Comparative Analysis of Inhaled Particles Contained in Human Bronchoalveolar Lavage Fluids, Lung Parenchyma and Lymph Nodes

P. Dumortier, P. De Vuyst, and J. C. Yernault

Research Unit on the Toxicity of Mineral Particles, Chest Department, Erasme Hospital, Brussels, Belgium

Translocation of inhaled particles from the alveolar spaces to lung parenchyma and lymph nodes is one of the mechanisms that determine the biopersistence of particles. This study compares the nonfibrous particulate burden in bronchoalveolar lavage (BAL) fluids, lung parenchyma, and thoracic lymph nodes and attempts to detect the degree of differentiation, if any, based on particle size or type. This comparison can only be done on BAL, lung parenchyma, and lymph node samples collected from the same subject over a short time. Patients undergoing surgical lung resection are suitable for this purpose. Particles recovered by digestion-filtration were counted, sized, and analyzed by analytical transmission electron microscopy. Total particle load ranges grossly between 10^2 to 10^3 p/ml in BAL, 10^9 to 10^{10} p/g dry tissue in parenchyma and 10^6 to 10^7 p/g dry tissue in lymph nodes. Diameters are log-normally distributed and mean diameters range between 0.5 to 0.9 μm. Nonlamellar silicate particles have a significantly larger diameter in lymph nodes. Differences in particle type between the three sampling sites are small and nonsystematic. — Environ Health Perspect 102(Suppl 5):257–259 (1994)

Key words: mineral particles, analytical electron microscopy, bronchoalveolar lavage, lung parenchyma, lymph node, clearance, biopersistence

Introduction

Deposition curves show that 10 to 60% of the inhaled particles within the range 0.1 to 5.0 μm reach the lung (1). The larger fraction of these particles undergoes rapid clearance, but a significant percentage remains trapped in the lungs. Particles may persist for many years in the alveolar spaces or be translocated and sequestered in lung parenchyma, lymph nodes or parietal pleura. This leads to the progressive buildup of a particle load, reflecting the different exposures of an individual.

Mineralogical analysis by analytical transmission electron microscopy has proved its usefulness to characterize the particulate burden of bronchoalveolar lavage (BAL) fluids, lung parenchyma, and lymph nodes (2–13). In a previous paper we discussed the necessity to correlate the information obtained on different types of samples (2). The present study has three aims: to assess the relationship between particle concentrations in BAL, lung parenchyma and lymph nodes; to test whether it is reasonable to analyze particles in BAL or lymph nodes, when it is known that diseases are mainly affecting lung parenchyma; and to determine if a detectable particle size or type differentiation occurs among the three types of samples. This information can only be obtained by analyzing samples collected from the same subject over a short time. The study population was composed of subjects undergoing surgical lung resection for lung cancer.

Materials and Methods

Population. Nine BAL, 10 lung parenchyma samples, and 10 lymph nodes collected from 10 subjects were investigated. All subjects were males and smokers or former smokers. Mean age was 62 ± 7 years (range: 31–72). Occupations of the subjects were varied. Nine were blue collar workers and one was a white collar worker. Duration of occupation was 40 ± 6 years (range: 31–48), delay since end was 6 ± 5 years (range 0–14).

Sample Preparation. BAL was performed within 10 days of surgery. From 10 to 40 ml of the second or third fraction were used. Lung tissue was sampled at distance from the tumor. Histologically cancer-free lymph nodes sampled during mediastinoscopy were used. Organic constituents were digested with sodium hypochlorite and particles collected on 0.45 μm Millipore filters. One hundred consecutive nonfibrous particles >0.1 μm were chemically analyzed and sized by transmission electron microscopy (TEM). Diameters were reported as visual estimations of the area equivalent diameter. All the particles present in a grid square were counted. Results were expressed as mineralogical profiles, reporting the relative percentage of each particle type, the total number of particles/ml BAL fluid or P/g dry lung tissue or lymph node, and the mean particle size.

Statistical Methods. Statistical analysis was performed with Wilcoxon nonpara-
metric tests. The Kolmogorov–Smirnov test was used to compare the particle size distribution.

**Results**

Particle sizes were log-normally distributed (Figure 1). There was no difference in the mean diameter of particles in BAL and lung tissue, but a significant difference ($p < 0.001$) was found between lung tissue and lymph nodes. This difference is due to the larger diameter of nonlamellar silicate particles in lymph nodes (geometric mean: 0.82 μm), as compared with BAL (geometric mean: 0.59 μm) and lung parenchyma (geometric mean: 0.54 μm) (Figure 2). Metallic compounds have a smaller diameter than nonlamellar silicates and phyllosilicates.

The absolute particle concentrations are summarized in Table 1 and the ratio of particle load between the three types of samples in Table 2. No regression line can be drawn, which would allow us to extrapolate from the measurement in one type of sample to the concentration in another. The ratios extend over two orders of magnitude for the lung parenchyma/BAL comparison. They are generally higher than 1000 for lung parenchyma/BAL and lower than 100 for lymph nodes/lung parenchyma.

| Table 1. Total particle load. |
|------------------------------|
| BAL | Lung$^9$ parenchyma | Lymph$^9$ nodes |
| Mean ± SD | 3.4 ± 6.0 | 48.2 ± 37.7 |
| Range | 0.04–1.85 | 6.6–123.3 |
| Geometric mean | 1.25 | 32.2 |

*10$^4$ particles/ml. $^9$10$^9$ particles/g dry tissue.

| Table 2. Particle load ratio. |
|------------------------------|
| Lung parenchyma/BAL | Lymph node/lung parenchyma |
| Mean ± SD | 6720 ± 7913 | 52 ± 63 |
| Range | 416–24407 | 6–180 |
| Geometric mean | 3162 | 26 |

| Table 3. Relative particle concentrations (%). |
|-------------------------------|
| BAL | Lung parenchyma | Lymph nodes |
| Silicates | 32.9 | 29.9 | 34.1 |
| Phyllosilicates | 26.6 | 37.9 | 30.1 |
| Metals | 32.1 | 9.4 | 3.6 |
| Others | 9.4 | 4.2 | 3.6 |

Silicates: nonlamellar silicates; silica, feldspar, siliceous flyash, other silicates. Phyllosilicates: lamellar silicates; kaolinite, illite, mica, talc, chlorite. Metals: compounds of metallic elements (Al, Fe, Ti, Cr, Sn, Ni, ...), pure or in combination.

No statistically significant difference in the relative particle concentration (%) appears among the three types of samples, except for kaolinite and iron compounds between BAL and lung parenchyma ($p < 0.05$). When grouping the particles into nonlamellar silicates, phyllosilicates, and metallic compounds, this difference disappears. However, the total silicates

Figure 2. Mean particle size in BAL, lung parenchyma, and lymph nodes as a function of particle type.

Figure 3. Comparative mineralological profiles for a coach builder and a dental technician.
(nonlamellar + phyllosilicates) are enriched in lymph nodes (66.3%) versus BAL (58.5) and lung parenchyma (57.1) (Table 3). This difference is not statistically significant due to the large variations that can exist in the individual mineralogical profiles. This latter point is illustrated in Figure 3. There are small differences in the profile of the first subject (a coach builder). For the second (a dental technician), there are no differences in the concentrations of the various silicates, but iron compounds are markedly enriched in lymph nodes, aluminium compounds are almost absent from lymph nodes, but present in 21% of the analyzed particles in BAL and 11% in lung parenchyma. Gold, which was related to the subject's occupation, was detected only in lung tissue. Although comparative mineralogical profiles are generally in good agreement, important variations can be observed in some subjects.

**Discussion**

There are no significant differences for particle sizes or relative concentrations between BAL and lung parenchyma. Particle sizes in lymph nodes are larger than in BAL and lung parenchyma. Relative concentrations seem higher for silicates and lower for metals in lymph nodes compared to BAL and lung parenchyma. Thus BAL analysis appears to reflect particulate content of lung parenchyma better than lymph node analysis. These conclusions, which are in agreement with a recently published paper by Chariot et al. (13), need to be refined by extending the studied population. Evaluating the lung concentration from the BAL analysis is of particular interest since the sampling technique is noninvasive and easily performed.

**REFERENCES**

1. Lippmann M, Yeates DB, Albert RE. Deposition, retention and clearance of inhaled particles. Br J Ind Med 37:337–362 (1980).
2. Dumortier P, De Vuyst P, Yernault JC. Non-fibrous inorganic particles in human bronchoalveolar lavage fluids. Scanning Microsc 3:1207–1218 (1989).
3. Gaudichet A, Pairol JC, Malandain O, Couste B, Brochard P, Bignon J. Etude minéralogique des particules non fibreuses du liquide de lavage broncho-alvéolaire. Rev Mal Resp 4:237–243 (1987).
4. Johnson NF, Halsam PL, Dewar A, Newman-Taylor AJ, Turner-Warwick M. Identification of inorganic dust particles in bronchoalveolar lavage macrophages by energy dispersive X-ray microanalysis. Arch Environ Health 41:133–144 (1986).
5. Rom WN, Churg A, Leapman R, Fiori C, Swyt C. Evaluation of alveolar macrophage particle burden in individuals occupationally exposed to inorganic dusts. J Aerosol Med 3:S43–S56 (1990).
6. Abraham JL, Burnett BR, Hunt A. Development and use of a pneumoconiosis database of human pulmonary inorganic particle burden in over 400 lungs. Scanning Microsc 5:95–108 (1991).
7. Churg A, Wiggs B. Mineral particles, mineral fibers, and lung cancer. Environ Res 37:364–372 (1985).
8. Churg A, Wiggs B. Types, numbers, sizes, and distribution of mineral particles in the lungs of urban male cigarette smokers. Environ Res 42:121–129 (1987).
9. Paoletti L, Batisti D, Caiazza S, Petrelli MG, Taggi F, De Zorzi L, Dina MA, Donelli G. Mineral particles in the lungs of subjects resident in the Rome area and not occupationally exposed to mineral dust. Environ Res 44:18–28 (1987).
10. Stettler LE, Platek SF, Riley RD, Mastin JP, Simon SD. Lung particulate burdens of subjects from the Cincinnati, Ohio urban area. Scanning Microsc 5:85–94 (1991).
11. Taikina-Aho O, Anttila S, Paakkö P, Sivonen SJ, Kalliomäki PL. Environmental pulmonary mineral burden correlated with smoking, pulmonary emphysema and lung cancer. In: Seventh International Pneumoconioses Conference, August 23–26, 1988, Pittsburgh, PA. Washington: U.S. Government Printing Office, 1990;1077–1082.
12. Tosi P, Francinielli A, Miracco C, Leoncini L, Minacci C, Baldelli C, Gotti G, Governa M. Silicotic lymph node lesions in non-occupationally exposed lung carcinoma patients. Eur J Respir Dis 68:362–369 (1986).
13. Chariot P, Couste B, Guillon F, Gaudichet A, Bignon J, Brochard P. Non-fibrous mineral particles in bronchoalveolar lavage fluid and lung parenchyma from the general population. Am Rev Respir Dis 146:61–65 (1992).