Influence of Particle Size and Chemical Composition on Efficiency of Clearance Mechanisms: Electron Microscopy Studies on Humans

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This article compares the presence of solid particles in lung parenchyma samples collected from accident victims and in bronchoalveolar lavage fluid taken from patients diagnosed with pulmonary carcinoma. Analysis by electron microscopy showed differences in particle size between the two groups, which could be attributable both to differences in original particle size and to their solubility in the biological environment. — Environ Health Perspect 102(Suppl 6):241–243 (1994)

Key words: mineral particles, smoke, clearance mechanisms, lung tissue, BAL, electron microscopy

Introduction

Biopersistence of solid particles in the respiratory system can be described schematically as depending on physicochemical characteristics of the inhaled particles, which determine the site of the deposition of particles and the efficiency of clearance mechanisms. Analytical electron microscopy (AEM) has been widely used (1–6) to investigate the fibrogenic or carcinogenic processes resulting from inhaled mineral particles.

Our studies have focused on the fine characterization of inhaled particles by AEM in subjects not professionally exposed to dusts, without obstructive pulmonary diseases, and living in an urban environment. We investigated the effects of exposure to urban air pollution on inhalation, retention, and translocation of mineral particles.

The results from two studies have been compared. The first (7) was performed on lung parenchyma samples of 85 subjects, not occupationally exposed or affected by chronic or serious diseases, but who had died by accident in Rome (Table 1). The second study was based on the analysis of bronchoalveolar lavage (BAL) fluids obtained from 30 patients, all with a diagnosis of suspected pulmonary carcinoma (Table 2).

Materials and Methods

Data on occupational history and smoking habits of subjects were collected on the basis of a standardized questionnaire (7). Autopsy samples were collected from the upper lobe of the right lung.

The 30 patients with suspected pulmonary carcinoma were subjected to BAL in both lungs. The lavage fluids from the lung, contralateral to the radiological lesion, were analyzed for particles. BAL was performed according to standard protocols (8) in the medium lobe and lingula. Half of the recovered fluid from each patient was used for cytological investigations, and the remaining portion was utilized for mineralogical analysis.

In the first study the organic fraction of the lung parenchyma sample was dissolved in atomic oxygen plasma; in the second, by treatment of the recovered fluid with sodium hypochlorite solution. The remaining inorganic fraction in each case was suspended in deionized water and filtered through cellulose nitrate filters of 0.45 μm pore size. Filters were then processed by the method of Paoletti et al. (9) and examined by transmission electron microscopy, equipped for X-ray energy dispersive spectroscopy.

Particles were identified by morphology, chemical composition and crystalline structure. Aerodynamic diameters (Dae) were evaluated taking into account Feret's diameters (Fd) of particles, volumetric weight (Po) (10) and volume coefficient (<k>) (11) according to the equation

\[ Dae = \frac{F_d \cdot (P_o / <k>)^{1/2}}{\text{std}} \]

Table 1. Lung parenchyma samples from residents of urban Rome.

| Category               | Percentage |
|------------------------|------------|
| Males                  | 81         |
| Females                | 19         |
| White-collar workers   | 41         |
| Blue-collar workers    | 59         |
| Smokers                | 80         |
| Nonsmokers             | 20         |

* n = 85, age, 46 ± 15 years. Subjects were taken from the upper lobe of the right lung. Subjects were not occupationally exposed or affected by chronic or serious disease.

Table 2. Bronchoalveolar lavage fluid samples taken from patients in Rome with pulmonary carcinoma.2

| Category               | Percentage |
|------------------------|------------|
| Males                  | 76         |
| Females                | 24         |
| White-collar workers   | 60         |
| Blue-collar workers    | 40         |
| Smokers                | 90         |
| Nonsmokers             | 10         |

Mean values from cytological analysis

- Cells/ml × 10^4: 11
- Cell type
  - Macrophages: 91
  - Neutrophils: 7
  - Lymphocytes: 1

* n = 30, age 55 ± 6 years. Subjects were not occupationally exposed. Samples were taken from the contralateral lung.
Table 3. Silicate particles.

| Minerals more frequently detected | Lung samples | % | BAL | Lung samples | % | BAL |
|-----------------------------------|--------------|----|-----|--------------|----|-----|
| Crystalline silica                 | 14           | 43 |     | 80           |    | 100 |
| Clays                             | 15           | 46 |     | 60           |    | 100 |
| Micas                             | 40           | 13 |     | 100          | 100|    |
| Talc                              | 16           | 10 |     | 100          | 89 |    |
| Chlorites                         | 5            | 2  |     | 30           | 58 |    |
| Undetected                        | 10           | 16 |     | 100          | 100|    |

Metal compound particles

| Elements as their compounds, more frequently detected | Lung samples | % | BAL | Lung samples | % | BAL |
|--------------------------------------------------------|--------------|----|-----|--------------|----|-----|
| Titanium                                               | 7            | 29 |     | 59           |    | 100 |
| Iron                                                   | 37           | 26 |     | 91           |    | 100 |
| Calcium                                                | 15           | 9  |     | 69           | 93 |    |
| Nickel                                                 | 2            | 7  |     | 15           | 63 |    |
| Steel                                                  | 6            | 5  |     | 65           | 69 |    |
| Barium sulfide                                         | 3            | 5  |     | 26           | 52 |    |
| Aluminium                                              | 2            | 3  |     | 33           | 76 |    |
| Lead                                                   | 0            | 1  |     | 0            | 41 |    |
| Others                                                 | 28           | 15 |     | 100          | 100|    |

Asbestos fibers

| Fiber type detected | Lung samples | % | BAL | Lung samples | % | BAL |
|---------------------|--------------|----|-----|--------------|----|-----|
| Chrysotile          | 68           |    | 100 | 16           | 41|    |
| Tremolite           | 19           |    |     | —            |   |    |
| Amosite             | 8            |    |     | —            |   |    |
| Crocidolite         | 5            |    |     | —            |   |    |

Data were generally log-normally distributed, so that statistical analysis could be performed with the Mann-Whitney U nonparametric test.

Results

Nonfibrous particles were identified in two groups: silicates, generally phyllosilicates and crystalline silica; and metal particles, sulphates, or alloys of a variety of elements. The detection frequencies of mineral species and the percentage of subjects in which the minerals were found were calculated separately for each study (Table 3).

Generally, the pattern of mineralogical types found in the two studies was similar in composition to that of the aerosol particles found in the urban atmosphere in Rome (12), but there were some important differences. Lead particles were present in BAL fluid but not in lung samples, and asbestos fibers (chrysotile, tremolite, amosite, and crocidolite) were detected in 16% of lung parenchyma samples, while chrysotile fibers alone were detected in 41% of BAL samples (Table 3). Moreover, the distribution of detection frequencies of silicate particles in the two studies were different; on average, a greater percentage of silicates was present in the samples from lung parenchyma than in the lavage from the bronchial tree (Figure 1).

Dae analysis showed silicate particles to be larger than metal particles (p = 0.0001) in the samples from both studies, but the particles detected in BAL samples were significantly larger than those in lung sample (p = 0.04). This difference appears to be attributable to differences in silicate dimensions (p = 0.004), while metal particles were not significantly different in size (Figure 2).

Discussion

In comparing the results of these two studies, we assumed that the two population samples, residing in the same urban area, were exposed to the same airborne pollution, that none of the persons had been professionally exposed to dust, and that none were affected by obstructive pulmonary diseases. BAL fluids were recovered from anatomic areas not affected by tumors present in the contralateral lung, nor by morphological or functional abnormalities of the clearance mechanisms. These limitations correspond to those applied in an earlier cytological study of "healthy" smokers and nonsmokers (13). A reliable evaluation of smoking habits of all the subjects was more difficult to assess, especially from the autopsy cases, but the percentage of reported smokers and nonsmokers in the two studies was similar. The observed differences in particle size between those detected in BAL and lung samples was possibly due to partial contamination of BAL fluid with the bronchoalveolar content. Alternatively, the smaller particles detected in lung...
Figure 2. Aerodynamic diameter of particles recovered from lung parenchyma samples and BAL fluids. Both silicate and all particles were significantly greater in diameter in BAL fluid. Metal particles were not significantly different in size in the two groups.

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