Hyaluronic acid in bronchoalveolar lavage in rats exposed to quartz

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ABSTRACT Hyaluronic acid, a connective tissue component, in bronchoalveolar lavage fluid (BAL) is correlated with decreased lung volumes in sarcoidosis. To investigate whether hyaluronic acid could be a marker of fibrosis in another interstitial lung disease, silicosis, the level of the substance in BAL fluid from rats exposed to crystalline silica (n = 3), amorphous silica (n = 3), and in one sham injected rat was measured. There was an increase in the total number of alveolar cells recovered in the rats exposed to crystalline silica and also a pronounced increase in the proportions of neutrophils and lymphocytes. In addition, the concentration of hyaluronic acid was high in this group of rats, and electron microscopic investigation of the lungs showed fibrosis. Thus hyaluronate in BAL fluid in rats exposed to crystalline silica seems to be a possible marker of fibrotic changes.

Patients with silicosis are staged according to the chest radiographs but there is no strict correlation between the x ray appearance and the degree of deterioration in lung function. In evaluating the activity of the disease measurement of lung function an evaluation of the radiographs must be performed on subsequent occasions. Thus it has been an urgent task to find better methods for evaluating the activity of the disease and to identify patients at risk of developing fibrosis. In the past few years analysis of the composition of the alveolar cell population recovered by bronchoalveolar lavage (BAL) has offered a new method to determine the activity of interstitial lung disease.1 Furthermore, increased concentrations of hyaluronic acid, hyaluronate, a connective tissue component, in BAL fluid correlates well with decreased lung volumes in patients with sarcoidosis, a disorder that also affects the alveoli and pulmonary interstitium.2 The present investigation was performed in order to study whether the level of hyaluronic acid in BAL fluid was correlated with the development of histologically proved pulmonary fibrosis in an animal model.

Material and methods

Crystalline silica, containing 98.3% quartz, was prepared from natural sand by sedimentation in water. The mean diameter of the particles was 1.2 μm and 90% had a diameter less than 3 μm. An amorphous silicon dioxide, with crystalline components less than 1%, was derived from the smoke of a ferrosilicon smelting furnace; the mean diameter of the particles was 0.05 μm.

Seven female Sprague-Dawley rats with an initial weight of 200 g were used in the investigation. Under shallow ether anaesthesia the trachea was exposed and punctured by an ejection needle that was directed downwards towards the bifurcation. The particles were suspended in physiological saline with a concentration of 40 mg/ml, and 1 ml was aspirated into an injection syringe and rapidly injected through the cannula into the trachea. Three animals received crystalline silica, three received amorphous silica, and one received 1 ml saline with no particles. Four months after the instillation the animals were killed by an overdose of ether.

The trachea was cannulated and the lungs lavaged with Hank’s balanced salt solution at 37°C. The lavage medium was introduced in aliquots of 5 ml into the lungs by gravity using 20 cm of hydrostatic pressure. After three minutes the lavage fluid was drained, also by gravity, into a siliconised tube placed on ice. The procedure was repeated 10 times. The recovered fluid was centrifuged at 400 g at +4°C for five minutes and the cell pellet was resuspended in 5 ml of Hank's...
solution. Total cell count was performed in a Bürker chamber. Cytocentrifugal smears were prepared on 8 × 10^4 cells per slide. Cytocentrifugation was carried out at 500 rpm for 10 minutes (Cytospin 2, Shandon, England). Smears were stained according to May-Grünwald Giemsa and 500 cells were analysed for differential count. The supernatant was stored at −70°C before analyses. Hyaluronate was analysed in duplicate in BAL fluid according to principles previously outlined.3

Pieces of the lungs were fixated in 3% glutaraldehyde solution buffered by Na-cacodylate to pH 7.4, embedded in Agar 100 and studied in Jeol electron microscope.

### Results

An average of 60% of the instilled fluid was recovered in the animals exposed to amorphous or crystalline silica, whereas the recovery in the non-exposed rat was 93%. As shown in the table, the recovery of alveolar cells was most pronounced in the rats exposed to crystalline silica. The percentages of lymphocytes and neutrophils were also increased in these rats. In addition, the concentration of hyaluronate was raised in this group when compared with animals exposed to amorphous silica and the non-exposed subject, respectively.

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**Table:**

<table>
<thead>
<tr>
<th>No</th>
<th>Treatment</th>
<th>Cells × 10^3/ml</th>
<th>Macrophages</th>
<th>Monocytes</th>
<th>Lymphocytes</th>
<th>Neutrophils</th>
<th>Eosinophils</th>
<th>Hyaluronate (μg/l)</th>
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<tr>
<td>1</td>
<td>Crystalline silica</td>
<td>265</td>
<td>18.0</td>
<td>—</td>
<td>24.0</td>
<td>58.0</td>
<td>—</td>
<td>50</td>
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<tr>
<td>2</td>
<td>Crystalline silica</td>
<td>330</td>
<td>46.3</td>
<td>—</td>
<td>27.7</td>
<td>27.8</td>
<td>—</td>
<td>67</td>
</tr>
<tr>
<td>3</td>
<td>Crystalline silica</td>
<td>275</td>
<td>30.5</td>
<td>—</td>
<td>32.8</td>
<td>37.2</td>
<td>—</td>
<td>46</td>
</tr>
<tr>
<td>4</td>
<td>Amorphous silica</td>
<td>70</td>
<td>60.8</td>
<td>—</td>
<td>13.0</td>
<td>26.2</td>
<td>—</td>
<td>7</td>
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<td>5</td>
<td>Amorphous silica</td>
<td>205</td>
<td>97.5</td>
<td>0.3</td>
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<td>—</td>
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<td>Amorphous silica</td>
<td>145</td>
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<td>—</td>
<td>1.2</td>
<td>2.0</td>
<td>—</td>
<td>9</td>
</tr>
<tr>
<td>7</td>
<td>Sham injected</td>
<td>110</td>
<td>95</td>
<td>—</td>
<td>3.5</td>
<td>1.2</td>
<td>0.3</td>
<td>2</td>
</tr>
</tbody>
</table>

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Fig 1  Electron microscopy from lung of sham injected rat shows normal picture with close connection between alveolar space and capillaries. Magnification × 3000.
Hyaluronic acid in bronchoalveolar lavage in rats exposed to quartz

Figure 1 shows typical electron microscopical pictures from the non-exposed and fig 2 those from a rat exposed to quartz. The rats exposed to amorphous silica showed a morphology similar to the non-exposed animal.

Discussion

The increased number of alveolar cells recovered by BAL in rats exposed to crystalline silica compared with controls makes a silica induced alveolitis in these subjects probable. This theory is supported also by an increase in the proportion of neutrophils and lymphocytes among the alveolar cells in this group of rats. Increased proportions of neutrophils in BAL from silicotic guinea pigs have been reported by Dauber et al.4 In addition, the concentration of hyaluronate found in BAL in this group was clearly higher than in the controls.

Hyaluronate is a connective tissue component present in the lung parenchyma, and it is released into the culture medium of growing fibroblasts.5 The fibroblasts in turn play a central part in the development of fibrosis. Thus it is tempting to speculate that the amount of hyaluronic acid found in BAL fluid in interstitial lung disease reflects an enhanced activation of fibroblasts or an increased number of fibroblasts, and that hyaluronate might be used as a marker for the development of fibrosis. The concomitant findings in the present study of cellular signs of an alveolitis, increased hyaluronate concentrations in BAL fluid, and histologically proved pulmonary fibrosis gives further support to this hypothesis.

In conclusion, the present investigation has shown that there seems to be a good correlation between a raised concentration of hyaluronic acid in BAL and the development of interstitial fibrosis induced by exposure to inorganic particles. Further investigations on an extended number of animals and at different time intervals after exposure are, however, needed before hyaluronic acid in BAL can be recommended as a biochemical marker of fibrosis in this animal model.

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References

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