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

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