



STATEMENT

Synopsis of Research Report 136

HEALTH
EFFECTS
INSTITUTE

Uptake and Inflammatory Effects of Nanoparticles in a Human Vascular Endothelial Cell Line

BACKGROUND

Epidemiologic and toxicologic studies indicate that ambient particulate matter (PM) — a complex mixture of solid and liquid particles suspended in air — has multiple effects on health. One of the key issues in assessing the health effects of PM is determining the physical characteristics (such as size and charge) and chemical characteristics most responsible for toxicity. Particles $\leq 10 \mu\text{m}$ in aerodynamic diameter (PM_{10}) are of most concern because these particles are respirable by humans. To protect the general population and groups considered most vulnerable to adverse effects from PM in the United States, the Environmental Protection Agency monitors PM_{10} levels and has promulgated National Ambient Air Quality Standards for particles $\leq 2.5 \mu\text{m}$ in aerodynamic diameter ($\text{PM}_{2.5}$, or fine particles). Some scientists believe that particles $< 100 \text{ nm}$ in diameter may be particularly toxic. These particles are referred to as ultrafine ($< 100 \text{ nm}$ in all dimensions) or nanoparticles (with at least one dimension $< 100 \text{ nm}$). Several studies have suggested that metals may be important toxic components of the PM mixture.

A further key issue is the identification of pathways by which particles interact with cells in the airways and other cells to exert toxic effects. Some studies suggest that after inhalation small particles move rapidly out of the lung, enter the bloodstream, and affect other tissues. Because endothelial cells — a layer of specialized cells that line the interior of blood vessels — serve as a barrier between tissues and the bloodstream, the passage of inhaled particles from lungs into the circulation and then into tissue implies that particles have the opportunity to interact with the endothelial layer. Because responses of endothelial cells also play a critical role in the development of atherosclerosis, and

because exposure to particles has been reported to affect the development of atherosclerosis, understanding the response of endothelial cells to particles may be important.

In response to HEI's Request for Preliminary Applications RFPA 04-6, Dr. Ian Kennedy and colleagues at the University of California–Davis, proposed to generate nanoparticles of the oxides of four different metals — iron, zinc, yttrium, and cerium. They chose these metals because iron and zinc are abundant in urban and diesel exhaust PM, iron and cerium are components of recently developed automobile technologies, and yttrium can be “tagged” with a fluorescent marker to identify the localization of particles taken up into cells. The investigators also proposed to evaluate the size and composition of the particles, and to study their potential to induce inflammatory effects in human aortic endothelial cells (HAECs). The investigators also proposed to identify where inside the HAECs the particles would be found after co-culture with the cells. The investigators hypothesized that the biologic effects of the particles would differ depending on their chemical composition and physical properties. The Health Research Committee recommended the study for one year of funding, to determine whether the proposed approaches would be successful.

APPROACH

Dr. Kennedy and colleagues used a flame combustion system to generate nanoparticles of the oxides of iron, zinc, yttrium, and cerium. They characterized several physical properties of the particles, using inductively coupled plasma–mass spectrometry, X-ray diffraction, transmission electron microscopy (TEM), and a scanning mobility particle sizer. They also calculated the particles' surface area.

The investigators incubated particles in solution in a range of concentrations with an HAEC line in vitro, for 4 hours in most experiments. The HAECs were evaluated for the induction of reactive oxygen species (ROS) and markers of oxidative stress and inflammation. Because their attempt to use a fluorescent tagging approach to identify particles within HAECs was not successful, the investigators used TEM on HAECs to identify the subcellular localization of the particles. Responses to cerium oxide particles were evaluated in only a few experiments.

RESULTS AND INTERPRETATIONS

The nanoparticles generated varied in morphology (spherical, cubic, or rod-shaped), agglomeration, and size range (from spherical particles of iron oxide [in two ranges — less than 5 nm in diameter and 30–90 nm diameter] to rod-shaped zinc oxide particles 100–200 nm long). The metal oxides showed a range of effects on HAECs. Of the particles tested, zinc oxide was associated with the greatest number of effects (increasing levels of some markers of inflammation and one measure each of ROS generation and oxidative stress); zinc oxide particles also affected the adherence of HAECs to the substrate and may have resulted in death of the HAECs that became nonadherent. Yttrium oxide was associated with changes in a few end points—specifically, increases in markers of inflammation and one measure of ROS generation. Iron oxide and cerium oxide had no biologic effects on any of the end points measured. The investigators calculated the surface areas of the different particles and found them to be in the following order: iron oxide (larger size range) > yttrium oxide > zinc oxide and cerium oxide.

Zinc, yttrium, and iron oxide particles were detected within HAECs (cerium oxide was not evaluated). The appearance and localization of the three metal oxides differed within the cells.

CONCLUSIONS

Kennedy and colleagues generated iron, zinc, yttrium, and cerium oxide nanoparticles and identified some of their important physical characteristics: size and shape, agglomeration, and calculated surface area. These properties differed for each type of particle. Preliminary studies to characterize effects of the different particles on inflammatory end points and the generation of ROS and oxidative stress in

HAECs showed that the different metal oxide particles induced different patterns of biologic responses. Zinc oxide affected more end points than yttrium oxide, whereas iron oxide and cerium oxide had no effects.

The Health Review Committee, which independently reviewed the study, agreed with the investigators' general conclusions about the biologic responses induced by the different particles: namely, that the effects induced by co-culture of HAECs with nanoparticles depended on the composition of the particles. However, the Committee thought that the differences in biologic responses may have resulted from differences among the particles not only in the physical properties the investigators reported, but also in other, unexamined physical properties, such as solubility and surface charge. Thus, the Committee thought that the investigators had developed a potentially useful model system with HAECs, but that the study's results would have been strengthened if some of these other particle characteristics had been further explored.

The investigators interpreted the findings to indicate that biologic activity was inversely correlated with surface area. The HEI Review Committee agreed that particle surface area is likely to be one important factor in determining biologic effects, but the Committee was not convinced by the investigators' interpretation because cerium oxide had the same surface area as zinc oxide but had no biologic effect.

Kennedy and colleagues showed that the particles were taken up by HAECs, with some evidence that the particles may have localized to different compartments in the cell. The Review Committee did not think this evidence was clear-cut, however, and was not convinced that the reported biologic effects were related to differential localization of the particles within HAECs.

Extrapolating the current study's findings to in vivo human responses is challenging. The physiological relevance of the responses of endothelial cells in vitro to the concentrations of metal particles used in the current study is uncertain. However, occupational exposure to high concentrations of inhaled metal particles has been associated with adverse respiratory and systemic inflammatory and cardiovascular effects. Thus, characterizing the physical and chemical properties of well-defined metal oxide nanoparticles and the effects they induce in vivo and in vitro merits further study.