Lung Lesions Induced by Intratracheal Instillation of Nickel Fumes and Nickeloxide Powder in Rats

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Abstract: Acute and subacute lung toxicity of nickel fumes was examined by single and repeated intratracheal instillation of nickel fumes and Ni_2O_3 and NiO powders in the rat. LD_{50} of nickel fumes was estimated as 38.2 mg/kg body weight (b.w.) according to the method of Litchfield and Wilcoxon. Body weight gain was retarded as in the order of a single dose of 13.0 mg Ni_2O_3 /kg > 14.3 mg nickel fumes/kg > 1.4 mg Ni_2O_3 /kg > 13.0 mg NiO/kg b.w. compared to controls. The histopathological changes in the lungs of the 14.3 mg nickel fumes/kg-dosed rats were milder than those induced by administration of 13.0 mg Ni_2O_3 /kg but severer than those induced by administration of 1.4 mg Ni_2O_3 /kg b.w. A single administration of NiO powder did not produce any histopathological effects on the lungs. The repeated administration of nickel fumes produced persistent edema and proteinosis in the alveoli. The nickel fumes, which were chemically composed of 97% of NiO and 3% of Ni_2O_3 , were very fine particles about 5–10 nm in diameter, partly aggregated into larger particles and spherical particles about 0.6 μm in diameter. Solubility in distilled water and saline was in the order of nickel fumes > Ni_2O_3 powder >> NiO powder. It was suggested that a toxic Ni_2O_3 component and very fine particles of nickel fumes are involved in the acute lung toxicity of nickel fumes. The epithelial injury induced by reactive oxygen and hydroxy radicals, which would be produced during the process of conversion of Ni(III) to Ni(II) and phagocytosis of nickel fumes by macrophages and polymorphonuclear cells, are presumed to be involved in the pathogenesis of nickel fumes-induced lung lesion.

Key words: Nickel toxicity, Ni(II)oxide, Ni(III)oxide, Nickel fumes, Dust, Intratracheal instillation, Lung lesions, Histopathological changes

Introduction

High nickel alloy has been widely used in industry, because of its excellent heat resistance and anticorrosion properties¹⁾. Many concerns have been raised concerning the health effects of nickel and its compounds to which workers are exposed in various working environments such as smelting and refining of nickel mat, in nickel foundries, welding, electroplating and Ni-Cd batteries. Different forms of nickel such as metallic nickel, Ni(III)oxide and Ni(II)oxide, di- and tri-valent ions and nickel salts have been reported to

adversely affect workers' health through different routes of entry into the body. Occupational inhalation exposure to nickel fumes has been reported to cause lung impairments²⁾ including asthma³⁾, pulmonary fibrosis⁴⁾ and pneumoconiosis⁵⁾ as well as carcinogenicity⁶⁾. Inflammatory changes⁷⁾ and asthma-like lung diseases⁸⁾ are common characteristics among humans acutely exposed to nickel dust. Acute or subchronic inhalation exposure of experimental animals to nickel dust has been demonstrated to cause histopathological changes in phagocytosis and size distribution of the alveolar macrophages in the rabbit⁹⁾, an increase in the size of alveolar type II cells and foamy macrophages in the rabbit¹⁰⁾, increased lung weight with

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minimal inflammatory reaction in the hamster¹¹⁾, development of pulmonary fibrosis in the mouse¹²⁾ and alveolar lipoproteinosis in the rat¹³⁾. Inhalation exposure of animals to Ni₂O₃ powder caused edema in the alveolar septa and lumen¹⁴⁾. Intratracheal instillation of particulate materials allows precise control of the dose to be administered to the animal's lung, leading to precise assessment of the dose-response or dose-effect relationship for the particulate materials, in comparison to inhalation exposure.

The present study was undertaken to evaluate the lung toxicity of nickel fumes in intratracheal administration in the rat. Nickel fumes which are generated at a high temperature are thought to be composed of various oxides. We also determined the amounts of NiO and Ni₂O₃ in nickel fumes by polarography, in order to find out what kinds of the oxidized components contribute to the lung toxicity of nickel fumes. Both Ni₂O₃ and NiO powders were also administered intratracheally to compare the lung toxicity of nickel fumes and their principal oxidized components. The following items were examined: mortality, growth rates and gross and histopathological changes in the lungs.

Materials and Methods

Animals

Male rats of the Sprague-Dawley strain (Crj:CD) were purchased from Charles River Japan at the age of 4 weeks and housed in wire-meshed cages (5 rats/cage) in an animal colony. The animal room was maintained at a temperature of $24 \pm 1^\circ\text{C}$, a relative humidity of $55 \pm 5\%$ and an automatic lighting schedule of a 12-hr light (8:00–20:00)/12-hr dark (20:00–8:00) cycle. The rats were allowed free access to autoclaved food pellets and tap water sterilized by ultraviolet irradiation.

Preparation of nickel fumes and nickel oxide powders instilled

Three kinds of particulate materials, nickel fumes, Ni₂O₃ and NiO powders were used in the present study. Nickel fumes was generated by feeding metallic nickel powder (56F, Nickel Powder Metco, USA) into a plasma flame metal sprayer^{15, 16)} with which molten nickel metal was released into an aerosol chamber. The fumes generated were collected on the surface of glass beads by means of a granular bed type fume collector. The nickel fumes collected were separated from the glass beads with a 250 mesh sieve. Ni₂O₃ and NiO powders were purchased from Kosou Chemicals (G2B081, G3H22906, respectively, Japan) and their nominal

particle sizes were reported as $2.0 \mu\text{m} \pm 1.69$ (geometric mean $\pm$ geometric SD) and $2.2 \mu\text{m} \pm 1.68$, respectively, according to the manufacturer. Each type of nickel fumes, Ni₂O₃ and NiO powders, was dispersed in a sterilized physiological saline, and sonicated for 15 min immediately before intratracheal instillation.

Characterization of the nickel fumes and nickel oxide powders

Size distributions of nickel fumes, Ni₂O₃ and NiO powders, were determined with a transmission electron microscope (H-8000, Hitachi Co., Japan). The particulates were dispersed in distilled water and sonicated for 30 sec. The particulate suspensions of 1 μl each were placed on an EM grid with carbon enforced collodion film. Transmission electron microscope (TEM) observations were performed to measure particulate sizes at various magnifications. For fine particles, the photograph was taken at a magnitude of $\times 100,000$, and for agglomerated particles, at $\times 600$. These TEM negative films were optically enlarged 5–10 times on the prints.

Amounts of NiO and Ni₂O₃ components in nickel fumes were determined with a PAR174A polarographic analyzer equipped with a model 303 dropping mercury electrode (Princeton Applied Research Co., U.S.A.). Polarographic analysis was done by the method previously reported¹⁷⁾.

Administration of fumes and powder

Each 8-week-old rat was anesthetized with ether. Under direct observation of the larynx with a dissecting microscope, the rat received intratracheal intubation to the region near the carina. The particulate suspension of 0.5 ml followed by injection of 0.5 ml expired gas was instilled out into the lungs in the inspiration phase. A different group of rats received intratracheal instillation of the vehicle solution in the same manner and served as a control. To determine the LD₅₀ of the nickel fumes, amounts of particulates ranging between 0.5 and 120 mg per animal were administered into the trachea. The doses of nickel fumes and nickeloxide powder and the numbers of rats used are shown in Table 1. A single instillation of 3.8 mg nickel fumes/kg was equivalent to the minimum lethal dose which was determined by the dose-lethality curve for nickel fumes.

In order to find out what kinds of the oxidized components contribute to the lung toxicity of nickel fumes, other groups of rats were given intratracheal instillation of Ni₂O₃ powder suspension at single doses of 1.4 and 13.0 mg/kg b.w. and NiO powder suspension at a single dose of 13.0 mg/kg b.w.

Table 1. Amounts of nickel fumes and nickeloxide powder instilled intratracheally into the lungs, and the number of rats used

Administrations	Substances	Doses (mg/kg)	No. of rats used
Single instillation	Nickel Fume	3.8	30
		14.3	25
	Ni ₂ O ₃	1.4	27
		13.0	23
	NiO	13.0	23
Repeated instillations	Control	0	15
	Nickel Fumes	5.9 × 4	31
		total 20.0	
		(once every two weeks)	
		0	15

Histopathological preparations of the lung

Five rats each were sacrificed for histopathological examination of the lungs and major visceral organs on Day 4 after instillation and Weeks 1, 2, 4 and 8 after the final instillation. Each animal was anesthetized with pentobarbital sodium (60 mg/kg, i.p.) and then bled to death by cutting the abdominal aorta. Before open thoracotomy, 1.5 ml of 10% phosphate-buffered formalin solution were instilled into the lung, and then the trachea was ligated. After gross observation, the lungs and major visceral organs were fixed in the same fixative for 2–3 days. About 3 mm thick slices of the right lung were cut along the longitudinal axis of the main bronchus and other slices were cut perpendicular to the bronchial axis. These slices were dehydrated and embedded in paraffin. The paraffin sections, 5 μ m thick, were stained with hematoxylin and eosin (H & E). Masson Trichrome stain and Periodic Acid Schiff stain were also used when needed.

Histopathological evaluation of the affected lungs

Histopathological changes in the lungs of the rats to which nickel fumes, Ni₂O₃ and NiO powders were administered were examined under a microscope to check for the following 9 items: 1) Cloudy swelling and mucoid degeneration, 2) Regeneration of the broncho-bronchiolar epithelium, 3) Reactive hyperplasia, 4) Exudation and accumulation of inflammatory cells and mobilization and destruction of macrophages, 5) Foreign-body granuloma, 6) Increased number and swelling of fibroblast cells, 7) thickening of the alveolar wall and ducts due to increased collagenous materials, 8) Proteinosis and 9) Edema. Each of the above mentioned histopathological changes was scored on a scale of increasing severity from 0 to 4: 0 indicates no change or the same changes as those observed in the lungs of control

rats given physiological saline. 1 indicates very slight or sporadic changes observed in a small, limited area. 2 indicates slight but unambiguous changes observed in the same area. 3 indicates moderate changes in the lesion restricted to an area smaller than 1/3 of the total area of the lobe. 4 indicates marked changes in a lung lesion that extended over an area larger than 1/3 of the lobe.

Solubility test

Ten milligrams of each of the three particulates, nickel fumes, Ni₂O₃ and NiO powders, were suspended in distilled water or saline of 10 ml, and then sonicated for 15 min. After filtration of the suspension with a millipore filter (0.3 μ m in pore diameter), the amount of nickel dissolved in water or saline was determined with a flameless atomic absorption spectrometer (Perkin Elmer Model Zeeman/5000 System, U.S.A.).

Results*Characterization of nickel fumes and nickel oxide powders*

Nickel fumes were composed mainly of very fine particles about 10 nm in diameter, partially agglomerated particles and spherical particles about $0.6 \pm 2.1 \mu$ m (geometric mean $\pm$ geometric SD) in diameter. NiO powder particles were $1.7 \pm 2.1 \mu$ m in diameter. The size distribution of Ni₂O₃ powder was similar to that of fine particles in nickel fumes.

The polarographic determination revealed that nickel fumes were chemically composed of two oxidized forms; 1.2–3.2% of Ni₂O₃ and 98.8–96.8% of NiO.

Mortality

Table 2 shows the relationship between a single dose of

Table 2. Mortality after intratracheal instillation of nickel fumes into Sprague-Dawley rats at different doses

Dose (mg/rat)	0.5	2	8	30	60	120
No. of rats instilled	7	7	7	9	7	6
No. of rats dead	0	1	3	7	7	6
Mortality %	0	14	43	78	100	100
Days survived after the instillation		3	3	2*	2	1

2*: Six out of 7 died on day 2, and another died on day 9.

nickel fumes intratracheally instilled and the mortality of the rats. Death due to toxic insult was observed during the 10 days after the intratracheal instillation. Death was caused by respiratory failure resulting from edema, hemorrhage and desquamation of the bronchiolar epithelium. A value of LD_{50} for nickel fumes was estimated to be 38.2 mg nickel fumes/kg b.w. according to the method of Litchfield and Wilcoxon¹⁸⁾. The instillation of nickel fumes at single doses of 3.8 and 14.3 mg/kg b.w., which corresponded to LD_6 and LD_{27} , respectively, actually caused 0% and 4% mortality, respectively. No death was observed during the 8 weeks after repeated instillations of nickel fumes at doses of 5.9 mg/kg once every 2 weeks (a total dose of 20 mg/kg b.w.). Sixty percent of the 23 rats instilled with a single dose of 13.0 mg Ni_2O_3 /kg died within 5 days and their death was caused by edema. A single instillation of either 1.4 mg Ni_2O_3 /kg or 13.0 mg NiO/kg b.w. caused no death.

Growth rate

Figure 1 shows the growth rates of the rats given single doses of 3.8 and 14.3 mg nickel fumes/kg, 13.0 mg and 1.4 mg Ni_2O_3 /kg and 13.0 mg NiO/kg b.w., in comparison with the growth rate of the rats given saline as the control. The administration of 14.3 mg nickel fumes/kg decreased the body weight for the first several days and the growth rate tended to be retarded thereafter, but there was no essential difference between the 3.8 mg nickel fumes/kg-instilled rats and the control in body weight. The rats given a single dose of 13.0 mg Ni_2O_3 /kg exhibited a pronounced decrease in body weight throughout the observation period of 7 days. Retardation of the growth rate of the 1.4 mg Ni_2O_3 /kg-instilled rats was similar to that of the 14.3 mg nickel fumes/kg-instilled rats. A single instillation of 13.0 mg NiO/kg did not produce any decrease in the growth rate throughout the observation period of 15 days. As shown in Figure 2, there was a slight but insignificant difference in the growth rate between the rats given repeated doses of 5.9 mg nickel fumes/

Fig. 1. Time course changes in growth rates of the rats after single intratracheal instillations of nickel fumes, Ni_2O_3 and NiO powders or physiological saline as the control

The growth rate was expressed as body weight after the instillation as a percentage of that before. The number of rats in each group is indicated in parenthesis.

Fig. 2. Time course changes in the growth rates of rats after repeated intratracheal instillations of nickel fumes or physiological saline as the control

The growth rate was expressed as body weight after the instillation as a percentage of that before. The number of rats in each group is indicated in the parenthesis. The arrows indicate the days when the rats received the instillations.

kg and the control.

Gross findings

On Days 4 to 7 after the instillation at a single dose of 14.3 mg nickel fumes/kg, the lungs of the rats were characterized by brown induration of the pulmonary hilus. These lesions disappeared by Weeks 4 to 8. The rats given a single dose of 3.8 mg nickel fumes/kg exhibited slighter changes and their lung lesions disappeared after Week 2. Repeated instillations of nickel fumes produced the same degree of lung lesions as observed in the 14.3 mg/kg-administered rats, but these lung lesions persisted for the first 4 weeks after the final administration and tended to be recovered afterward.

The lungs of the 13.0 mg Ni_2O_3 /kg-administered rats which died within 5 days were characterized by consolidation, red coloration, diffuse atelectasis and exudation of serous liquids into the thoracic cavity. The lesions induced by administration of 13.0 mg Ni_2O_3 /kg were far severer than those induced by 14.3 mg nickel fumes/kg-administration. The surviving rats that had received 13.0 mg Ni_2O_3 /kg exhibited marked diffuse hemorrhage and crater-like induration on the surface of the lobes. The 1.4 mg Ni_2O_3 /kg-administered rats exhibited hemorrhagic patches on the entire lobes and edematous swelling on Days 4 to 7. These lesions became slighter on Weeks 4 to 8 than on Days 4 to 7. There were no observable changes in the lungs of the rats given 13.0 mg NiO/kg except for slightly red coloration over the lobes.

Histopathological examinations of the lungs

As shown in the microphotographs (Fig. 3), the lung lesions induced by the single instillation of nickel fumes were characterized by goblet cell hyperplasia, perivascular inflammatory cells and edema in the alveolar space (in A), whereas repeated instillations produced a secretion of proteinaceous materials in the alveolar space (in B). Ni_2O_3 administration produced goblet cell hyperplasia, focal granuloma, inflammatory cells in the alveolar space and thickening of the alveolar wall (in C), but NiO did not produce any apparent histopathological change in the lungs (in D).

Severity scores for the histopathological changes in the lungs of the rats given nickel fumes, Ni_2O_3 and NiO powders are shown in Tables 3 and 4. The single and repeated instillations of nickel fumes were found to produce the following characteristic changes with varying degree of severity (Table 3): Hypersecretion and reactive hyperplasia of the broncho-bronchiolar epithelium as well as the

alveolus was observed on Days 4 to 7 after a single dose of 14.3 mg/kg. Marked mobilization of the macrophages into the alveoli, the destruction of macrophages, granuloma and thickened alveolar septa resulting from infiltration of inflammatory cells were also observed. After 4 to 8 weeks, the lesions became slighter, but the number of foamy, particulate-laden macrophages increased and mild fibrosis developed in the alveolar duct. In comparison to the above mentioned histopathological changes induced by the administration of 14.3 mg/kg, a single instillation of 3.8 mg nickel fumes/kg produced slight changes by Days 4 to 7 and both abundant debris of the dead macrophages and mobilization of the foamy macrophages into the alveoli during Weeks 4 to 8. The repeated administration of nickel fumes at a total dose of 20 mg/kg caused proteinosis, edema and slight fibrosis in the alveoli by Days 4 to 7 after the final instillation. By Weeks 4 to 8, the proteinosis and fibrosis were observed in addition to the development of the above mentioned histopathological changes in the macrophages.

The single instillations of 13.0 and 1.4 mg Ni_2O_3 /kg and 13.0 mg NiO/kg were characterized by the following histopathological changes with various degrees of severity (Table 4): The instillation of 13.0 mg Ni_2O_3 /kg produced edema in the alveolar spaces and around the vessels, necrosis, desquamation and regeneration of the broncho-bronchiolar epithelium and an early stage of thickening in the alveolar ducts and wall. The lesions induced by the 1.4 mg Ni_2O_3 /kg administration were far slighter than those induced by the 13.0 mg/kg administration. The changes in of the lungs observed in Weeks 4 to 8 after the 1.4 mg Ni_2O_3 /kg administration were characterized by mobilization of the alveolar macrophages, an increase in the number of foamy cells, proteinosis in the alveoli and fibrosis in the alveolar ducts and wall. The instillation of 13.0 mg NiO/kg did not produce any histopathological changes except a small number of foamy macrophage cells containing NiO particles and the sporadic appearance of granuloma surrounded by bronchiolar epithelia in 3 out of the 24 instilled rats.

Solubility

Nickel fumes were solubilized into distilled water and saline by 1.8 and 2.1 wt %, respectively. Ni_2O_3 powder was dissolved into distilled water and saline by 1.2 and 0.35 wt %, respectively, but NiO powder was not dissolved in either of the two liquids.

Fig. 3. In A, goblet cell hyperplasia, and perivascular inflammatory cells and edema in the alveolar space in the rat lung on Day 4 after a single instillation of 14.3 mg nickel fumes/kg b.w. In B, exudate of proteinaceous materials in the alveolar space on Day 7 after the 4th instillation of 5.9 mg nickel fumes/kg. In C, goblet cell hyperplasia, inflammatory cells in the alveolar space, and thickening of the alveolar wall and minimal fibrous reaction cells on Day 7 after a single instillation of 1.4 mg Ni_2O_3 /kg. In D, no apparent change in the lungs on Day 7 after a single instillation of 13.0 mg NiO /kg

All staining by H & E, magnification at $\times 33.3$ and the bars = 50 μm .

Table 3. Lung lesions induced by intratracheal instillation of nickel fumes into Sprague-Dawley rats

Site	Intratracheal instillation Dose/body weight Type of lesion	Single 14.3 mg/kg		Repeated 5.9 mg/kg	
		4 d-1 wk	4 wk-8 wk	4 d-1 wk	4 wk-8 wk
Bronchus	Degeneration	0-1	0	0-1	0
	Desquamation/Regeneration	0-1/0	0/0	0/0	0/0
	Reactive hyperplasia	1	0	0	0
	Inflammation	1-2	1	1	1
Bronchiole and Alveolar duct	Degeneration	0-1	0	0	0
	Desquamation/Regeneration	0-1/0	0/0	0-1/0-1	0/0
	Reactive hyperplasia	2	0-1	0	0
	Fibrosis of Alv. duct	0	0	1-2	1-2
	Inflammation	1-2	0	2	2
Alveolus	Mobilization/Destruction of MΦ	4/4	2/2	3/3	3/4
	Increase/Destruction of Foamy cells	2/2	3/2	3/3	4/4
	Reactive hyperplasia	2	0	0	0
	Granuloma/Fibrosis	1-3/1	1/0	2/1	2/0-1
	Thickening/Fibrosis of Alv. wall	2-3/1	2-3/2	4/1-2	4/2
	Edema	2-4	1-2	3	3
	Proteinosis	0	0	2-3	3

0: Negative 1: Very slight 2: Slight 3: Moderate 4: Marked.

Table 4. Lung lesions induced by intratracheal instillation of Ni₂O₃ and NiO power into Sprague-Dawley rats

Site	Intratracheal instillation Dose/body weight Type of lesion	Ni ₂ O ₃		NiO
		13.0 mg/kg 1 wk	1.4 mg/kg 4 wk	13.0 mg/kg 4 wk
Bronchus	Degeneration	4	0	0
	Desquamation/Regeneration	2/2	0/0	0/0
	Hyperplasia	3	0	0
	Inflammation	2	0	0
Bronchiole and Alveolar duct	Degeneration	2	0	0
	Desquamation/Regeneration	2/3	0	0
	Hyperplasia	3	0	0
	Fibrosis of Alv. duct	1	2	0
	Inflammation	2	0	0
Alveolus	Mobilization/Destruction of MΦ	3/3	2/0-1	1/0
	Increase/Destruction of Foamy cells	2/2	2/1-2	0/0
	Hyperplasia	2	0	0
	Granuloma/Fibrosis	3/1	2-3/1	0/0
	Thickening/Fibrosis of Alv. wall	2/1	1/0-1	0/0
	Edema	3	0	0
	Proteinosis	0	1	0

0: Negative 1: Very slight 2: Slight 3: Moderate 4: Marked.

Discussion

In the present study comparison of the lung toxicity of nickel fumes and their principal oxidized components, Ni_2O_3 and NiO powders, was made in terms of growth rate, gross findings in the tissues and histopathological examination of the lungs. The administration of Ni_2O_3 powder produced the least gain in body weight and the severest changes in the lungs as detected by both gross and histopathological observations, followed by nickel fumes. However, the NiO powder produced neither any significant pathological change nor growth rate change.

The histopathological changes resulting from the single instillation of 14.3 mg nickel fumes/kg were milder than those induced by 13.0 mg Ni_2O_3 /kg but severer than those induced by a single instillation of 1.4 mg Ni_2O_3 /kg. In sharp contrast to the significant histopathological changes in the lungs induced by both nickel fumes and Ni_2O_3 powder, a single instillation of NiO powder did not produce any detectable effects on the lungs at least up to 8 weeks. The present findings on the absence of acute and subchronic effects of NiO are consistent with the results reported by Wehner *et al.*⁵⁾ and Dunnick *et al.*¹²⁾ who demonstrated no significant histopathological changes in the lung after exposure of hamsters, rats and mice to the inhalation of NiO particles. It can therefore be inferred that acute or subacute lung toxicity resulting from exposure to nickel fumes is mediated primarily through the Ni_2O_3 component in the nickel fumes rather than NiO.

In the present study, it was found by polarographic analysis that the nickel fumes generated from the plasma flame metal sprayer were chemically composed of two oxidized forms: 1.2–3.2% of Ni_2O_3 and 98.8–96.8% of NiO. Assuming 3% to be the Ni_2O_3 content in nickel fumes, the two oxidized forms of Ni_2O_3 and NiO in the 14.3 mg nickel fumes/kg administration can be estimated to be composed of 0.43 mg Ni_2O_3 /kg and 13.9 mg NiO/kg, respectively. On the basis of the present histopathological findings, it can be noted that the lung toxicity induced by the single instillation of 14.3 mg nickel fumes/kg, which is estimated to contain 0.43 mg Ni_2O_3 /kg, was severer than that induced by the 1.4 mg Ni_2O_3 /kg administration. This means that, in addition to the Ni_2O_3 component, another factor may be involved in the lung pathogenesis of nickel fume toxicity. It was found by TEM observation that the size distribution of Ni_2O_3 powder was similar to that of the fine particles in nickel fumes and that there was a great difference between the size distribution of Ni_2O_3 powder detected in the present TEM observation

($0.42 \pm 2.0 \mu\text{m}$) and the nominal size ($2.0 \pm 1.69 \mu\text{m}$) according to the manufacturer. Nevertheless, the possibility that the size distribution of Ni_2O_3 determined by the TEM observation may not reflect the real size distribution of the powder in the aqueous solution cannot be ruled out. It can therefore be inferred on the basis of the study by Oberdöster *et al.*¹⁹⁾ that the ultrafine particle size as well as the active component of Ni_2O_3 in nickel fumes plays an important role in nickel fumes-induced lung lesions, because the ultrafine particles were agglomerated in nickel fumes. One plausible explanation for the enhanced lung toxicity is that the surface area which interacts with surrounding biomolecules would be much larger for nickel fumes than for NiO powder.

The present study demonstrated that solubility of the three particulates in physiological saline was in the order of nickel fumes > Ni_2O_3 > NiO. The greater solubility of nickel fumes and Ni_2O_3 powder in saline is presumed to be attributable to the larger surface area of nickel fumes and Ni_2O_3 powder, both of which were composed of abundant ultrafine particles. The mechanism by which nickel fumes adversely affect the lung remains unclear, but it can be surmised that the acute and subacute lung toxicity induced by nickel fumes is mediated at least through both very toxic Ni_2O_3 and ultrafine particles. Sawatari²⁰⁾ reported that reactive oxygen and hydroxy radicals were generated during the process of conversion of Ni(III) to Ni(II) ions, when Ni_2O_3 powders were dissolved in an aqueous solution. Kawanishi *et al.*²¹⁾ suggested that the production of these reactive oxygen and hydroxy radicals in the presence of Ni(II) and H_2O_2 damaged biomolecular components such as DNA. It is therefore tempting to speculate that the epithelial injury induced by the reactive species of oxygen and hydroxy radicals, which would be produced by both the process of conversion of Ni(III) and the phagocytosis of nickel fumes, is involved in the pathogenesis of nickel fumes-induced lung lesions. Further study will be clarify the effects of very fine nickel fumes on phagocytic activity.

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