Mapk Pathways Activation Induced By Nanosilver And Tio_2: Role Of Ros

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Abstract

In recent years, large quantities of engineered nanoparticles (NPs) have been rapidly produced and widely applied, leading to an increased exposure of workers and consumers to various kinds of manufactured NPs. Despite many benefits of nanotechnology, there is increasing concern about potential adverse effects on humans and the environment. NPs differ from the corresponding bulk material; because of their size, they have unique physiochemical properties which may contribute to more aggressive forms of long-term toxicity. The interaction with several macromolecules could have many consequences such as mutational alteration, signalling effects, enzyme inhibition and oxidant injuries.

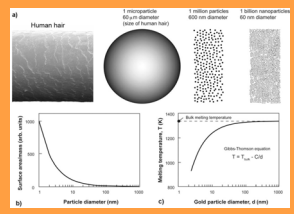
NPs have broad applications from food to electronics and they are present in cosmetics, detergents, cars, fuels, textiles, pesticides, glass and various other materials.

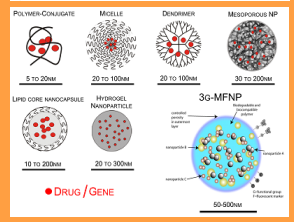
One of the mechanisms proposed for NPs toxicity is their ability of generate ROS and oxidative stress. The main characteristics determining ROS-generating capability are size, shape, chemical composition and surface reactivity, but little is known about the possible molecular mechanisms that link ROS to the biological effects of NPs. Although previous studies have proposed a role for ROS in NP-induced toxicity, the downstream pathways through which NPs signal in human cells and induce cytokine production and DNA damage are unclear.

Considering the increasing use of nanosilver (NS) and TiO_2 it has become crucial to develop a fundamental understanding of the cellular responses to these NPs. Despite the widespread use of NS and TiO_2 products relatively few studies are available about their biological effects.

In this study we looked at the possible molecular mechanisms underlying the biological effects of NS and TiO2. We found that exposure to both NS and TiO2 were able to induce ROS production in human embryonic epithelial EUE cells and that this production was an early event after the exposure. Furthermore, two of three MAPK investigated were activated by both NPs. Interestingly we observed correlation between the time points of ROS production and MAPK activation. This may suggest that MAPK activation is regulated by the ROS generation in EUE. Moreover, we found that the time frame of MAPK activation vary in NS and TiO2 exposure, suggesting that there may be differences in toxicity outcomes of these two NPs.

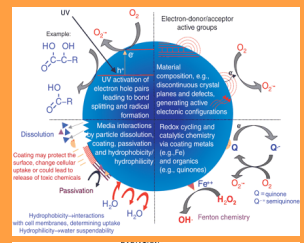
Introduction

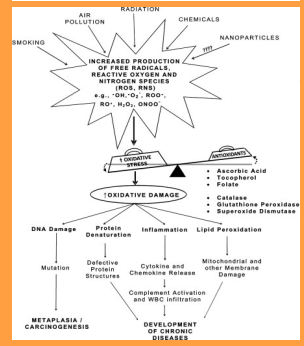




Nanoparticles - particle with one or more dimensions of the order of 100 (200) nm or less.

Relatively large surface and and the number of particles per unit mass - increased interactions between NPs and biological tissue compared to larger particles.





❖Toxicology of a variety of particles including environmental and manifactured nanoparticles shows a clear link between oxidative stress and diseases including asthma, cancer and cardiovascular disfunction.

❖It has been suggested oxidative stress is a suitable measure for comparing and discriminating the toxic effects of different nanoparticles.

❖Identifying molecular an biochemical mechanisms by which nanoparticles induce toxic effects oxidative stress-mediated and, consequently, design tests that can be used for predict nanoparticle toxicity, would allow scientists to generate new and safer nanoparticle knowing structure-toxicity information.

Results

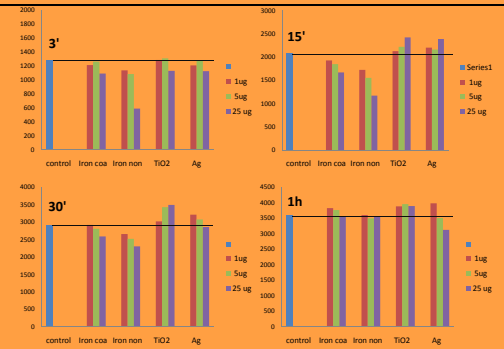


Fig. 1 Comparison of intracellular ROS production after treatment with different NPs. EUE cells were incubated for 40 min with 2μM cell permeable fluorophore dihydrodichlorofluorescein (DCFDH) before treatment with different NPs. DCFDH is oxidized by ROS to dichlorofluorescein (DCF) which can be detected by fluorescence using an ex λ 488nm and em λ 510nm.

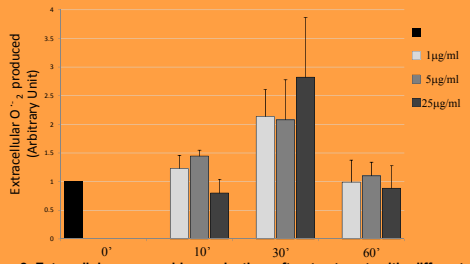


Fig. 2 Extracellular superoxide production after treatment with different concentration of NanoSilver. EUE cells were incubated for 30 min with 2.5mM nitrotetrazolium blue (NBT), a cell not permeable compound, before treatment with different concentrations of Silver nanoparticles. NBT reacts specifically with extracellular produced-superoxide resulting in the formation of insoluble blue formazan deposit. The insoluble blue formazan was solubilised by adding 2 M KOH, and dimethyl sulphoxide and measured at 630nm by spetrometry.

Early response

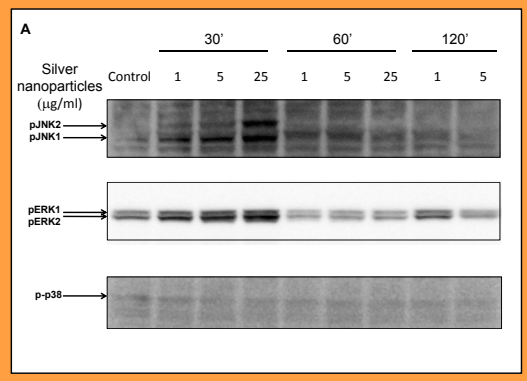
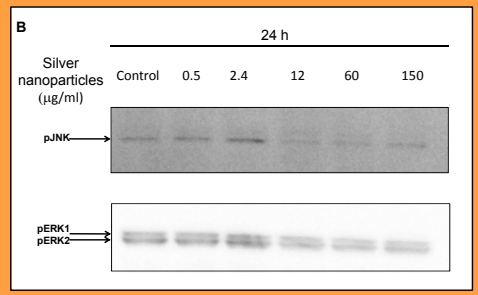
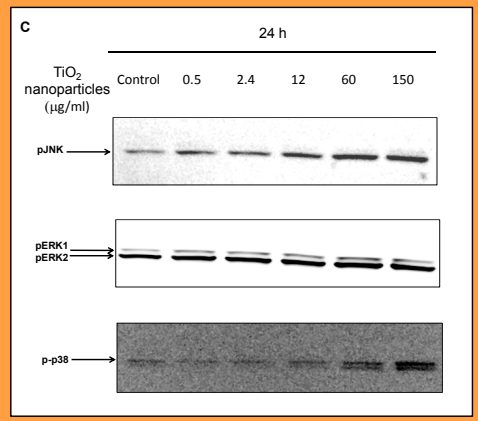


Fig. 3 Mapks activation upon treatment with Silver nanoparticles and TiO_2 Cells were seeded 24h before treatment with NPs. After 24h the media was replaced and EUE, near confluency, were treated with different concentration of NPs for the indicated time, and JNK, ERK and p38 phosphorylation levels were determined by western blot with the appropriate antibody (A-C).

Late response





Conclusions

▪Exposure to both NS and TiO_2 were able to induce ROS production in human embryonic epithelial EUE cells.

▪Extracellular superoxide production was an early event after the exposure to silver nanoparticles in EUE .

▪ERK and JNK activation was an early response but not sustained in time to nanosilver exposure. ROS generation might be involved in such activation.

▪In contrast JNK and p38 activation was a late response to TiO_2 exposure.

▪ Activation of different MAPK might play an important role on the toxicity outcomes of nanoparticles and to understand this process may be helpful for the identification of nanoparticles toxicity biomarkers.