Industrial Health 2011, **49**, 47–55 **Original Article**

Safety Evaluation of Rock Wool after Nasal Inhalation in Rats

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Received August 11, 2009 and accepted May 6, 2010
Published online in J-STAGE September 1, 2010

Abstract: Asbestos is reported to cause pulmonary fibrosis, and its use has been banned. We examined the biopersistence and histopathological effect of rock wool (RW) fibers in rat lungs by a nose-only inhalation exposure study. Twenty-four rats were exposed to RW fibers for 6 h daily for 5 consecutive days. Six rats each were sacrificed shortly and 1, 2, and 4 wk after exposure, and the fiber numbers and sizes in lungs were determined. The fiber number in the lungs 4 wk after exposure significantly decreased. The clearance half time was 28 d for fibers with L>20 μm and 50 d for World Health Organization fibers. The reasons for the decrease in number and size of fibers include: 1) discharge outside of the body by mucociliary movement, 2) dissolution by body fluid, and 3) phagocytosis and digestion by alveolar macrophages. Twelve rats were used for histopathological examination, and the pathological changes were classified by Wagner's criteria. As a result, changes up to only Grade 2 were observed. The reason for the increase in macrophage number was considered to be a transient reaction of body defense. These results suggest that RW has low biopersistence and only a limited pathological effect.

Key words: Rock wool, Nose-only inhalation, Biopersistence, Histopathological effect, Wagner's criteria, Clearance half time

Introduction

Rock wool (RW), a kind of asbestos substitute that is classified as a synthetic vitreous fiber (SVF), excels in heat resistance, heat insulation, and sound absorption, and is used as a fire-resistant material, heat-insulation material, and sound-absorption material[1)].

At present, the International Agency for Research on Cancer classifies RW as Group 3: having limited evidence of carcinogenicity in experimental animals, and inadequate evidence of carcinogenicity in humans[2)]. The chemical composition of RW differs according to the type of RW, and RW with different chemical compositions is reported to have different effects on the respiratory system.

The fiber size and biopersistence of asbestos or SVFs have been shown in many previous epidemiological, physicochemical, and animal studies to be important factors in their adverse health effects, especially carcinogenicity. With regard to inhaled fibers, which were 5 μm or longer in length and 3 μm or shorter in width, these previous studies reported that the thinner and longer the fiber is, the more carcinogenic it becomes. In addition, regarding biopersistence, fibers that remain in the lung tissues for a long period of time without being dissolved or discharged to outside of the body are considered to be more carcinogenic[2)]. It is said that fibers with a length of 20 μm or longer having a long half-life tend to cause fibrosis or cancer because of their low dissolution in the living body[2, 3)].

Based on these findings, we have investigated the biopersistence of RW by conducting nasal inhalation exposure experiments in rats for 3 h daily for 5 consecutive days[4–6)]. This time, we conducted a nasal inhalation exposure experiment in rats for 6 h daily for

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5 consecutive days to further evaluate the safety of RW using the same RW material as that used in 2005, since experiments of 6 h are more common in Europe and the US[7].

At the same time, we investigated the pathological effects as well as the biopersistence of RW: number and change in length and width of fibers in the lungs.

Materials and Methods

The present study was performed in accordance with the Ethical Guidelines for Animal Experimentation adopted by the Institutional Review Board of Kitasato University School of Medicine (Approval No. 2004022).

Materials

As the analyte material, we used an RW sample manufactured by NT Co. Ltd. and provided by the Rock Wool Association, Japan. Fluorescent X-ray spectroscopy showed that the RW sample used in the study was chemically composed of 40% SiO_2, 37% CaO, 14% Al_2O_3, 6% MgO, 0.9% S, 0.6% MnO, 0.6% TiO_2 and 0.3% Fe_2O_3.

Originally, RW is present in the form of lumps of fibers of different sizes (length and width). Because the biological effect of fibers is known to vary depending on the size, the fiber size should be adjusted to obtain the maximum harmful effect. Therefore, we adjusted the size of RW fibers in accordance with the method by Kohyama *et al*[8]. As a result, the geometric mean length (geometric standard deviation, GSD) was 14.28 (2.25) μm, and the geometric mean width (GSD) was 1.76 (2.08) μm (Fig. 1). Then, to make it easier to generate RW fibers in the nose-only inhalation exposure study system, pulverized RW fibers were mixed with glass beads (BZ-02, AS ONE Corp.) at a weight ratio of 1 (RW fibers) to 39 (glass beads).

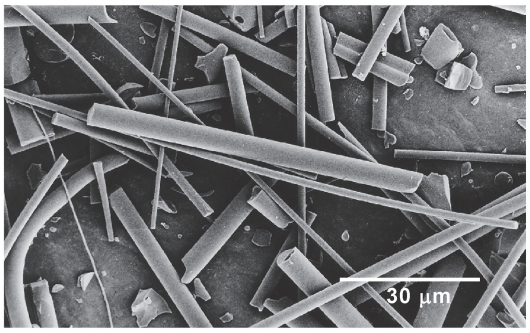

Fig. 1. Electron microscopic image of fiber before generation (× 1,000).
Geometric mean length and width (GSD) were 14.28 (2.25) μm and 1.76 (2.08) μm, respectively. (bar=30 μm).

Nose-only inhalation exposure study method

The materials prepared according to the procedure already mentioned were generated in the same way as we have reported[4–6].

Twelve male Fischer 344 rats (6 to 10 wk old) were used for each experiment, and the experiments were performed three times (24 rats for biopersistence study and 12 rats for pathological study, 36 rats in total). Twelve rats that were not exposed to RW fibers were used as a control group to evaluate the pathological effect. To acclimatize the rats to the environment of the laboratory, they were first housed in cages for about one week with free access to food and water. The temperature was kept at 22°C and humidity at 40%, with a continuous supply of fresh filtered air. The experiment was conducted by continuously exposing the rats to RW fibers for 6 h a day for 5 consecutive days, and the target fiber concentration was set at 100 fibers/cm^3 for fibers with L>20 μm.

During the exposure period, the fibers in the exposure chamber were collected in a plastic holder 5 times a day (every one hour after the start of the exposure experiment), using an electric suction pump (GilAir-5: Gilian, USA) at a suction speed of 500 ml/min, in order to confirm the fiber concentration. Air sampling was performed using a membrane filter (MF, pore diameter: 0.8 μm and diameter: 25 mm, Millipore Corp.) and a T60A20 filter (T60A20, diameter: 25 mm, Tokyo Dylec Corp.). Sample fibers were collected on MF for 1 min and on T60A20 for 10 min. The fiber concentration was confirmed by measuring the fiber number concentration (fibers/cm^3) and mass concentration (mg/m^3).

The body weights of the rats were measured once a week, and their appearance and condition were intermittently monitored for any change during and after the exposure period. Shortly after the exposure, 6 rats were sacrificed ("shortly-after group"). Six rats each were also sacrificed one week ("1-week-after group"), 2 wk ("2-weeks-after group"), and 4 wk ("4-weeks-after group") after the end of the exposure period.

Resection and processing of rat lungs

Rats were anesthetized by intraperitoneal administration of Nembutal (0.15 ml/100 g). Then, they were sacrificed by making a midline incision in the abdomen and bleeding from the abdominal aorta. The diaphragm was cut open and the lungs were resected. The resected lungs were weighed and stored at –80°C. Subsequently, the lung tissue was lyophilized for about two days and the weight was measured. The weight after lyophilization was regarded as the weight of dried lungs. The lyophilized lungs were ashed in a low-temperature plas-

ma asher (Plasma Asher LTA-102, Yanaco Corp.) for 24 h.

After ashing, a nuclepore filter (NF, pore diameter: 0.2 μm, Nomura Micro Science) was set to the suction filter, fibers were collected and allowed to dry, and photomicroscopy was performed using a scanning electron microscope.

Judgment criteria of fibers

A fiber is defined as one whose aspect ratio (ratio of length to width) is 3 or higher. Among them, fibers longer than 5 μm in length and shorter than 3 μm in width are called World Health Organization (WHO) fibers. In our study, the number and size (length and width) of fibers deposited in the lungs were measured using an electron microscope according to the measurement method stipulated by WHO (1985)[9]. The total fiber number, number of fibers by size (L≤5 μm, 5 μm < L ≤ 20 μm, L>20 μm), and WHO fiber number were counted.

Monitoring of fiber concentration in exposure chamber

After collection of fibers, the MF was put on a slide glass and exposed to acetone vapor using Quick Fix, making it transparent. These samples were used for measurement of fiber concentration (fibers/cm^3) in the chamber. After confirming that fibers were uniformly collected on the MF, using a phase-contrast microscope (BX41, Olympus Corp.) and WinRoof (image analysis software, Mitani Corp.) at low magnification (× 100), measurement was performed at × 400 magnification. Fibers were measured using a light microscope in accordance with the criteria in the "Guidebook for Working Environment Measurement I" (Japan Association for Working Environment Measurement, 2000)[10].

For measurement of mass concentration (mg/m^3) of fibers in the chamber, the weight of the filter (T60A20) before and after collection of fibers was weighed with an electronic balance, and mass concentration was calculated.

Measurement of fiber number and fiber sizes in rat lungs

The fibers in rat lungs were measured at × 2,000 – 20,000 magnification, and at least 400 fibers were counted in each rat. The fiber number obtained was converted to the fiber number per weight of dried lung. The number and size of fibers were measured using an electron microscope according to the measurement method stipulated by WHO (1985)[9].

Clearance half time

The clearance half time of fibers in rat lungs was calculated from the exponential approximation curve on the assumption that the geometric mean of fiber number in the lungs in the shortly-after group was 100%. In accordance with the description of Bernstein *et al*. and Hesterberg *et al*., a single exponential function was employed for calculation of clearance half time when the regression corresponds 80% or more of the variability (for fibers with 5 μm < L ≤ 20 μm, L>20 μm and WHO fibers), and a double exponential function was employed for other fiber sizes (L≤5 μm)[3, 7].

Statistical analysis

The geometric mean and standard deviation of the fiber number and fiber size (length and width) were calculated. One-way analysis of variance and multiple comparisons by Scheffe's test were performed.

Pathological evaluation

Rat lungs in the shortly-after, 1-week-, 2-weeks- and 4-weeks-after groups and those in the control group (3 rats each) were resected in the same way as already described. The lung samples obtained were processed by fixation in 10% formalin, alcohol dehydration, xylol substitution, paraffin embedding and hematoxylin-eosin staining. Lung slices were prepared by site, distinguishing the left and right lobes, and pathological examination of the hilar and peripheral lungs was performed by optical microscopy. To classify the pathological changes in tissues, we employed the histopathological criteria (Grade 1–8) of Wagner *et al*. used in a previous study[11]. The grading scale is shown in Table 1. In this system, Grade 1 is considered normal and Grades 2–3 are considered to be evidence of focal cellular change, while Grades 4–8 represent the former lesions plus increasing degrees of fibrosis. Further, we confirmed the activity of alveolar macrophages to phagocytose RW fibers, using transmission electron microscopy.

Table 1. Pathology grading scale

Grade		
		Cellular change
Normal	1	No lesion
Minimal	2	Macrophage response
Mild	3	Macrophage/bronchiolization
		Fibrosis
Minimal	4	Fibrosis restricted to terminal bronchioles/proximal alveoli
Mild	5	Interlobular linking
Moderate	6	Early consolidation
Severe	7	Marked fibrosis/consolidation
	8	Complete obstruction of airways

Note from McConnell *et al*. (1984)[11].

Table 2. Number of fibers in rat lungs

Observation period	Total fiber number	L≤5	5 < L ≤ 20	20<L	WHO fibers
Shortly-after group	58.94 (1.16)	24.40 (1.23)	29.16 (1.15)	5.13 (1.20)	34.30 (1.15)
1-week-after group	57.23 (1.14)	28.46 (1.22)	25.09 (1.10)	3.95 (1.16)	29.07 (1.10)
2-weeks-after group	54.55 (1.20)	25.31 (1.18)	25.51 (1.25)	3.52 (1.40)	29.07 (1.26)
4-weeks-after group	43.65 (1.10)[ab]	20.35 (1.14)[b]	20.58 (1.08)[a]	2.66 (1.18)[ab]	23.28 (1.07)[a]

Values are geometric mean × 10^5/lung (geometric standard deviation), [a]Comparison with shortly-after group (p<0.05), [b]Comparison with 1-week-after group (p<0.05), L=Length (μm), n=6, The total fiber number, fibers with 5 < L ≤ 20 μm, fibers with L>20 μm, and WHO fibers in the 4-weeks-after group significantly decreased (p<0.05). (Multiple comparison by Scheffe's test).

Results

Monitoring of fiber concentration in exposure chamber

In the biopersistence study, the arithmetic mean (standard deviation, SD) of the fiber concentration in the exposure chamber was 420.84 (103.08) fibers/cm^3, and 104.39 (31.93) fibers/cm^3 for fibers with L>20 μm. The weight concentration (SD) was 51.92 (14.12) mg/m^3. The geometric mean length (GSD) was 11.38 (2.02) μm, while the geometric mean width (GSD) was 1.34 (1.60) μm.

In the pathological evaluation study, the arithmetic mean (SD) of the fiber concentration in the exposure chamber was 345.88 (118.03) fibers/cm^3, and 83.17 (22.41) fibers/cm^3 for fibers with L>20 μm. The weight concentration (SD) was 42.64 (15.47) mg/m^3. The geometric mean length (GSD) was 10.60 (2.18) μm, while the geometric mean width (GSD) was 0.18 (1.65) μm.

Changes in fiber number in rat lungs

Table 2 and Fig. 2 show the time course changes in the number of RW fibers accumulated in the lungs and their proportions on the assumption that the geometric mean value shortly after exposure was 100%. The mean of the total fiber number, the number by size and the number of WHO fibers tended to decrease, from shortly after exposure to 4 wk after exposure, though an intermittent increase was noted. Fibers with L>20 μm showed a greater decrease than other sizes (Table 2). Multiple comparison by Scheffe's test showed that the total fiber number, fibers with 5 < L ≤ 20 μm, fibers with L>20 μm, and WHO fibers in the 4-weeks-after group significantly decreased from those in the shortly-after group (p<0.05) (Table 2).

Clearance half time

The clearance half time of fibers in rat lungs, calculated from the exponential approximation curve on the assumption that the geometric mean of the fiber number shortly after exposure was 100%, was 60 d for fibers with L≤5 μm, 56 d for fibers with 5 μm < L ≤ 20 μm, 28 d for fibers with L>20 μm, and 50 d for WHO fibers. The clearance half time of fibers with L>20 μm, which are identified as carcinogenic, specifically was shorter (Fig. 3)

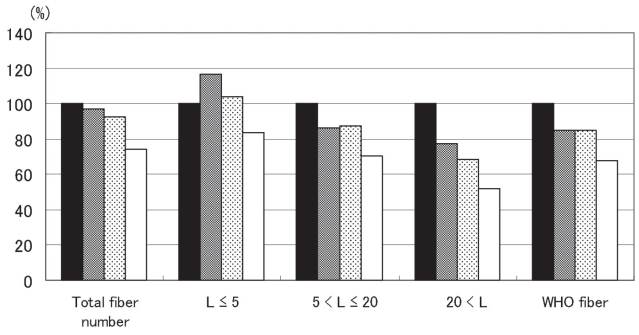

Fig. 2. Changes of fiber number in lungs.
Total fiber number, fibers with 5 < L ≤ 20 μm, fibers with L>20 μm, and WHO fibers in the 4-weeks-after group significantly decreased.
■ Shortly-after group
▨ 1-week-after group
⁝ 2-weeks-after group
□ 4-weeks-after group
Percentage when the value in the shortly-after group is assumed to be 100%.
n=6, L=Length of fiber (μm).

Changes in fiber size in lungs

Table 3 shows the time course changes in the length and width of fibers. In multiple comparisons by Scheffe's test, the geometric mean value of the length showed a significant decrease in the 4-weeks-after group compared to the shortly-after group, though an intermittent increase was noted (p<0.05). The geometric mean value of the width decreased from shortly after to 4 wk after exposure, and the rate of decrease was significant in the 4-weeks-after group compared to the shortly-after group (p<0.05). Figure 4A–D shows the fibers in ashed lung tissue from shortly after to 4 wk after exposure (scanning electron micrographs). These figures suggest that the fibers were dissolved, broken and folded in the lungs.

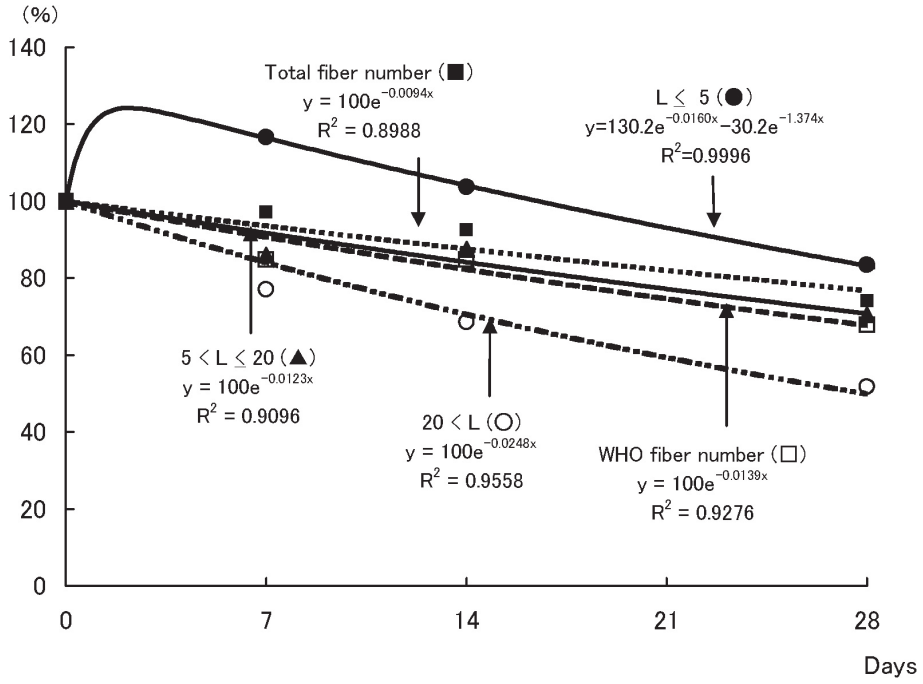

Fig. 3. Clearance of RW fibers from rat lungs.
The clearance half time of fibers with L>20 μm specifically decreased (28 d).
(%): Calculated assuming that the value in the shortly-after group is 100%.

Table 3. Changes in fiber size

Observation period	Length	Width
Shortly-after group	6.36 (2.47)	0.47 (1.66)
1-week-after group	5.25 (2.58)[a]	0.44 (1.65)[a]
2-weeks-after group	5.62 (2.50)[a]	0.43 (1.69)[a]
4-weeks-after group	5.64 (2.38)[a]	0.40 (1.78)[abc]

Values are geometric mean (geometric standard deviation) (μm), [a]Comparison with shortly-after group (p<0.05), [b]Comparison with 1-week-after group (p<0.05), [c]Comparison with 2-weeks-after group (p<0.05), The geometric mean values of the length and width significantly decreased from shortly after to 4 weeks after exposure (p<0.05).

Pathological evaluation

The lung architecture was appropriately retained throughout the observation period. However, an increase of macrophages in the alveolar space was observed from 1 wk after exposure (Fig. 5C), showing a peak increase at 2 wk after (Fig. 5D), and a decrease at 4 wk after exposure (Fig. 5E). Transient infiltration of neutrophils was observed in the shortly-after group (Fig. 5B), and granuloma formation was observed in the shortly-after, 1-, 2-, and 4-weeks-after groups (Fig. 5B–E). Pathological changes up to Grade 2 of Wagner's histopathological criteria were frequently observed in the lungs at 1, 2 and 4 wk after exposure (Table 4). Grade 1 was observed in the control group (Fig. 5A), the shortly-after group (Fig. 5B). Further, no difference in findings was observed between the left and right lobes, or between the hilar and peripheral lungs. No fibrotic change was observed in bronchi or bronchioles. Swelling was observed in the pulmonary interstitium from shortly after to 1 wk after exposure (Fig. 5B, C), suggesting that acute inflammation occurred in the pulmonary parenchyma and interstitium. No pathological change was observed in the pleura. Transmission electron micrographs showed phagocytosed RW fibers within the cytoplasm of macrophages (Fig. 6A, B).

Discussion

In the present study, we set the fiber concentration higher than that in our previous studies (3 h of exposure, target fiber concentration of 50 fibers/cm^3). The experiment was performed continuously for 6 h, at a fiber concentration of 100 fibers/cm^3 or more for fibers with L>20 μm. The experiment results showed that fibers were generated at the target fiber concentration. The observation period of this study was set at 4 wk: the reason was that we planned to establish a method to assess the pulmonary biopersistence of fibers, as a screening inhalation study, for a shorter period than that recommended in a previous study (at least 3 months)[7].

The total fiber number and fiber number by sizes (length) tended to decrease from the shortly-after group to the 4-weeks-after group. In preceding studies, where the same exposure method was used, the number of

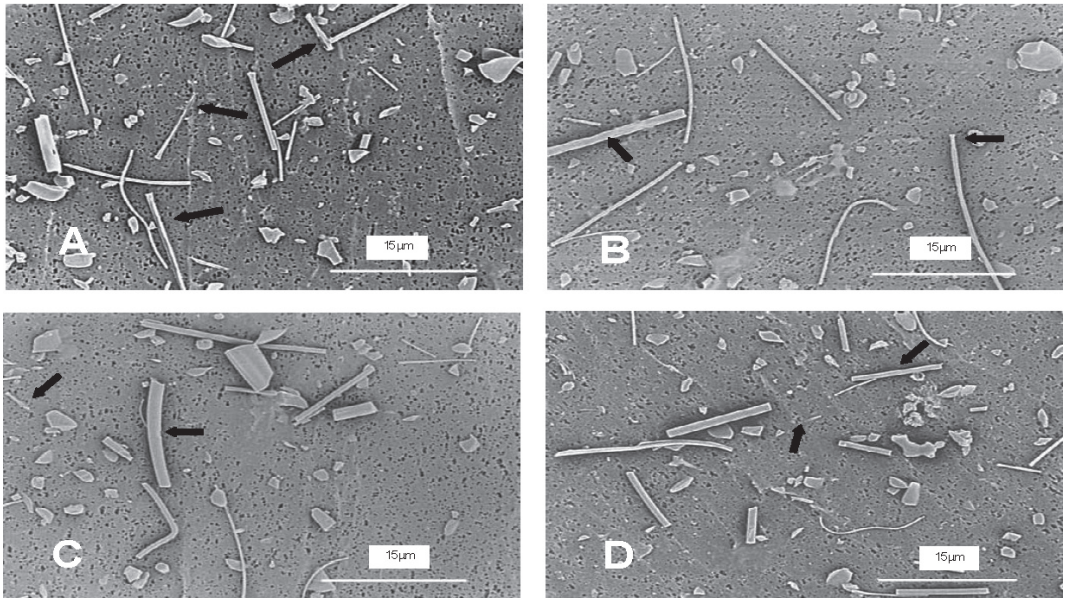

Fig. 4. Scanning electron microscopic images of fibers in ashed lung tissue (× 2,000).
Arrows show dissolved, broken and folded fibers.
A: Shortly-after group, B: 1 week-after group, C: 2-weeks-after group, D: 4-weeks-after group. (bar=15 μm)

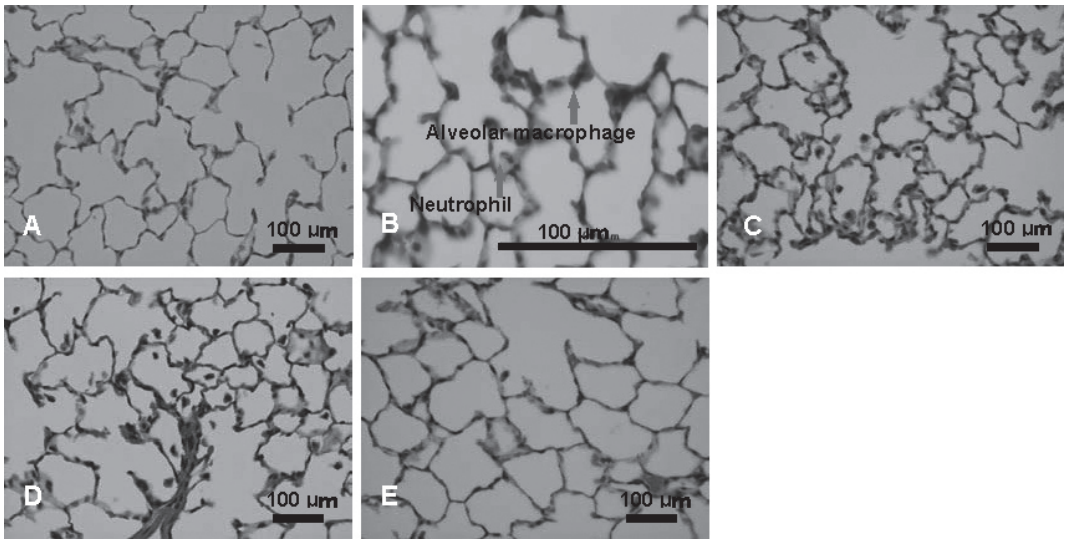

Fig. 5. Pathological change observed in hematoxylin-eosin-stained lungs by optical microscopy, by Wagner's histopathological criteria (× 200).
A: Control group (Grade 1); No pathological change, B: Shortly-after group (Grade 1); No pathological change, C: 1-week-after group (Grade 2); Increase of macrophages in the alveolar space, D: 2-weeks-after group (Grade 2); Increase of macrophages in the alveolar space, E: 4-weeks-after group (Grade 1); No pathological change. (bar=100 μm).

fibers of all sizes decreased by 30–50% after 30 d of exposure[12, 13].

The reason for the reduction in the number of fibers inhaled is as follows: fibers deposited in the bronchioles are transferred to the pharynx by mucociliary movement and discharged from the body[2, 3]. Fibers deposited in the alveoli were phagocytosed by alveolar macrophages (Fig. 5A, B), and then transferred to the bronchioles by mucociliary movement, and discharged from the body. Whether a fiber is phagocytosed or not depends on its length. Fibers with L≤20 μm were phagocytosed (Fig. 5A, B) and digested by alveolar macrophages[2, 3], while those with L>20 μm cannot be completely phagocytosed by alveolar macrophages. These fibers were dissolved by body fluid, folded transversely and crushed to be shortened in length and then phagocytosed and

Table 4. Histopathological findings classified by Wagner's criteria

	Control group			Shortly-after group			1-week-after group			2-weeks-after group			4-weeks-after group		
Lung sample	A	B	C	No. 4	No. 7	No. 11	No. 1	No. 5	No. 9	No. 3	No. 8	No. 12	No. 24	No. 6	No. 10
Right hilar lung	1	1	1	1	1	1	2	2	1	2	2	2	1	2	2
Right peripheral lung	1	1	1	1	1	1	1	1	2	2	1	1	1	2	2
Left hilar lung	1	1	1	1	1	1	2	1	2	2	2	2	1	1	2
Left peripheral lung	1	1	1	1	1	1	1	1	2	2	2	2	1	1	2
Pleura	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Pathological changes up to Grade 2 of Wagner's histopathological criteria were observed.

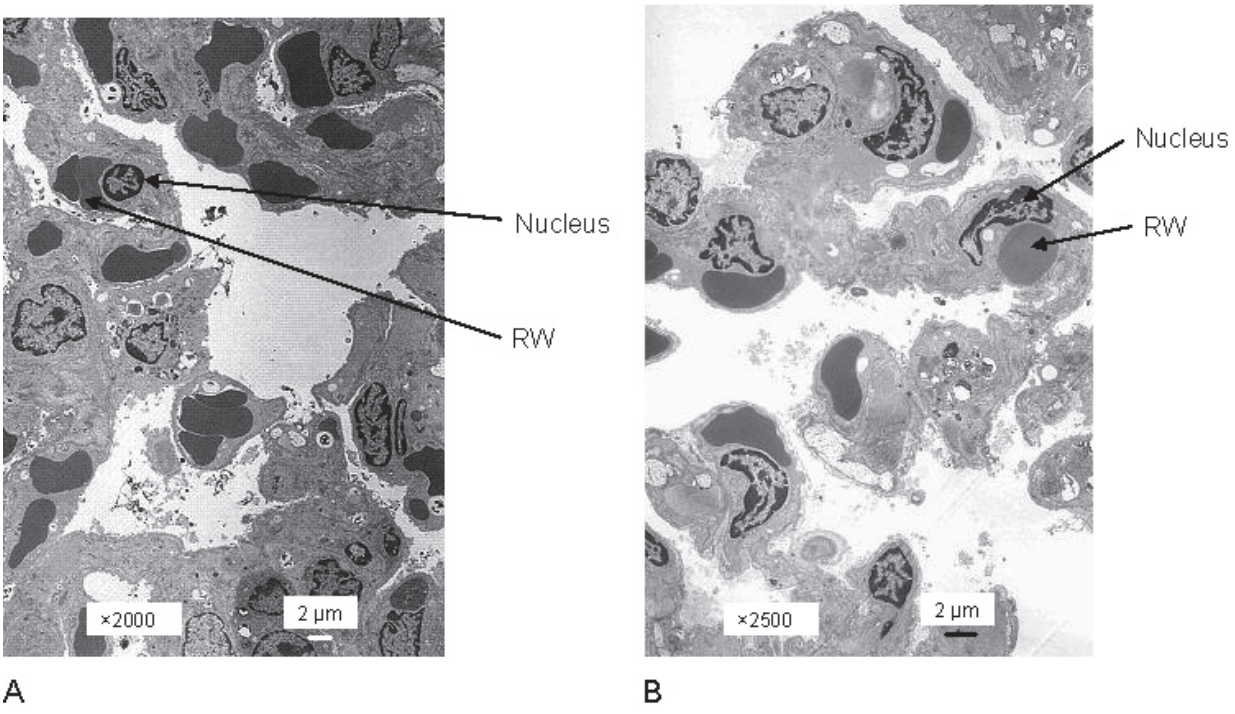

Fig. 6. Transmission electron microscopic images of alveolar macrophages. Alveolar macrophages that had phagocytosed RW fibers.
A: 2-weeks-after group (× 2,000), B: 4-weeks-after group (× 2,500), (bar=2 μm).

digested by alveolar macrophages, or taken into pulmonary epithelial cells and transferred to the pulmonary interstitium and local lymph nodes or pleura, thus being discharged from the body[2, 3]. The number of fibers with L≤5 μm increased in the 1-week-after group, and that of fibers with 5 μm < L ≤ 20 μm temporarily increased in the 2-weeks-after group compared to the 1-week-after group. A possible reason for this phenomenon is that fibers longer than 20 μm were dissolved by extracellular fluid and folded transversely, thus increasing the number of shorter fibers[2, 3].

In a preceding study (Hesterberg *et al*., 1998), biopersistence of fibers in the lungs was evaluated by exposing animals to fibers (L>20 μm) for 6 h daily for 5 consecutive days, at a fiber concentration of 100 fibers/cm^3 or above, and weight concentration of 30 mg/m^3, counting at least 400 WHO fibers[13]. In that study, the clearance half time of fibers with L>20 μm was 67 d, when the number of fibers counted on Day 1 after exposure was assumed to be 100%. In other reports in which animals were exposed by a similar method, the clearance half time of fibers with L>20 μm was 92 d[14] or 53 d[12]. On the other hand, the clearance half time was 28 d for fibers with L>20 μm in our present study, where animals were exposed to fibers for 6 h daily, and at least 400 fibers were counted in accordance with the criteria for fiber measurement[9, 10] and with the conditions described in preceding studies[13, 14]. However, the results of the present study cannot be directly compared with those of the preceding studies

because the calculation method of clearance half time and the observation period differed from those of the preceding studies. Therefore, the results obtained from the present study should be carefully evaluated.

The clearance half times of fibers with L>20 μm and WHO fibers (28 and 50 d, respectively) were shorter than those of crocidolite, a kind of asbestos, reported in a previous study (986 and 234 d, respectively)[13]. Among them, the clearance half time of fibers with L>20 μm was particularly short.

The mean values of size, length and width of fibers in the lungs in our study decreased in the 4th week compared to shortly after exposure. The reason for this is considered to be that fibers were phagocytosed by alveolar macrophages, and dissolved by body fluid. The geometric mean value of the length intermittently increased from shortly after to 4 wk after exposure. The reason for this is considered to be that fibers with L>20 μm were broken, increasing the number of fibers with 5 < L≤20 μm. On pathological evaluation, a possible reason for the increase in macrophages from the first week in this study was considered to be a transient reaction of body defenses. Macrophages immediately take in and phagocytose foreign substances invading the body. The number of macrophages in the lung alveolar spaces increased in this study, since macrophages reacted to RW fibers that reached the lungs as being foreign substances, and thus phagocytosed them. In the early stage of exposure, acute inflammation occurred, and granuloma formation was observed throughout the observation period, suggesting that repair of lung tissue had occurred.

In a preceding study (Kamstrup *et al*., 1998), animals were exposed to fibers (L>20 μm) for 6 h daily for 5 consecutive days for two years at a fiber concentration in the exposure chamber of 100 fibers/cm^3 or higher, and a weight concentration of 30 mg/m[14]. During the experiment, the animals were examined by autopsy after 3, 6, 12, 18 and 24 months of exposure. The results of this study showed the grade to be 4 of Wagner's histopathological criteria from 3 months onward. In another report (Kamstrup *et al*., 2004), animals were exposed to fibers (L>20 μm) for 6 h daily for 5 consecutive days for 3 months at a fiber concentration in the exposure chamber of 150 fibers/cm^3 or higher, and a weight concentration of 37 mg/m[15]. They reported that the grade was 3–4 of Wagner's histopathological criteria from 1 wk after exposure onward, in autopsies conducted 1 wk, 1.5 and 3 months after termination of exposure. The results of our present study cannot be directly compared with those of the preceding studies since the exposure and observation periods differed. However, we presume that there was no pathological effect in our present study. The reason seems to be that the exposure period (5 d) and observation period (4 wk) were shorter than those of preceding studies[14, 15]. It is necessary to further examine the pathological effect in studies with extended exposure and observation periods.

Regarding the safety of SVF, it has been reported that fibers with L>20 μm, having higher biopersistence in the lungs, are less likely to be dissolved by body fluid in the lungs and may lead to fibrosis and carcinoma[2]. We calculated the dissolution rate of RW fibers used in our study based on the equation of Eastes *et al*.[16], and found it to be 2,451 ng/cm^3/h. This value is higher than that of RW fibers used in the preceding study (25 ng/cm^3/h)[13]. These results suggest that the RW fibers used in our study are easier to dissolve and faster to clear from the lungs than those used in the preceding studies.

In the European Union (EU) classification system, SVF wools with widths greater than 6 μm are exempt from carcinogenicity classification. Untested SVF wool compositions with widths less than or equal to 6 μm are categorized as 3 or 2 depending on their Soluble Components Index (also known as KNB). For fibers with width < 6 μm; if the index is > 18%, the fiber is placed in Category 3; if the index is ≤ 18%, the fiber is placed in Category 2. Based on this index, traditional refractory ceramic fibers and E glass microfibers are placed in Category 2 (probable carcinogen), and standard glass and mineral (rock/stone/slag) insulation wools are placed in Category 3 (possible carcinogen) until they are tested[3]. The KNB value of the RW fibers used in our study was calculated as 43%, which can be placed in Category 3; equal to those of the RW fibers used in the preceding studies[11–15]. Category 3 is described as "a substance that is of concern as a possible human carcinogen, but available information is not adequate for a valid assessment"[3]. Therefore, further evaluation of RW fibers is required in the future regarding biopersistence in the lungs for a longer period of time.

Conclusions

This study indicated that the biopersistence of RW is lower than that of crocidolite, and no pathological fibrosis was observed. The reason seems to be that the experimental period of our present study was shorter than those of preceding studies[14, 15]. Based on these results, it can be said that RW has a limited harmful effect in a short-term exposure experiment of 5 d and observation period of 4 wk. Further, we found that our aim to establish a method to assess the pulmonary biopersistence of fibers, as a screening inhalation study, for a shorter period than that recommended in a previous

study, was achieved. It will be necessary in the future to further confirm the long-term safety of RW fibers in our ongoing study, by assessing the biopersistence of fibers in the lungs and their pathological effects, together with asbestos as a control.

Acknowledgements

We express our gratitude to Ms. Yumiko Sugiura, Ms. Michiyo Koyama, and Ms. Etsuko Ohta, Department of Preventive Medicine and Public Health, Kitasato University School of Medicine, as well as to Ms. Noriko Nemoto, Electron Microscopy Center for their precise and enthusiastic advice. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan in 2008.

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