and serum levels of cortisol, estradiol and testosterone were assessed in 8-wk-old pups. Levels of IgG1, IgG2a and IgE were measured in serum and BAL in pups enrolled in HDM asthma study. Copper burden in lung tissues of adults and offspring was determined by ICP-MS. **Results:** Pups exposed to CuO NPs exhibited signs of pulmonary inflammation during early life. Number of total cells, neutrophils, and pro-inflammatory cytokines in BAL were higher in CuO NP-exposed pups (males and females) than controls. Significant hormone levels disruption was observed after CuO NP exposure in pups, which was sex-dependent. Shams as well as CuO NP exposed pups enrolled in HDM asthma model both displayed worse pulmonary mechanics parameters, increase in eosinophils in BAL fluid, and higher concentrations of Th2-cytokines, however, these parameters were worse in asthmatic pups exposed to CuO NP when compared to sham exposed pups in asthma model alone. **Conclusions:** CuO NP inhalation exposure caused pulmonary inflammation that resolved after exposure stopped. Although lung inflammation resolved, the impacts of the perinatal exposure was shown in modulation of immune responses in pups when exposed to HDM. Our findings demonstrate that exposure to ultrafine inflammatory particulates before and during gestation as well as in early life in offspring may induce higher risk for developing asthmatic phenotype if exposed to airborne allergens. This work was funded by NIH U01 ES027252 and NIH P30 ES005605.

PS 3715 Inflammatory Response Dynamics to Copper Oxide Nanoparticle Inhalation in an Obese Mouse Model

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Background and Purpose: Obesity is a serious disease that affects 41.9% of the US population with estimated healthcare costs of almost $200 billion annually. Copper oxide nanoparticles (CuO NP) are among the most toxic metal nanoparticles, and with increased production, the risk of exposure due to their release into the environment is increased in the population. Although the proinflammatory effect of the CuO NPs has been extensively investigated, the modulation of immune responses by obesity, a proinflammatory state itself, remains poorly understood. Thus, this study aimed to delineate the inflammatory outcomes after inhalational exposure to CuO NPs within an obese murine model, thereby enriching the understanding of nanotoxicity in the context of pre-existing metabolic disorders. **Methods:** Obesity was induced in C57BL/6J mice via a two-week high-fat/high-sucrose (HFHS) diet regimen, confirmed by insulin and glucose tolerance tests, complemented by body mass assessments. Subsequently, both the induced obese and lean control mice were exposed to CuO NP aerosols with a geometric standard deviation (GSD, 1.8) particle size distribution of 40 nm. The inhalation exposure was conducted in a dynamic whole-body chamber to either CuO NP aerosols or filtered air (for control groups) for two weeks (4 h/day, 5 d/week), with an average mass concentration of 3.51 ± 0.16 mg/m^3. Post-exposure assessments were conducted for days 1, 3, 4, and 14 to evaluate both immediate and prolonged effects. Samples including bronchoalveolar lavage fluid (BALF), blood, and adipose tissues were collected during necropsies. The analysis encompassed a comprehensive profile of total cell counts, differential counts for macrophages, neutrophils, and lymphocytes, total protein levels, and a spectrum of cytokine/chemokine profiles, in addition to cytotoxicity evaluations. **Results:** Insulin and glucose tolerance profiles, as well as body mass measurements, remained consistent before and after exposure, indicating that CuO NP inhalation did not significantly alter metabolic function. However, notable changes in pulmonary inflammatory responses were observed. The immediate post-exposure assessment showed increased numbers of early markers of inflammation such as neutrophils and increased

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