Phospholipid content of bronchoalveolar lavage fluid in granite workers with silicosis in Quebec

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Abstract

**Background**—Some of the prominent features of silicosis are hyperplasia and hypertrophy of epithelial type II cells, which in experimental animals are often accompanied by accumulation of phospholipids in the lung.

**Methods**—The total phospholipid content of lung lavage fluid and its composition in 28 granite stone cutters with long term exposure to silica dust (23 with radiological silicosis) was compared with that of lavage fluid in 15 normal volunteers, 15 patients with untreated idiopathic pulmonary fibrosis, and 19 patients with untreated stage 2 or 3 sarcoidosis. All lavage fluid was obtained at the time of first pulmonary investigation, which also included lung function tests.

**Results**—In the normal subjects total phospholipid content was 1-13 (0-16) µg phosphorus/ml of lung lavage, in the patients with idiopathic pulmonary fibrosis 0-52 (0-07) µg/ml (p < 0.05), and in the patients with sarcoidosis 1-02 (0-20) µg/ml composition being in the range reported in humans. In the patients with silicosis total phospholipid content was significantly decreased to an average of 0-46 (0-08) µg/ml compared with the findings in normal subjects and patients with sarcoidosis. Within the group exposed to silica changes in total phospholipid content did not correlate with the severity of the radiographic disease, changes in lung function, the cellularity of lung lavage fluid, or hyaluronate concentrations. The secretory capacity of rat epithelial type II cells was not significantly different when cultured with bronchoalveolar lavage fluid from all four groups of subjects.

**Conclusions**—Total phospholipid content in lung lavage fluid was significantly reduced in granite workers with radiological evidence of lung disease, but showed no correlation with radiological or functional markers of disease severity.

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Reductions in the amount and composition of surface active materials in alveolar lining fluids have been observed in the adult respiratory distress syndrome and idiopathic pulmonary fibrosis. These observations contrast with results in animals with experimental acute lung injury—for example, with silica—in which the total phospholipid content of bronchoalveolar lavage fluid is usually increased.

In humans the accumulation of phospholipids in the lung is part of the repair process following silica inhalation, being found in the acute form of silicoproteinosis or in the nodular form of the disease associated with increased numbers of epithelial type II cells in the lavage fluid.

Other aspects of silicosis in humans have been studied. Subclinical alveolitis has been reported in granite workers, characterised by excessive accumulation of lymphocytes and immunoglobulins and associated with normal phagocytic function and viability of dust laden alveolar macrophages. The subclinical alveolitis was associated with inhibitory activity of lung lavage fluid against fibroblast proliferation, but fluid from patients with the alveolitis of simple or confluent silicosis up-regulated the growth of fibroblasts. We have documented a direct proliferative action of silica on type II cells and observed a proliferative activity for type II cells in the bronchoalveolar lavage fluid from normal human lung. This activity was further upregulated early in the silicotic process in contrast to the fibroblastic activity, which is increased only after clinical disease is detected.

We measured the phospholipid content of lung lavage fluid in humans exposed to silica, comparing the findings with those in normal subjects and patients with idiopathic pulmonary fibrosis or sarcoidosis and correlating the findings with functional markers of severity of disease. We also tested a possible mechanism for the regulation of the accumulation of phospholipids in lavage fluid and evaluated the capacity of fluid from controls and patients with idiopathic pulmonary fibrosis to induce the secretion of phospholipids and proliferation of type II rat epithelial cells.

**Methods**

**PATIENTS**

The 15 normal subjects were paid volunteers who were free of symptoms and disease. The 15 patients with idiopathic pulmonary fibrosis and the 19 patients with sarcoidosis were
diagnosed on the basis of established criteria. At the time of investigation all patients with idiopathic pulmonary fibrosis and with sarcoidosis had had the disease for less than one year, as determined by the patients' symptoms. The 28 cases of silicosis were in men exposed in the granite cutting industry of the eastern townships of Quebec for an average of 30 years (range 18–35). They had no history of other pulmonary diseases. The granite industry of the eastern townships of Quebec has been characterised. In the patients with silicosis the disease was symptomatic in most cases and investigation was carried out at the time of the first abnormal results in a chest radiograph for the cases of simple silicosis and at the time of recognition of large opacities for the cases of confluent silicosis. All patients and subjects had been non-smokers for at least two years before investigation. The study was approved by the ethical committee of our hospital and written informed consent was obtained from each subject.

**LUNG FUNCTION TESTS**

All patients underwent pulmonary function tests including lung volumes, single breath carbon monoxide diffusion capacity, gas exchange at rest and after exercise, and lung compliance as previously described.

**BRONCHOALVEOLAR LAVAGE**

Bronchoalveolar lavage was performed in each subject as previously described. The albumin concentration in the lavage fluid was determined by laser nephelometry (Behring LN modular system, Hoechst Behring, Frankfurt, Germany). Lactate dehydrogenase activity was measured as a marker of non-specific cell damage. An aliquot of the cell free supernatant of the lavage fluid was used for the assay of hyaluronate concentrations according to the technique of Laurent and Tengblad.

Phospholipids were extracted from a 3 ml sample of supernatant in 10 vol chloroform/methanol solvent (2:1) and collected according to the method of Folch et al. The lower chloroform layer was evaporated to dryness and suspended in a small volume of chloroform. Total phospholipid concentration was measured by the method of Bartlett. Individual phospholipids were separated by thin layer chromatography on silica gel H plates (Merck Chemical Company, Montreal) using a mixture of chloroform, ethanol, and triethylamine (30:34:8.5). The lipids were detected under ultraviolet light after being sprayed with rhodamine. Phosphorus was estimated on gel scrapings by the method of Rouser et al.

**TYPE II EPITHELIAL CELL STUDIES**

Pneumocytes from fetal rats were obtained as reported. The secretion of phosphatidylcholine by type II cells was evaluated by determining the release of tritiated phosphatidylcholine. The growth effect of bronchoalveolar lavage fluid on rat epithelial type II cells in vitro was evaluated by the incorporation of tritiated thymidine into type II epithelial cells at 24 hours and counting the monolayers at 48 hours, given the phase lag between DNA synthesis and cell proliferation.

For these studies bronchoalveolar lavage fluid (10 ml of acellular supernatant) was concentrated by 48 hours of lyophilisation (Greece mobile 24, Virtis, Gardines, New York) with subsequent resolubilisation and application to G25 chromatography columns (PD10, Pharmacia Canada, Baie D'Urfe, Quebec) previously saturated with Eagles minimum essential medium (Gibco, Grand Island, New York) to obtain a resulting volume 10 times the original effluent concentration in lavage fluid. Dilutions to 20% of the concentrated volumes of lavage fluid for normal subjects and patients with idiopathic pulmonary fibrosis were also tested. The procedures were controlled by total protein determination each time.

**STATISTICAL ANALYSIS**

Data are means (SE). Statistical significance was evaluated with an analysis of variance with an a of 0.05 and a b of 0.20 (power 0.80) and a Dunnett t test for comparison with control data. Relations were tested by the Spearman procedure.

**Results**

**LUNG FUNCTION TESTS**

Table 1 shows the results of lung function tests for each group. The most severe changes were in patients with idiopathic pulmonary fibrosis and the least in patients with silicosis, which included five cases of long term exposure with normal results in chest radiographs, 14 cases of simple silicosis, and nine cases of confluent silicosis. Within the silicosis group patients with more severe radiological disease had more loss of lung function. The sarcoidosis group had lung function changes intermediate between the idiopathic pulmonary fibrosis and the silicosis group.

**ANALYSES OF LUNG LAVAGE FLUID**

Table 2 shows the content of bronchoalveolar lavage fluid. All groups of patients had significantly increased cellularity, with changes in the subsets of cells as in previous reports. Albumin, lactate dehydrogenase, and hyaluronate concentrations were increased in all
Table 2  Mean (SE) contents of lung lavage fluid

<table>
<thead>
<tr>
<th>Patients with:</th>
<th>Normal subjects (n = 15)</th>
<th>Silicosis (n = 28)</th>
<th>Idiopathic fibrosis (n = 15)</th>
<th>Sarcoidosis (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cells (1 × 10⁶/ml)</td>
<td>12.6 (1.2)</td>
<td>28.0 (5.1)*</td>
<td>35.3 (4.6)*</td>
<td>30.1 (4.1)*</td>
</tr>
<tr>
<td>Macrophages: 1 × 10⁶/ml</td>
<td>8.99 (1.1)</td>
<td>21.8 (4.5)*</td>
<td>23.9 (2.6)*</td>
<td>16.1 (2.2)</td>
</tr>
<tr>
<td>%</td>
<td>69.1 (2.3)</td>
<td>78.2 (3.1)</td>
<td>77.3 (2.9)</td>
<td>55.8 (2.7)*</td>
</tr>
<tr>
<td>Lymphocytes: 1 × 10⁶/ml</td>
<td>3.4 (0.2)</td>
<td>4.7 (1.4)</td>
<td>8.6 (2.9)</td>
<td>10.5 (1.6)*</td>
</tr>
<tr>
<td>%</td>
<td>28.1 (2.1)</td>
<td>17.8 (3.6)</td>
<td>20.7 (4.1)</td>
<td>36.0 (3.2)</td>
</tr>
<tr>
<td>Neutrophils: 1 × 10⁶/ml</td>
<td>0.3 (0.07)</td>
<td>0.8 (0.7)</td>
<td>1.77 (0.6)</td>
<td>2.7 (1.3)</td>
</tr>
<tr>
<td>%</td>
<td>1.7 (0.4)</td>
<td>2.2 (1.2)</td>
<td>4.6 (1.2)</td>
<td>6.1 (2.9)</td>
</tr>
<tr>
<td>Albumin (µg/ml)</td>
<td>33.1 (2.7)</td>
<td>74.0 (15.7)</td>
<td>121.9 (35.4)*</td>
<td>98.4 (23.4)</td>
</tr>
<tr>
<td>Total phospholipid content (µg/ml)</td>
<td>1.13 (0.16)</td>
<td>0.47 (0.08)*</td>
<td>0.52 (0.07)*</td>
<td>1.02 (0.20)</td>
</tr>
<tr>
<td>Phospholipids(%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphatidycholine (PC)</td>
<td>58.8 (3.2)</td>
<td>51.3 (4.5)</td>
<td>53.9 (4.2)</td>
<td>50.1 (4.0)</td>
</tr>
<tr>
<td>Phosphatidylglycerol (PG)</td>
<td>27.4 (3.9)</td>
<td>25.3 (4.9)</td>
<td>22.0 (1.9)</td>
<td>30.0 (5.0)</td>
</tr>
<tr>
<td>Phosphatidylinositol (PI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphatidylserine (PS)</td>
<td>3.4 (0.3)</td>
<td>4.2 (0.6)</td>
<td>6.9 (1.6)</td>
<td>5.6 (1.0)</td>
</tr>
<tr>
<td>Phosphatidylethanolamine</td>
<td>5.6 (0.9)</td>
<td>13.4 (1.4)*</td>
<td>10.2 (1.6)</td>
<td>6.2 (1.4)</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>2.5 (0.2)</td>
<td>2.6 (0.4)</td>
<td>3.5 (0.8)</td>
<td>4.1 (1.0)</td>
</tr>
<tr>
<td>Lyso phosphatidylcholine</td>
<td>2.2 (0.1)</td>
<td>2.9 (0.6)</td>
<td>3.5 (0.8)</td>
<td>3.0 (0.9)</td>
</tr>
<tr>
<td>PC/PG ratio</td>
<td>0.49 (0.09)</td>
<td>0.57 (0.18)</td>
<td>0.43 (0.07)</td>
<td>0.63 (0.19)</td>
</tr>
<tr>
<td>PG/PI + PS ratio</td>
<td>8.2 (0.9)</td>
<td>6.3 (1.1)</td>
<td>3.4 (0.9)</td>
<td>6.4 (2.2)</td>
</tr>
</tbody>
</table>

*p < 0.05 compared with normal subjects.

Discussion

We have confirmed previous reports in patients with idiopathic pulmonary fibrosis of low total phospholipid concentrations in bronchoalveolar lavage fluid compared with normal subjects and patients with sarcoidosis.1,3 Robinson et al2 and Hughes et al3 found proportionate reductions in phosphatidylglycerol, which we did not confirm. In agreement with Robinson et al we did not find a significant relation for cellular components and phospholipids of lavage fluid or a significant relation between pulmonary function test variables of disease severity and the concentrations of phospholipid in lavage fluid. Furthermore in our patients with idiopathic pulmonary fibrosis there was no effect of lavage fluid on the secretion or proliferation of epithelial type II cells, which contrasts with the effect of lavage fluid of patients with idiopathic pulmonary fibrosis on the proliferation of fibroblasts.

In silicosis we observed significant decreases in the total phospholipid content of...
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Phospholipid of bronchoalveolar lavage fluid is present in high nodules, as radiographic simulation. Finally, to the tribute the alveolar space may accelerate to be process associated with phospholipids accumulated by lipids. Fourthly, within the alveolar space, phospholipids may translocated rapidly in idiopathic fibrosis. Whereas altered surfactant abnormalities in respiratory failure have been reported, altered phospholipids, surface activity, phospholipase activity, and plasma myoinositol.

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18. Rousier G, Fleischner S, Yamamoto A. Two dimensional thin layer chromatographic separation of polar lipids and determination of phospholipids by phosphorus


