

UK Alumina Nanoparticles Induce Endothelial Cell Dysfunction: Implications in Vascular Disease UK

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Introduction

Manufactured nanoparticles are being increasingly used in a variety of industrial and commercial applications including cosmetics, abrasives, and drug delivery. Because of the high surface-to-volume ratio of nanomaterials as compared to bulk/micro scale materials, nanomaterials exhibit increased reactivity, as well as unique chemical, mechanical, optical, and electrical properties.

Concerns have been raised that the same properties of nanomaterials that make them so attractive to industry could potentially lead to as yet unpredicted environmental or health hazards.

Why study toxicity of nanomaterials ?

Nanoparticles can enter the systemic circulation and reach luminal cell types such as the vascular endothelium causing inflammation and increased adhesiveness initiating cardiovascular diseases, such as atherosclerosis. This is due to the increased expression of adhesion molecules such as vascular cellular adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and endothelial leukocyte adhesion molecule-1 (ELAM-1).

✓ Nanoparticles of TiO₂, Fe₂O₃, Y₂O₃, and ZnO have been shown to cause toxic effects in endothelium, leukocytes, macrophages, etc.

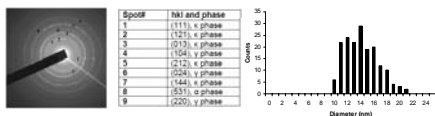
✓ Alumina nanoparticles have been shown to initiate inflammatory events in macrophages, including secretion of proinflammatory cytokines and interaction with neighboring cell types that could lead to activation of the endothelium.

Goals of Our Research

Study the toxicity of manufactured nanoparticles, such as alumina, that cause increased endothelial cell inflammation, evidenced by increased adhesiveness

Experimental Results and Discussions

Microscopic Characterization and Electron Diffraction Data



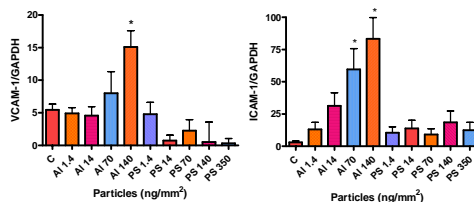
Alumina nanoparticles were dispersed in deionized water and analyzed on a 200 mesh Cu-lacy carbon transmission electron microscopy (TEM) grid performed on a JEOL 2010F instrument with operating voltage of 200 kV.

Electron diffraction data indicated the presence of three different phases of polycrystalline alumina nanoparticles.

TEM characterization indicates nanoparticles with size range from 10 – 20 nm.

The diffraction pattern and indexing was obtained using Digital Micrograph imaging software interfaced with the microscope (Standard Joint Committee on Powder Diffraction - International Centre for Diffraction Data, JCPDS-ICDD).

Alumina, but not polystyrene, dose-dependently increases ICAM-1 and VCAM-1 protein expression



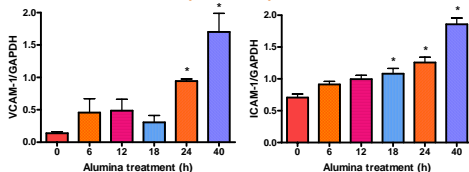
Human umbilical vein endothelial cells (HUVECs) were treated with alumina (Al) or polystyrene (PS) particles at varying concentrations for 24h. Polystyrene particles were 50-100 nm in diameter.

Whole cell lysates were analyzed by immunoblot for VCAM-1, ICAM-1, or GAPDH. Equal amounts of protein (30 µg) were resolved by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes.

Primary antibodies were incubated at a 1:1000 dilution in 5% nonfat milk in TBS-Tween. Horseradish peroxidase-conjugated secondary antibodies were incubated at a 1:3000 dilution. Bands were quantified using UN-SCAN-IT gel Version 5.1 and normalized to GAPDH protein expression.

Bars represent average and SEM of at least three independent groups. * p<0.05 compared to control cultures analyzed by One Way ANOVA followed by Tukey post hoc test.

Alumina time-dependently increases ICAM-1 and VCAM-1 protein expression



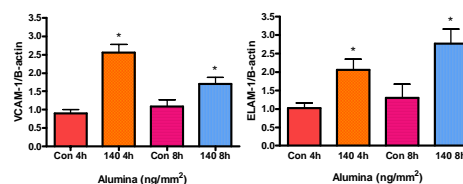
HUVECs were treated with alumina particles (140 ng/mm²) for varying times.

Whole cell lysates were analyzed by immunoblot for VCAM-1, ICAM-1, or GAPDH. Equal amounts of protein (30 µg) were resolved by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes.

Primary antibodies were incubated at a 1:1000 dilution in 5% nonfat milk in TBS-Tween. Horseradish peroxidase-conjugated secondary antibodies were incubated at a 1:3000 dilution. Bands were quantified using UN-SCAN-IT gel Version 5.1 and normalized to GAPDH protein expression.

Bars represent average and SEM of at least three independent groups. * p<0.05 compared to control cultures analyzed by One Way ANOVA followed by Tukey post hoc test.

Alumina increases VCAM-1 and ELAM-1 mRNA expression

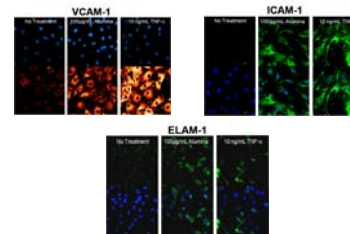


Primary porcine arterial endothelial cells (PECs) were treated with 140 ng/mm² alumina particles for 4 and 8 h.

Total RNA was extracted from cells using Trizol reagent and reverse transcribed into cDNA using the Promega Reverse Transcription System. VCAM-1 and ELAM-1 gene expression changes were analyzed using quantitative real-time PCR and SYBR Green technology.

Sample concentrations were calculated based on a standard curve and normalized to β-actin expression. Bars represent average and standard error of the mean (SEM) of at least three independent groups. * p<0.05 compared to corresponding time control cultures analyzed by One Way ANOVA followed by Tukey post hoc test.

Alumina increases VCAM-1, ICAM-1, and ELAM-1 protein expression

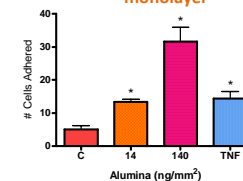


PECs and human umbilical endothelial cells (HUVECs) were treated with 100 µg/mL alumina particles or 10 ng/mL TNF-α for 24 h.

Cells were treated with 50:50 acetone:methanol and blocked of nonspecific binding. Cells were incubated with VCAM-1 or ELAM-1 primary antibodies (for 30 min at 37 °C, dilution of 1:50) followed by Alexafluor antibodies.

Samples were visualized and captured using an Olympus BX61WI confocal microscope. Images are representative of multiple treatment groups in both cell types.

Alumina increases monocyte adhesion to endothelial monolayer



HUVECs were treated with 14 ng/mm² or 140 ng/mm² alumina particles for 8 h.

Human THP-1 monocytes were activated with TNF-α (10 min) and loaded with the fluorescent probe calcein. Monocytes were added to treated endothelial cell monolayers and incubated, allowing for monocyte adhesion. Unbound monocytes were washed away, and the monolayer was fixed.

Attached fluorescent monocytes were counted using a fluorescent microscope (Olympus IX70). * p<0.05 compared to control cultures analyzed by One Way ANOVA followed by Holm-Sidak post test.

Conclusions

It was demonstrated that manufactured alumina nanoparticles cause dose- and time- dependent increased endothelial adhesiveness as shown by induction of VCAM-1, ICAM-1, and ELAM-1, as well as the increased adhesion of monocytes to the vascular endothelium.

These data suggest that nanoparticles may pose a risk in altering the typical function of the vascular endothelium and thus allowing for the development of inflammatory diseases, such as atherosclerosis.

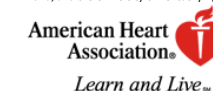
Future Directions

The mechanisms involved in the increased inflammation of the endothelium from alumina exposure are unknown, however it is possible that these particles may interact with intracellular signaling molecules involved in the transcriptional regulation of these molecules.

It is important to understand how these particles are internalized by cells and interact with intracellular organelles.

Acknowledgements

We would like to thank Dr. Tom Curry and UK Labor & Delivery for help obtaining human umbilical cords. This research was supported by grants from NIEHS/NIH (P42ES07380), AHA Pre-doctoral Fellowship (06132168), the University of Kentucky Research Support Fund, and the University of Kentucky Agricultural Experiment Station.



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