1745 Comparison of Toxicity of Bare- and Phospholipid-Coated-Iron Oxide Nanoparticles in Different Cell Lines.

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We pursue a better quality of life as well as a rich life through the development of science and technology. However, the scientific principles and its application threaten our health sometimes. In this study, we compared the toxicity of phospholipid coated-iron oxide nanoparticles (PLC-FeNPs) and bare-FeNPs in different cell lines. PLC-FeNPs showed higher negative charge than that of bare-FeNPs in the vehicle, but not in the cell culture media containing fetal bovine serum. In addition, the trend of uptake in each cell lines was very similar between both types of FeNPs, despite differences in their diameters. However, cell cycle changes varied according to the cell line and the type of FeNPs. We performed further study using two macrophage cell lines. At 1 h after exposure, MH-S cells exposed to both types of FeNPs showed a strong correlation between gene expression changes despite the uptake process was different, but RAW264.7 cells exposed to both types of FeNPs showed a weak correlation between gene expression changes despite the uptake process was similar. At 24 h after exposure, two macrophage cells exposed to PLC-FeNPs and RAW264.7 cells exposed to bare-FeNPs induced the increase in the number of autophagosome-like vacuoles. While, the cytosolic component of MH-S cells exposed to bare FeNPs completely disappeared, although the membrane remained intact. The predominant gene in MH-S and RAW264.7 cells was also different. Furthermore, when treated with both types of FeNPs, RAW264.7 cells secreted TNFα only, whereas MH-S cells secreted IL-1β and IL-6 along with TNFα. Based on these results, choice of cell line is very important to improve the reliability of in vitro toxicity data. Further, we think that the increase of autophagosome-like vacuoles may be an important cause of cell death which is caused by nanoparticles.

1746 Mechanisms of Silica Nanoparticle-Induced Interleukin-8: Requirement of p38/TACE/TGF-α/EGFR-Cascade-and NF-κB Signalling in Lung Epithelial Cells.

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Nanoparticles (NPs) of non-crystalline (amorphous) silica particles (SiNPs) are used in a large range of products. Inhalation of NPs represents a potential health hazard and may induce inflammation in lung tissues. We have previously shown that SiNPs induced marked cytokine responses independently of particle uptake in human bronchial epithelial cells (BEAS-2B). In the present study the mechanisms involved in SiNP-induced IL-8 responses were further examined. SiNP-exposure induced an early increase in phosphorylation of p65 (NF-κB) as well as the three main MAP-kinases ERK1/2, p38 and JNK, concurrent with an early up-regulation of IL-8 mRNA. SiNP also induced a time-dependent increase in phosphorylation of the epidermal growth factor receptor (EGFR) and release of the EGFR-ligand transforming growth factor (TGF)-α. SiNP-induced IL-8 responses were attenuated by the p38-inhibitor SB202190, the NFkB-inhibitor PDT-p65 and siRNA against p65, as well as the EGFR-inhibitor AG1478, a TGF-α-neutralizing antibody and TAPI-1 (inhibitor of the metalloprotease TACE which cleaves pro-TGF-α to TGF-α). However, inhibitors of ERK and JNK did not exert any effect on SiNP-induced IL-8. Moreover, SiNP-induced EGFR-phosphorylation was inhibited by AG1478 and TAPI-1, and SB202190 reduced the SiNP-induced TGF-α response. The SiNP-induced phosphorylations of p38 and p65 were not affected by TAPI-1 or AG1478. Thus, SiNP appeared to induce EGFR-phosphorylation through a p38- and TACE-dependent cleavage/release of TGF-α. Interestingly, EGF and TGF-α induced little effect on IL-8 release compared to SiNP, suggesting that EGFR-signalling alone is an insufficient stimuli for IL-8 induction. In conclusion, SiNP-induced IL-8 responses seemed to require activation of p38/TACE/TGF-α/EGFR-cascade, presumably acting in concert with the classical NF-κB pathway in BEAS-2B cells.

1747 Role of Tungstate Nanoparticles in the Production of ROS and Induction of Cellular Damage.

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Alkaline-earth metal tungstate AWO_4 (A= Ca, Ba, Sr) nanoparticles are currently being used in a variety of applications including use as components of medical equipment, optical fibers, gas sensors, and scintillator detectors. Due to tungstate nanoparticle versatility, their manufacturing is expected to increase within the next 10 years. Our ongoing study is designed to examine the effects of tungstate nanoparticle exposure in order to develop safe workplace practices to limit exposure. Electron Spin Resonance (ESR) was used to measure hydroxyl radical (·OH) production of tungstate nanoparticles following incubation with either hydrogen peroxide (H_2O_2) or RAW 264.7 cells. Additionally, enhanced dark field microscopy was used to assess nanoparticle association and engulfment by RAW cells over multiple time points up to 3 hours. Assays measuring H_2O_2 production, oxygen consumption, DNA damage and lipid peroxidation were used to assess possible cellular injury following RAW cell incubation with tungstate nanoparticles. Data showed that tungstate nanoparticles are capable of producing ·OH in the presence of H_2O_2 and RAW cells. Further, tungstate nanowires produced significantly greater ·OH compared to nanospheres as shown through ESR measurements. Initial dark field microscopy data showed an increase in association between tungstate nanoparticles and cells and a decrease in non-specific binding after 3 hours of exposure. Cellular damage results in conjunction with ESR data will promote an understanding of tungstate nanoparticle toxicity. It is important to understand the damaging effects and free radical production produced by tungstate nanoparticle exposure in order to ensure that accurate toxicity models are developed and to promote proper training in nanoparticle inhalation prevention.

1748 Comparative *In Vitro* Toxicity Study of Bismuth and Bismuth-Derivatives Nanoparticles.

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Bismuth and bismuth-derived nanoparticles (Bi-NP) were developed for safer consumer applications as bioprobes, photocatalysts, piezoelectrics and contrast agents. A comparative in vitro study was performed to test the biointeraction and toxicity of Bi-NP - bismuth trioxide (Bi_2O_3), bismuth vanadate ($BiVO_4$), bismuth antimonium (Bi-Sb), bismuth-sodium-barium-titanate (BNT-BT), and zero-valent colloidal Bi (ZV-Bi). Dispersion and stability in biological fluids and in vitro toxic effects in target cells (liver-HepG2, kidney-LLCPK1, lung-A549 and brain-SHSY5Y) were evaluated using a nanometer fractionized suspension (<~200 nm) achieved through sonication, centrifugation, and addition of bovine serum albumin (BSA) to reduce agglomeration. Scanning electron microscopy (SEM) confirmed morphological analysis and dispersion of Bi-NP. Cytotoxicity was observed at high concentrations (100 μg/ml) at 24 to 72 h, using well established LDH and MTT assays and High Content Analysis. Lung and brain cells were more susceptible, the main form of cell death was necrosis withoutinvolving reactive oxygen species (ROS) generation. Cell cycle analysis showed arrest at G0-G1 phase with BNT-BT and Bi-Sb. With $BiVO_4$ treatment an increase in S-phase and significant DNA damage assessed with the comet assay, were observed. No relevant acute cytotoxicity was observed in the exposed cell systems from the initial screening of Bi-NP. The severity of biological outcomes of Bi-NP in descending order were $BiVO_4$>Bi_2O_3>Bi-Sb>BNT-BT>ZV-Bi. Further work is underway to elucidate the mechanisms and nuclear effects, and to connect the biological effects with the physicochemical properties of the Bi-NP. Funding from the European Community Seven Framework Programme and CONACYT (Grant agreements #263878 and 12514).

The Toxicologist

Supplement to *Toxicological Sciences*

52nd Annual Meeting and ToxExpo™

March 10–14, 2013 • San Antonio, Texas

An Official Journal of the Society of Toxicology

SOT | Society of Toxicology

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www.toxicology.org

OXFORD UNIVERSITY PRESS

ISSN 1096-6080
Volume 132, Issue 1
March 2013

www.toxsci.oxfordjournals.org