Clearance of Inhaled Potassium Octatitanate Whisker from Rat Lungs

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Abstract: Clearance of Inhaled Potassium Octatitanate Whisker from Rat Lungs: Hiroshi Yamato, et al. Department of Environmental Health Engineering, University of Occupational and Environmental Health, Japan—The clearance rate of inhaled man-made fibers (MMFs) is one of the important factors for predicting health hazards in human. Potassium octatitanate whisker is a newly developed MMF in Japan with strong resistance to heat and chemicals. Two types of commercially sold potassium octatitanate whisker were used in this study. In order to examine the rate of clearance of this whisker, rats were exposed to whisker aerosol for 4 weeks and the rate of clearance of inhaled whiskers from rat lungs was examined in terms of the fiber mass clearance rate and dimensional change in whiskers. The decrease in fiber mass retained in rat lungs was expressed with a two-compartment model. As for the dimensional change in whiskers, no decrease was observed in diameter and no surface change due to dissolution was detected over the 12-month observation period. Macrophage mediated clearance was thought to be the main mechanism for the clearance of potassium octatitanate whiskers.

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Key words: Man-made fibers, Whisker, Inhalation, Clearance, Solubility

Occupational exposure to asbestos is thought to produce pulmonary fibrosis and lung cancers. Various types of man-made fibers (MMFs) have been developed as substitutes for asbestos or as newly developed materials. It has been pointed out that some kinds of MMFs possess similar adverse biological effects to those of asbestos.

Biopersistence of inhaled fiber is one of the most important factors for predicting the health effects of fibers. Biopersistence is thought to depend on the solubility of fibers in body fluid and the rate of macrophage-mediated clearance. Most inhaled MMFs, such as glass fiber and ceramic fiber are cleared through these two mechanisms so that it would sometimes be difficult to estimate the rate of macrophage-mediated clearance.

The purpose of this study is to determine the rate of macrophage-mediated clearance by using almost non-soluble MMF, which are two types of potassium octatitanate whiskers (PT1 and TW). The word “whisker” is used for thin natural fiber and MMFs with a crystalline structure. PT1 is one of the standard reference fibrous minerals prepared by the Japan Fibrous Material Research Association. TW is also a type of potassium octatitanate whisker with the same crystalline structure as PT1 from a different factory. Small particles in TW were removed by a water elutriation method. Short-term inhalation studies of two types of potassium octatitanate whiskers were performed in rats. Macrophage-mediated clearance of PT1 and TW from rat lungs was analyzed in terms of the change in fiber dimensions and the change in fiber mass in rat lungs.

Materials and Methods

Two types of potassium octatitanate whiskers (PT1 and TW) were used in this study. They are commercially available from two different factories in Japan. Both of them have the same crystalline structure and the chemical composition is 100% K2O·8TiO2. Details of our exposure system and apparatus were reported in our previous paper. The experimental conditions of inhalation studies are summarized in Table 1. Geometric mean length
(GML) and diameter (GMD) in the exposure chamber were determined with a scanning electron microscope (S-900, Hitachi, Japan).

Five or six Wistar rats (male) for each group were sacrificed at 3 d, 1, 3, 6 and 12 months after the end of short-term inhalation exposure for 4 wk (6 h/d, 5 d/wk). The two right lower lobes were used for measuring the diameter and length retained in lungs. The left and other right lobes were used for measuring the titanium content in lungs in order to calculate the mass of whiskers retained in lungs.

**Determination of dimensions of whisker recovered from lungs**

Details of the method for measuring the dimensions of recovered whiskers were described in our previous study. Briefly, PT1 and TW were extracted onto a 0.2 µm pore sized filter by low temperature ashing of lungs, then sputtered with platinum. Photomicrographs of the recovered fibers were taken and printed on enlargements at a final magnification of ×7,000. An example photo of PT1 recovered from lungs is shown in Fig. 1. The whisker dimensions were measured on the photographs with a backlight digitizer or directly measured in a computer by using NIH-image software. The dimensions of whiskers were estimated as the geometric mean diameter and length.

**Observation of the surface structure at high magnification**

To observe the surface structure of PT1 and TW in rat lungs, some whiskers recovered after 3 d and 12 months were investigated at high magnification (×80,000) by using scanning electron microscope without sputtering with platinum.

**Determination of the mass of PT1 and TW retained in lungs**

To the left lung and remaining right lobes were added 2.5 ml of reagent solution and 4.0 ml of H₂O₂ then they were wet-ashed with Microdigest (Model 300, Prolabo, France). The composition of the reagent solution was 40 g of (NH₄)₂SO₄ and 100 ml of H₂SO₄. The residual solutions were diluted to 50 ml with distilled water and the amount of titanium was analyzed with an ICP atomic emission spectroscope. The mass of whiskers retained in the lungs was calculated from the amount of titanium. The results of deposition of whiskers were expressed as the total mass of whiskers in all the lungs of each rat.

**Results**

**Change in whisker dimensions**

Tables 2 and 3 show the change in dimensions of PT1 and TW recovered from rat lungs. There was no change in the geometric mean diameter (GMD) of whiskers throughout the 12-month clearance period. No erosion was observed on the PT1 surface of those recovered in the 12-month clearance period (Fig. 2). These results are compatible with the high resistance of potassium octatitanate whiskers observed in vitro.

Figures 3 and 4 show the change in frequency of whisker length. The frequency of short fibers decreased with the clearance period. On the other hand, the frequency of long fibers increased. The percentage of whiskers longer than 20 µm was only 3.8% for PT1 and 3.6% for TW at 3 d after the inhalation, and those percentages increased to 12.4% for PT1 and 12.3% for TW at 12-month clearance period, respectively. As the result of these changes in whisker dimensions, the geometric mean length (GML) of whiskers became lengthened from 3.6 µm to 5.7 µm for PT1 and 3.2 µm to 6.0 µm for TW after 12 months’ clearance (Tables 3 and 4).

**Deposition of PT1 and TW**

The mass concentrations of PT1 and TW were 1.1 and 1.7 mg/m³, respectively. But the fiber concentrations in the chamber were 137 fiber/ml for PT1 and 73 fiber/ml for TW as shown in Table 1. Although the mass concentration of TW was higher than PT1, the longer whiskers with a large mass were thought to be filtered through the upper respiratory tracts. The initial deposition of PT1 was higher than that of TW (Figs. 5 and 6).

| Table 1. Experiment conditions |
|-------------------------------|-----------------|
|                               | PT1             | TW              |
| Chemical composition          | K₂O·8TiO₂       | K₂O·8TiO₂       |
| MMAD in chamber (µm)          | 1.9 (2.2)       | 2.2 (2.0)       |
| Fiber diameter (SD)*          | 0.44 (1.5)      | 0.39 (1.5)      |
| Fiber length (SD)*            | 3.4 (2.7)       | 5.6 (2.3)       |
| Exposure concentration (mg/m³)| 1.1 ± 0.3       | 1.7 ± 0.6       |
| Exposure concentration (fiber/m³)| 137 ± 53 | 73 ± 16 |
| Exposure Duration             | 4 wk            | 4 wk            |

*: Geometric means and standard deviations.
Table 2. Change in PT1 diameter and length

<table>
<thead>
<tr>
<th></th>
<th>Number counted</th>
<th>Fiber diameter (SD)* (µm)</th>
<th>Fiber diameter (SD)* (µm)</th>
</tr>
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<tbody>
<tr>
<td>3 d</td>
<td>741</td>
<td>0.45 (1.5)</td>
<td>3.6 (2.3)</td>
</tr>
<tr>
<td>1 month</td>
<td>682</td>
<td>0.43 (1.4)</td>
<td>3.5 (2.5)</td>
</tr>
<tr>
<td>3 months</td>
<td>496</td>
<td>0.43 (1.5)</td>
<td>3.5 (2.4)</td>
</tr>
<tr>
<td>6 months</td>
<td>500</td>
<td>0.44 (1.4)</td>
<td>4.8 (2.5)</td>
</tr>
<tr>
<td>12 months</td>
<td>235</td>
<td>0.43 (1.5)</td>
<td>5.7 (2.8)</td>
</tr>
</tbody>
</table>

*: Geometric means and standard deviations.

Table 3. Change in TW diameter and length

<table>
<thead>
<tr>
<th></th>
<th>Number counted</th>
<th>Fiber diameter (SD)* (µm)</th>
<th>Fiber length (SD)* (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 d</td>
<td>342</td>
<td>0.39 (1.4)</td>
<td>3.3 (2.5)</td>
</tr>
<tr>
<td>1 month</td>
<td>326</td>
<td>0.34 (1.3)</td>
<td>3.1 (2.4)</td>
</tr>
<tr>
<td>3.5 months</td>
<td>394</td>
<td>0.42 (1.6)</td>
<td>3.5 (2.5)</td>
</tr>
<tr>
<td>6 months</td>
<td>237</td>
<td>0.39 (1.6)</td>
<td>4.7 (2.8)</td>
</tr>
<tr>
<td>12 months</td>
<td>231</td>
<td>0.43 (1.6)</td>
<td>6.0 (3.2)</td>
</tr>
</tbody>
</table>

*: Geometric means and standard deviations.

Fig. 1. TW recovered from rat lungs and photographed through a scanning electron microscope.
Fig. 2. Surface structure of PT1 recovered from rat lungs (Left: 3 days, Right: 12 months).

Fig. 3. Change in whisker length of PT1.

Fig. 4. Change in whisker length of TW.

**Mass clearance**

The mass of inhaled PT1 and TW in rat lungs decreased with time as shown in Figs. 5 and 6, but the rate of decrease in PT1 and TW was not linear on the logarithm axis so that a two-compartment model is applied for mass clearance\(^3,14\). The equation is expressed as follows:

\[
m = m_{01}e^{-\alpha t} + m_{02}e^{-\beta t} \quad (1),
\]

where \(m\) is the mass of fibers (\(\mu g\)) in each rat, \(t\) is clearance period in months, \(\alpha\) and \(\beta\) are the rate constants for faster and slower clearance, respectively.

Regression lines for Eq(1) are shown in the figures with solid lines.
Discussion

In our previous study, we confirmed that the clearance of inhaled fibers which dissolve in lungs like ceramic fibers could be estimated by two factors: one is the dissolution rate of fibers in body fluid and the other is the rate of clearance by means of macrophage mediated transportation\textsuperscript{12–15}. We found the eroded appearance of ceramic fibers when observed at high magnification ($\times$ 80,000) as the result of dissolution in body fluid. These soluble fibers can easily break down in the lungs. As a result of these phenomena, both fiber diameter and length decreased in the inhalation study on ceramic fibers in rats\textsuperscript{6}. These factors should be taken into consideration when we estimate the rate of clearance of soluble MMFs.

Nevertheless, potassium octatitanate whisker is a fiber with high resistance to chemicals, so the influence of dissolution or breakage of whiskers should be minimum \textit{in vivo}. There was no erosion on the PT1 surface recovered after 12 months' observation period after the end of inhalation. And there was no decrease in GMD during the 12 months' observation period. These facts could be explained by the low rate of dissolution of whiskers. It is also expected that those fibers with a low dissolution rate would not break up in lungs. It is reasonable that the GML of fibers increased as the result of macrophage mediated clearance because short fibers would be easily phagocytized and cleared by macrophages. It is considered that less soluble whiskers are suitable for assessing the clearance of inhaled asbestos.

As for the mass clearance of fibers, the decreasing rates for PT1 and TW were not linear on the logarithm axis. There seemed to be two different clearances, one is rapid clearance with a half-life of 2–3 months explained by the macrophage mediated clearance. The other is the slower clearance with a long half-life of more than 12 months. The longest whisker recovered from rat lungs in the 12-month clearance period was 41 $\mu$m in this study. As mentioned above, long whiskers with a low dissolution rate would not be easily cleared by macrophages if the length of fiber were longer than the diameter of macrophages and the diameter of the long fibers tends to be greater than that of the short fibers. This means that the mass of whiskers increases in proportion to the length and the square of the diameter. In view of these considerations, the long and thick whiskers with a large mass would persist for a longer period than the short and thin fibers. Figure 7 is a scanning electron microscope photograph of the long fibers observed in BAL fluid after inhalation exposure to ceramic fibers in rats in another experiment\textsuperscript{15}. It is considered that, as with asbestos, the long and less soluble whiskers would remain in the lungs for a long time.

The shape of the clearance curves of PT1 and TW are different (Figs. 5 and 6). The concave part of the clearance curve of TW is more distinct than that of PT1. One possible reason is the difference in the initial amount of whiskers deposited. It is reported that the delayed clearance of inhaled particles was observed as the exposure level increased\textsuperscript{16}. The initial deposition of TW was almost half that of PT1, so that similar phenomena might occur in these experiments.

Conclusion

It is generally considered that rapid clearance of short fibers by macrophages and slow clearance by fiber dissolution in body fluid occurs when man-made fibers are inhaled into the lungs. Man-made fibers that are inhalable and longer than macrophages were thought to persist in the lungs for a long period like asbestos. It is suggested that fibers or whiskers with very low solubility such as PT1 and TW should be carefully handled like asbestos.
Fig. 7. MMFs engulfed by macrophages (Y. Morimoto).

References


