STATEMENT

Synopsis of Research Report 151

HEALTH EFFECTS INSTITUTE

Biologic Effects of Inhaled Diesel Exhaust in Young and Old Mice

BACKGROUND

Exposure to particulate matter (PM) has been associated with increases in cardiopulmonary morbidity and mortality, with elderly people particularly susceptible. However, biologic pathways that might explain why the elderly are more susceptible than younger people to the effects of PM have not been examined extensively. In response to HEI's Request for Preliminary Applications 05-3, issued in 2005, Dr. Debra L. Laskin of Rutgers University and the Environmental and Occupational Health Sciences Institute and colleagues submitted an application for a study to explore possible differences in the responses of young and old mice exposed to diesel exhaust. The investigators proposed to evaluate the hypothesis that the increased susceptibility of elderly animals to PM results from impairment of the capacity of lung cells — alveolar macrophages, specifically — to produce the cytokine tumor necrosis factor α (TNF-α), as compared with lung-cell production of TNF-α by young animals. Laskin and colleagues reasoned that although TNF-α is proinflammatory — that is, it plays a central role in the induction of oxidative stress (a pathway emerging as a plausible mechanism to explain the adverse effects of exposure to PM) and inflammatory responses — TNF-α is also thought to induce protective, antioxidant defenses and tissue-repair mechanisms, and thus it may play a role in limiting the extent of inflammatory responses and injury.

The investigators proposed to test this hypothesis by comparing the production of TNF-α, other markers of the inflammatory response, and molecules involved in antioxidant defenses in young and elderly mice exposed to diesel exhaust emissions, a component of PM found in urban air.

APPROACHES

Laskin and colleagues developed and characterized an animal inhalation exposure system, using diesel exhaust from a Yanmar 406-cc diesel-powered electric generator operated with diesel fuel containing < 500 ppm sulfur and 40-weight motor oil. Young (2-month-old) and old (18-month-old) male CB6F1 mice were exposed for 3 hours on one day ("single exposure") or for 3 hours on each of three consecutive days ("repeated exposure") to diesel exhaust at a concentration of 300 μg/m^3 or 1000 μg/m^3 PM or to filtered air.

The investigators assessed the lungs of mice histologically immediately after and 24 hours after the end of exposure for qualitative changes in markers of inflammation (such as edema and numbers of macrophages and neutrophils) and expression of the manganese-dependent isoform of superoxide dismutase, an enzyme involved in antioxidant defenses. They also measured messenger RNA (mRNA) and protein levels of TNF-α and several molecules associated with inflammation and injury in lung tissue and bronchoalveolar-lavage fluid. These molecules included interleukin-8 (a cytokine that recruits neutrophils into tissues in response to an inflammatory stimulus), interleukin-6 (an acute-phase protein, a component of the rapid systemic response to infectious or other agents), cyclooxygenase-2 (an enzyme that synthesizes prostaglandins, which are involved in inflammatory responses), and lipocalin 24p3 (another acute-phase protein). They also measured levels of TNF-α in the blood.

This Statement, prepared by the Health Effects Institute, summarizes a research project funded by HEI and conducted by Dr. Debra L. Laskin of the Department of Pharmacology and Toxicology, Rutgers University, Piscataway, New Jersey, and colleagues. The complete report, *Pulmonary Effects of Inhaled Diesel Exhaust in Young and Old Mice: A Pilot Project* (© 2010 Health Effects Institute), can be obtained from HEI or our Web site (see next page).

RESULTS AND INTERPRETATION

The original hypothesis of the study was that TNF-α production would be impaired in old animals exposed to diesel exhaust. In their final submitted report, the investigators extended their hypothesis to suggest that exposure to diesel exhaust may differentially affect molecules involved in inflammation and protective antioxidant pathways in young and old mice. However, after single and repeated exposures of young and old mice to diesel exhaust, the pattern of changes in TNF-α levels did not differ between the young and old mice in a straightforward, easily interpreted way. Some qualitative differences were found in inflammatory endpoints measured histologically in lung tissue, with more changes detected in the old than the young mice. However, this pattern of inflammatory changes in the lung was not consistent with the pattern of changes in bronchoalveolar-lavage fluid. The investigators also found that the effects of diesel exhaust exposure on expression of the manganese-dependent isoform of superoxide dismutase differed between young and old mice, as did mRNA levels of cyclooxygenase-2. The mRNA expression of lipocalin 24p3 in the lung increased in more diesel exposure scenarios in old than in young mice.

In its independent evaluation of the study, the HEI Review Committee thought that Laskin and colleagues had succeeded in creating a diesel exposure system and generating preliminary data on multiple endpoints in response to the inhalation of diesel exhaust in young and old mice. The Committee further thought that the investigators' hypothesis to explain the greater susceptibility of the elderly to adverse cardiovascular effects after exposure to PM was novel and interesting. However, the original hypothesis — that TNF-α production would be impaired in elderly animals exposed to diesel exhaust — was not supported by the findings, and the complexity of the pattern of changes in other endpoints in young and old animals exposed to diesel exhaust made it challenging to interpret the study findings in a clear-cut fashion. Thus, it was difficult to make links between changes in expression of the molecules measured in the study and age-related changes in susceptibility to diesel exhaust exposure. The Review Committee further noted that whereas the investigators interpreted the increases in lipocalin 24p3 mRNA expression in the lungs of old mice as representing an increase in oxidative-stress responses, the Committee regarded this molecule as being only peripherally related to oxidative stress. Finally, the Review Committee noted that strides have been made in diesel particulate control technology and that the sulfur content of diesel fuel has been reduced very substantially in recent years. Thus, future emissions of PM from diesel engines are expected to be much lower than those found in the past. Hence, the relevance of the emissions derived from the diesel generator used in the current study to emissions from future diesel engines is uncertain. Further studies are needed to assess hypotheses and biologic response pathways that may explain why the elderly are more susceptible to exposure to PM than the young and healthy.

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