

Determining Expression Levels Of The Inflammatory Marker IL-8 In Human Alveolar Basal Epithelial A549 Cells Following Exposure To Amorphous Silicon Dioxide Micro- And Nanoparticles In The Presence Of IL-1 β

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Introduction

Exposure to nano- and microparticles can have adverse health effects, particularly for the increasing population with pre-existing inflammatory lung conditions (COPD, asthma, etc.). This is of concern as there is an increased usage of small particles in the workplace and in consumer products (1). Large particles are cleared from the lung via the mucociliary escalator (the cilia of the airways, pushing the particles up and out of the lung). Nanoparticles in particular reach the peripheral lung, where the mucociliary escalator is not present (1). We studied how particle exposure affected inflammation in cell cultures of A549 cells, including cells pre-exposed to inflammation. A549s are immortalized human alveolar basal epithelial cells that closely resemble Type II lung epithelial cells (2). Interleukin(IL)-1 β was used to induce an inflammatory response and assessed by measuring IL-8 (3). IL-8 is an important pro-inflammatory cytokine expressed in respiratory cells of the human lung, induced upon environmental

insults by particles and is associated with neutrophil recruitment (4). The particles used were amorphous silicon dioxide (silica) nano- and microparticles. Earlier we determined that the nanoparticles form agglomerates similar in size to individual silica microparticles. The nanoparticle agglomerates are vigorously up-taken, but by a different pathway (5).

Methods

Microparticle and Nanoparticle Exposure

Procedure 1: A549 cells were cultured on 96-well plates in F12 media supplemented with 10% fetal bovine serum (FBS). Particles were serially diluted three times with IL-1 β (1 μ g/ml) media containing 10% FBS for a total of four concentrations per particle type. Particle solutions and standards with or without IL-1 β were added to the plates for an exposure length of 2 hours. Note: 2 and 6 hours were most commonly used standard procedure time for ELISA analysis.

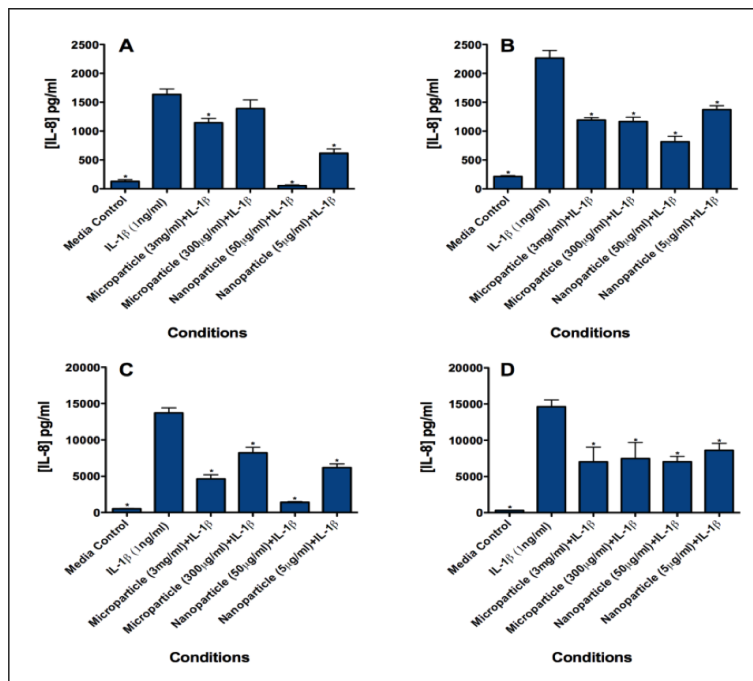


Figure 1:

Effect of long and brief exposure of nanoparticles and microparticles on IL-8 expression of cells, stimulated by IL-1 β . A549 cells in (A) and (C) were exposed to particles and IL-1 β (1 ng/mL) for 2 and 6 hour incubations, respectively. (B) and (D), cells were pre-incubated for 30 minutes with IL-1 β , washed, exposed to particles for 5 minutes and incubated for 2 and 6 hours respectively with IL-1 β . N=12. Even a short exposure to particles strongly dampens A549s inflammatory response. SEM were measured using Prism Software. Represents conditions significantly different than IL-1 β using a one-way ANOVA. Statistics were also carried out to compare against the negative media control. All sets were significantly different than media except nanoparticles (50 μ g/mL) in both A and C.

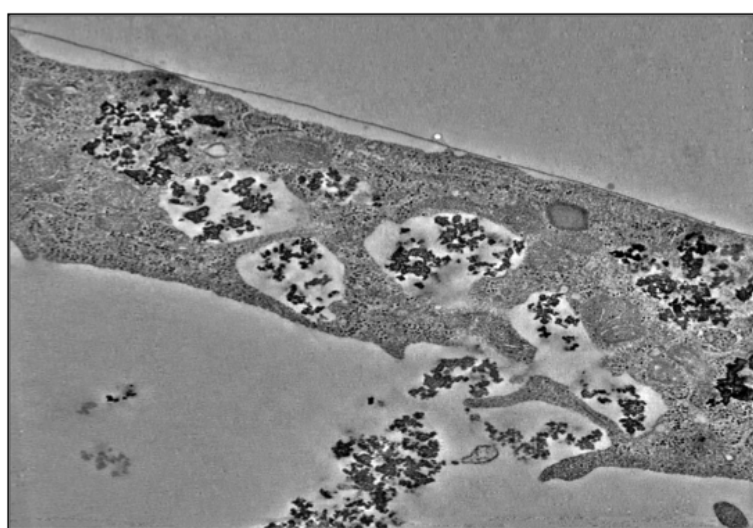


Figure 2:

A549 cell exposed to silica nanoparticles. The picture above shows the formation of nanoparticle agglomerates. The agglomerates are taken up by the cells and encapsulated in endosomes.

Procedure 2: Two concentrations of silica nanoparticles ($5\mu\text{g/mL}$ and $50\mu\text{g/mL}$) or microparticles ($300\mu\text{g/mL}$ and 3mg/mL) were diluted in media containing 10% serum for treatment of A549 cells by 2 separate treatment methods:

1. Cells were exposed to particles for 5 minutes and then washed 3 times with media. Fresh media containing 10% serum with or without IL-1 β was added for 2-hours.
2. Cells were treated with IL-1 β for 30 minutes. Then, cells were washed once prior to particle exposure for 5 minutes followed by three additional washes. Fresh media with or without IL-1 β was added to the cells for an exposure length of either 2 or 6 hours.

All supernatants were harvested for analysis by IL-8 ELISA assay. Transmission electron microscopy techniques were used to characterize the uptake of particles.

Detection of IL-8 Levels

Silica microparticle and nanoparticle exposure was performed on A549 cells, and cell supernatants were removed and stored at -20° . An ELISA kit from R&D Systems was used following the manufacturers method. Absorbance levels at a wavelength of 450nm were measured using a FluroStar plate reader to determine IL-8 concentrations. SEM were calculated using Prism software and one-way ANOVA was performed to determine statistical significance.

Cell Viability

A549 cells were exposed to silica microparticles and nanoparticles for 2 hours and 6 hours. The A549 cells were then trypsinized and stained with either Erythrocin B stain or Trypan Blue solution. Cells were counted using a hemocytometer.

Results

Results are shown in Figures 1 and 2.

Discussion and Conclusions

Silicon dioxide nano- and microparticles alone did not elicit the release of IL-8, which leads to

inflammation, in A549 cells. Surprisingly, the particles were anti-inflammatory. After 6 hours, particles strongly dampened the release of IL-8 in IL-1 β -triggered cells. This was not related to cell death, which was overall quite low (less than 20%) in all treatments. Even brief exposure (5 minutes) of cells to particles strongly dampened the inflammatory response triggered by IL-1 β , for both the 2 and 6 hour time points (Figure 1). Nanoparticles appeared to dampen inflammation slightly more than microparticles. Electron microscopy showed that particles were strongly taken up and transcytosed by A549 cells. To explain our results, we speculate that uptake and transcytosis are an efficient clearance mechanism for particles that reach the peripheral lung, where the mucocilliary elevator is absent. Transcytosis is not associated with inflammation and is even anti-inflammatory, thus preventing unnecessary damage to the lung in the process

Future Directions

It would be interesting to examine the activation of transcription factors such as nuclear factors, NF kappa beta and activator protein-1, that control pro-inflammatory genes. Also, it is a valuable experiment to perform treatment with selected inhibitors to gain an understanding of the relation between particle uptake in the presence of inflammation and cellular response to inflammation. Finally, the next step would be to perform Fluorescence Microscopy to investigate the kinetics of real time particle uptake in primary cell cultures.

References

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