4720 Effects of Carbon Nanodots on Mice Liver and Heart Tissue

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Cardiovascular disease affects many people around the world, and atherosclerosis is one of the main causes of cardiovascular disease. Atherosclerosis is the hardening of blood vessels and is strongly regulated by various pro-inflammatory molecules such as macrophage chemoattractant protein-1 (MCP-1), interleukin 1 beta (IL-1 beta), interleukin 6 (IL-6). The liver is closely related to the metabolism of abnormal lipids and complex inflammatory disease and is the organ we have been studying. There is a new class of nanoparticles, called Carbon Nanodots (CNDs), which have been noted as potential candidates for bioimaging, biosensing, and drug delivery. However, there is not much research on the effects of CNDs on inflammation in the liver and heart. In this study, I studied the impact of CNDs on TNF alpha mediated expressions of pro-inflammatory genes in mouse in both of the previously mentioned tissues. C57BL/6 mice tissues have been treated with either TNF alpha (25µl/kg bw), CNDs (2.5 mg/kg CNDs), both TNF alpha and CNDs, or neither to serve as the control. The real-time PCR performed shows that the TNF alpha increased the expression of MCP-1, IL-1 beta, and IL-6 beta in the liver tissues studied. Other experimental data are still in progress. Thisstudy will gain a better understanding of the actions of the CND on TNF alpha-induced inflammation *in vivo*.

4721 Delphinidin Alleviates the Adverse Metabolic Effects of Polystyrene Exposure in Mice

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Microplastics (MPs), with a diameter less than 5 mm, have become widespread contaminants in the environment, where human consumption is inevitable. While the health consequences of MP inhalation or ingestion are largely unknown, our previous work has demonstrated that the consumption of polystyrene (PS) beads by mice promotes adiposity and indices of insulin resistance. To develop an understanding for the basis of these outcomes, we supplied male C57BL/6 mice at 13 weeks of age with normal water or that containing polystyrene beads (5 µm or 0.5 µm; 1 µg/ml) for 14 wk and assessed adipose immune cell infiltration, adipokine levels, as well as metabolic and inflammatory markers in the plasma and liver. We observed a significant increase in adipose tissue macrophage abundance in those mice drinking PS-containing water compared to mice drinking normal water. This was accompanied in the same group by increases in the expression of adipose Ccl2 and Irs2 and in plasma by increased leptin levels. The livers of PS-exposed mice demonstrated increases of cholesterol and protein CysSSG, but no changes in triglycerides and a decrease in the levels of carnitine palmitoyltransferase-2 (Cpt2). In additional mechanistic studies we tested if delphinidin, a plant- and berry-derived anthocyanidin with antioxidant and anti-inflammatory properties, could alleviate PS-effects. Thus, groups of PS-exposed mice received either intraperitoneal injections of DMSO or delphinidin (20mg/kg; 3x per week). After 4wk we assessed weight gain, and body composition, We observed that mice receiving consuming PS beads and receiving the delphinidin injections gained significantly less weight and had a smaller percentage of body fat, compared with those mice receiving consuming PS beads and receiving DMSO injections. These results suggest that ingestion of PS beads promotes metabolic disturbances likely through mechanisms involving oxidative stress and inflammation, which could be alleviated by supplementation with delphinidin.

4722 Application of the Local Lymph Node Assay: 5-Bromo-2-Deoxyuridine Flow Cytometry Method for Prediction of Skin Sensitization Potential of Silicon Dioxide and Titanium Dioxide Nanoparticles

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Nanometals are being used in various fields including cosmetic, medicinal, agricultural, or consumer products. Nanometal oxides such as zinc oxide, aluminum oxide, manganese oxide, copper oxide, titanium oxide (TiO_2), or silicon dioxide (SiO_2) are consistently used in cosmetics and consumer products due to their unique characteristics of large surface area-to-volume ratio, electronic properties, optical properties, and antimicrobial properties. While the consideration of nanometal oxides use is increasing, concerns have been raised regarding their potential negative impacts. Although used in dermal products, the skin sensitization (SS) potential of nanometal oxides has not been well investigated. In the present study, we employed local lymph node assay: 5-bromo-2-deoxyuridine flow cytometry method (LLNA:BrdU-FCM) to screen the skin sensitization potentials of TiO_2 and SiO_2 nanometals. SiO_2 (2.5, 5, 10%) nanometal was suspended uniformly in *N,N*-dimethylformamide and TiO_2 (5, 10, 25%) nanometal was suspended in dimethyl sulfoxide for experimentation. AOO (acetone: olive oil=4:1) and α-hexyl cinnamaldehyde were used as negative and positive control, respectively. The stimulation index (SI) values of SiO_2 were 1.2, 1.3, and 1.6 at 2.5%, 5%, and 10% test concentrations, respectively. Similarly, SI values of TiO_2 were 0.9, 0.8, and 0.9 at 5%, 10%, and 25% test concentrations, respectively. Since SI ≤ 2.7 is considered a non-skin sensitizer, both nanometals were predicted as non-skin sensitizers. According to the present study, cosmetics or dermal products containing those nanometals are considered safe regarding skin sensitization potential. However cautious use is recommended as various studies have revealed their toxicity *in vitro*. *Supported by grant #2020R1I1A3A04036509 11, National Research Foundation of Korea, and the Ministry of Environment-Chemical hazards and risk educational training program.*

4723 Combined Long-Term Effects of Metal Nanocatalysts and UVB on Human Epidermal Keratinocytes

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Transition metal ferrites (MFe_2O_4) are widely used for various industrial catalytic processes due to their extraordinary properties and stability thus representing potential human health risk. The health effects arising from exposure to nano-catalysts depend on their composition and the extent of exposure. In addition to inhalation exposure route, workers may also be exposed through dermal contact where keratinocytes are main cellular constituents of the epidermis and highly active sentinel cells. Further, the study of interactions of nanoparticles (NPs) with the skin cells, in particular after the environmental stress like UVB exposure, are essential. The aim of this study was to investigate the cytotoxicity, oxidative stress, genotoxicity, cytokine responses and potential to induce cell transformation following long-term (8 weeks) exposure of human epidermal keratinocytes (HEK) to a sub-toxic dose of two spinel ferrite NPs, $NiFe_2O_4$ or $CoFe_2O_4$, with or without UVB (2 kJ/cm^2) pre-treatment. Long-term exposure to NPs caused structural alterations in cells that were enhanced by co-exposure with UVB. Significant oxidative modification of proteins - accumulation of carbonyls - was induced only by combined exposure with UVB, while an increase in lipid peroxidation products and phosphorylated H_2AX protein was induced by both NPs alone and co-exposure to UVB. UVB alone caused marked amplification of the observed responses. Moreover, NPs alone induced significant changes in cell invasion (except $NiFe_2O_4$), migration and anchorage-independent growth stimulated by UVB pre-treatment. Nickel NPs, known to cause cell transformation, were used as a positive control. These findings are further supported by observed levels of cytokines/chemokines/growth factors secretion related to inflammatory and T_H2-type/regulatory immune responses. Exposure to $NiFe_2O_4$ alone caused release of IL-8 and IL-12p70, while RANTES was specific only for $CoFe_2O_4$. Secretion of IL-9, IL-15, IL-17, G-CSF and MIP-1b was induced by both NPs and significantly amplified by UVB pre-treatment, while VEGF, IL-2, IL-5, IL-8, IL-12p70, GM-CSF and Eotaxin were released by cells exposed to both, NPs and UVB. Release of IL-4, RANTES and MIP-1a were unique for $CoFe_2O_4$/UVB co-exposure. Altogether, these results clearly indicate that spinel ferrite NPs alone or combined with UVB pre-treatment can induce cytotoxicity, oxidative stress, and inflammation, and may potentially influence neoplastic-like transformation in HEK. Moreover, such effects are dependent on the composition of NPs. Further long-term studies focusing on understanding molecular mechanisms and likelihood to induce tumorigenic effects *in vivo* are necessary.

4724 Physicochemical Characterization and Pulmonary *In Vitro* Toxicity Screening of Different Categories of Two-Dimensional (2D) Nanomaterials

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Two-dimensional (2D) nanomaterials are a large class of engineered nanoparticles with a multitude of applications in electronics, biosensors, and more. Increased demand for these materials, including graphene, nanoclay, transition metal dichalcogenides (TMDs), such as WS_2 and MoS_2, and hexagonal boron nitride (hBN), has elevated the potential for occupational exposures during manufacturing, notably respiratory exposure. Although graphene has been well investigated, there are relatively few toxicity studies of this class of materials as a whole. Existing studies indicate these materials may have the propensity to induce inflammation and cytotoxicity; however, some results are contradictory and comparison across the entire highly variable class remains difficult. The goal of the current study was to conduct a comparative toxicity study of representative 2D materials for the different categories listed above using high throughput *in vitro* screening assays. The five materials were thoroughly characterized for size, density, surface area, hydrodynamic diameter, and more. A battery of toxicity assays was performed using human bronchial epithelial cells (BEAS-2B) and human THP-1 monocytes in doses ranging from 1-100 µg/ml. Cytotoxicity and cell proliferation were assessed using WST-1 and Alamar blue for each cell type. Significant reduction in cell viability was found to occur with graphene at doses ≥ 12.5 µg/ml. Nanoclay and hBN had significant changes at doses ≥ 25 µg/ml, while little to no changes were seen, even at the highest doses (100 µg/ml) for TMDs. Inflammasome activation was assessed in THP-1 cells. IL-1b was found to be significantly increased at an average of 3.6 (6.25 µg/ml) and 4.8 (25 µg/ml) times the control level following nanoclay exposure, and a significant 1.8-fold change occurred following hBN (6.25 µg/ml) exposure. A 4-fold change in Caspase-1 also resulted from exposure to 25 µg/ml nanoclay. In

BEAS-2B cells, there was a trend for cell cycle arrest in G0/G1 following the 25 µg/ml exposure to nanoclay. These initial findings suggest that the TMD category is relatively less toxic than the other classes of 2D materials and nanoclay may be of greater toxicological concern overall. Future work will further screen these materials for genotoxicity, oxidative stress, and inflammatory effects, as well as conduct statistical analyses of the relationship of material properties to these outcomes.

PS 4725 Development of Toxicity Evaluation Method for Nanomaterials Using Activation of THP-1 Cell as an Index

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The development and international standardization of a test method that can efficiently and accurately evaluate the toxicity of various nanomaterials (NMs) is an urgent issue. We have a hypothesis that activation of antigen presenting cell is a trigger of the toxic effect caused by NMs. In this study, therefore we focused on the effects of various NMs on antigen-presenting cells (APCs), which play a fundamental role in the recognition of foreign substances and aimed to elucidate the mechanism of toxicity and develop an *in vitro* test method. We evaluated various NMs using the human Cell Line Activation Test (h-CLAT), a skin sensitization test adopted by the Organisation for Economic Co-operation and Development guideline that measures activation of APCs as an indicator. Our goal is to clarify the mechanism of the toxicity caused by NMs, and to standardize our *in vitro* evaluation system. All five nano silicas examined in this study were positive for h-CLAT. Some samples increased the expression of CD54 by over 20-fold that of the control. The potency of THP-1 cell activation was varied depending on nano silicas. For silver nanoparticles, both CD86 and CD54 expression by THP-1 cells were enhanced as well as allergenic silver ions. The activation of THP-1 cells by silver nanoparticles was considered to be due to silver ions that leached from the nanoparticles into the culture medium. In the case of titanium dioxide NMs, CD54 expression was mildly increased. In addition, some carbon nanotubes strongly enhanced CD54 expression at low concentrations. As results, many of them including silver nano particles, titanium dioxide and silicon dioxide NMs were resulted in positive. But the potency was varied depending on the type of NMs. In general, the activation potency of the silicon dioxide NMs was higher than that of titanium dioxide NMs. Among the same manufacturing method, the potency of THP-1 activation was depended on the primary particle size of silicon dioxide. Furthermore, the effects of mixed exposure to a nano silica along with LPS, which can activate THP-1 cells, was investigated. A synergistic effect was observed on mixed exposure to LPS and nano-silica. These results suggest the usefulness of the h-CLAT test as a screening test for the evaluation of NM immunotoxicity. We are grateful to the TAYCA Corporation and the JRC Nanomaterials Repository for being the supplier of materials in this study. *This research was supported by the Ministry of Health, Labor and Welfare Science Research Grant, Chemical Substance Risk Research 20KD1004.*

PS 4726 Nanotoxicity of Engineered Nanoparticles following Acute and Chronic Exposures of the Small Intestinal Tissue Model

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Despite the expanding number of applications for engineered nanoparticles (ENPs), human health concerns associated with ingested nanoparticles are poorly understood. In this study we utilized 3D human small intestinal (SMI) tissue model to develop a physiologically relevant test system to assess nanotoxicity of ingestible nanomaterials. For dose response experiments, we tested ENPs of various sizes: 1) Copper oxide (CuO), 2) Zinc oxide (ZnO), 3) titanium oxide (TiO_2), 4) silver (Ag), 5) Aluminum (Al), 6) iron oxide (FeO), and 7) single wall carbon nanotubes (SWCNT) at 4 different concentrations. Nanotoxicity was monitored using the following endpoints: 1) tissue viability (MTT), 2) barrier integrity (TEER), 3) structural damage (histology), 4) cytokine release (BioPlex ELISA) and 5) DNA damage (Comet assay) to monitor genetic perturbations in the gut epithelium. In all the experiments, tissues were exposed to 40 ul of different doses of sonicated nanoparticles under rocking condition for 4 hr. After 4hours, dosed tissues were further cultured for overnight under static condition. For chronic exposure, tissues were exposed every other day and cultured for a total of 7- 18 days (4-8 repeat dose applications). Using IC_{15} (concentration that reduces barrier function or tissue viability by 15%) as a cut off, we observed a dose response reduction of barrier integrity and tissue viability for CuO and ZnO following acute exposure. However, acute exposure of titanium oxide did not induce toxicity for the concentrations tested. Furthermore, culture supernatants collected at 24 hr of the culture period were also analyzed for inflammatory cytokines and the result showed a dose dependent release of IL-8 for CuO and ZnO. Tissues exposed to lower doses of nanoparticles that were not found to be toxic in acute exposure studies showed toxicity following repeat applications. For instance, repeated exposure of tissues to CuO (50 nm; 125 ug/ml) showed toxicity at days 7, 14, and 18. FeO (5 nm, 900 ug/ml, gold (2 um, 125 ug/ml), and TiO_2 (15 nm, 900 ug/ml) showed toxicity at days 14 and 18. Analysis of the comet assay of the chronic exposure showed an increase in DNA tail length in tissues treated with SWCNT (125 and 900 µg/mL) and CuO (125 and 900 µg/mL). Overall, the TEER measurement was a sensitive endpoint compared to the MTT tissue viability assay. In summary, the use of the SMI tissue model to examine the toxicity profile of ingested nanotoxicity will also enhance our understanding of nanoparticle cellular deposition and absorption/permeation through the intestinal tissues. The acute and chronic exposure results will play a key role in dose/design studies to generate a physiologically relevant data set for the food and pharmaceutical industries and to provide greater insight into *in vivo* responses.

PS 4727 Nanoparticle Synthesis and Toxicological Characterization of Distinct Size Populations of Stabilized Zinc Colloids

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Engineered nanoparticles are precisely designed during the synthesis process to exploit unique properties based on small size and high surface area for use in agricultural, biomedical, and environmental applications. Generally, the narrower the size distribution of a particle systems, the higher the intensity of the unique property. The size distribution of agglomerated nanoparticles broadens after internalization within an organism and can dampen efficacy, such as targeted delivery or magnetic resonance. For example, tailored size populations (such as 20 nm) increase deposition and accumulation because particles can enter cells passively. Because zinc-based theragnostic agents are gaining popularity in biomedical science for drug delivery and imaging purposes, and because the safety of zinc colloids in organismal circulation is relatively unknown, surface-stabilized water-suspendable zero-valent zinc clusters were selected for this study. The aim was to produce spherical zinc particles stabilized with polyvinylpyrrolidone (PVP) that exist in three distinct and stable size populations: 35±5 nm, 300±50 nm, and 600±100 nm in diameter. PVP was chosen because it confers a neutrally charged particle surface and is not known to induce cytotoxicity effects at the concentrations used in our formulation. Four different synthesis methods were performed and optimized for low dispersity indices, and the toxicological properties were examined. To evaluate the toxicological effects of the nanoparticles to a mammalian ingestion scenario, human intestinal epithelial (Caco-2) cells were exposed. Inductively coupled plasma mass spectrometry was utilized to analyze the uptake efficacy of the cells. The smallest particles were taken up into cells in largest amounts and induced the highest cytotoxicity. In addition, the smallest particles induced the greatest amount of oxidative stress. The medium and large size populations followed similar trends, thus showing that, for zinc colloids, size of the nanoparticle affects toxicological effects.

PS 4728 MMP-3–Mediated Cleavage of OPN Is Involved in Copper Oxide Nanoparticle–Induced Activation of Fibroblasts

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Copper oxide nanoparticles (Nano-CuO) are one of the most produced metal oxide nanomaterials, which have been widely used in various areas such as catalysts, wood protection, coating, etc. Previous studies have shown that exposure to Nano-CuO caused epithelial cell injury, pulmonary inflammation, and even lung fibrosis. However, the mechanisms underlying Nano-CuO-induced lung fibrosis are still unclear. We hypothesized that exposure of human lung epithelial cells and macrophages to Nano-CuO would cause increased expression and activity of MMP-3 as well as increased production of MMP-3-cleaved OPN, which may further contribute to the activation of fibroblasts, leading to lung fibrosis. At first, the cytotoxicity of Nano-CuO in cells was determined by MTS assay and alamarBlue assay. And we found that exposure to non-cytotoxic doses of Nano-CuO (0, 0.5, and 1 µg/mL) caused a dose-dependent increase in MMP-3 expression and activity and OPN expression in both human lung epithelial cells BEAS-2B and PMA-differentiated U937 macrophages (U937*), but not in human MRC-5 fibroblasts. Nano-CuO also caused increased production of MMP-3-cleaved OPN fragment in BEAS-2B and U937* cells, which was abolished by MMP-3 siRNA transfection. In addition, conditioned media from Nano-CuO-exposed BEAS-2B, U937*, or the co-culture of BEAS-2B and U937* caused activation of unexposed MRC-5 fibroblasts, which were reflected by increased expression of α-SMA, COL1A1, and fibronectin. However, direct exposure of MRC-5 cells to Nano-CuO did not induce the activation of MRC-5 fibroblasts. We then established a triple co-culture model and found that exposure of BEAS-2B and U937* cells to Nano-CuO caused activation of unexposed MRC-5 fibroblasts. However, transfection of MMP-3 siRNA into BEAS-2B and U937* cells significantly inhibited the activation of MRC-5 fibroblasts, suggesting that MMP-3 played a key role in Nano-CuO-induced activation of MRC-5 fibroblasts. Taken together, our results demonstrated that exposure of lung epithelial cells and macrophages to Nano-CuO caused MMP-3 production and MMP-3-mediated cleavage of OPN, which led to the activation of fibroblasts, finally resulting in lung fibrosis.

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