Dynamic Changes of Constituents in Bronchoalveolar Lavage Fluid in Experimental Silicotic Rats

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Abstract: Bronchoalveolar lavage (BAL) is a useful and safe method for sampling cellular and biochemical components from the lung. Analysis of bronchoalveolar lavage fluid (BALF) constituents is useful for defining the stage of disease, and for assessing disease progression and the response to therapy in lung disorders. We studied the dynamic changes in various indices for BALF and the accompanying silicotic changes in the lungs of rats at different times after quartz instillation. Total cell counts, LDH activity, protein concentration, and lipoperoxide (LPO) in the BALF of experimental silicotic rats were significantly higher than those of control rats (p < 0.05 or 0.01). After instillation, quartz content, total cell counts, LDH activity and protein concentration in BALF tended to decrease over time. These findings suggested that in acute silicosis, quartz can induce serious inflammation and damage the lung, with acute lung proteinosis seen as the main change in this stage.

Key words: Quartz — Experimental silicosis — Bronchoalveolar lavage — Bronchoalveolar lavage fluid

INTRODUCTION

Pneumoconiosis is the most hazardous occupational disease in workers exposed to dust. Presently, there are more than 0.4 million pneumoconiotic patients in China1. Although respiratory protection and dust suppression are basic measures
against pneumoconiosis, this disease has become so prevalent that it is necessary to investigate its pathogenesis and develop effective methods for treating patients and ameliorating the development of silicosis. Bronchoalveolar lavage (BAL) is a safe and relatively non-traumatic diagnostic method\textsuperscript{2-6}, which has also been used for treating diffuse lung diseases including alveolar proteinosis\textsuperscript{7-12}. The present study observed time-related changes in various components of the bronchoalveolar lavage fluid (BALF), and pathology in rats at different times after quartz instillation. The data obtained from this study are helpful in determining the degree of lung damage in acute or subacute silicosis.

\textbf{MATERIALS AND METHODS}

\textit{Quartz}

The purity of the quartz used was more than 99\%. The size distribution was as following: particles with a size of < 1 µm, 45.4\%; > 1 µm to < 2 µm, 37.0\%; > 2 µm to < 3 µm 13.1\%; > 4 µm to < 5 µm, 1.0\%; > 5 µm to < 6 µm, 0.1\%. Particles of < 0.5 µm had a distribution of more than 99.9\%; those of < 2 µm, more than 82\%. Quartz was newly ground by the Beijing 605 factory according to the method of Fu\textsuperscript{3} and provided by the Institute of Occupational Health, Chinese Preventive Medical Sciences, Beijing. This quartz has been widely used in People’s Republic of China as a standard for many scientific studies.

\textit{Animals and Treatment}

SPF, male Wistar rats, 180–200 g and 7–8 weeks of age were supplied by the Center for Laboratory Animals in Zhejiang Medical University. Rats were divided randomly into ten groups. Each experimental rat was injected intratracheally with 40 mg/ml of quartz in 1 ml of physiological saline. Each control rat was injected with 1 ml physiological saline. Ninety-four rats were sacrificed by overdose of phenobarbital at 7, 15, 30, 90, and 180 days after exposure to dust, respectively. Sixty-nine rats underwent BAL, and twenty-five rats were used for histopathological examination.

\textit{Biochemical and cytological evaluation of BALF}

After sacrifice, the rats’ heart-lung blocks were removed. Lungs were lavaged 4 times with 8 ml of physiological saline at 37°C. Cells were removed by centrifugation (1500 rpm for 10 min. at 5°C) and pooled for cytological evaluation. Fluid from the first lavage was pooled for evaluation of biochemical changes after cell removal. We determined the activity of LDH in BALF according to the method of Babson and Phillips\textsuperscript{14}. The total protein in BALF was determined by Lowry’s method\textsuperscript{5}, and LPO content by fluorescence\textsuperscript{6}. BALF cell counts were enumerated with a haemocytometer by the conventional method. We measured the content of quartz in BALF by infra-red spectrophotometry\textsuperscript{7}. 
Histopathology

Animals for histopathological examination were sacrificed as described above; their lungs were then removed and fixed by intratracheal instillation of a 4% neutral buffered formalin solution, embedded in paraffin, and sectioned at 5 µm. Five paraffin sections (from each lobe) were prepared for each rat and stained with hematoxylin and eosin, then examined microscopically. Pathological changes were evaluated according to the method of King. The appraisal standard was as follows:

Grade I: Numerous nodular lesions through the lung; silicotic nodule composed of cellular compounds; dust cells have clear membranes and nuclei. There are few fibroblasts around the nodule.

Grade II: The silicotic nodule is composed of cellular components; dust cells have unclear membranes and nuclei. There are more fibroblasts around the nodule.

Grade III: The silicotic nodule is also composed mainly of cellular components, but few collagenous fibers are formed in its intermedium.

Grade IV: The silicotic nodule is composed mainly of collagenous fibers, and the collagenous fibers are condensated. The nucleus can be seen, and there is a cellular compound in the margin of nodule.

Grade V: The silicotic is composed of collagenous fiber and collagenous fibers are formed. There are very few or no cellular components. Hyaline degeneration appears in the intermedium of a nodule.

Statistical analysis

Values were expressed as means and standard deviations. Differences between the experimental groups and the controls were tested by t-test. Differences among groups exposed to dust for different durations were tested by Wilcoxon’s rank test. The relationships between the content of quartz and total protein, the total cell counts, or LDH activity in BALF at different exposure times were analyzed by linear correlation.

RESULTS

Recovery of BALF

The recovery of BALF did not differ significantly either between the experimental groups and the control group or among groups taken at different times after quartz instillation (Fig. 1).

Quartz content, total cell counts, LDH activity, total protein, and LPO content in BALF

Substantial quantities of dust could be measured in BALF obtained from quartz-injected rats, as seen in Figure 2. No significant differences among groups were noted at 7, 15, 30 days after quartz injection, but the content of quartz in each
Fig. 1. The recovery (%) of bronchoalveolar lavage fluid (BALF) at different times after injection of quartz.
Values are means ± SD from 7–8 rats in each treatment group and 6–7 rats in each control group.

Fig. 2. Content of quartz in BALF in rats at different time after exposure to quartz.
Values are mean ± SD taken from 7–8 rats in each treatment group. A: significantly higher than the group 90 days after exposure to quartz (p < 0.05); B: significantly higher than the group 180 days after exposure to quartz (p < 0.05).
of these exposure groups was significantly higher than those at 90 or 180 days after instillation. The results also indicated that quartz content in BALF clearly trended downward over the experiment’s duration (R = -0.5274, p < 0.001) (Fig. 2).

BALF cell counts of quartz-injected rats were significantly higher than those in control rats (p < 0.001), and showed a significantly decreasing trend within the period after quartz injection (R = -0.7059, p < 0.001) (Fig. 3).

In the BALF of quartz-injected rats, the LDH activity was remarkably higher than in that of the control rats (p < 0.001). No significant differences among groups were observed at 7, 15 or 30 days after quartz instillation, but values at each period were significantly higher than for groups at 90 and 180 days after injection (p < 0.001). There was also a significant decreasing trend within the experimental duration (R = -0.8294, p < 0.001) (Fig. 4).

Quartz-injected rats showed significantly higher protein content than control rats (p < 0.001). The results also showed that protein contents was significantly higher in all groups at 7, 15, and 30 days than at 90, 180 days after instillation. Total protein concentration showed a clearly decreasing trend within the experimental period (R = -0.7107, p < 0.001) (Fig. 5).

![Fig. 3. Total number of cells in BALF in rats at different times after exposure to quartz.](image)

Values are mean ± SD taken from 7–8 rats in each treatment group and 6–7 rats in each control group. A: significantly higher than the group 90 days after exposure to quartz (p < 0.01); B: significantly higher than the group 180 days after exposure to quartz (p < 0.01); C: significantly higher than the control group (p < 0.001).
Fig. 4. LDH activity in BALF in rats at different times after exposure to quartz.
Values are mean ± SD taken from 7–8 rats in each treatment group and 6–7 rats in each control group. A: significantly higher than the group 90 days after exposure to quartz (p < 0.01); B: significantly higher than the group after 180 days exposure to quartz (p < 0.01); C: significantly higher than the control group (p < 0.001).

Fig. 5. Protein content in BALF in rats at different times after exposure to quartz.
Values are mean ± SD taken from 7–8 rats in each treatment group and 6–7 rats in each control group. A: significantly higher than the group 90 days after exposure to quartz (p < 0.01); B: significantly higher than the group 180 days after exposure to quartz (p < 0.01); C: significantly higher than the control group (p < 0.001).
There was a significant difference in LPO content between the quartz-injected groups and controls. The LPO content of the quartz-injected rats reached a maximum level at 15 days after instillation and declined thereafter (Fig. 6).

**Pathological findings**

Light microscopy indicated that the lung structure of the control rats was normal. In quartz-injected rats at 7, 15, and 30 days after instillation, the silicotic nodules were composed of cellular components. Some dust-cell membranes and nuclei in the rats at 7, 15, and 30 days after injection were not clear, there were fibroblasts around the nodule up to 30 days after injection, and a little collagenous fiber was formed. At 7, 15, and 30 days after instillation, cellular or granulomatous nodules were predominant. Collagenous nodules then tended to gradually replace cellular nodules over time. At 90 and 180 days after instillation, collagenous nodules and collagenous fibrosis were predominant (Table 1).

**DISCUSSION**

Quartz dust is the main pathogenic factor in the development of silicosis. It induces destruction and disintegration of the macrophages; once injured the macrophages release chemokines and proteases, which mobilize inflammatory cells in

![Fig. 6. LPO content in BALF in rats at different time after exposure to quartz.](image) Values are mean ± SD taken from 7–8 rats in each treatment group and 6–7 rats in each control group. A: significantly higher than the control group (p < 0.01); B: P < 0.05.
the lung, thus leading to more damage of the lung tissue\textsuperscript{19-20}. There is a dose-response relationship between the morbidity of silicosis and the environmental concentration of dust and between silica dust content in the lung and the pathological change characteristic of the silicosis\textsuperscript{21}. Lowering environmental dust concentration in the workplace would prevent the occurrence of silicosis. On the other hand, among silicotic patients, if the progress of lung fibrosis is blocked by removing the silica dust, the progression of silicosis would be delayed or prevented. The assessment for BALF from exposed animals has proven to be useful as a rapid screening method for lung injury due to inhaled particles. Both cellular and liquid components of BALF can be changed by inhaling dust\textsuperscript{2, 4, 6, 22-24}. In the early stage of silicosis, there is alveolitis associated with recruitment of macrophages, lymphocytes and neutrophils\textsuperscript{2-4, 6, 24-27}. Lipid peroxidation of the pneumocyte membranes may be caused by continuous stimulation with silica dust-derived free radicals or inflammatory cell-derived oxidant\textsuperscript{28}. In addition, LDH which exists in the cytoplasm is released after pneumocytes or leukocytes die\textsuperscript{29}. Protein can leak into the alveolar space because of loss of the epithelial integrity. These reactions can be reflected by increasing the cell counts, LDH, and concentrations of total protein and LPO in BALF in the rats injected with quartz at different times compared to control rats, especially at 7, 15 and 30 days after injection of quartz. One of the early responses of the lung to quartz is the formation of protein-rich edematous fluid in the alveolar surface\textsuperscript{30}. It remains unclear whether the accumulation of alveolar surface protein has a direct role in fibrogenesis and its mechanism, though the accumulated protein in the lung will affect the lung function\textsuperscript{31}. Therefore, silicosis may be ameliorated if inflammatory products, such as the inflammatory cells or accumulated protein, are washed out as a result of BAL.

At the early stage of silicosis in our model, when the lesion was at only Grade

<table>
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<tr>
<th>Days after exposure to quartz</th>
<th>Total number of lung lobes examined</th>
<th>Number of cellular or collagenous nodules (% in any category)</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
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<tr>
<td>7</td>
<td>20*</td>
<td>210* (94.45%)\textsuperscript{a} 10 (4.55%)</td>
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<td>15</td>
<td>20</td>
<td>116 (38.93%) 82 (27.52%) 57 (19.13%) 26 (3.72%) 17 (5.7%)</td>
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<td>30</td>
<td>20</td>
<td>21 (8.43%) 91 (36.55%) 73 (29.32%) 41 (16.47%) 23 (9.23%)</td>
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<tr>
<td>90</td>
<td>20</td>
<td>6 (2.08%) 42 (14.58%) 97 (33.68%) 113 (39.24%) 30 (10.42%)</td>
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<tr>
<td>180</td>
<td>20</td>
<td>6 (1.52%) 7 (1.77%) 124 (31.39%) 187 (47.34%) 71 (17.97%)</td>
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Note: *: 4 silicotic lungs were examined under microscope, giving a total of 20 lobes in each treatment group.  
\(a\): Number of cellular or collagenous nodules in 20 lobes.  
\(b\): The percentage of lobes affected in each grade.
I, considerable quartz dust, dust-laden macrophages, LPO, and proteins were present in BALF than at later stages. These findings suggest that at the early stage of silicosis, the quartz may be more toxic to the lung in term of inflammatory reaction than at later stages. Alveolar macrophages can release chemotactic factors that attract polymorphonuclear leucocytes and/or monocytes, and stimulate fibroblast proliferation. Our research showed that as much as 5.75 mg of quartz from the lung was washed out by BAL in dust-exposed rats. Although considerable quartz was left in the lung which could continue to damage it, removing quartz from lung may ameliorate lung damage. Application of BAL applied to early-stage silicosis may thus delay or prevent the progression of silicosis by removing additional quartz and toxic substances from the lung.

On the other hand, it is well known that the quartz can be removed by migration to the upper respiratory tract, sequestered in small amounts in the interstitium, or moved by the lymph system\(^1\). From the results of our animal models, at the later stage, the histopathological assessment revealed that collagenous nodules predominated in rat lungs, and that the content of inflammatory materials in BALF was much less than at earlier stages. This suggests that at the later stage of silicosis, the BAL can remove only limited contents of quartz and toxic products from injured lung tissue.

On the whole, this study has shown the process of lung damage in the development of silicosis in rats. The data obtained have highlighted the need for research into the treatment of early silicosis by BAL, and suggest a need for further research for its clinical application.

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REFERENCES