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Short Term Effect of Silicon Carbide Whisker to the Rat Lung

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Abstract: We studied the short-term effect of silicon carbide whisker (SiCW) *in vivo* by instillation and inhalation to the rat lung. SiCW was instilled low dose (2 mg/0.5 ml saline) or high dose (10 mg/0.5 ml) intratracheally into the lungs of 25 rats. SiCW was also inhaled to another 25 rats at the average concentration of 10.4 mg/m^3 for 1 month. In instillation study, the lung had focal alveolitis with the destruction of alveolar wall especially at 3 days after the instillation, and the lesion remained as an aggregated foci of SiCW at 6 months. The 'inflammation-score' of the instilled group by point counting method of the specimen correspondingly decreased gradually. In inhalation group, a minimum inflammatory change was observed. Collagen deposition in the aggregated foci of SiCW with accumulated alveolar macrophages and neutrophils was not progressive during the observed period. These findings suggest that SiCW may cause a minor effect to the rat lung in 6 months after exposure.

Key words: Silicon carbide whisker, Lung pathology, Inhalation, Instillation

Introduction

Occupational and environmental exposure to asbestos is thought to produce pulmonary fibrosis and lung cancers. Various types of man-made mineral fibers (MMMF) have been developed as substitutes for asbestos, and the demand for these products is increasing. Some of these fibers are thought to possess the same adverse biological effects as asbestos because of similar physicochemical properties. Silicon Carbide whisker (SiCW) is produced by the heating of rice hulls in a reducing atmosphere. Although isogeometric SiC was generally considered no worse than a "nuisance dust", evidences appeared that workers exposed during the manufacturing process might suffer chronic lung damage[1–3]. The advent of controlled SiCW manufacture and industrial use is much more recent and no epidemiological information is yet available in the literature.

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In this study, we examined the short-term effect of SiCW *in vivo* by instillation and inhalation to the rat lung.

Material and Methods

Fiber preparation

Silicon carbide whisker used in this study was Toka Whisker (Tokai Carbon co., Tokyo Japan). The mean fiber length of the bulk material was approximately 5.1 μm (SD 2.3) and the mean width was 0.3 μm (SD 1.5).

Animals

Male Wistar rats (9 weeks old at the start of exposure) were used in this study. The rats were fed and housed under conventional laboratory conditions. All of the experimental procedures were approved by the Experimentation Committee of the University of Occupational and Environmental Health, Japan.

Intratracheal instillation

SiCW was suspended in saline solution. Low dose (2 mg/0.5 ml saline) or high dose (10 mg/0.5 ml) of the suspension were injected intratracheally into the lungs of 25 rats. Five rats were sacrificed at the time of 3 days, 1 week, 1 month, 3 months and 6 months after instillation. For the control group, only saline solution was injected and sacrificed in same manners as exposure group.

Inhalation study

The exposure system and the experimental procedure have been described elsewhere[4, 5]. Exposure concentration in the chamber was monitored continuously by a light-scanning method (Dust Monitor AP-632, Shibata Sci. Tech. Japan). The mass concentration of the SiCW was measured gravimetrically each day by a glass fiber filter. The average exposure concentration was controlled at 10.4 mg/m^3 during the exposure period. The mass median aerodynamic diameter (MMAD) of SiCW in the exposure chamber was 2.5 μm, measured by using an Andersen cascade impactor (AN-200 Sibata Sci. Tech. Japan). The rats were randomly allocated to control and exposed groups. Twenty-five rats in the test groups were exposed to SiCW for 6 hours per day, 5 days per week by inhalation. The rats were sacrificed at 3 days, 2 week, 1 month, 3 months and 6 months at 1 month after the start of exposure. Twenty-five control rats were exposed clean air in identical, adjacent chambers under similar conditions. The controls were similarly sacrificed at the same time.

Pathological procedures

At each sacrifice time, the lung was inflated and fixed by intratracheal infusion with 10% buffered formalin at 25 cmH_2O pressure for one night. The left lung was sectioned into 3 blocks and embedded in paraffin. The paraffin sections of 3 μm thickness were stained with Hematoxylin-Eosin and van Gieson.

Morphological procedure

The examined images were digitally captured randomly from each specimen for at least 6 portions by CCD camera (DXC-950, Sony Co., Tokyo, Japan) under the light microscopy at the magnification of $\times$ 100. Inflammatory area in each image was examined by the point counting method. Briefly, the transparent sheet with a grid of 300 (15×20) points was put on the image, then the numbers of interesting points, that fall on the accumulated SiCW fibers or inflammatory cells of the alveolar wall, alveolar duct, and respiratory bronchiole other than blood vessels and bronchi, were determined. We categorized these results of point-counting into four groups and set the score as the rate of the lesion; score 0: no inflammation, score 1: less than 5% points of inflammation, score 2: 5% to 20%, score 3: 20% to 50%, score 4: more than 50% (Table 1).

Table 1. Inflammation score

Score	Inflammatory area* in captured image	Category
0	No inflammation (0%)	none
1	Less than 5%	slight
2	5% to 20%	mild
3	20% to 50%	moderate
4	More than 50%	severe

*Inflammatory area was determined by point counting method (see details in Material and methods). The numbers of interesting points, that fall on the inflammatory area in the alveolar wall, alveolar duct, respiratory bronchiole other than blood vessels and bronchi, were determined. Data was expressed as the ratio against the total points (300 pts.) on the captured image.

To evaluate the collagen deposition, the images of van Gieson-stained specimens were also captured digitally in the same manners as HE section. Point counting method was also performed for the collagen (red area), parenchyma, and the air apace. The ratio of collagen count for each rat was expressed by the division:

$$\frac{\text{Count of collagen (red) points in parenchyma}}{\text{Total points in the lung parenchyma}}$$

Statistics

All the data was expressed as mean value ± SEM. The comparison between two categories was evaluated by two-way repeated-measures ANOVA. P-value was examined by Fisher's PLSD or Scheffe's F-test. Statistical significance was defined as a value of $p<0.01$.

Results

Pathological findings

In inhalation study, the rats that received SiCW showed sparse foci of thickened alveolar walls throughout the 1-month exposure and 6-month recovery periods. Most of the alveolar walls enclosing fiber-laden alveolar macrophage (AM) maintained normal structure during the observed period (Fig. 1a-d).

Figure 2a to 2d showed the pathological features of 2 mg-instilled rat lung. The foci of aggregated SiCW and fiber-laden AMs covered with thin fibrous sheath scattered in the parenchymal tissue of the terminal bronchiole or

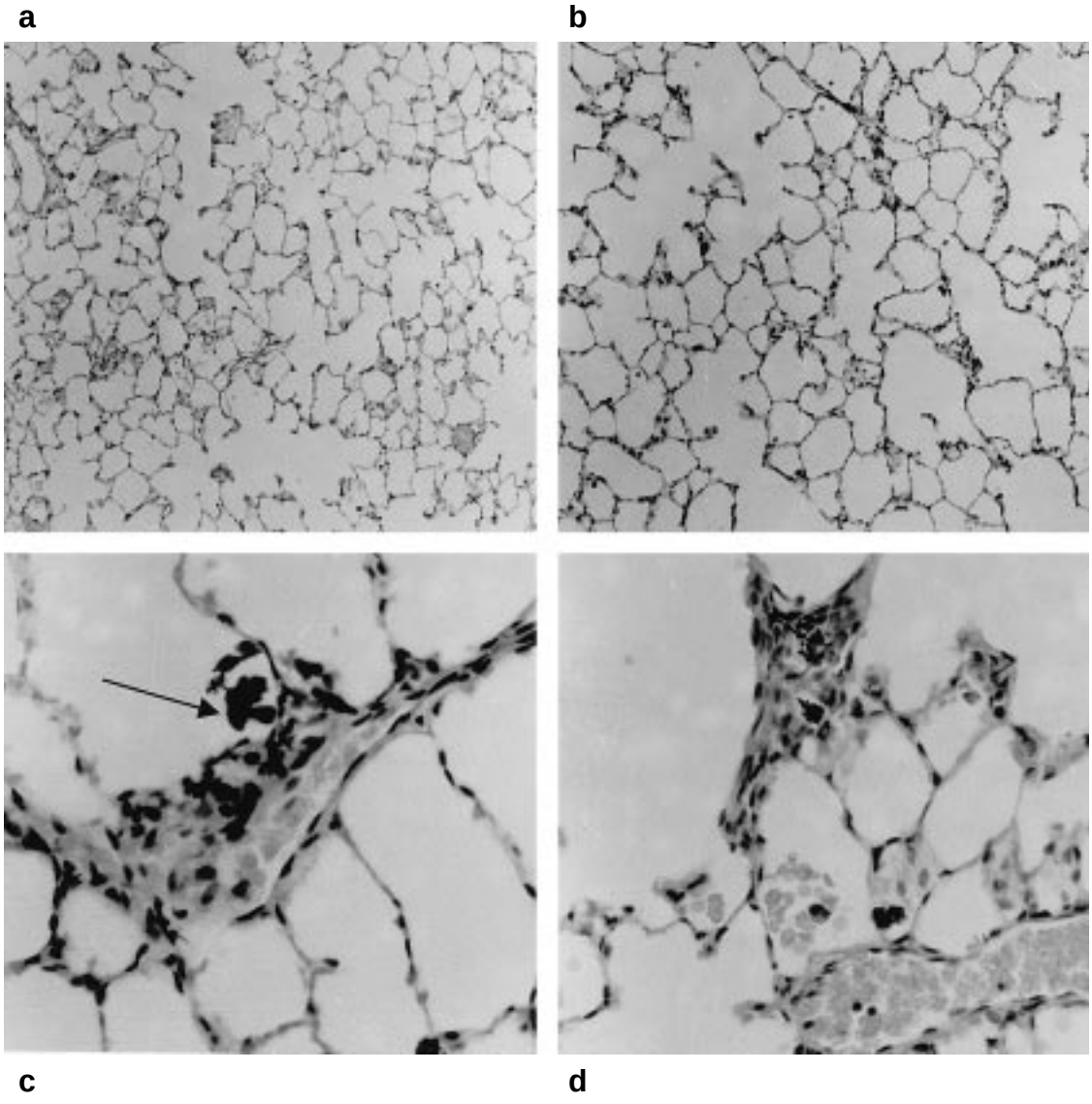

Fig. 1. Lung tissue after 1 month inhalation of SiCW.
a) 3 days after the termination of 1 month inhalation. b) 6 months after the termination of 1 month inhalation. × 100, H&E stain. c) higher magnification of a), d) higher magnification of b), × 400, H&E stain. A sparse foci of thickening alveolar walls are observed throughout the 1-month exposure and 6-month recovery periods. Most of the alveolar walls enclosing fiber-laden AMs (arrow) maintained normal structure during the observed period.

alveolar duct region. However, these lesions occupied a small area of the lung specimen, and most of the alveoli were relatively free of fiber deposition and aggregation. Solitary SiCW fibers were observed in the interstitium as well as free in the alveolar spaces. Figure 3a to 3d showed the pathological features of 10mg-instilled rat lung. Focal alveolitis with destructed alveolar wall were prominent especially in the alveolar duct region at 3 days. In the foci, fiber-laden AM, lymphocytes, and polymorphonuclear leukocytes (PMN) infiltrated and aggregated. Slight amounts of collagenous material occasionally deposited in some of the thickened alveolar ducts and adjoining alveoli together with the aggregates of AM. These aggregated foci were not recognized to be adenomatous hyperplasia. None of the groups showed any pleural changes (i.e., thickening of pleural lining, fibrosis, proliferation of mesothelium, etc.) compared with the controls.

The changes of inflammation and 'inflammation score'

The inflammation such as focal alveolitis was remarkable

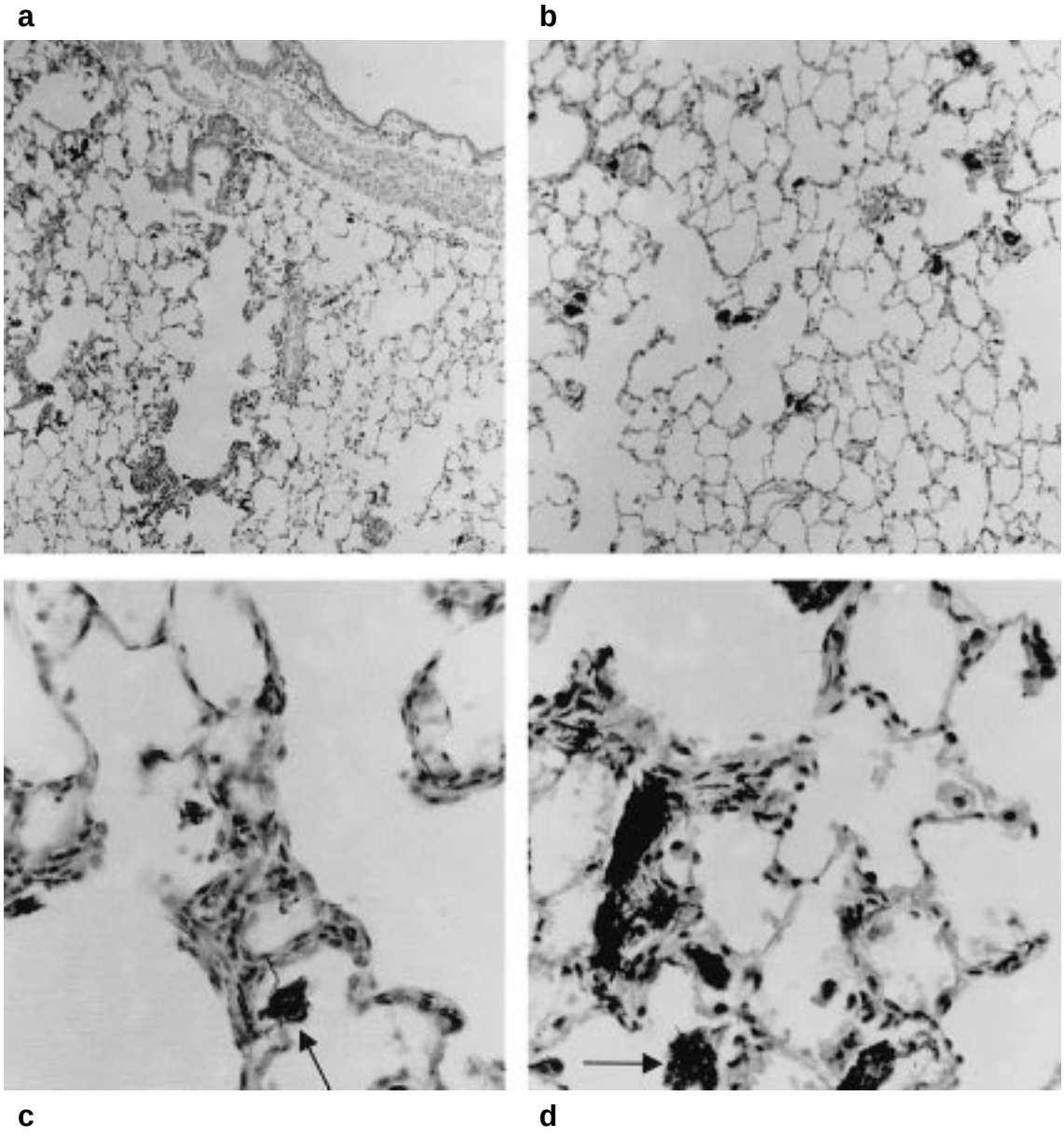

Fig. 2. Lung tissue after instillation of 2 mg SiCW.
a) 3 days after instillation of 2 mg SiCW. b) 6 months after instillation of SiCW, × 100, H&E stain. c) higher magnification of a). Alveoli were relatively free of fiber deposition and contained a few fiber-laden AMs (arrow). d) higher magnification of b), × 400, H&E stain. Fiber-laden AM (arrow) accumulated in the slightly thickened alveolar ducts and adjoining alveoli.

at 3 days by the instillation, but was not so progressive at 6 months. As the AM and PMN became fewer in the aggregated foci, the inflammatory area decreased both in the 2mg-instilled lung and the 10 mg-instilled lung (Fig. 2c, 2d, Fig. 3c, 3d).

'Inflammation score' by point counting of the images showed that, the lesions of the 10 mg-instilled group scored maximum at the time of 3 days after instillation, then, gradually decreased to around the score of 1 after 3 months (Fig. 4). The 10 mg-instilled group scored higher than other groups within 6 months. The inhalation group scored less than the score of 1 during the observed period.

Collagen deposition of instilled group

The 10 mg-instilled group showed a significant higher collagen deposition than 2 mg or control group (Fig. 5). The collagen deposition of 2 mg-instilled group showed similar pattern as that of the control group during the observed period.

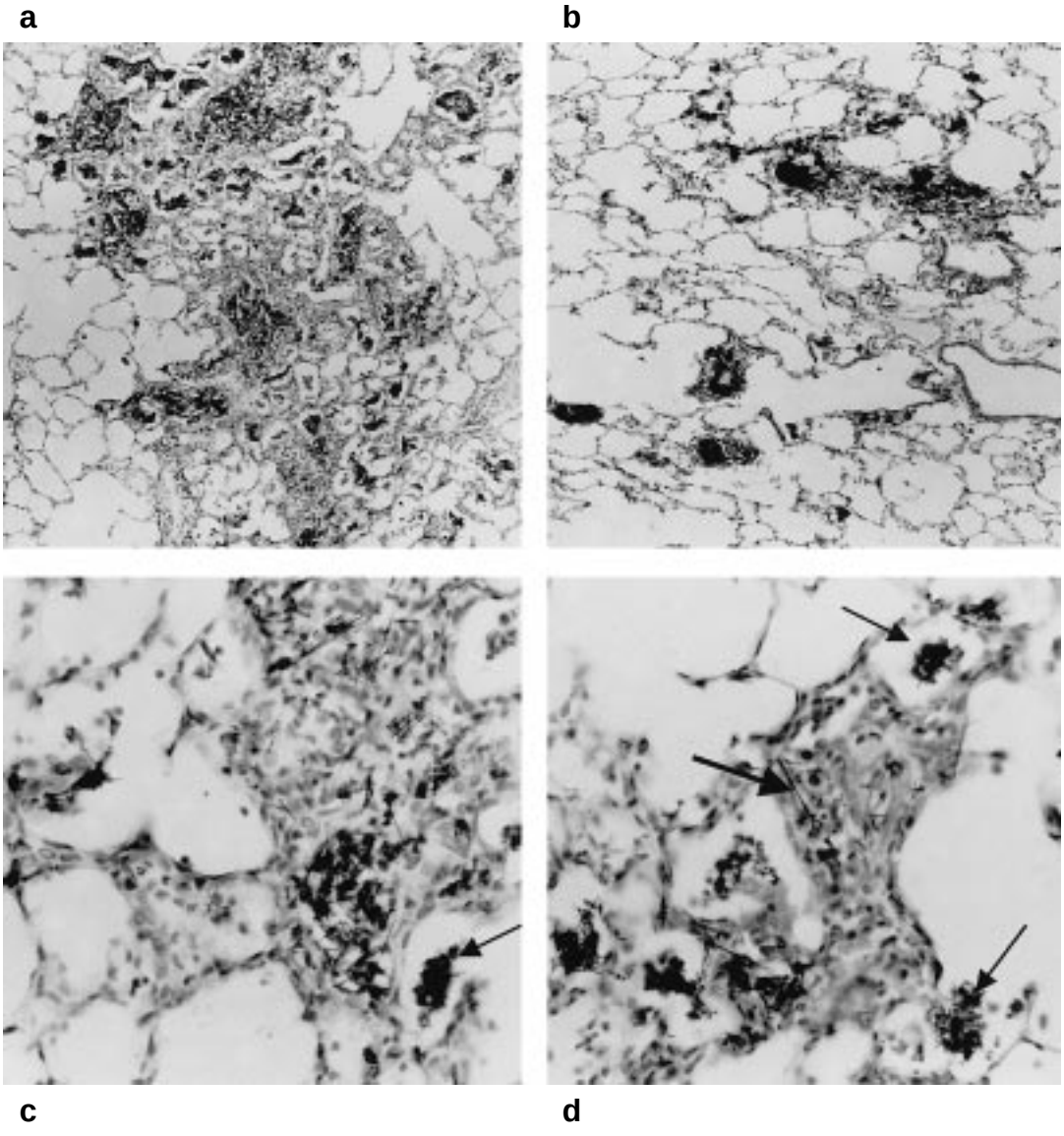

Fig. 3. Lung tissue after instillation of 10 mg SiCW.
a) 3 days after instillation of 10 mg SiCW, focal alveolitis with destructed alveolar wall is prominent especially in the alveolar duct region. b) 6 months after instillation of 10 mg SiCW, × 100, H&E stain. The foci of aggregated SiCW and fiber-laden AMs covered with thin fibrous sheath remained. c) higher magnification of a), the aggregated foci consists of fiber-laden AMs (arrow) or other inflammatory cell infiltrates. d) higher magnification of b), × 400, H&E stain. Solitary SiCW (bold arrow) is distributed in the interstitium, and some fiber-laden AMs (arrow) locate in the alveolar spaces. A small amount of collagenous material occasionally deposit in the thickened alveoli together with the aggregates of AM.

Discussion

The current study showed the short-term effect of SiCW by instillation or inhalation to the rat lung. By instillation of 2 mg or 10 mg of SiCW, inflammatory response such as focal alveolitis, infiltration of PMN and lymphocytes were remarkably observed maximum at 3 days. Although the aggregation of SiCW which was distributed in the parenchymal region remained even at 6 months after instillation, this lesion was not so progressive and the rest of the alveolar wall resembled as normal. These data suggest that SiCW may cause a minor effect to the rat lung in short-term.

The substance Silicon carbide dust is deposited practically inert in the lung from the results *in vivo*, and considered as inert from the experiments *in vitro*[6, 7]. However, SiCW with

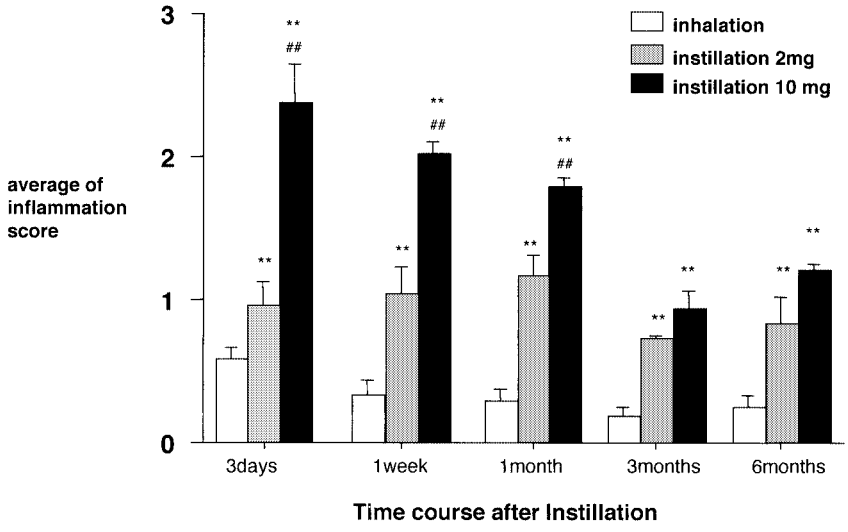

Fig. 4. Inflammation score
Inflammation score was determined as described in Table 1. **p<0.01 vs control. ##p<0.01 vs SiCW 2 mg-instillation.

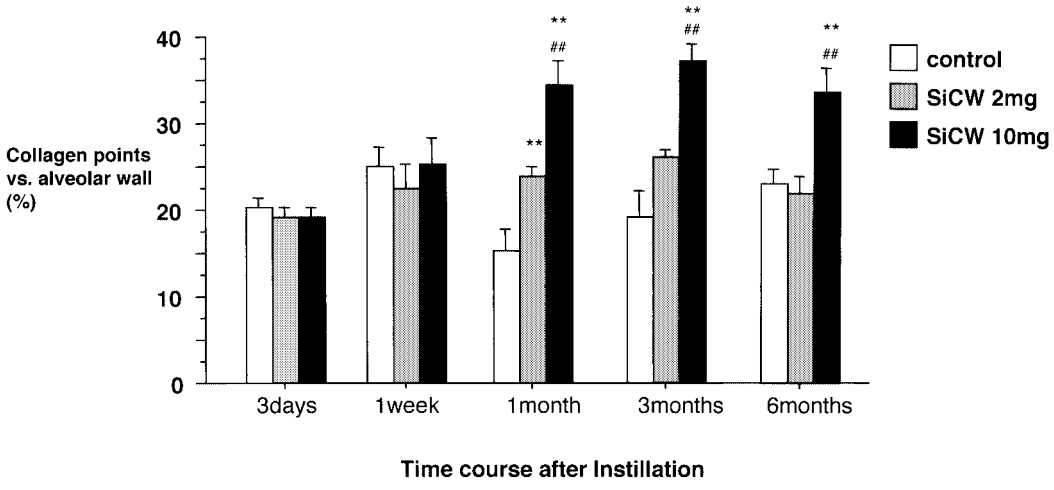

Fig. 5. Deposition changes of collagen by instillation of SiCW.
To evaluate the amount of collagen deposition, van Gieson-stained specimens of instillation group were also captured as digital image in the same manners as HE section. Point counting method was also used and performed (see Material and Methods) for the collagen (red area), parenchyma, and the air apace. The ratio of collagen point for each rat was expressed by the division:

$$\frac{\text{Count of collagen (red) points in parenchyma}}{\text{Total points in the lung parenchyma}}$$

**p<0.01 vs control. ## p<0.01 vs SiCW 2 mg.

long length and high aspect ratio has a toxic effect. Vaughan *et al.* reported that the most common findings in histological preparations of tissues taken from animals 18 months after instillation to SiCW was a high incidence of multiple pulmonary granulomas which occasionally occluded airways, and the lesions caused by it were similar to those found with crocidolite[8]. Few studies have been reported about the toxicity of SiCW *in vivo*. Lapin *et al.* reported that, after 13 week inhalation of SiCW, histopathological changes including mobilization of alveolar macrophages, thickening of the alveolar wall and pleura lining, alveolitis, and mild focal fibrosis of pleura were observed in the lungs of treated

animals associating with the deposited fibers[9]. *In vitro* studies, SiCWs with longer length and higher aspect ratio than Toka whisker showed a high DNA breaking potential and H_2O_2 formation[10].

However, there are some points to be considered in comparing with other studies. First, the material used in this study has a different dimension from that used in others. Mean length of bulk Toka Whisker used in this study was 5.1 μm. In Vaughan's study, however, those of SiCW-1 and SiCW-2 were 18.1 μm and 19.0 μm respectively. As Stanton and Leyard concluded that the carcinogenecity of fibriform materials relates more with a function of dimension and durability than with a physicochemical nature, the importance of the dimension of the fiber has been well-documented[11, 12]. Thus, SiCW with shorter fiber length in this study may cause only a mild biological effect. Second, the exposed dose may be lower comparing with other studies especially in inhalation study. The residual amount of SiCW by inhalation in the current study was 0.60 mg, and much less inflammatory changes were observed than those by instillation. Our preliminary data showed that the half-clearance time ($t_{1/2}$) of SiCW by inhalation is estimated about 3.6 months (data not shown). In this experimental design of inhalation, most of the fibers may be cleared from the lung during the observed period, and resulted in the minimum effect to the lung.

Another question arises whether the observed period in the current study may be rather short to show the development of fibrosis or neoplasm. In the experimental model of asbestosis, rats exposed to the high crocidolite concentrations by inhalation for 20 days exhibited inflammation and focal fibrotic lesions with a slight thickening of alveolar septa[13]. Other study using mouse showed that at four weeks after the instillation of long crocidelite, collagen levels were significantly increased and fibrosis was seen in alveolar walls[14]. Thus, 6 months may be not short time period to show the development of fibrosis or the fibrotic changes. In the current study of instillation, deposition of collagen was not progressive in the aggregated foci at 6 months, and these foci were not recognized to be adenomatous hyperplasia. These findings show that SiCW is less fibrogenic than crocidelite within 6 months after instillation.

The pathological features of lung after the intratracheal instillation of MMMF differ with the type of fiber. Lemaire reported that, Fiberfrax (an aluminium silicate) caused significant granulomatous reactions and the appearance of early fibrosis in the rat lung at 1 month after intratracheal instillation (dose: 1, 5, and 10 mg)[15]. They also examined attapulgite and xonotlite (a calcium silicate), but they caused much less inflammatory reaction. The intratracheal instillation of potassium titanate whisker did not cause neoplasm or fibrosis in 6 months after single 2 mg or 10 mg injection to the rats (preliminary data, not shown). Adachi *et al.* reported that tumors (squamous cell carcinoma and neuroblastoma) were observed in hamsters that had intratracheally received magnesium sulfate fiber, metaphosphate fiber, calcium sulfate fiber, and fiberglass at 2 years after the first instillation[16]. However, they also stated that potassium titanate whisker did not cause tumors but inflammation and pleural thickening. Comparing with these MMMFs, the data in this study imply that the short-term effect of SiCW to the rat lung is less toxic.

The case of probable toxicity of SiCW must be built upon the results of tests in the several types of animal model systems and of tests using *in vitro* cell culture systems. Although low dose or a certain type of SiCW as used in this study may cause a minor effect to the rat lung in 6 months observation period, inhalation or instillation studies with longer term should be needed for the determination of a "no effect" level for workers handling with SiCW.

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