affin-embedded small intestinal sections were collected by laser microdissection, deparaffinized, and RNA was extracted. Libraries for RNA sequencing were prepared using the SMART-Seq Stranded Kit. **Results:** The median particle sizes for the 6, 30, and 180 nm crystallites were 178.4, 362.1, and 252.3 nm with nanoparticle ratios of 27.2, 11.5, and 13.2%, respectively. Regarding general toxicity parameters, no treatment-related changes were observed in mortality, general conditions, body weights, food intake, urinalysis, macroscopic findings, organ weights, hematology, or serum biochemistry. In histopathology, TiO_2 agglomerates were observed in PP across all TiO_2-treated groups, with occasional phagocytosis by macrophages but no associated inflammation or tissue injury. RNA sequencing analysis of PP revealed no toxicologically relevant changes in gene expression, including immune-related genes such as cytokines. No significant changes in titanium content were observed in the kidney or spleen, although a significant increase in the liver was observed in the 180 nm group. However, the difference in the titanium concentration between the control and 180 nm groups was only 7.6 ng/g liver, and no signs of hepatotoxicity or γ-H2AX expression were observed. **Conclusions:** No toxic effects were observed in rats following 90-day repeated oral administration of TiO_2 with three different crystallite sizes across general toxicity parameters. TiO_2 agglomerates were deposited in PP regardless of their crystallite sizes; however, these deposits did not induce inflammation or notable gene expression changes including immune-response genes. The slight but significant increase in titanium content in the liver of 180 nm group suggests trace amounts of TiO_2 can be absorbed from the gastrointestinal tract into the liver, though without causing hepatotoxicity or DNA damage. Correctively, our findings support an absence of toxicity from oral exposure of TiO_2 regardless of particle sizes tested.

4866 Activation of NLRP3 inflammasome via HDAC6 is involved in nickel nanoparticle-induced pulmonary inflammation and fibrosis

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Background and Purpose: Nickel nanoparticles (Nano-Ni) have wide and increasing industrial and biomedical applications, thus, their potential adverse health effects are getting more and more attention. Our previous studies have shown that Nano-Ni exposure caused severe, extensive, and persistent pulmonary inflammation and fibrosis. However, the underlying mechanisms are still obscure. NLRP3 inflammasome is a critical component of the innate immune system and inflammatory signaling. In this study, we explored whether and how Nano-Ni exposure activated NLRP3 inflammasome and whether its activation was involved in Nano-Ni-induced pulmonary inflammation and fibrosis. **Methods:** Mice were exposed to Nano-Ni by intratracheal instillation, and lavaged to determine the cellular and biochemical parameters in BALF after exposure. Lung histology was examined by H&E and Trichrome stainings. Mouse alveolar macrophages (MH-S) were also used. The mRNA and protein expressions of various genes were determined by RT-qPCR and Western blot, respectively. IL-1β protein concentration in the BALF or cell culture medium was measured by ELISA kit. Knockout mice such as *Nlrp3*$^{-/-}$ and *Il-1r1*$^{-/-}$ mice and HDAC6 siRNA were used for mechanism studies. **Results:** Our results showed that intratracheal instillation of wild-type mice (C57BL/6J) with 50 μg per mouse of Nano-Ni caused NLRP3 inflammasome activation, IL-1β production, and extensive pulmonary inflammation and fibrosis. However, Nano-Ni exposure only caused mild pulmonary inflammation and fibrosis in *Nlrp3*$^{-/-}$ (dysfunction of the NLRP3 inflammasome) or *Il-1r1*$^{-/-}$ (not respond to IL-1) mice, indicating that Nano-Ni-induced activation of NLRP3 inflammasome was involved in Nano-Ni-induced pulmonary inflammation and fibrosis. In addition, mouse alveolar macrophages (MH-S) were used to explore how Nano-Ni activated NLRP3 inflammasome. We found that during the activation process of NLRP3 inflammasome, Nano-Ni served as a second activation signal; Nano-Ni activated NLRP3 inflammasome in LPS-primed but not unprimed cells. Using a siRNA strategy, we found that activation of NLRP3 inflammasome was dependent on Nano-Ni-induced HDAC6 upregulation. **Conclusions:** Nano-Ni activates NLRP3 inflammasome as a second activation signal via HDAC6, leading to IL-1β production, which may further induce pulmonary inflammation and fibrosis. Our results provide a further understanding of the molecular mechanisms underlying Nano-Ni-induced inflammation and fibrosis.

4867 Gene expression changes in the lung following exposure to different forms of graphenes

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Background and Purpose: Nanostructured materials display interesting and valuable properties. This has sparked a massive expansion in the production and use of engineered nanomaterials over the last 20 years. Due to this expansion, there is a need to assess the hazards of exposure to such materials to gain insight on their potential harm to human health. One of the newest and most highly utilized nanomaterials is graphene. Graphene has unique electrical and mechanical properties, a large specific surface area and potential biocompatibility. As a result, it is widely used in materials, electronics, energy, optics, and biomedical fields, for applications such as cell imaging, drug delivery and biosensing. Respiratory exposure in workers during the production and handling of graphene has been identified as the main concern for human exposure, but molecular mechanisms driving adverse outcomes in the lung related to exposure to different types of graphene remain to be determined. We previously characterized gene expression in lung tissue following in vivo exposure to graphene nanoplates of differing sizes. The goal of the study was to identify molecular changes in the lung associated with exposure to graphenes at different stages of oxidation and reduction. **Methods:** Three graphenes were provided by Cabot Corp. (Billerica, MA); graphene nanoplates ~5 μm lateral dimension (Gr5), graphene oxide (GO), and reduced graphene oxide (rGO). To assess gene expression changes in the lung initiated by these differing types of graphene, an *in vivo* acute bolus dose study was conducted. For this study, mice (male, C57BL/6J, ~8 weeks old) were dosed by oropharyngeal aspiration to these 3 graphenes (Gr5, GO, or rGO) or to multi-walled carbon nanotubes (MWCNT; Mitsui-7) at doses of 4 or 40 μg/mouse. At 4 h, 1 day, 7 days, 1 month, and 2 months post-exposure the left lung was ligated and frozen in liquid nitrogen for RNA isolation. Evaluation of gene expression was determined using the lung RNA and standard 96-well technology using the StepOne™ (Applied Biosystems, Carlsbad, CA, USA) with TaqMan® probes and primers. Assessed genes in the lung included IL-6, MIP-2 (*Ccl2*), MCP-1 (*Cxcl2*), MDC (*Ccl22*); all of which play a role in inflammation and macrophage activation; and osteopontin (*Spp1)* which is a gene indicative of tissue remodeling and possible granuloma and fibrosis formation. Relative gene expression was calculated using the comparative threshold method ($2^{-\Delta\Delta Ct}$) with vehicle-treated mice serving as the reference group. Data from Taqman® arrays and qPCR were log transformed and analyzed by a Kruskal-Wallis Rank Sum Test with a Dunn's Test for pairwise comparison in *R*. Differences were considered statistically significant at $p < 0.05$. **Results:** The mRNA expression of genes encoding proinflammatory and tissue remodeling factors showed little to no change in the lungs of mice receiving the low dose of Gr5, GO, or rGO at any post-exposure timepoint. Increased mRNA expression of the proinflammatory genes in the lungs of the mice exposed to the high dose of Gr5 and GO was greatest at 4 hours post-exposure for *IL-6*, *Cxcl2*, *Ccl2*, and *Ccl22*. However, this expression declined by 7 days post-exposure and beyond. The expression of these genes persisted the longest in the high dose of rGO exposed mice. Beyond 1-day post-exposure (7-days, 1-month, and 2-months post-exposure) levels remained significantly elevated but did begin to decline, with the exception of *Spp1*. A similar pattern to rGO-exposed mice was also shown in mice exposed to a fibrogenic form of multiwalled carbon nanotube **Conclusions:** Taken together, the results suggest that all three types of these graphene nanoplates utilized in this study promote mRNA expression of genes involved in inflammation and tissue remodeling. However, effects resolved over time in Gr5- and GO-exposed mice; whereas alterations in gene expression were greatest in rGO-exposed mice and persisted throughout the study's time course. These study findings highlight property-dependent toxicological differences in materials within the graphene nanomaterial family.

4868 Pulmonary Nanoparticle-Biomolecule Interactions Modified during Metabolic Syndrome Disease Progression Contributing to Susceptibility

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Background and Purpose: Metabolic disease enhances the toxic effects of inhaled particulates. Nanosized particles compose a significant portion of inhaled pollution while exposures to engineered nanoparticles (NPs) are increasing due to applications in manufacturing, biomedical applications, and consumer products. NPs have unique properties influencing their toxicity. When NPs are introduced into the body, they are rapidly coated with biomolecules (proteins and lipids), forming a biocorona. The biocorona modifies NP characteristics and cell interactions resulting in altered functionality and toxicity. The formation of the biocorona is dependent upon the NP's physicochemical properties, and biological environment composition. Variations in the biocorona may contribute to the susceptibility observed following inhalation exposures. Previously we have determined NPs form unique biocoronas in bronchoalveolar lavage fluid (BALF) collected from a mouse model of metabolic syndrome (MetS), which exhibited distinct inflammatory consequences compared to healthy mouse model. However, MetS is a progressive disease resulting in alterations in biomolecules over time which may influence exposure susceptibility. Hence, we hypothesize to elucidate NP-biomolecule interactions during MetS disease progression. **Methods:** To evaluate this hypothesis, we used C57BL/6J mice at 6 weeks of age that were randomly assigned to healthy or MetS groups and fed a control diet or a high-fat western diet (HFWD), respectively. BALF was collected via lavage after 2, 4, 8, 12, 16, 20 or 24 weeks on the diets. BALF samples were used to form biocoronas on 20 nm iron oxide NPs then examined by a proteomic/lipidomic approach. **Results:** Biomolecules interacting with the NPs were compared based on time course and between healthy and MetS. BALF differences in protein and lipid biomolecular content were confirmed demonstrating variations based on age and disease progression. Unique biocoronas were determined to form at each time point which was indicative of aging for the healthy and disease progression for the MetS mice. Comparisons between healthy and MetS biocoronas demonstrated distinct biomolecule interactions. These comparisons included both unique proteins and lipids as well as quantitative differences. Protein enrichment and pathway analysis using KEGG and Reactome databases determined proteins were involved in various pathways that are altered during disease progression. Additionally, proteins such as histocompatibility class 1, interleukin enhancer-binding factor 2, immunoglobulin J chain and lipids such as di-

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