The Effect of Lung Burden on Biopersistence and Pulmonary Effects in Rats Exposed to Potassium Octatitanate Whiskers by Intratracheal Instillation

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Abstract: The Effect of Lung Burden on Biopersistence and Pulmonary Effects in Rats Exposed to Potassium Octatitanate Whiskers by Intratracheal Instillation: Takako OYABU, et al. Institute of Industrial Ecological Sciences, University of Occupational and Environmental Health—In our previous inhalation studies on health effects of the asbestos substitute, potassium octatitanate whiskers (POW), we showed that an excess amount of POW deposition in the rat lung increased biopersistence resulting in fibrotic changes. The critical deposition amount which induced the higher biopersistence was estimated to lie between 1.5 mg and 2.4 mg. In order to find the exact amount, the relationship between the lung POW burden and biopersistence was investigated by the intratracheal instillation method. The chemical formula of POW is $\text{K}_2\text{Ti}_8\text{O}_{17}$ and the geometric mean fiber diameter (geometric standard deviation, GSD) and geometric mean fiber length (GSD) are 0.35 $\mu$m (1.6) and 4.4 $\mu$m (2.7), respectively. Rats were intratracheally instilled with 0.5 mg, 1.0 mg, 2.0 mg or 5.0 mg of POW and sacrificed at 1 day and 1, 3, 6 and 12 months after the instillation. The POW amount in each lung was chemically analyzed by ICP-AES after microwave digestion and the biological half time (BHT) of each POW dose was calculated. The BHTs of each group were 10, 15, 20 and 42 months for 0.5, 1.0, 2.0 and 5.0 mg of POW, respectively, and BHT showed a linear dose-dependent increase, but without a threshold within the range of 0.5 mg to 5.0 mg, which was recognized in our earlier inhalation studies. In the histopathological photograph just after the instillation, many macrophages, which had phagocytized many more fibers, existed around the bronchiole compared with the earlier inhalation study at almost the same deposited amount. The relationship between POW amount and biopersistence in this intratracheal instillation study was different from that of our previous inhalation studies, probably due to the unnatural method of the fiber introduction to the lung, which in turn led to a different fiber distribution. It is suggested that an intratracheal instillation study is not an appropriate method for estimating excess deposition amounts of POW and an inhalation study will be needed. However, this intratracheal instillation study clarified that the clearance of POW was delayed as compared with previous inhalation studies at similar deposition amounts and this result has importance for the hazard assessment of dusts in animal experiments. (J Occup Health 2006; 48: 44–48)

Key words: Lung burden, Biopersistence, Pulmonary effect, Potassium octatitanate whisker, Intratracheal instillation
in inhalation studies using the biopersistence of deposited dusts in the lung as an index\(^{1-3}\). The biopersistence is an useful index of lung injury and it is known that higher biopersistence results in greater health effects. Our studies on health effects by inhalation exposure showed that an excess amount of POW, which impairs the migration of alveolar macrophages due to the excess uptake of the fiber in the rat lung, increased biopersistence resulting in fibrotic changes around aggregated dust cells, and the threshold deposition amount inducing the higher biopersistence was estimated to lie between 1.5 mg and 2.4 mg\(^{3,4}\). Even low toxicity particles such as TiO\(_2\) experimentally lead to the fibrotic changes and tumors in cases of large lung burden\(^{5-7}\), thus it is important for the prevention of lung impairment to clarify the minimum deposition that leads to the higher biopersistence and to avoid this excess deposition for each fiber and dust.

This study used an intratracheal instillation method to investigate the relationship between POW amount in the lung and biopersistence at four dose levels around 2.0 mg, which is the critical dosage inducing prolongation of clearance, in order to find the exact excess deposition amount of POW.

**Materials and Methods**

*Intratracheal instillation*

The POW used in this study was the JFM standard reference sample named PT1 from the Japan Fibrous Materials Research Association (JFMRA). The geometric mean fiber diameter (geometric standard deviation, GSD) and geometric mean fiber length (GSD) are 0.35 µm (1.6) and 4.4 µm (2.7), respectively. The chemical formula is K\(_2\)Ti\(_8\)O\(_{17}\). The geometric morphology is shown in Fig. 1.

Doses of POW administered to each rat lung were 0.5 mg, 1.0 mg, 2.0 mg or 5.0 mg. Each dose of POW was dispersed in 0.4 ml of saline solution and intratracheally instilled into the tracheal lumen of Kud:Wistar male rats (total 140 rats, aged 10 wk old) after anesthesia by diethyl ether. At 1d and 1, 3, 6 and 12 months after the instillation 5 rats of each group were sacrificed by an intraperitoneal injection of pentobarbital for the determination of lung burden along with 1 or 2 rats for the pathological examination. The body weight and wet organ weights (lungs, livers, kidneys and spleens) were measured at the same time.

*Determination of POW in rat lung*

POW fibers in the lung were digested with lung tissues into the element with H\(_2\)SO\(_4\), (NH\(_4\))\(_2\)SO\(_4\) and H\(_2\)O\(_2\) by microwave (mls 1200 mega, Milestone, Italy) under high temperature and high pressure conditions for 18 minutes and Ti amounts were determined by an inductively coupled plasma-atomic emission spectrometer (ICP-AES, SPS1500R, SII Japan). The mass of POW retained in the lung was calculated from the Ti content (52.2%) by percentage.

*Histopathological procedures*

The rat lungs were inflated and fixed with 10% buffered formalin by intratracheal infusion at 25 cm H\(_2\)O pressure, then sectioned and embedded in paraffin. The paraffin sections of 3 µm thickness were stained with hematoxylin and eosin.

*Statistical analysis*

Student’s t-test for the mean was used to analyze the statistical significance of differences.

**Results**

*Body and wet organ weights*

There were no significant differences in body and wet organ weights among the four groups except lung weight. Figure 2 shows the wet lung weight at each sacrifice time. Values are expressed as the mean ± standard deviation.
At 1 day and 1 month after instillation the wet lung weights in the 1.0, 2.0, 5.0 mg groups were significantly increased compared with those in the 0.5 mg group. After 3 months, only the wet lung weights of the 5.0 mg group were significantly increased.

**Biopersistence of POW in lung**

Figure 3 shows the temporal change of POW amounts in lungs at each time point. The POW amounts exponentially decreased at every dose level. The solid lines are the regression lines based on a single compartment model. The biological half time (BHT) linearly increased with the increase of the instilled dose. The BHTs of each group were 10, 15, 20 and 42 months in 0.5, 1.0, 2.0 and 5.0 mg groups, respectively.

**Histopathological findings**

Figure 4 shows the histopathological features of each dose at 12 months after the instillation. Aggregated POW were partly observed in the terminal bronchiole or alveolar ducts. The fibrotic changes around the foci and thickness of the alveolar wall seemed to be more obvious as the POW dose increased.

**Discussion**

This intratracheal instillation study for investigating
the effects of instilled POW amount on the lung showed dose-dependent lung weight increase, prolonged biopersistence and histopathological changes, therefore the instilled POW amount was an important factor for the effects on the lung. Figure 5 shows the quantitative relationship between the biopersistence, which is an index of lung injury, and POW amount in the lung in the present intratracheal instillation study; the corresponding relationship obtained by our past POW inhalation studies is also added to Fig. 5. There was no threshold POW amount in the lung within the range of 0.5 mg to 5.0 mg in the present intratracheal instillation study, contrary to the results of our previous inhalation studies, and the dose-response curve of POW amount with biopersistence was nearly linear. At comparable amounts of POW in the lung the clearance was delayed in the present intratracheal instillation study as compared with our previous inhalation studies.

Higher biopersistence in intratracheal instillation studies than in inhalation exposure studies has been reported for other asbestos substitutes8, 9). Since dissolution in the lung fluids and transfer by macrophages are major mechanisms of fiber clearance in the alveolar region, POW which is poorly soluble10) is likely to be removed from the lung by macrophages. Clearance of poorly soluble fibers by macrophages mainly depends on the length of fibers and phagocytic capacity of macrophages. Although it is generally known that a longer fiber increases the difficulty of uptake and removal by macrophages, the geometric morphology of the POW in this intratracheal instillation study was very short and similar to those of the fibers in the chamber or the lung of inhalation studies2, 3). Thus, the length of the fiber was not considered to be a cause of the prolongation of the clearance in this intratracheal instillation study. For phagocytosis by macrophages, the larger the dust volume the macrophages take up, the higher the biopersistence becomes, generally due to decreased macrophage migration11). Figure 6 shows a histopathological photograph of a lung in which (a) 1.5 mg of POW was deposited after 4 wk of inhalation exposure5) and (b) after 1 d of intratracheal instillation with 1.0 mg (the present study). In the inhalation exposure study, many macrophages phagocytized a small number of POW distributed extensively in the alveolar space, while in the present intratracheal instillation study, many macrophages had phagocytized many fibers accumulated around the bronchiole. Despite the smaller amount of POW in the lung in the present intratracheal instillation study, 1.0 mg deposition, biopersistence was higher possibly due to the lower migration of these macrophages with many fibers.

These findings suggest that the relationship between POW amount in the lung and biopersistence in the present intratracheal instillation study is different from that of
the inhalation studies and it is probably due to the unnatural method of fiber introduction to the lung, which in turn leads to a different fiber distribution. Since it is difficult to distribute fibers uniformly in the alveolar space in an intratracheal instillation study, inhalation exposure is likely to be necessary to estimate the excess exposure amount.

The intratracheal instillation study as well as the inhalation study are commonly used methods to evaluate lung injury. Inhalation exposure is more suitable because its condition is closer to the actual exposure experienced by workers, however, it takes longer, costs more and the precise deposition amount in the lung is not easy to predict. On the other hand, since the intratracheal instillation takes less time, costs less and permits the instillation of a known amount of fibers, it is very useful for the comparison of effects on health between different fibers. Based on this advantage, the intratracheal instillation method is employed in the exclusion conditions of MMVF for carcinogenesis in the EU (EU Directive 97/69/EC), and is distinguished clearly from the inhalation method. Its bio-assessment criteria adopts the biopersistence (biological half time) of long fibers, which is 10 d for an inhalation exposure study and 40 d for an intratracheal instillation study, taking into account the more prolonged clearance in intratracheal instillation study. Consequently, although the intratracheal instillation study is not appropriate for estimating the excess deposition amount, it can be a useful bio-assessment approach for assessing the health effect; therefore it is important to choose the exposure method most relevant to the objective.

Conclusion

The effects of deposited POW amount in the lung on POW clearance and on lung tissue were investigated in an intratracheal instillation study. The results showed a linear dose-response relationship between POW amount in the lung and biopersistence. No threshold was recognized in the present intratracheal instillation study, contrary to the results of earlier inhalation studies, and therefore, an inhalation study will be necessary to estimate the excess exposure amount which induces higher biopersistence and lung injury.

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