PULMONARY DEPOSITION AND EFFECTS OF INHALED SILICA PARTICLES AFTER SHORT-TERM EXPOSURES IN THE RAT

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Abstract—The present study was designed to evaluate the pulmonary deposition and the effects of inhaled silica particles in the rat model. Wistar (W/M strain) rats were exposed to silica aerosols generated from a fluidized bed dust generator for 1 hr a day, intermittently for 6 days, using a "nose-only" inhalation chamber. After the cessation of the exposures, analysis of lavaged bronchoalveolar cells (BAC) and histological examinations of lungs and tracheobronchial lymph nodes (TBLN) were performed during a period of 6 months. Total cell yields and the proportions of alveolar macrophages (AM) in BAC were not altered, whereas the proportions of lymphocytes in BAC were significantly increased in the exposed animals. Although the proportions of silica-laden AM in BAC were gradually decreased during the 6 months, particle-laden AM were predominantly and persistently observed in the alveoli under light microscopy. Silica particles were also identified in macrophages of granulomatous nodules in pulmonary peribronchial lymphoid tissues (PBLT) and TBLN, indicating the translocation of particles via the lymph. Associated with pulmonary particle deposition, some characteristic histopathological features were evident, including thickening of alveolar duct bifurcations and lymphocyte infiltrations both in the alveolar sacs and around the interstitial blood vessels. At later months after the exposures, the alveolar interstitium was thickened with the increase of fibroblasts and collagen. These results implicate that short-term exposures of silica particles in the rat can evoke histopathologic changes in the lungs and lymphatic tissues, associated with their deposition and translocation.

Key words: Pulmonary deposition, inhalation, silicosis, rats.
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INTRODUCTION

It has long been recognized that many air pollutants or industrial dusts can evoke pulmonary disorders leading to fibrosis after inhalation exposures. Fibrotic process with respirable dust particles, like silica and asbestos, is of interest because they are still major hazardous materials in industrial and occupational fields (Ziskind, Jones, and Weill, 1976; Becklake, 1976). Although the pathogenesis remains unclear, factors affecting the pathogenicity of these dust particles are physical or chemical properties, particle size, and concentration in air (Reiser and Last, 1979; Craighead and Mossman, 1982). Initial pulmonary events would occur immediately after the particle deposition, which can be dependent on the above factors, and this deposition and subsequent translocation of particles may affect the pathologic changes. Despite a number of studies, it is not known whether differences in deposition and translocation patterns exist among various types of dust particles. In the previous study, intrapulmonary distribution was compared between both types of asbestos, crocidolite and chrysotile (Oghiso, Kagan, and Brody, 1984). After a prolonged inhalation, both of asbestos particles were identified within pulmonary interstitial, whereas crocidolite asbestos was also detected in alveolar macrophages, especially of subpleural areas. Initial particle deposition of silica as well as chrysotile asbestos after a short-term exposure was described to occur at the site of alveolar duct bifurcations (Brody et al., 1981; Brody and Roe, 1983; Warheit et al., 1984). Nevertheless, no detailed information is available on deposition and translocation patterns of these dust particles at the levels of pulmonary and lymphatic tissues during a relatively longer period.

The present study was designed to evaluate the pulmonary deposition and translocation to the regional lymph nodes (tracheobronchial lymph nodes, TBLN) of inhaled silica particles, as well as their effects on pulmonary and lymphatic tissues. We have accomplished "nose-only" exposures to silica aerosols by a repeated and short-term inhalation regimen instead of whole body exposures or intratracheal instillation. Histological examinations of lungs and TBLN were performed from 1 to 6 months after the exposures, as well as analysis of bronchoalveolar cells (BAC) obtained by bronchoalveolar lavage to determine the proportional changes of particle-laden alveolar macrophages (AM) and other cell populations.

MATERIALS AND METHODS

Animals: Male inbred Wistar (W/M strain) rats of 350-400 g were obtained from animal facility of our institute. Only animals without spontaneous pulmonary infections were selected before use, and housed under barrier conditions.

Silica samples: Silica particles used for the present inhalation exposures were obtained from Dust for Industrial Testing (No. 3, JIS Z8901-1979), which consisted of 97% or more of SiO₂ and 3% or less of other elements (Fe₂O₃, Al₂O₃, TiO₂ and MgO). Particle size distribution was the followings: approximately 40% less than 5 μm, 20%
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between 5-10 µm, and 40% more than 10 µm.

**Exposure conditions**: Inhalation exposures were performed using a "nose-only" type vertical flow inhalation chamber. Each rat was placed into each of plastic plethysmography boxes under unanesthetized condition. All animals were then exposed to silica aerosols for 1 hr a day, intermittently for 6 days. Silica aerosols were generated by passing compressed and filtered air through the silica samples contained in a fluidized bed dust generator (Model 3211, Nihon Kogaku, Japan). The air-dust mixtures entered the exposure chamber through the superiory located central entry part and were exhausted inferiorly. Air flow was monitored continuously by noting pressure differences from a minihelic pressure gauge. The time-weighted average concentration of silica particles in air was monitored by a digital dust indicator (Model p-5, Sibata Chemical, JAPAN) and also determined as 120-150 mg/m³ for 1 hr-exposures by measuring gravimetric glass fiber filters on a cascade impactor. The aerodynamic mass median diameter (MMD) of particles within the generated cloud was determined as 4.4 µm with a standard deviation (σ) of 2.12 by an Andersen sampler (Model 3351, Nihon Kogaku, JAPAN). The respiratory volume of each animal during the 1 hr-exposures was calculated as 105-150 ml/min by plethysmography method (Kubota et al., 1984). The control animals were defined in the present study as non-exposed rats of the same age, housed in the same animal room under barrier conditions before use.

After inhalation exposures, both of the exposed and control animals were kept under barrier isolation for 6 months.

**Analysis of bronchoalveolar cells (BAC)**: The BAC were obtained from each rat by the previously described method (Kagan, Oghiso, and Hartmann, 1983). Briefly, 6 exposed and 2 control animals were exsanguinated under anesthesia with ketamine chloride (Ketalar®, Sankyo, JAPAN) respectively at each month after the cessation of the exposures. Bronchoalveolar lavage was then performed for each animal by the repeated instillation of 10 ml aliquots of Ca²⁺-and Mg²⁺-free Hanks' balanced salt solution (HBSS; GIBCO, Grand Island, NY) prewarmed to 37°C in a water bath. After washing BAC with HBSS and resuspended in HBSS containing 10% fetal calf serum (GIBCO), total cell yields were counted by a hemocytometer. The viability was also determined by trypan blue exclusion tests (Flow Laboratory, McLean, VA). The proportions of cell populations of BAC were examined by a differential cell count of smear preparations stained with Giemsa. The proportions of silica-laden alveolar macrophages (AM) in BAC were also counted under a phase-contrast light microscopy.

**Histological examinations of lungs and TBLN**: At each month after the cessation of the exposures, the lungs from 6 exposed and 1 control animals were respectively inflation-fixed in situ, by the intratracheal instillation of approximately 6 ml of Bouin's fixative after animals were exsanguinated (Oghiso, Kagan, and Brody, 1984). The fixative was introduced at a pressure of 20 cm through a tracheal cannula without opening the chest. Lung tissues including TBLN were then removed from the chest, and fixed in 70% ethanol for 6 hr. For histological examinations of the lungs, sagittal sections of each lobe (right anterior, right middle, right posterior, accessory and left
lobe) were dissected with sharp razor blades. TBLN were also detached carefully from tracheobronchial regions, and dissected into a few pieces. Tissue samples were all embedded in paraffin and routinely processed for histological analysis. Five-micron-thick sections were prepared, and stained with hematoxylin and eosin (H & E), Prussian blue and Masson's trichrome stains.

RESULTS

Changes of cell yields and populations in the BAC after silica-exposures.

BAC were obtained from animals at each month after the cessation of silica-exposures. The viability of BAC was consistent (90-95%) both in the silica-exposed and control animals during the 6 months. The total cell yields of BAC were not significantly different among the exposed and control animals, and not altered during the post-exposure period (Table 1). The proportions of alveolar macrophages (AM) and polymorphonuclear leukocytes (PMN) in BAC from the exposed animals were not different from the control animals, whereas the proportions of lymphocytes in BAC from the exposed animals were significantly higher than those from the control animals during the 4 months after the exposures (p<0.05 or 0.005).

The proportions of silica-laden AM in BAC from the exposed animals were altered during the post-exposure period (Fig. 1). At 1 month after the exposures, approximately 52.0% of AM, ranging 35-60%, were observed to phagocytose silica particles. The proportions were thereafter decreased, and finally reduced to 2.5-9.5% at 5 or 6 month after the exposures, indicating the clearance of particles from the alveolar levels.

Deposition of silica particles in the lungs and histopathologic features.

The follow-up histological examinations were performed on the lungs from the exposed and control animals during the post-exposure period. Silica particles were predominantly and persistently seen in the clusters of AM aggregated in alveoli even at 6 months after the exposures. Such aggregates of silica-laden AM were especially

<table>
<thead>
<tr>
<th>Post-Exposure Months (No. Tested)</th>
<th>Yield of BAC (×10⁴)</th>
<th>Proportion of Cell Populations (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AM</td>
<td>Lymphocytes</td>
</tr>
<tr>
<td>1 (n=9)</td>
<td>4.8±1.3</td>
<td>89.6±8.5</td>
</tr>
<tr>
<td>2 (n=9)</td>
<td>5.2±0.9</td>
<td>88.9±9.7</td>
</tr>
<tr>
<td>3 (n=9)</td>
<td>5.2±1.4</td>
<td>87.5±4.9</td>
</tr>
<tr>
<td>4 (n=6)</td>
<td>5.7±0.7</td>
<td>85.3±5.3</td>
</tr>
<tr>
<td>5 (n=6)</td>
<td>5.9±1.8</td>
<td>92.3±4.1</td>
</tr>
<tr>
<td>6 (n=6)</td>
<td>4.2±0.9</td>
<td>92.9±3.0</td>
</tr>
<tr>
<td>Control (n=15)</td>
<td>4.7±1.4</td>
<td>94.5±3.9</td>
</tr>
</tbody>
</table>

*: p<0.05, compared to lymphocyte proportions of the control.

**: p<0.005, compared to lymphocyte proportions of control.
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![Graph showing proportional changes of silica-laden AM in BAE from silica-exposed rats.](image)

**Fig. 1** Proportional changes of silica-laden AM in BAE from silica-exposed rats. Percentage of the proportion from each animal is plotted monthly during a period of 6 months after the cessation of the exposures.

distributed in the subpleural alveolar sacs and also in the alveoli close to the alveolar duct bifurcations (Photo. 1). Alveolar duct bifurcations were thickened with increase of cellular components, although particles were not observed (Photo. 2). Associated with accumulations of particle-laden AM, a number of lymphocytes and scattered PMN were observed in the alveoli, especially of subpleural areas (Photo. 3). Some macrophages contained hemosiderin granules in these alveoli. In addition to these cellular infiltrates, foamy cells were often seen in subpleural alveolar sacs (Photo. 4). These cells were also observed in the lungs from the control animals, although their incidence was much lower (17% of total control rats) than the exposed animals (74% of total exposed rats). In some exposed rats, subpleural alveolar sacs were filled with acidophilic proteinous granules, cholesterol clefts and cellular debris as well as foamy cells (Photo. 5).

Around the small blood vessels of the alveolar septum, prominent infiltrations of lymphocytes with scattered PMN or histiocytes, were present, although silica particles were not identified (Photo. 6). This perivascular cuffing was seen in about 60% of the exposed animals, whereas it was not observed in the control animals. At later months after the exposures, perivascular infiltrates sometimes contained fibroblasts, and thickening of alveolar interstitium with increased fibroblasts and collagen was prominent (Photo. 7). Such interstitial thickening was observed in smaller areas of about 56% of
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the exposed animals, whereas it was not seen in the control animals.

Although silica particles were not identified in the alveolar interstitium, they were occasionally observed in the peribronchial lymphoid tissues (PBLT), in which granulomatosus nodules, composed of silica-laden macrophages and fibroblasts, were developed (Photo. 8). PBLT were often hyperplastic in the exposed animals, compared to the control animals.

Translocation of silica particles into TBLN and histopathologic features.

The size of TBLN from the exposed animals was not grossly altered, compared to the control animals, throughout the post-exposure period.

Interestingly, granulomatous nodules similar to those seen in PBLT of the lungs were noted in TBLN from about 70% of the exposed animals, which were sacrificed at 2 months or later after the exposures (Photo. 9A). None of the TBLN from the control animals had such granulomatous nodules. These nodules were distributed in the para-cortical and medullary areas, and were composed of macrophages, fibroblasts, and thin collagen. Silica particles were prominently identified in macrophages of these nodules (Photo. 9B). No noticeable histopathologic changes, like lymphoid hyperplasia, were, however, observed.

Photo. 1 Alveolar deposition of silica particles in the rat lungs. (A). Lung from a rat 5 months after the exposures. Particle-laden AM aggregates are seen in the subpleural alveolar sacs. (H & E, ×200). (B). Lung from a rat 3 months after the exposures. Particle-laden AM are seen to accumulate in the alveoli close to the alveolar duct bifurcation. (H & E, ×200).
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Photo. 2 Lung from a rat 4 months after the exposures. Note the thickened alveolar duct bifurcation with increased cellular components. (H & E, ×100).

Photo. 3 Lung from a rat 2 months after the exposures. A number of lymphocytes and PMN are observed in the subpleural alveoli. (H & E, ×100).
Photo. 4 Lung from a rat 2 months after the exposures. Foamy alveolar macrophages are present in the subpleural alveolar sacs. (H & E, ×200).

Photo. 5 Lung from a rat 6 months after the exposures. Note cellular debris, proteinous granules, foamy cells, and cholesterol clefts in the alveolar sacs. (HE, ×200).
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Photo. 6 Lung from a rat 3 months after the exposures. Perivascular cuffing of a number of lymphocytes is seen around the blood vessels in the alveolar interstitium. (H & E, ×100).

Photo. 7 Lung from a rat 5 months after the exposures. Alveolar interstitium is thickened with increase of fibroblasts and collagen as well as lymphocyte infiltration. (H & E, ×200).
Photo 8   Lung from a rat 4 months after the exposures. Granulomatous nodules are present in PBLT around the pulmonary bronchial tissue. (H & E, ×100).

Photo 9   particle deposition and lesions in TBLN from the exposed rats. (A). TBLN from a rat 6 months after the exposures. A number of granulomatous nodules are present in the paracortical and medullary areas. (H & E, ×100). (B). TBLN from another rat 6 months after the exposures. Note the particle deposition in the macrophages of a granulomatous nodule. (H & E, ×400).
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DISCUSSION

Despite a numerous studies on pulmonary disorders by fibrogenic silica or asbestos, the relationship between the pathogenicity of these dust particles and their deposition patterns is not fully elucidated. On asbestos, some studies have shown differences in the clearance and retention patterns of inhaled amphiboles and chrysotile asbestos (Morgan, Evans, and Holmes, 1977; Middleton, Beckett and Davis, 1977). One of the authors have also implicated the different distribution patterns and pathologic features between crocidolite and chrysotile asbestos, even though both of them are known to be equally fibrogenic (Oghiso, Kagan, and Brody, 1984). To our knowledge, information on the deposition patterns of silica particles is only that initial deposition occurs at the level of alveolar duct bifurcations, associated with accumulations of pulmonary macrophages (Brody et al., 1982; Brody and Roe, 1983).

The present study revealed histologically pulmonary deposition of silica particles and their translocation into lymphatic tissues during a longer period (6 months) after short-term exposures. Thus, silica particles were predominantly and persistently identified in AM aggregated in the alveoli up to 6 months after the exposures. Bronchoalveolar lavage analysis of the exposed animals, however, revealed the gradual decrease in silica-laden AM during the 6 months, although the total cell yields as well as AM proportions in BAC were not altered. It is, therefore, suggested that the clearance and translocation of silica particles could occur after the exposures, while a smaller amount of particles can be retained in AM for a longer period (Lippman, Yeates, and Albert, 1980). While the present histological analysis of the lungs could not identify the interstitial localization of silica particles, particles were noted in peribronchial lymphoid tissues (PBLT), indicating the intrapulmonary translocation from the airways during the 6 months after the exposures. Particle deposition in PBLT has been observed on silica (Heppleston, 1963; Reiser et al., 1983) and on asbestos (Lee et al., 1983; Oghiso and Kagan, unpublished). Although the present results did not make clear transmigration process of particles into PBLT through the lymphatic or blood vessels as implicated by Lee et al. (1983), it is an important finding that silica particles were also identified in the regional lymph nodes, TBLN. Thus, it is indicated that free particles or particle-laden macrophages may penetrate into the interstitial lymphatic circulation and transmigrate into the lymphatic tissues (Lee et al., 1983).

Although a typical pulmonary fibrosis was not observed in the exposed animals during a period of 6 months in the present experiments, some characteristic histopathologic features were evident in the lungs and TBLN. Associated with accumulation of particle-laden AM in the alveoli, a number of lymphocytes were observed in the alveolar sacs. Perivascular cuffing of lymphocytes was also prominent, although interstitial localization of particles was not clearly seen. These findings can explain the significant increase of lymphocyte proportions in lavaged BAC from the exposed animals. The alveolar sacs sometimes contained foamy cells, proteinous granules, and
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cholesterol clefts. The foamy alveolar cells have also been documented to appear in the lungs after treated with a variety of inhalants (Innes et al., 1955; Svendsen, 1977). It has also been described that silica-instillation in the rat causes lipoproteinosis characterized by proteins and cholesterol clefts (Reiser et al., 1983). These findings may reflect a similar effect of inhaled silica particles.

One of the characteristic features was thickening of alveolar duct bifurcations with cellular increase. This has also been noted both in the asbestos-inhalations (Brody et al., 1981; Oghiso, Kagan, and Brody, 1984) and in the silica-inhalations (Brody and Roe, 1983), and therefore might reflect the immediate cellular responses at these sites after the initial particle deposition (Brody et al., 1982).

Alveolar interstitium was also thickened with the increase of fibroblasts and collagen at later months after the exposures. These features, however, appeared less severe than the reported experimental silicosis which have been performed by a direct intratracheal instillation (Reiser et al., 1983; Lugano, Dauber, and Daniele, 1982). Because the present experiments were undertaken by short term inhalation exposure regimen, the differences in severity of interstitial pathologic features may be attributable to a variety of administration method and dose used.

It is of interest that granulomatous nodules associated with particle deposition in macrophages, were noted both in PBLT and TBLN, as described also by Heppleston (1963) and Reiser et al. (1983). These findings implicate strongly not only the translocation of silica particles via lymph but also their fibrogenic effects on lymphatic tissues.

The present study has shown that even short-term exposures by our inhalation regimen can evoke histopathologic changes in pulmonary and lymphatic tissues, associated with particle deposition. It is, however, unknown whether or not the pathogenicity of inhalation of dust particles may be attributable directly to deposited particles, because some investigations have implicated other biologic mechanisms, such as immunologic derangements, in silicosis or asbestosis (Reiser and Last, 1979; Pernis and Vigliani, 1982). Fibrotic process and granulomatous reaction both in the lungs and TBLN may be related to such immunological mechanism (Kagan, Oghiso, and Hartmann, 1983). Further studies should be needed on the mechanism affecting pulmonary disorders by inhaled particles.

REFERENCES

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