Sites of Particle Retention and Lung Tissue Responses to Chronically Inhaled Diesel Exhaust and Coal Dust in Rats and Cynomolgus Monkeys

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The usefulness of pulmonary carcinogenicity data from rats exposed to high concentrations of particles for quantitatively predicting lung cancer risk in humans exposed to much lower environmental or occupational concentrations has been questioned. The results of several chronic inhalation bioassays of poorly soluble, nonfibrous particles have suggested that rats may be more prone than other rodent species to develop persistent pulmonary epithelial hyperplasia, metaplasia, and tumors in response to the accumulation of inhaled particles. In addition, rats and primates differ in their pulmonary anatomy and rate of particle clearance from the lung. This paper reviews results of recent Lovelace Respiratory Research Institute (Albuquerque, NM) investigations that directly compared the anatomical patterns of particle retention and the lung tissue responses of rats and monkeys exposed chronically to high occupational concentrations of poorly soluble particles. Lung sections from male cynomolgus monkeys and F344 rats exposed 7 hr/day, 5 days/week for 24 months to filtered ambient air, diesel exhaust (2 mg soot/m³), coal dust (2 mg respirable particulate material/m³), or diesel exhaust and coal dust combined (1 mg soot and 1 mg respirable coal dust/m³) were obtained from a study conducted at the U.S. National Institute for Occupational Safety and Health and examined histopathologically and morphometrically. Within each species, the sites of particle retention and lung tissue responses were the same for diesel soot, coal dust, and combined material. Rats retained a significantly greater portion of the particulate material in the lumens of alveolar ducts and alveoli than monkeys. Conversely, monkeys retained a significantly greater portion of the particulate material in the interstitium than rats. Rats, but not monkeys, had significant alveolar epithelial hyperplastic, inflammatory, and septic fibrotic responses to the retained particles. These results suggest that anatomical patterns of particle retention and lung tissue reactions in rats may not be predictive of retention patterns and tissue responses in primates that inhale poorly soluble particles at concentrations representing high occupational exposures. — Environ Health Perspect 105(Suppl 5):1231–1234 (1997)

Key words: diesel soot, diesel exhaust, coal dust, rats, monkeys, inhalation, inhalated particles, particle retention in lung, interspecies comparisons

Introduction

Several chronic inhalation bioassays of poorly soluble, nonfibrous particles in rats, Syrian hamsters, and mice have shown that rats are generally more sensitive than mice or hamsters to the induction of persistent pulmonary epithelial hyperplasia, metaplasia, and tumors in response to chronically inhaled particles at high exposure concentrations (1). In addition, there is limited evidence for the carcinogenicity of coal dust in rats (2), yet coal dust exposure alone does not significantly increase the risk for lung cancer in humans (3).

The rate of particle clearance from the alveolar region differs among species. Rats and mice clear particles from the lung relatively quickly, whereas monkeys and humans clear particles more slowly (4). Anatomical differences between the faster-clearing and slower-clearing species could affect particle deposition, retention, and clearance. Because mice and rats lack respiratory bronchioles, they have simple acini. Macaque monkeys and humans have similar numbers of respiratory bronchiole generations between the terminal bronchiole and alveolar ducts (5,6), and have larger alveoli and alveolar ducts than rats (7). Therefore, monkeys and humans have more complex, larger acini than rats. The amount of alveolar sepal connective tissue in the lung also differs; small rodents have less and primates more (8–10). Lastly, rats have thin pleura and relatively few pleural lymphatics; nonhuman primates have thicker pleura with more lymphatics than rat pleura; and humans have thick pleura and relatively abundant pleural lymphatics (11–14).

The scientific and regulatory communities are currently debating the usefulness of pulmonary carcinogenicity data from rats exposed to high concentrations of particles for the prediction of lung cancer risk in humans exposed to much lower environmental or occupational concentrations. The purpose of the investigation (15) summarized in this paper was to directly compare patterns of particle retention and lung tissue responses of rats and monkeys exposed chronically to diesel exhaust, coal dust, or diesel exhaust combined with coal dust; at exposure concentrations equivalent to the current permissible airborne concentration in underground coal mines in the United States (2 mg respirable particulate/m³).

Materials and Methods

Lung tissues examined were from a 2-year bioassay by Lewis et al. (16) of cynomolgus monkeys and F344 rats exposed to filtered, conditioned, ambient air (FA) as controls, or to one of three exposures to particulate material: diluted whole diesel exhaust (DE) at a target particle concentration of 2 mg/m³; coal dust (CD) aerosolized in air at a target respirable (particles < 7 μm) particle concentration of 2 mg/m³; or a combination of 1 mg/m³ diesel soot with the same gaseous or vapor airborne dust.
Results
Histopathology
The lungs of DE-, CD-, and DECD-exposed rats exhibited the same histopathology. Overall, the particles were observed mainly within multifocal collections of alveolar macrophage(s) (AM). Most commonly these macrophage aggregates were located within centriacinar alveoli, but the alveoli immediately adjacent to the pleura was another common location. A lesser portion of the retained particles was located in the interstitium. The characteristic tissue response to densely aggregated AM was alveolar epithelial hyperplasia. Other responses were particle-associated inflammation and a local septal fibrotic reaction.

Most of the control monkey lungs contained nonsoot, noncoal dust particulate material. This material consisted of endogenous pigments, materials inhaled and retained during the lifetime of the animal, and, in some monkeys, debris from pulmonary mites. The incidence of pulmonary acariasis was the same across all exposure groups. Soot and coal dust were also present in exposed monkey lungs.

The predominant sites of particle retention and the characteristic tissue responses were the same in DE-, CD-, and DECD-exposed monkeys. The retained particles had a multifocal distribution in the monkey lungs, but, in contrast to the rat, more of the particulate material was located in the interstitium than in the alveoli. Most commonly, interstitial particle-laden macrophages were a) within the alveolar septa; b) within interstitium of respiratory bronchioles; c) within the adventitia and lymphatic capillaries surrounding arterioles and veins within the pulmonary parenchyma; and d) in the pleura. The interstitial particulate material did not seem to elicit a tissue response. The portion of the particulate material within intraluminal collections of AM was smaller than in rats, and the aggregates of particle-laden macrophages elicited much less of a tissue response in the monkeys than in the rats.

Tables 1 and 2 show the incidences and average severity scores of key categories of lesions in rats and monkeys, respectively. Rats had a significantly greater alveolar epithelial hyperplastic response to particle exposure than monkeys (p < 0.001). Alveolar epithelial hyperplasia was significantly greater in particle exposed than in control rats (p < 0.001), but there was no difference in the hyperplastic response due to type of particle exposure (p = 0.3). The

Table 1. Incidences and average severity scores of key particle associated or possibly particle-associated histopathologic diagnoses in rats.

| Diagnosis                              | Exposure group | Filtered air | Diesel exhaust | Coal dust | DECD |
|----------------------------------------|----------------|--------------|----------------|-----------|------|
| Number of rats examined                 |                | 15           | 15             | 15        | 15   |
| Alveolar epithelial hyperplasia         |                | 1 (1.0)      | 15 (1.7)       | 14 (1.5)  | 15 (1.2) |
| Particle-associated inflammation        |                | 0 (0.0)      | 10 (1.1)       | 7 (1.0)   | 7 (1.0)  |
| Septal fibrotic reaction                |                | 0 (0.0)      | 7 (1.1)        | 4 (1.0)   | 4 (1.0)  |

*Histopathologic diagnoses whose incidence or severity suggested a possible particle association in any exposure group in either rats or monkeys. °Rats were exposed to FA as controls or DE, CD, or DECD at target concentrations of 2-mg respirable particulate material/m. First number is the number of rats with the diagnosis. The number in parenthesis is the average severity score calculated as sum of severity scores/number of rats with the diagnosis. Lesions were scored as 1, slight; 2, minimal; 3, mild; 4, moderate; 5, marked.

Table 2. Incidences and average severity scores of key particle associated or possibly particle-associated histopathologic diagnoses in monkeys.

| Diagnosis                              | Exposure group | Filtered air | Diesel exhaust | Coal dust | DECD |
|----------------------------------------|----------------|--------------|----------------|-----------|------|
| Number of monkeys examined              |                | 14           | 15             | 14        | 15   |
| Alveolar epithelial hyperplasia         |                | 2 (1.5)      | 4 (1.5)        | 3 (1.0)   | 4 (2.0)  |
| Particle-associated inflammation        |                | 1 (1.0)      | 3 (1.0)        | 4 (1.0)   | 4 (1.2)  |
| Septal fibrotic reaction                |                | 3 (1.3)      | 0 (0.0)        | 0 (0.0)   | 1 (1.0)  |

*Histopathologic diagnoses whose evidence or severity suggested a possible particle association in any exposure group in either rats or monkeys. °Monkeys were exposed to FA as controls or DE, CD, or a DECD at target concentrations of 2-mg respirable particulate material/m. First number is the number of monkeys with the diagnosis. The number in parenthesis is the average severity score calculated as sum of severity scores/number of rats with the diagnosis. Lesions were scored as 1, slight; 2, minimal; 3, mild; 4, moderate; 5, marked.
monkeys did not have a significant alveolar epithelial hyperplastic response to particle exposure ($p = 0.4$).

Rats had a significantly greater inflammatory response to particles than monkeys ($p = 0.02$). Particle-exposed rats had significantly greater inflammation than control rats ($p < 0.001$). There was no significant difference in the inflammatory response due to the type of particle exposure in rats ($p = 0.5$). In monkeys, there was no significant effect of particle exposure ($p = 0.1$).

The septal fibrotic reaction in rats occurred as collagen increased within alveolar septa in foci of AM aggregation, alveolar epithelial hyperplasia, and particle-associated inflammation. The fibrosis did not occur independently of these other reactions. In monkeys, a septal fibrotic reaction was not associated with diesel soot or CD particles. Rats had a significantly greater septal fibrotic reaction to particles than monkeys ($p = 0.006$). In rats, this reaction was significantly greater in particle exposed than in control rats ($p = 0.001$). There was no significant difference in the septal fibrotic reaction due to the type of particle exposure in rats ($p = 0.15$). There was less septal fibrotic reaction in particle exposed than in control monkeys ($p = 0.02$).

Morphometry

Approximately 73 and 43% of the particulate material in exposed rats and monkeys, respectively, was in the lumens of alveoli and alveolar ducts (Figure 1). Approximately 27 and 52% of the particulate material in exposed rats and monkeys, respectively, were in the interstitium. As shown in Figure 1, particles in the pleural lymphatics and connective tissue were grouped with the interstitial particles for this analysis. Interspecies comparison showed a significantly greater volume percentage of the total particulate material in the lumens of alveoli and alveolar ducts in exposed rats than in exposed monkeys ($p < 0.001$). Conversely, a significantly greater volume percentage of the total particulate material was in the interstitium of exposed monkeys than in exposed rats ($p < 0.001$). Within each species, there were no statistical differences between DE, CD, and DEC/D animals for the volume percentage of the total particulate material in the lumens of alveoli and alveolar ducts or in the interstitium.

Discussion

The morphometry showed clear differences between rats and monkeys in the predominant sites of particle retention. In rats, more than 70% of the particulate material was retained in the lumens of alveoli and alveolar ducts. In monkeys, the particulate material was almost equally divided between these luminal compartments and the interstitium, but more of the material tended to be in the interstitium. Within each species, the predominant site of particle retention did not vary by exposure material. The

![Figure 1](image.png)

**Figure 1.** Volume percentages of particulate material in the lumens of alveoli and alveolar ducts (LU) versus interstitium and pleura (IP) as mean percentages of total particulate material (± SE) for animals exposed to DE, CD, or DEC/D.

The present findings, combined with the known anatomical differences and data showing that primates clearly deposit particles more slowly than rats (4), suggest that a greater proportion of particles or particle-laden macrophages penetrate the airway epithelium and enter the interstitium in primates than in rats and that the particles in the interstitium may be cleared more slowly than those cleared via mucociliary action. If the presence of respiratory bronchioles, the amount of interstitial and pleural tissue, and the thickness of the alveolar septa are important determinants of the sites of particle retention, the differences between rats and humans might be even greater than the differences between rats and monkeys. Human lungs have more extensive interlobular septa, thicker pleura, and wider interstitial spaces in the alveolar septa than monkeys (12, 19, 20).

The response to particles, including alveolar epithelial hyperplasia, inflammation, and focal septal fibrosis, was significantly greater in rats than monkeys. Epithelial hyperplasia concomitant with the aggregation of particle-laden macrophages in alveolar lumens is a characteristic response to many poorly soluble particles in the rat lung, both at exposure concentrations that result in lung tumors (17, 21–23) and at exposure concentrations below those resulting in lung tumors (21–24). Hyperplasia of the surrounding epithelium in response to accumulation of particulate material in focal aggregates of AM was not characteristic of the response to diesel soot or CD in monkeys in this investigation (15), nor is it characteristic of coal workers' pneumoconiosis (3, 25, 26), silicosis (27), or talc pneumoconiosis (28) in humans.

If human lungs respond to poorly soluble particles in a manner more like monkey lungs than rat lungs, perhaps the pulmonary response of rats to particles may not be predictive of the response in human lungs at concentrations representing high occupational exposures. Consideration should also be given to the question of whether carcinogenicity data from rats exposed to high concentrations of particles, which greatly exceed expected human exposure concentrations, should be used to quantitatively predict carcinogenicity in humans exposed at lower rates. Particle-induced inflammatory and epithelial proliferative responses that seem critical to carcinogenicity in rats may not occur in primate lungs exposed at environmental or occupational concentrations.
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