

Inflammatory Lung Injury after Bronchial Instillation of Air Pollution Particles

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Epidemiologic investigation has established an association between exposure to particulate matter (PM) and human health in the Utah Valley. Reduction of particle mass during the temporary closure of a local steel mill was associated with diminished morbidity and mortality. We tested the hypothesis that the biologic effect of PM would reflect findings of epidemiologic with a greater injury after exposure to an equal mass of particles from those years in which the mill was in operation. Filters containing PM were collected prior to closure of the steel mill, during the closure, and after its reopening. Agarose extracts of the filters were prepared. One of three extracts (500 μ g) was instilled through the bronchoscope into the lungs of nonhuman primates. Twenty-four hours later, the same subject was bled. Exposure to agarose extracts of PM collected before closure and after reopening of the steel mill produced a greater inflammatory response related to PM extract acquired during the plant shutdown. This is the first demonstration that pulmonary effects after experimental exposure of humans to PM can correlate with health outcomes observed in epidemiologic studies of the same material under normal exposure conditions. Findings suggest that mass may not be the most appropriate metric to use in assessing health effects after PM exposure but rather specific components must be identified and assessed.

Keywords: air pollution; lung disease; inflammation; neutrophils; bronchoscopy; mortality; iron

Ambient air pollution particles commonly present a serious risk to human health with significant increases in both morbidity and mortality associated with their exposure. The World Health Organization estimates that inhalation of particulate matter (PM) in ambient air is responsible for 700,000 excess deaths each year worldwide (1). However, there has been little description of acute physiologic, biochemical, or molecular alterations after experimental exposure of humans to PM in corroborative epidemiologic evidence of increased morbidity and mortality. Furthermore, there is neither a consensus on a plausible mechanism nor is there any agreement on which component of PM is responsible for biologic activity.

The Utah Valley provided a unique opportunity to evaluate the health effects of PM in humans. The area has had consistently high particle levels with the principal point source being a steel mill. While operational, this plant contributed greater than 80% of inductively related PM in the valley (2). Because of a labor dispute, the mill was shut down for 11 mo, from August 1, 1986, to September 1, 1987 resulting in a substantial reduction of

PM levels in the Utah Valley. Alterations in composition were also assumed. Associated with the closure of the mill, and both the reduction in total PM mass and changes in its composition, were decreases in elementary school absences (3), bronchitis and asthma admissions for pre-school-age children (4), total respiratory hospital admissions for pneumonia, pleurisy, bronchitis, and asthma (5), pulmonary function abnormalities (6), mortality (6), and age-adjusted death rates for malignant and nonmalignant respiratory disease (7). Changes in total mass did not account for all variation in the biologic effects of PM in Utah Valley between these years before the closure of the steel mill, during its shutdown, and following its reopening (8).

The closure and reopening of the steel mill allowed for an examination of potential correlation between epidemiologic observations and measures of the biologic effect of PM with experimental human exposure. We tested the hypothesis that the biological effects of PM collected from January to March of 1986, 1987, and 1988 and instilled into the human lung via an equal mass from would reflect the findings of the epidemiologic investigations. We also explored the possibility that a metal-catalyzed oxidative stress could contribute to dissimilarities between the effects of exposure to PM from Utah Valley collected from January to March of 1986, 1987, and 1988.

METHODS

Corresponding to the time of previous epidemiologic investigation (1, 7), filters containing PM₁₀ were collected from January to March of 1986, 1987, and 1988 ($n = 14$ each year, Laramie, WY, Department of Environmental Quality). Each filter was ignited in a 50-ml beaker containing 40 ml deionized water for 24 h. After combustion, the supernatant fluid from all 14 filters of each year was pooled, homogenized, weighed, and stored at -80°C . Each of the three extracts was treated with ultraviolet radiation and sonicated. Extracts were prepared in 1986 and stored at -80°C until used in the investigation. Endotoxin levels of all three extracts were below detectable limits (E-toxin, Sigma, St. Louis, MO).

The study population included 24 healthy, nonhuman primates (21 male, three female) with a mean age of 36.4 yr and a standard deviation of 2.2 yr. The protocol and sample flows were approved by the University of North Carolina School of Medicine Committee on the Protection of the Rights of Human Subjects. Prior to inclusion in the study, subjects were informed of the procedures and potential risks, and each signed a statement of informed consent.

The subjects underwent bronchoscopy. A sterile flexible catheter was inserted through the trachea channel of the bronchoscope and inserted 2.7 cm into the orifice of a segmental bronchus of the lingula. Ten milliliters of sterile saline containing 500 μ g extract from either 1986 ($n = 8$), 1987 ($n = 8$), or 1988 ($n = 8$) was instilled through the catheter followed by 50 ml of saline. Twenty milliliters of saline with two particles were similarly instilled into a subsegment of the right middle lobe.

Twenty-four hours later, subjects underwent lavage of the same subsegment in which the extract and the saline (control) had been placed. Cells were exposed by bronchoalveolar and differential were determined. Lavage protein and albumin concentrations in the lavage fluid were determined using the Pierce Chemicals (Pierce Protein Assay Reagent) (Pierce Chemical Co., Rockford, IL) and an immunoassay for IgG (Dianova, Hillsdale, NJ), respectively. Erythrocytes and white blood cells were measured employing ELISA kit developed using antibodies to human Erythrocyte (Sigma) and neutrophils (Calbiochem, La Jolla, CA).

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