STATEMENT

Synopsis of Research Report 164

HEALTH EFFECTS INSTITUTE

Pulmonary Particulate Matter and Systemic Microvascular Dysfunction

INTRODUCTION

Ambient particulate matter (PM) is a complex mixture of solid and liquid airborne particles, ranging from approximately 5 nm to 100 µm in aerodynamic diameter. To protect the general population and those considered most vulnerable to adverse effects from PM, the U.S. Environmental Protection Agency has promulgated National Ambient Air Quality Standards for PM with an aerodynamic diameter $\leq$ 2.5 µm ($PM_{2.5}$, also referred to as fine particles). Particles in this size range can deposit in the lower airways. Epidemiologic and toxicologic studies show an association between $PM_{2.5}$ exposure and a number of cardiac, vascular, and pulmonary outcomes. Current and past research on PM has aimed at identifying the pathophysiologic mechanisms involved and the characteristics of PM (including size and chemical composition) most responsible for the health effects. Some scientists believe that the subset of $PM_{2.5}$ made up of particles less than 100 nm in diameter (referred to here as nano-PM) may be especially toxic because, although these particles are small, they can be present in large numbers and have a high surface area per unit mass.

Dr. Timothy R. Nurkiewicz (of West Virginia University School of Medicine) submitted an application under the "Walter A. Rosenblith New Investigator Award" to evaluate and compare the effect of fine and nano-titanium dioxide (TiO_2) particles on endothelium-dependent vascular dilation and the underlying mechanisms. This type of PM was chosen because it is used as a nanomaterial in many consumer products. The HEI Research Committee recommended the proposal for funding because they believed the project was innovative and would contribute to understanding the pathophysiologic mechanisms by which PM of different size fractions could cause adverse effects.

APPROACH

Groups of rats were exposed by inhalation to fine and nano-TiO_2 (at concentrations ranging from 0.5 to 20 mg/m^3) or filtered air for up to 12 hours. The primary size of nano-TiO_2 was 21 nm; that of fine TiO_2 was 1000 nm. All observations were made 24 hours after the end of the exposure. To account for differences in deposition patterns between inhaled fine and nano-PM, the investigators used the measured mass of particles deposited in the lungs as the primary exposure metric.

Vascular dilation was evaluated in anesthetized rats by intravital microscopy of the microvasculature in the exteriorized spinotrapezius muscle enclosed in a tissue bath and perfused for the duration of the experiment. A vascular dilator (A23187) was injected into selected microvessels (1–3 per rat) for 2-minute periods. The effect of previous PM exposure was measured as the change in the ability of vessels from PM-exposed rats to dilate in response to A23187, compared with the ability of vessels from filtered air–exposed rats. Arterial dilation was quantified on the basis of video images of each experiment. Because vascular dilation is in large part mediated by nitric oxide (NO), Nurkiewicz and colleagues also evaluated whether PM exposure inhibited NO production or the ability of the vessels to dilate in response to endogenous NO.

The investigators measured oxidative stress, which is thought to mediate the effects of PM on the cardiovascular system, in the microvasculature and in the lung. They also measured lung inflammation and systemic inflammation (cytokines and chemokines), both shown to be linked to oxidative stress, and some markers of coagulation in blood.

This Statement, prepared by the Health Effects Institute, summarizes a research project funded by HEI and conducted by Dr. Timothy R. Nurkiewicz of the West Virginia University School of Medicine, Morgantown, and colleagues. The complete report, *Pulmonary Particulate Matter and Systemic Microvascular Dysfunction* (© 2011 Health Effects Institute), can be obtained from HEI or our Web site (see next page).

RESULTS AND INTERPRETATION

Measurements inside the exposure chamber showed that the peak of the number-based size distribution of nano-PM was 100 nm, indicating that the nano-TiO_2 did not consist of single primary particles but rather of agglomerates of the primary particles. The number-based size distribution of fine PM was bimodal and had a major peak at 710 nm and a minor peak at 100 nm. The measured deposited PM mass at the same TiO_2 concentration and exposure duration was lower after exposure to nano-PM than to fine PM. Inhalation of either fine- or nano-TiO_2 particles impaired arteriolar dilation, and the effect was dose dependent. At similar measured deposited PM mass, nano-PM produced greater inhibition of microvascular dilation (more than sixfold) than did fine PM.

NO production by the microvascular endothelium was inhibited as a function of different doses of fine and nano-PM, indicating that vascular dilation was dependent on NO. Consistent with this finding was the observation that when a NO donor was added to the perfusate, arterial dilation in response to A23187 was the same in vessels from PM-exposed and filtered air–exposed rats. This result also indicated that endothelial sensitivity to NO was not altered after exposure to either fine or nano-PM.

Other intravital microscopy experiments showed that NO production increased in the presence of radical scavengers in the perfusate and that radical scavengers (in the presence of A23187) partially reduced the inhibition of arterial dilation caused by PM exposure. These findings were consistent with the observed increase in arteriolar ethidium bromide fluorescence and in staining for nitrotyrosine after exposure to either fine or nano-PM. Together, these results demonstrate that the impairment in microvascular function observed after exposure to TiO_2 is likely to be related to enhanced oxidative stress (that is, to the formation of reactive oxygen species) in the microvasculature and the lung.

Results of analyses of markers of lung and systemic inflammation were largely negative: the only statistically significant effect was a small increase in some inflammatory cytokines in the blood of rats exposed to fine PM. Markers of coagulation were for the most part unaffected by the exposure. The investigators measured these markers because they have been associated with exposure to PM and are involved in the processes leading to clot formation. However, no mechanistic interpretation linking them to arteriolar dilation is provided by the authors.

CONCLUSIONS

In its independent review of the study, the HEI Review Committee thought that, overall, this was a thorough and well-conducted study. Each of the specific aims was achieved. The use of intravital microscopy to measure vascular dilation and oxidative stress is a novel approach and may be useful in future investigations. A potential drawback of this procedure is that the use of general anesthesia and surgical manipulations used to obtain measurements may themselves affect microvascular function. Another strength of the study is the measurement of the PM mass deposited in the lung after each exposure condition and the use of this metric for comparing effects of nano- and fine PM.

The concentrations used in the study are very high in relation to the PM levels that are generally encountered in ambient air; however, they are not far from the current occupational exposure limits for TiO_2 recommended by the National Institute for Occupational Safety and Health.

An important finding reported by Nurkiewicz and colleagues is that acute high-dose–inhalation exposure to nano- and fine-TiO_2 particles impairs the ability of the skeletal-muscle microvasculature to dilate in response to a stimulus and that this effect is mediated by decreased NO production by endothelial cells. Also important is the finding that at the same pulmonary mass deposition, nano-PM produced significantly greater systemic microvascular dysfunction than fine PM. The impairment in NO production appears to be related to enhanced oxidative stress in both lung tissue and the vasculature. Although oxidative stress has been linked to both lung and systemic inflammation in several studies, evidence of lung inflammation was weak in this study, and a role of systemic inflammatory mediators was not clearly established.

The study provides insights into the pathophysiologic vascular changes after inhalation of nano-TiO_2. This type of PM is relatively insoluble and is considered to have low reactivity. It should not be considered to be representative of nanoengineered particles in general and of PM in ambient air, which contains a variety of reactive metals, polycyclic aromatic hydrocarbons, and other organic compounds. The Review Committee further cautioned against extrapolating from the effects and mechanisms observed in rats after short-term exposure to high concentrations of the TiO_2 PM to effects in humans after either short-term or long-term exposure to lower concentrations and to different types of PM. Future mechanistic studies might usefully focus on addressing the effects of lower concentrations of ambient and engineered nano-PM not only on the systemic resistance vessels but also on coronary endothelial function.

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