Experimental Study on Fibrogenic Effect of Fur Dust on Rat Lung

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Abstract:

Objective: The fibrogenicity of fur dust was studied in rat lung tissues.

Methods: Intratracheal instillation of fur dust, morphologic examination of lungs and analysis of collagen content were performed in Wistar rats.

Results: Morphologic examination revealed that the earliest changes consisted of alveolar edema, increased numbers of intraalveolar macrophages, and marked thickening of interalveolar septa with mixed cellular infiltrate. After sixth months, there was moderate thickening of the alveolar walls and the peribronchioli. After 12 months, interstitial positive fibrosis of the alveolar wall and the peribronchioli were weakly seen. In the carding dust group (silica content 17.6%), interstitial nodules were observed composed of fibroblasts, reticular fibers, and collagen fibers. Electron microscopic examination also showed that alveolar walls became thickened and collagen fiber bundles were seen around bronchioles and small vessels in the carding groups after 12 months. At all stages of analysis, the collagen content in lungs of the fur dust groups was significantly higher than that of the control group.

Conclusions: Our study suggested that fur dust might induce weak interstitial fibrosis in the lung.

Key words: fur dust, lung interstitial fibrosis, experimental study

Introduction

In China, the fur processing industry has recently developed rapidly and fur processing workers are exposed to high levels of dust. Such exposure has been reported to be associated with respiratory health problems in fur processing workers (1–5). The studies indicated that fur dust exposure causes acute and chronic respiratory function loss and increases chronic respiratory symptoms. The problem of dust in the fur processing workplace is complex. The fur dust itself, mineral impurities, microorganisms and their metabolic products are commonly considered to be the main etiologic factors. It is still controversial whether organic dust exposure induces lung interstitial fibrosis or not (6, 7). We conducted an experimental study in rats by intratracheal instillation and collagen content analysis to detect whether there is a fibrogenic potential from fur dust exposure.

Material and Methods

Dust samples

The fur processing factory where samples were collected is located in Jinzhou City, Liaoning Province, northeastern China.

Table 1 Silica content and particle size distribution of the fur dust samples and quartz

Groups	Silica content (%)	Particle size distribution			
		<2 μm	2 μm ~	5 μm ~	10 μm ~
Carding	17.6	94.8	2.0	2.8	0.6
Sewing 1	0.6	84.7	8.4	4.1	2.8
Sewing 2	0.7	76.8	20.3	2.3	0.6
Quartz	>97	84.0	14.0	2.0	—

Three fur dust samples were tested. The fur dust samples were collected from the surfaces of the machines in the work areas for the carding (wool dust) and two sewing (rabbit fur dust and wool dust) procedures in the factory. These dust samples were repeatedly cut with scissors and then pestled finely in an agate mortar.

Quartz dust was provided by the Institute of Industrial Hygiene and Occupational Disease, Chinese Academy of Preventive Medicine. The silica content analyses and particle size distribution of the fur dust samples and quartz dust are given in Table 1.

Animals and experimental design

A total of 200 Wistar rats, males and females in equal proportion, weighing 180–220 g, were used. These rats were provided by the Center of Experimental Animals at China Medical University. They were randomly divided into five groups: carding group (wool dust), sewing group1 (rabbit fur dust), sewing group 2 (wool dust), quartz group and control group (saline).

Three fur dust samples and quartz dust were suspended in

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saline, then sterilized by autoclave (120°C, 20 pound, 30 min). Before administration, the suspension was shaken vigorously first by hand, then by a vortex shaker. Rats, lightly anesthetized with ether, were instilled intratracheally under direct observation with the aid of laryngoscope. The total dose (100 mg for each fur dust sample) was administered in 3 instillations given over a period of 45 days (40 mg the first time, the second time and third time 30 mg each). The quartz dust was instilled intratracheally once for a dose of 40 mg. 1 ml of saline was administered in the control group. Four to six rats per group were sacrificed by decapitation after being anesthetized with ether at 1, 3, 6 and 12 months after the last administration.

Light microscopy procedure

After the gross inspection, small pieces of lung tissue from the middle part of the lobes of each rat and hilar lymph nodes were routinely fixed with 4% formaldehyde, embedded in paraffin, sectioned at 5 µm, then stained with hematoxylin and eosin (HE), Foot for reticulate fibers, and van Gieson stains (VG) for collagen fibers.

Scanning and transmission electron microscopy procedure

12 months after instillation, some lung tissues were rinsed in phosphate buffer. For scanning electron microscopy, lung tissues were fixed using a glutaraldehyde solution buffered with 0.05 M sodium cacodylate. The tissues were then dried in carbon dioxide at critical point, mounted, and coated with a thin layer of gold. Observations were made using a TSMT-300 scanning electron microscope. For transmission electron microscopy, the tissues were postfixed in 1% osmium tetroxide, dehydrated in graded ethanol solutions, and embedded in Epon812. Ultrathin sections were cut with a diamond knife, double stained with uranyl acetate and lead citrate, and observed with a JEM-1200 transmission electron microscope.

Determination of collagen content

The remaining lung tissues of each group, at 1, 3, 6, and 12 months after instillation, were dried to a constant weight. Three aliquots per lung were measured for hydroxyproline concentration using chloramine-T method, modified according to Woessner JF Jr.'s method (8). Collagen content was calculated and expressed as milligrams per lung. Data in each stage of study for each group were averaged and the standard deviation was calculated. The significance of the differences between values obtained from experimental and control groups was tested using Student's *t* test.

Results

Morphologic examinations

Fur dust groups:

The histological findings of the three fur dust groups persisted with similar characteristics at all stages after instillation although the extent of lung interstitial fibrosis in the sewing groups was lesser than that in the carding group.

Gross inspection: The lungs of rats were smooth, soft and enlarged 1, 3, 6 months after instillation. Tiny spotty dust foci, brown or grey, were scattered on the surface and across the sections of lungs. The quantity of dust foci decreased with time after instillation. 12 months after treatment, the lung was slightly

hardened while the surface was smooth. At every stage, hilar lymph nodes were swollen, dark grey, and slightly hardened.

Light microscopic examination: 1 and 3 months after instillation, there were severe inflammatory reactions. These reactions consisted of alveolar edema, increased numbers of intraalveolar macrophages, and marked thickening of interalveolar septa with mixed cellular infiltrate in which monocyte-macrophages accounted for about 50–60%, and plasma cells were about 20%. Only a few lymphocytes and granulocytes were present. Free dust particles and dust-laden phagocytes were observed, and lymphocytes proliferated abundantly around bronchioli. The alveolar reaction remained constant during the whole observation period, but at later stages, the reaction became weak. Macrophages and dust particles were still found in the alveoli at later times. After 6 months, granuloma lesions were formed by epithelioid cells, multinuclear cells, fibroblasts, and dust particles around bronchioli and small vessels. After 12 months, there was moderate thickening of the alveolar walls and the peribronchioli. The fibrosis of alveolar walls and peribronchioli were weakly positive on VG stain for collagen. In the carding group, interstitial nodules were observed, mainly composed of fibroblasts, reticular fibers, and collagen fibers. In all stages, interspersed dust could be seen in hilar lymph nodes and no fiber proliferation was found.

Electron microscopic examination: Under scanning electron microscope, the structure of lung tissue in rats at 12 months after dust exposure was markedly destroyed and the alveolar type II pneumocytes proliferated. The alveolar walls were thickened and collagen fiber bundles were seen around bronchioli and small vessels in the carding groups. Under transmission electron microscope, the proliferative alveolar type II epithelial cells had numerous lamellar bodies in the cytoplasm and the alveoli had macrophages with abundant lysosome.

Quartz group:

Only 1 month after quartz dust instillation, cellular fibrotic nodules formed in lungs. After 6 months, fibrotic nodules diffusely formed. After 12 months, some silicotic nodules had fused and formed massive fibrosis. The changes in lymph nodes at all stages were similar to those of lung tissue.

Control group:

No abnormality was observed, except for inflammatory alteration in a few pulmonary lobes at the early stage.

Determination of collagen content

At all stages of analysis, the collagen content in the lungs of the quartz group was the highest. Collagen content of rat lungs in the fur dust groups was significantly higher than that of control group (Table 2). There was no significant difference between fur dust groups.

Discussion

Three fur dust samples were studied by performing intratracheal instillations in rats totally 100 mg per rat, using dust collected from the surfaces of the machines in the work areas for carding (wool dust, silica content 17.6%) and two sewing (rabbit fur dust and wool dust, silica content 0.6–0.7%) procedures in a fur processing factory. The aim of this study was to see whether fur dust had a fibrogenic potential. Quartz served as a positive

Table 2 Lung collagen content(mg/lung) at different stages after instillation

Time	Control group	Quartz group	Carding group	Sewing group 1	Sewing group 2
1 month	21.4±5.8 (6)	42.19±10.9 (6)	26.2±6.4 (5)	34.1±8.5* (5)	29.9±4.8* (5)
3 months	23.2±6.4 (5)	63.98±21.6 (5)	32.8±8.6* (5)	31.6±4.4* (6)	35.9±8.9* (5)
6 months	26.4±6.2 (5)	74.17±19.1 (5)	40.9±14.1* (6)	40.1±8.8* (4)	30.7±3.4 (6)
12 months	25.4±6.0 (4)	79.13±20.5 (6)	50.8±13.0** (5)	54.2±18.4* (5)	51.4±24.3* (6)

Values shown are mean±SD (n). Significantly different from control group (* p<0.05, ** p<0.01)

control.

The results showed that the earliest changes consisted of alveolar edema, increased numbers of intraalveolar macrophages, and marked thickening of interalveolar septa with mixed cellular infiltrate. After six months, there was moderate thickening of the alveolar walls and the peribronchioli. After 12 months, interstitial fibrosis of alveolar walls and peribronchioli was weakly observed. In the carding group, interstitial cellular nodules were observed, mainly composed of fibroblasts, reticular fibers, and collagen fibers. At all times of analysis, the collagen content in lungs of the fur dust groups was significantly higher than that of the control group. Pimental identified reticulate and collagen fiber proliferation in lungs from the biopsy of fur processing workers (9). Xing reported atypical fibrotic foci in the lungs of fur processing workers from autopsy samples (10). Accordingly, our experimental study also indicated that fur dust could weakly induce lung inter-

stitial fibrosis.

In our field studies, an industrial hygiene investigation showed that the procedures in fur processing produce high levels of dust that contain substantial amounts of mineral ash and silica (11). A few fur processing workers who worked at carding and wearing procedures were exposed to dust containing above 16% silica. Most were exposed to fur dusts that contained less than 2.5% silica. This experimental study showed that the degree of lung interstitial fibrosis in sewing groups was lesser than that in the carding group, although the histological findings of the three fur dust groups showed similar characteristics at all stages after instillation. The different silica content of the three fur dusts might play a role in causing different degrees of lung interstitial fibrosis. Although fur dust only induced lung interstitial fibrosis weakly in this experimental study, the harmful effects of high levels of fur dust should not be neglected and deserve further study.

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