DAMAGING EFFECT OF CADMIUM OXIDE DUST TO THE LUNG AND ITS RELATION TO SOLUBILITY OF THE DUST

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The effects of cadmium oxide dusts on the rat lung were studied after exposure of 90 min with its concentration of 19.5 mg/m³, and for the comparison the [similar experiments were carried out on calcium carbonate and polyvinyl chloride dusts with different concentrations.

Alveolar macrophages, protein and phospholipid in the lung washing fluids of rats as well as the lung weights were determined 1 hr and 21 hr after exposure to each dust.

Remarkable points in the results were the decrease in number of macrophages and increase of protein 21 hr after exposure to cadmium oxide dusts. The decrease of macrophages was observed also 1 hr after exposure to cadmium oxide dusts.

Cadmium oxide dusts were dissolved easily in water or Tyrode's solution added with protein or amino acid as in the case of cadmium fume reported previously. Cadmium oxide dusts added in the washing fluid from the lung of rats 21 hr after exposure showed a higher solubility compared with that added in the washing fluid from non-exposed (control) rat lung.

Cadmium which was dissolved from cadmium oxide dusts in albumin or glycine solution showed a strong toxic action on the intraperitoneal macrophages and 3T6 cells.

From these results, decrease of macrophages and increase of protein in the lung washing fluid by exposure to cadmium oxide dusts was assumed to be evoked by the toxic action of cadmium of which solubility was enhanced by the biological medium in the lung containing protein and others.

It has been shown by many investigators^{1,2)} that the acute exposure to cadmium oxide showed damaging effect on the lung of experimental animals. In the present paper, the response of lung of rats to short-term exposure of cadmium oxide dusts was studied on the number of macrophage, protein and phospholipid content in the lung washing fluid as well as the lung weight in comparison with these responses of lung to short-term exposure of polyvinyl chloride dusts, calcium carbonate dusts and cadmium oxide dusts mixed with polyvinyl chloride dusts. In addition, the solubility of cadmium oxide dusts and cell toxicity of dissolved cadmium were also studied.

EXPERIMENTALS AND RESULTS

1. Exposure Experiment

Animal and exposure groups

The animal used in the experiment were Sprague-Dawley male rats weighing between 260 g and 280 g (about 8 weeks old). These rats were bred in the all fresh air conditioned room with $23 \pm 1^\circ\text{C}$ and $50 \pm 5\%$ relative humidity and were given sterilized commercial pellet diet and sterilized tap water freely. Five groups of animals were exposed to dusts of CdO (19.5 mg/m^3), CdO (0.8 mg/m^3) +PVC (4.8 mg/m^3), PVC I (40.7 mg/m^3), PVC II (120.0 mg/m^3) and CaCO_3 (81.2 mg/m^3) respectively. Six rats were used for each group.

Dust particles

(1) *Cadmium oxide.* Cadmium oxide (CdO) was purchased from Kanto Chemical Co. Inc. (Guaranteed Reagent)

(2) *Polyvinyl chloride particles.* Polyvinyl chloride (PVC) particles were produced by emulsion polymerization procedure. (Nippon Carbide Industries Co. Inc.) These particles contained no plasticizer.

(3) *Calcium carbonate.* Calcium carbonate (CaCO_3) was purchased from Wako Pure Chemical Industries Ltd. (Special Grade)

Dusting method of particles

The dustings of the particles of CdO and CdO mixed with PVC were carried out with the drum type feeder. The dustings of the particles of PVC and CaCO_3 were carried out with the centrifugal type dust feeder which was remodeled from an electric

Table 1. Concentration and size of dust particles used in inhalation experiments.

	Mean concentration of dust mg/m^3	Particle size			
		AMD	MMD	CMD	σ_g
Control		—	—	—	—
CdO	19.5	4.0	1.2	0.7	1.5
CdO	0.8	3.8	—	—	2.2
+PVC	4.8				
PVC I	40.7	2.8	2.9	1.0	1.8
PVC II	120.0	3.7	3.8	1.8	1.7
CaCO_3	81.2	2.6	1.6	0.4	1.9

AMD: Aerodynamic median diameter

MMD: Mass median diameter

CMD: Count median diameter

σ_g : Geometric standard deviation

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juicer. The generated dusts were introduced into the exposure system shown in the previous paper.³⁾ Dusts were kept within the mean concentration of $\pm 10\%$. The particles that passed through the exposure chamber were collected by Andersen sampler. The particle size distribution of dusts was estimated by measuring weight of the particles in each stage of Andersen sampler. The concentration and size of dust particles during exposure were shown in Table 1.

Method of exposure

The characteristics of the exposure chamber used in this experiment were described previously.⁴⁾ When the dust concentration reached to the stable state in the mean concentration in Table 1, six rats were charged in the inhalation chamber. Exposure time was ninety minutes. After exposure the rats were kept in an air conditioned room and given again the pellet diet and water until the sacrifice.

Washing procedure of lungs rats

Three rats were killed after 1 hr and the remaining three were also killed 21 hr after the exposure. Rats were lightly anesthetized with ethyl ether and then were sacrificed by cutting the abdominal aorta. The chest cavity was opened and the trachea was ligated to prevent the entry of blood into the lung and then the lung and trachea were removed from the thoracic cavity. A vinyl chloride cannula was introduced into the trachea and a syringe containing a measured volume (5 ml per g lung) of isotonic saline solution was connected to the cannula. The solution was allowed to run into the lung and then the lung was inverted to collect the washed solution. The washing procedure was repeated four times.

Counting of cell number

Cells in aliquot of the washings were counted in hemocytometer and the total number of cells in the washing was calculated.

Protein and phospholipid content in the washing fluid

Washing fluid was centrifuged at 1000 r.p.m. for five minutes and the supernatant was obtained. The protein content in the supernatant was determined by the method of Lowry⁵⁾ and the phospholipid in the supernatant was first extracted by the method of Folch⁶⁾ and then determined by the method of Bettle.⁷⁾

Results

Table 2 shows the weight of the lung, the number of macrophages in the washing fluid, the protein and phospholipid content in the centrifuged washing fluid 1 hr and 21 hr after exposure in each exposure group. The lung weights of CaCO_3 , PVC and CdO (low concentration) mixed with PVC groups showed no significant difference. The weights of the lungs in CdO group (high concentration) increased 21 hr after exposure compared with control group.

The number of macrophages lavaged from the lungs of rats after CdO dust (high concentration) exposure significantly decreased 1 and 21 hr after exposure in comparison

Table 2. The lung weight and the number of macrophages, the protein and phospholipid content in the washing fluid from lungs of rats exposed to various dusts.

hr after exposure	Weight of lung		Macrophage number		Protein content		Phospholipid content	
	1 hr	21 hr	1 hr	21 hr	1 hr	21 hr	1 hr	21 hr
Control	1.19 ±0.02	1.18 ±0.02	2.5 ±0.3	2.5 ±0.7	2.6 ±0.7	3.0 ±1.1	1.02 ±0.45	1.30 ±0.10
CdO (19.5 mg/m ³)	1.23 ±0.11	1.67 ±0.05	0.5 ±0.1	0.8 ±0.8	2.8 ±0.9	17.7 ±2.3	0.95 ±0.09	1.58 ±0.25
CdO (0.8 mg/m ³) +PVC (4.8 mg/m ³)	1.05 ±0.01	1.29 ±0.02	3.3 ±1.0	2.0 ±0.2	2.6 ±0.8	8.2 ±1.1	0.96 ±0.01	2.63 ±0.24
PVC I (40.7 mg/m ³)	1.17 ±0.01	1.17 ±0.01	2.7 ±0.3	2.1 ±0.3	2.6 ±0.7	3.0 ±0.9	0.99 ±0.3	1.01 ±0.1
PVC II (120.0 mg/m ³)	1.35 ±0.06	1.28 ±0.00	2.9 ±0.1	3.1 ±0.8	2.5 ±0.1	2.9 ±0.1	1.39 ±0.4	2.52 ±0.31
CaCO ₃ (81.2 mg/m ³)	1.11 ±0.10	1.08 ±0.04	3.2 ±0.1	3.1 ±0.3	4.1 ±0.7	4.0 ±0.4	1.84 ±0.23	2.58 ±0.63

with that of control rats, but such a change was not observed in the other exposure groups. Many destroyed cells and erythrocytes were observed in the washings from lungs of rats exposed to a high concentration of CdO. The content of protein in CdO and CdO mixed with PVC groups significantly increased 21 hr after exposure. The protein in CaCO₃ and PVC groups was not markedly different from control group.

The phospholipid in CaCO₃ and PVC (high concentration) groups slightly increased 21 hr after exposure. CdO group did not show a noticeable change on phospholipid content 1 and 21 hr after exposure.

II. Dissolution of CdO Dust

Procedure of solubility experiment

Water solution of various concentrations of albumin, glycine, and fetal calf serum were used as solvent. The albumin or glycine concentrations were 0.001, 0.01, 0.1, 0.3, 1, 3 and 10% and fetal calf serum concentrations were 6.25, 12.5, 25, 50 and 100%.

Four milligrams of CdO dusts were suspended in 20 ml of each solution and then the suspension was dispersed for 2 min by ultrasonic vibration (29 kHz, 45 W). These dispersed suspensions were transfused into centrifuge tube and centrifuged at 40,000 r.p.m. for 1 hr to separate the supernatants. The cadmium content of each supernatant was determined by an atomic absorption spectrometer with air acetylene flame. (Hitachi Type 308, two wavelengths). Pulmonary washing fluid was also used as solvent. The total pulmonary washing fluid in each lung was about 25 ml. Four mg of CdO was suspended in 20 ml of pulmonary washing from lungs of control group and CdO (high concentration) group 21 hr after exposure. Here, sodium chloride solution of 0.9% was used for comparison. These 20 ml suspensions were dispersed by ultrasonic vibration for 2 min and 5 ml in each suspension was separated for the determination of

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immediately dissolved Cd. Then 5 ml was separated successively after 2 hr and 24 hr incubation at 37°C.

Procedure for the solubility experiment of CdO dusts by shaking:

Four milligrams of CdO was added into 20 ml of Tyrode's solution and 2% glycine or albumin Tyrode's solution and then each mixture was shaken at 100 times per minute at 25°C instead of ultrasonic vibration. After 1, 2, 4, 6 and 8 hr shaking the suspensions were ultracentrifuged. Cadmium values in each supernatant were determined.

Results.

(1) *Solubility of cadmium in albumin, glycine and fetal calf serum solutions.* The experimental results are shown in Fig. 1. As seen in the figure, dissolution curves of Cd against concentrations of glycine and albumin show different patterns. In the glycine solution, dissolution of Cd rapidly increased from 0.01% and was saturated over 3%. In albumin solution, however, dissolution was very rapid over 1% while it was slow under 1%. In 10% solution of both albumin and glycine, dissolved Cd was about 150 $\mu\text{g/ml}$, which seems very high solubility since the amount of added cadmium was about 175 $\mu\text{g/ml}$. In the case of fetal calf serum the dissolution of cadmium increased with increasing serum concentration to 50% and then was saturated.

Fig. 1. Solubility of cadmium oxide dusts in albumin, glycine or fetal calf serum solution of various concentrations.

(2) *Solubility of CdO in pulmonary washing fluid.* Results were shown in Fig. 2. As shown in the figure, the supernatant from the suspension of CdO in 0.9% sodium chloride solution contained very small amount of cadmium in all incubation times. Cd content in the supernatant of lung washing fluid added with CdO dusts increased with incubation time. When pulmonary washing fluid from lungs of control group was

Fig. 2. Solubilities of cadmium oxide dusts in pulmonary washing fluids of rat and in saline.

- Pulmonary washings from lungs of rats 21 hr after exposure to CdO dusts.
- △——△ Pulmonary washings from lungs of control rats.
- ×---× Saline (0.9% sodium chloride solution)

Fig. 3. Solubility of cadmium oxide dusts in glycine or albumin Tyrode's solution by shaking.

used as solvent, the amount of cadmium in the supernatant was about four times as much as that in 0.9% sodium chloride solution after 2 hr incubation. In the case of pulmonary washing fluid in CdO exposure, the cadmium was about ten times as much as that in 0.9% sodium chloride solution after the same incubation time. In this case,

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as the washing fluid from lungs of CdO group contained $0.11\text{ }\mu\text{g}$ per ml of cadmium, this value was deducted from the dissolved cadmium.

(3) *Solubility of CdO by shaking procedure.* The results were shown in Fig. 3. As shown in the figure, in the case of Tyrode's solution the dissolved cadmium was very small by 8 hr shaking. The dissolution of cadmium in 2% albumin Tyrode's solution increased with shaking time to 4 hr and it was saturated by 6 hr shaking. When glycine Tyrode's solution was used as solvent, dissolution of cadmium dusts increased rapidly with shaking time till 1 hr and then was saturated.

The amount of dissolved cadmium, when 2% glycine Tyrode's solution containing CdO dusts was shaken until saturation, was about $145\text{ }\mu\text{g/ml}$ as in Fig. 3 and the cadmium dissolved in glycine water solution with 2% glycine by ultrasonic vibration was about $150\text{ }\mu\text{g/ml}$ as Fig. 1. That is, almost equal value of dissolved cadmium was obtained by either shaking and ultrasonic vibration. This relation was also observed between albumin Tyrode's solution by shaking and albumin water solution by ultrasonic vibration.

III. Cell Toxicity of Dissolved Cadmium

Procedure of cell toxicity experiment

Macrophages. The macrophages were obtained from intraperitoneal exudate induced in rats by intraperitoneal injection of sterile Tyrode's solution containing 0.001% glycogen and then the cells were washed twice by Tyrode's solution. The cell were suspended in Tyrode's solution in the concentration of 1×10^7 cells/ml. The macrophages were cultivated in Tyrode's solution containing 20% fetal calf serum.

3T6 fibroblast. The mouse embryo fibroblast of line 3T6 was cultivated in Dulbecco-Vogt medium supplemented with 10% fetal calf serum.

The supernatants containing $10\text{ }\mu\text{g}$ or $5\text{ }\mu\text{g}$ dissolved cadmium in 2% albumin or glycine solution were added to macrophages (5×10^6 cells) or 3T6 cells (2×10^5 cells) cultures and there were cultivated for 2 days in humidified CO_2 incubator. After Cultivation these cultures were observed microscopically.

Results

The macrophages or 3T6 fibroblast were observed under microscope to be damaged completely when they were added with $10\text{ }\mu\text{g}$ dissolved cadmium but the damage appeared moderately by the addition of $5\text{ }\mu\text{g}$.

DISCUSSION

The lungs of rats exposed to the high concentration of CdO dusts showed a slight increase of their weight 21 hr after exposure, and the washing fluids from these lungs showed a decrease of macrophages and increase of protein 21 hr after exposure.

There was no significant change of phospholipid in the lung washing 1 and 21 hr after CdO dusts exposure. In the PVC exposure groups, there was no noticeable change except increase of phospholipid content in the lung washing fluid of rats exposed to the high concentration. In the CaCO_3 exposure group, increase of phospholipid was remarkable. In the CdO with PVC exposure group, increase of protein and phospholipid were observed 21 hr after exposure.

Among these obtained results, the decrease of macrophages and increase of protein by exposure to a high concentration of CdO dusts were predominant changes.

There may be some question that protein increase was due to the bleeding from bleeding flecks on the lung surface observed makroskopically. But the protein content in washing fluid was about ten times as much as the plasma protein value corresponding the number of erythrocyte. Therefore, increase of protein seems to be due to exudate from circulatory system which was attacked by dissolved cadmium. These two effects evoked by CdO dusts suggest that CdO dust has a toxic action to the macrophages and some irritative action to the circulatory system. It might be reasonable to assume that these actions were produced not by CdO dust itself but rather by dissolved cadmium. Dissolution of CdO dust in the lung was assumed not to be slow since it was observed that macrophages decreased 1 hour after the exposure. However, it has been recognized that water solubility of CdO dust is very slight. Concerning this point, the present experiment showed that CdO dust was easily dissolved in albumin or glycine solution and further that it was dissolved more easily in the lung washing fluid from non exposed rats than in the sodium chloride solution.

Higher dissolution of CdO dust in the lung washing fluid from animal exposed to CdO dust compared with that from non exposed animals may be explained by the difference of protein content in these fluids. In the former, protein was 0.07% and 0.014% in the latter.

Concerning the cell toxicity of dissolved cadmium, it was found in the present study that cadmium dissolved from CdO dust in albumin or glycine Tyrode's solution damaged the rat peritoneal macrophages and 3T6 fibroblasts. In the previous study,⁸⁾ Cd fume or CdO dust to the intraperitoneal macrophages was shown to have a stronger toxic action in Tyrode's solution containing 20% calf serum than in only Tyrode's solution.

Rhoades⁹⁾ already presented the information that the lung washings of rats exposed to carbon dusts for 16 days showed a significant increase of phospholipid and lecithin which relate to pulmonary surface properties. In this study, phospholipid content in the lung washing showed high values 21 hr after exposure in every exposure group of CdO with PVC, PVC II (high concentration) and CaCO_3 , but clear increase of phospholipid was not seen in CdO dust and PVC I (low concentration) exposure group. For the reasoning of these results, we must wait for future study.

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REFERENCES

- 1) Prodan, L. (1932). *J. Ind. Hyg.*, **14**, 174.
- 2) Paterson, J.C. (1947). *J. Ind. Hyg. Toxicol.*, **29**, 294.
- 3) Koshi, K., Homma, K. and Sakabe, H. (1975). *Ind. Health*, **13**, 37.
- 4) Homma, K. (1975). *Jap. J. Hyg.*, **30**, 323. (in Japanese).
- 5) Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). *J. Biol. Chem.*, **193**, 265.
- 6) Folch, J., Lees, M. and Sloane Stanley, G.H. (1957). *J. Biol. Chem.*, **226**, 497.
- 7) Bettel, G.R. (1959). *J. Biol. Chem.*, **234**, 466.
- 8) Koshi, K., Homma, K., Kohyama, N. and Sakabe, H. (1975). *Ind. Health*, **13**, 253.
- 9) Rhoades, R.A. (1972). *Life Sci.*, Part I, **11**, 33.