ORIGINAL

Pulmonary Deposition and Clearance of Inhaled or Instilled ^{198}Au -Colloid in the Rat after the Induction of Pulmonary Delayed Type Hypersensitivity Reactions

Yoshihisa KUBOTA*,*¹ Sentaro TAKAHASHI,**,*¹ Hiroshi SATO,***,*¹
Yuji YAMADA****,*¹ and Osamu MATSUOKA*****,*¹

(Received February 16, 1988)

(Accepted June 15, 1988)

The deposition and clearance of inhaled or instilled colloidal ^{198}Au -particles were studied in rats with pulmonary lesions induced by delayed type hypersensitivity (DTH) reactions. The increased amount of radioactivity was detected in the lung associated lymph nodes and the kidney of DTH rats on day 4 after intratracheal instillation of ^{198}Au -particles. The initial lung deposition of inhaled ^{198}Au -particles in the lung lobes with DTH lesions was approximately 55% of normal lobes. Early mucociliary clearance of ^{198}Au -particles was impaired in DTH rats. These results suggest that the deposition pattern and the subsequent movements of inhaled particles in the respiratory tract might be considerably influenced by pathologic conditions in the lung.

KEY WORDS: DTH, ^{198}Au -colloid, intratracheal instillation, inhalation, rat

I INTRODUCTION

In order to assess the toxicity of inhaled radioactive particles, it is essential to know their movement and metabolism in the respiratory system. The fate of airborne particles deposited in the lung has been the subject of a number of investigations.^{1,2)} In those studies, experimental animals used were usually under normal conditions or free from any respiratory diseases. In the practical cases, however, a significant portion of human population is spontaneously being affected by respiratory disorders such as pneumonia and asthma. Nevertheless, only a limited number of studies has been focused on the deposition and clearance of radioactive particles in the respiratory

system under the pathologic conditions.³⁻⁵⁾ The present study was therefore undertaken to compare the deposition and clearance of radioactive ^{198}Au -particles in the respiratory tracts of rats having a pulmonary disease with those of normal animals. A pulmonary disease was induced by delayed type hypersensitivity (DTH) reactions using the previously described method. This process induces pulmonary alveolitis and granuloma formation. Our results indicate that such conditions of pulmonary disorders could affect the deposition and/or clearance of inhaled particles.

II MATERIALS AND METHODS

1. Induction of pulmonary DTH reaction

Schematic experimental protocols are shown in Fig. 1. Wistar rats of both sexes, aged 12-16 weeks and weighing 300-390 g, were supplied from the animal breeding facility of this institute. The animals were divided into three groups. The first group was immunized by subcutaneous injection of 2-3 ml methylated bovine serum albumin

* 久保田善久, ** 高橋千太郎, *** 佐藤 宏, **** 山田裕司, ***** 松岡 理

^{*1} Division of Comparative Radiotoxicology, National Institute of Radiological Sciences; 9-1, Anagawa 4-chome, Chiba, 260 Japan.

放射線医学総合研究所内部被ばく研究部; 千葉市穴川 4-9-1 (〒260)

Fig. 1 Schematic experimental protocols on the induction of pulmonary DTH.

(MBSA, Sigma Co., Ltd.) solution (1-2 mg) emulsified in complete FREUND'S adjuvant. The MBSA solution was injected bilaterally into separate sites on the dorsal flanks of the rats. Ten days after immunization, two groups of rats, one group immunized and the other not immunized, were instilled intratracheally with 0.3 ml suspension of MBSA coupled to cyanogen bromide-activated Sepharose 4B beads (Pharmacia Fine Chemical Co., Ltd.) at a concentration of 3×10^5 beads ml^{-1} . The coupling of MBSA to cyanogen bromide-activated Sepharose 4B beads was carried out according to the direction of Pharmacia Fine Chemicals. The rats in each experimental group were referred to as the following, rats immunized and instilled intratracheally with Sepharose 4B beads as DTH rats, rats not immunized but instilled with beads as beads injected rats, and non-treated rats as control rats. Two days after the intratracheal instillation of Sepharose 4B beads, all the rats were administered with ^{198}Au -colloid by intratracheal instillation or inhalation.

2. Preparation and intratracheal injection of ^{198}Au -colloid

The method for preparing the colloidal gold labelled with ^{198}Au was detailed elsewhere.⁶⁾ The radioactivity of the solution was 7.4×10^5 Bq ml^{-1} and the concentration of gold was 0.12 mg ml^{-1} . The particle size distribution was found by electron microscopy to be log-normal, with a count median diameter of $0.02 \mu\text{m}$. The colloidal gold suspension at a dose of 0.5 ml per rat was instilled into the rat lung by intratracheal cannulation method as reported previously.⁷⁾ In brief, the rats were anesthetized and a nylon cannula was passed to the first bifurcation of the trachea. The ^{198}Au -colloidal solution was vigorously instilled during a spontaneous inspiration by the rat in order to disperse the colloid particles throughout the lung as uniformly as possible.

3. Inhalation of ^{198}Au -colloid

Inhalation of ^{198}Au -colloid was performed with a small animal exposure apparatus of which outline has been reported previously.⁸⁾ This apparatus can expose up to 20 animals simultaneously to highly radioactive aerosols. The animals were

Table 1 Experimental design of this study.

Exp. No	Induction of DTH	Administration of ^{198}Au colloid	Purpose
Exp. 1	Whole lung	Lung instillation	Retention up to 4 day and systematic distribution
Exp. 2	Individual lung lobe(s)	Inhalation	Initial lung deposition
Exp. 3	Whole lung	Inhalation	Early clearance

placed in specially designed holders to allow the exposure of only the nasal area of the animal head. Fifty milliliters of the same colloidal gold suspension (total ^{198}Au activity; 3.7×10^7 Bq) as used in the intratracheal injection study was aerosolized by aerosol generator, and 20 rats were exposed for about one hour. It had been confirmed in preliminary trials that there was no significant external contamination, except for a slight contamination in the nasal area.

4. Experimental procedure

The present study consists of three separate experiments as shown in **Table 1**. Experiment 1 was designed to investigate the retention and systematic distribution of ^{198}Au after the intratracheal instillation of ^{198}Au -colloid particles. In this experiment, intratracheal instillation was used in preference to inhalation because it allowed the administration of enough activity and defined amount of particles. In Experiment 2, the effect of DTH on the initial lung deposition of inhaled colloidal gold was studied. Experiment 3 was carried out to investigate early clearance of inhaled colloidal gold. In both Exp. 2 and Exp. 3, the initial lung burden of ^{198}Au -colloid by inhalation was much less than 10^4 Bq per rat, so that the movement of colloidal gold from the lung to the other organs except for gastro-intestinal tract could not be measured. In Exp. 1, rats were administered with ^{198}Au -colloid by intratracheal instillation as described above, on the 2nd day after the intratracheal instillation of Sepharose 4B beads. Total body burden was measured by whole body counter immediately and on day 2 after instillation of ^{198}Au -colloid. Rats were anesthetized and killed by exsanguinating on day 4. The lungs were lavaged with about 10 ml of Ca^{2+} , Mg^{2+} free phosphate buffered saline. Lavage fluids recovered were centrifuged to separate the supernatants from the cell pellets which almost consisted of bronchoalveolar macrophages. The

radioactivity of ^{198}Au in these samples and other organs was counted by auto γ well counter. In Exp. 2, the rats were instilled with Sepharose 4B beads intratracheally into either left or right lung lobe(s) by inserting the intratracheal cannula into the individual lung lobe(s) through the trachea, resulting in the induction of DTH lesions in the one or two lung lobe(s) in DTH rats. Small amount of colloidal carbon was mixed with Sepharose 4B beads so that it could be easily identified with naked eyes which lung lobe was loaded with Sepharose 4B beads. Rats were exposed to the aerosol of ^{198}Au -colloid as described above on the 2nd day after the beads instillation. Immediately after the inhalation, the animals were sacrificed and the radioactivity of ^{198}Au in each lung lobe was counted by auto γ well counter. In Exp. 3, Sepharose 4B beads were instilled into the whole lung as uniformly as possible. This treatment induced DTH lesions throughout the lung in DTH rats. The animals were exposed to ^{198}Au -colloid 2 days after the beads instillation. The radioactivities in the thoracic and abdominal region of the rats were measured by external scanning with a collimated detector 3–5 h after the inhalation. The rats were sacrificed on day 2 and the lung lobes, trachea and gastro-intestinal tract were dissected free. After measuring the radioactivities, paraffin sections of these organs were prepared for light microscopy.

III RESULTS

1. Histopathology of DTH induced pulmonary lesions

The pulmonary broncho-alveoli in DTH rats were being filled with exudated macrophages, lymphocytes and a few neutrophils surrounding Sepharose 4B beads. These cellular reactions were considered to be a characteristic of DTH reactions. Clear-cut cellular accumulation was observed only around Sepharose 4B beads. These

Fig. 2 Histopathology of pulmonary delayed type hypersensitivity (DTH) induced in Wistar rats by intratracheal injection of antigen coated Sepharose 4B beads (hematoxylin and eosin staining, $\times 100$). a) Section from the lung of a control rat. b) Section from the lung of a beads injected rat sacrificed 4 days after intratracheal instillation of Sepharose 4B beads. The presence of Sepharose 4B beads are indicated by the letter, B. Beads are surrounded by a very mild infiltrate of macrophages. c) Representative pulmonary DTH seen in a DTH rat sacrificed 4 days after intratracheal challenge with MBSA-coupled Sepharose 4B beads. The inflammatory infiltrate surrounding the beads is composed of macrophages, lymphocytes, scattered neutrophils.

Fig. 3 Whole-body and lung retention of colloidal ^{198}Au following the intratracheal instillation. Rats were sacrificed on day 4 and their lungs were lavaged with PBS. The lavage fluids recovered were centrifuged to separate the supernatants from the cellular fraction, which almost consisted of broncho-alveolar macrophages. A. The ratio of whole-body count (WB) on day 2 to that on day 0 after intratracheal instillation of colloidal ^{198}Au . B. The ratio of lung retention of ^{198}Au on day 4 to whole-body count on day 2. C. The ratio of the activity of ^{198}Au in the cell pellet (broncho-alveolar macrophages) to that recovered from whole lung lavage fluid.

Fig. 4 Tissue distribution of colloidal ^{198}Au following the intratracheal instillation. Rats were sacrificed on day 4 after the intratracheal instillation of colloidal ^{198}Au . The accumulation of colloidal ^{198}Au in the lung-associated regional lymph nodes (A) and the kidney (B) were expressed as percentage of lung retention on day 4. No significant accumulation of ^{198}Au was observed in the other organs.

Table 2 The distribution of colloidal ^{198}Au among lung lobes in the inhalation study.

Lung lobe	Control rats ^{#1}	DTH rats ^{#1}	
		Example 1)	Example 2)
Left	30.9 $\pm$ 4.6 (100) ^{#2}	8.6* (27.8) ^{#2}	45.4
Right apical	11.7 $\pm$ 1.9	8.5	12.1
Right cardiac	12.5 $\pm$ 1.0	22.7	10.8
Right diaphragmatic	27.6 $\pm$ 2.9 (100) ^{#2}	37.5	16.2* (58.7) ^{#2}
Right intermediate	14.2 $\pm$ 2.7	22.0	14.2
Tracheo-bronchi	3.1 $\pm$ 1.4	0.7	1.3
Control rats		Beads injected rats	
100 ^{#3}		103.8 $\pm$ 9.7 ^{#3}	
		DTH rats	
		54.7 $\pm$ 21.8 ^{#3}	

^{#1} The deposition of ^{198}Au in the individual lung lobes of control rats and two representative DTH rats expressed as the percentage of whole lung deposition. The values of control rats are mean $\pm$ SD.

* The asterisk indicates the lung lobe into which Sepharose 4B beads and colloid carbon were instilled.

^{#2} Values in the parentheses are the deposition of ^{198}Au in the lung lobe expressed as the percentage of that in the corresponding lobe of control rats.

^{#3} Values are inhaled ^{198}Au deposition in the lung lobes injected with Sepharose 4B beads of DTH and beads injected rats expressed as the percentage of that in the corresponding lobes of control rats and are mean $\pm$ SD of the values in the above parentheses^{#2} obtained from all the rats examined.

histological features were also referred to as granulomatous delayed type hypersensitivity (Fig. 2c). In contrast, only a moderate foreign body type response was seen in the lungs of the beads injected rats (Fig. 2b). The lungs from control rats appeared to have little or no inflammatory lesions (Fig. 2a).

2. Intratracheal instillation of ^{198}Au -colloid (Experiment 1)

The ratios of whole body retention of ^{198}Au -colloid on day 2 to that on day 0 after intratracheal instillation of ^{198}Au -colloid are shown in Fig. 3. There were no significant differences in these ratios among three groups of rats. Approximately 60–70% of initial lung burden were still remained in all groups of rats even on day 2 when almost all mucociliary clearance appeared to have been completed. The lung retention of ^{198}Au on day 4 was approximately 95% of the whole body count on day 2 in all groups. The activity of ^{198}Au in the cell pellets recovered from the lung lavage was 98–99% of whole lavage fluid in all three groups, suggesting that virtually all the colloidal gold particles were almost phagocytosed mainly by bronchoalveolar macrophages.

Regarding the translocation of colloidal gold from the lung to the other organs, no significant accumulation of colloidal gold was observed in

the other organs except for the regional lymph nodes and the kidney. The distribution of ^{198}Au to the above two organs were very small, *i.e.*, less than 0.1% in all three groups, but there were significant differences among the three groups of rats (Fig. 4). The distribution of ^{198}Au to these organs, which was expressed as percentage of the lung retention on day 4, was considerably higher in DTH rats than that in beads injected and control rats. There were no significant differences between beads injected and control group.

3. The effect of DTH on the initial lung deposition of inhaled ^{198}Au -colloid (Experiment 2)

In Experiment 2, ^{198}Au -colloid was administered by an inhalation to DTH and beads injected rats which were previously received intratracheal instillation of MBSA coupled Sepharose 4B beads into either left or right lung lobe(s), in order to study the effect of DTH on the initial lung deposition of inhaled particles. As known, the rat has five lung lobes, *i.e.*, left, right apical, right cardiac, right diaphragmatic and right intermediate lobe. In control rats, the inhaled colloidal gold was deposited in each lung lobe at constant ratios (Table 2). The deposition of ^{198}Au in the individual lung lobes of two DTH rats is shown for representative examples in Table 2. The deposition of colloidal gold was much less in the lung

Fig. 5 The clearance of colloidal ^{198}Au from lung in the inhalation study. Left. The ratio of the retention of ^{198}Au in the thoracic regions to that in abdominal regions 3–5 h after the end of inhalation was obtained by external scanning with a collimated detector. Right. The lung retention of ^{198}Au activity was expressed as the percentage of the sum activities of the lung, gastro-intestinal tract and feces on day 2 after the inhalation.

lobes where DTH lesions were induced by the administration of Sepharose 4B beads, in comparison with that in the corresponding lung lobes of control rats. For quantitative analysis, the amount of colloidal gold deposited in the lung lobes of DTH and bead injected rats was expressed as the percentage of the amount in the corresponding lobe of control rats (Table 2). The deposition ratio in beads injected lobe(s) of DTH rats was approximately 55% of that in the corresponding lobe(s) of control rats, while that of beads injected rats was similar to the control.

4. The effect of DTH on early clearance of inhaled ^{198}Au -colloid (Experiment 3)

The results of Experiment 3 are shown in Fig. 5. The ratio of ^{198}Au retention in the thoracic region to that in the abdominal region 3–5 h after the end of inhalation was much higher in DTH rats than that in control or beads injected rats, as shown in the left part of Fig. 5. On the 2nd day after the inhalation when almost mucociliary

clearance was considered to have been completed, the lung retention of ^{198}Au in DTH rats was also much higher than that in the other two groups, when the lung retention was expressed as the percentage of the sum activities of the lung, gastro-intestinal tract and feces (right part of Fig. 5).

IV DISCUSSION

Severe granulomatous delayed type hypersensitivity was induced in the lung of DTH rats by the immunization with MBSA and the subsequent intratracheal challenge with the same antigen insolubilized by the coupling to Sepharose 4B beads. In contrast, only a moderate foreign body type response was developed in beads injected rats. Pulmonary DTH lesions in DTH rats observed by light microscopy appeared very similar to those induced in mice by the same methods.^{9,10} By using these DTH rats as experimental model animals with a respiratory disease, the initial lung

deposition and the subsequent movement of colloidal gold administered by intratracheal instillation or inhalation were examined in three different lines of experiments. The results obtained in the present study might be applied to the risk estimation of radiation hazard from inhaled radioactive particles in the population with lung disorders.

As shown in Fig. 2, when the colloidal gold particles were administered by the intratracheal instillation, there was no significant difference in the lung retention among three groups of rats. It was an unexpected result that the presence of severe granulomatous lesions did not influence the lung retention of ^{198}Au -colloid at all. There is no explanation for this finding at the present. The ratio of ^{198}Au activity of the cell pellet and the supernatant in the lung lavage fluid was also not influenced by DTH or beads instillation. ^{198}Au activity in the lung lavage fluid was present exclusively in the cell pellet in all rats. This fact indicates that colloidal gold existed in the lung as very insoluble particles, and as intracellular particles phagocytized by bronchoalveolar macrophages.

On the other hand, the translocation of colloidal gold from the lung to the regional lymph nodes and the kidney was much enhanced in DTH rats in comparison with that in the other two groups of rats. Although the mechanism of this difference is not fully apparent from the present results, some immunological inflammatory reactions might be involved.¹¹⁻¹³ The severe increment in the number of macrophages might have resulted in the enhancement of phagocytosis and transportation of particulate materials from alveolar space to some other organs, and the increment in the number of lymphocytes probably activated macrophages to enhance phagocytosis and transportation of particles.

Experiment 2 clearly showed that initial deposition of inhaled ^{198}Au -colloid in the DTH lung lobe was much less than that in normal ones. It has been reported that experimentally induced emphysema or cigarette-smoking caused lower initial lung deposition of inhaled particles in rats and beagle dogs.³⁻⁵ Those observations are well consistent with the present result. The reason is not well known why initial lung deposition becomes lower in the lung with obstructive lung disease such as DTH or emphysema. LUNDGREN *et al.*³ speculated that the reason for the lower

deposition of PuO_2 particles in the rat with emphysema centered around the decrease in alveolar wall area, altered breathing pattern and changes in gas mixing in the lung.

It is well known that considerable fraction of inhaled particles is cleared rapidly from the lung to the gastro-intestinal tract in normal animals. Therefore, the ratio of inhaled radioactive particles retained in lung to that cleared to the gastro-intestinal tract can be used as a indicator to estimate the extent of early clearance. A high ratio of ^{198}Au retention in the thoracic region to that in the abdominal region a few hours after the inhalation means that the early clearance is depressed. It is reasonable to conclude that early clearance of ^{198}Au -colloid was depressed in DTH rats. Possible interpretations for these findings might be as follows. The first interpretation is the alternation of the particle deposition pattern in the respiratory tracts of DTH rats as a result of the changes in their respiratory pattern. The fraction of ^{198}Au -colloid deposited in distal lung regions, where inhaled particles could not be removed readily by mucociliary clearance, might be increased in DTH rats. Second, the particles deposited in DTH lesions might escape from mucociliary clearance and stay there for a longer period. Third, the mucociliary movement by which particles are carried to the upper respiratory regions might be itself impaired in DTH rats. If the mucociliary escalator could not well clear the particles deposited in DTH lesions as the second interpretation, or if the mucociliary movement was impaired in DTH lung as the third, the early clearance of ^{198}Au -colloid after intratracheal instillation should be interfered in DTH rats. In the intratracheal injection studies (Exp. 1), there was no significant difference between normal and DTH rats in the early clearance of colloidal gold from the lung. Therefore the first assumption appeared to be more reasonable in the present case. Further experiment would be needed for determining the accurate mechanisms.

V CONCLUSIONS

The lung deposition and clearance of inhaled or instilled colloidal ^{198}Au -particles were studied in normal rats and in rats with pulmonary lesions induced by delayed type hypersensitivity (DTH) reactions. The deposition pattern, early clearance and redistribution of inhaled or instilled ^{198}Au -

particles in the respiratory tract was considerably influenced by the respiratory disease. This suggests that in the practical cases the radiation dose from inhaled radioactive materials and their risks are altered in the population with lung disorders.

REFERENCES

- 1) ICRP Publication 30; Part 1 (Annals of the ICRP vol. 2 No. 3/4), Limits for intakes of radionuclides by workers, Pergamon Press, Oxford (1978).
- 2) ICRP Publication 48; The metabolism of plutonium and related elements, Pergamon Press, Oxford (1986).
- 3) D.L. LUNDGREN, D.L. DAMON, J.H. DIEHL and F.F. HAHN; The deposition, distribution and retention of inhaled $^{239}\text{PuO}_2$ in the lung of rat with pulmonary emphysema, *Health Phys.*, **40**, 231 (1981).
- 4) R.E. FILIPY, L. PAPPIN, D.L. STEVENS and W.J. BAIR; The impairment of pulmonary clearance of $^{239}\text{PuO}_2$ in rats by prolonged exposure to cigarette smoke, Pacific Northwest Laboratory Annual Report for 1980 to the DOE Office of Energy Research, Part 1, PNL 3700, p. 110 (1981).
- 5) R.E. FILIPY, L. PAPPIN, D.L. STEVENS and W.J. BAIR; The effect of cigarette smoke on clearance of $^{239}\text{PuO}_2$ from the lungs of beagle dogs, Pacific Northwest Laboratory Annual Report for 1980 to the DOE Office of Energy Research, Part 1, PNL 3700, p. 107 (1981).
- 6) S. TAKAHASHI, S. ASAHO, Y. KUBOTA, H. SATO and O. MATSUOKA; Distribution of ^{198}Au and ^{133}Ba in thoracic and cervical lymph nodes of the rat following the intratracheal instillation of ^{198}Au -colloid and $^{133}\text{BaSO}_4$, *J. Rad. Res.*, **28**, 227 (1987).
- 7) S. TAKAHASHI and G. PATRICK; Patterns of lymphatic drainage to individual thoracic and cervical lymph nodes in the rat, *Lab. Anim.*, **21**, 31 (1987).
- 8) Y. KUBOTA, Y. YAMADA, S. TAKAHASHI and O. MATSUOKA; Estimation of the amount of material inhaled by rats in the inhalation studies, *Exp. Anim.*, **33**, 535 (1984).
- 9) K. KOBAYASHI, C. ALLRED, S. COHEN and T. YOSHIDA; Role of interleukin 1 in experimental pulmonary granuloma in mice, *J. Immunol.*, **134**, 358 (1985).
- 10) D.O. SLAUSON and M. DAHLSTROM; The pulmonary inflammatory response: cellular events in experimental pulmonary arterial hypersensitivity disease, Inhalation Toxicology Research Institute Annual Report for 1973–1974, p. 206 (1974).
- 11) A.G. HARMSSEN, B.A. MUGGENBURG, M.B. SNIPES and D.E. BICE; The role of macrophages in particle translocation from lung to lymph nodes, *Science*, **230**, 1277 (1985).
- 12) B.E. LEHNERT, Y.E. VALDEZ and C.C. STEWART; Translocation of particles to tracheobronchial lymph nodes after lung deposition: kinetics and particle-cell relationships, *Exp. Lung. Res.*, **10**, 245 (1986).
- 13) A.G. HARMSSEN, N.A. GILLET and B. STEGELMEIR; Translocation of particles by macrophages from lung to lymph nodes of rats, Inhalation Toxicology Research Institute Annual Report for 1985–1986, p. 101 (1986).