Toxicity of Ultrafine Nickel Particles in Lungs after Intratracheal Instillation

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Abstract: Toxicity of Ultrafine Nickel Particles in Lungs after Intratracheal Instillation: Qunwei ZHANG, et al. Department of Environment Health, Fukui Medical University—To study the lung acute and subacute toxicity of ultrafine nickel particles, rats were intratracheally instilled with 0, 0.1, 0.5, 1 and 5 mg ultrafine nickel (Uf-Ni), respectively. At 3 days after injection, the body weight and wet lung weight were determined. At the same time, bronchoalveolar lavage fluid (BALF) was analyzed for lactate dehydrogenase (LDH), total protein (TP), and total cell and differential cell counts. The results showed that indicators of lung injury and inflammation in BALF were markedly raised with increased Uf-Ni from 0 to 1 mg, and there were no differences in the indices between injection of Uf-Ni at 1 mg and at 5 mg. Rats were intratracheally instilled with 1 mg Uf-Ni, and wet lung weight, and bronchoalveolar lavage fluid (BALF) profiles were analyzed 1, 3, 7, 15 and 30 days later. The effects of Uf-Ni on indices that can be presumed to reflect epithelial injury and permeability (LDH or TP) were dramatically increased from day 1 up to 30 days after injection. Lung histology findings generally confirmed the BALF data, showing severe lung inflammation at 1 day after injection of Uf-Ni, and epithelial hyperplasia and inflammation still present at 30 days after injection. Our findings suggest that Uf-Ni causes persistent inflammation following instillation of a small dose.

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Key words: Ultrafine nickel, Bronchoalveolar lavage, Lung toxicity

The toxicity of metal particles in lung in both occupational and environmental settings is not only related to exposure but also to the size of the particles. For instance, titanium dioxide (TiO2) is classified as a ‘nuisance’ dust and so it is considered to have very little adverse effect on lungs except at high exposure concentration, but there are now reports showing that ultrafine titanium dioxide (Uf-TiO2) particles with a diameter of 20 nm can induce a lung inflammatory reaction to a greater extent than 250 nm TiO2 particles1–6. With the development of science and technology of materials, other ultrafine products are now being produced which may induce new health problems. For example, ultrafine metallic nickel (Uf-Ni) with a mean particle diameter of 20 nm, is now made by the process of vacuum vapor deposition. Its characteristics, including a high level of surface energy, high magnetism and low melting point, are expected to allow its use in magnetic tape, conduction paste, chemical catalysts, and sintering promotion. To date, however, there have been no reports on the toxicity of Uf-Ni in the lungs. The aim of this study was to use the technique of BAL to study the dose response and time course of the effects of Uf-Ni on lungs, and, concomitantly, pathological findings were observed in rats at 1, 3, 7, 15 and 30 days after nickel instillation. The data obtained from a time and dose response study are helpful in predicting the acute or subacute effects of Uf-Ni on lungs.

Materials and Methods

Laboratory animals

Wistar male rats (specific pathogen-free), weighing 180–200 g and at 7–8 weeks of age were supplied by Clear Japan, INC (JCL). The rats were housed in the animal center of Fukui Medical University, Japan. They were fed conventional laboratory diet and had free access to food and tap water.

Particles

Nickel particles with an average diameter of 20 nm, called ultrafine nickel (Uf-Ni) (Ultra fine powder, Lot No. 2237, Inabta and Co, Ltd. Vacuum Metallurgical Co.,
Histopathology was determined by the Wright-Giemsa method. The size distribution of Uf-Ni was detailed previously. The particles were suspended in physiological saline. The concentrations were 0.1, 0.5, 1 and 5 mg/ml. The particle suspensions were ultrasonicated for about 30 min and then sterilized at 0.5 kgf/cm² and 120°C for 15 min.

Treatment

Dose-response: Twenty-seven rats were divided randomly into 5 groups of 5 to 6 rats. The rats were instilled intratracheally with 0.1, 0.5, 1.0 and 5.0 mg of Uf-Ni in suspensions in 1 ml of physiological saline under diethyl ether anaesthesia. Hereinafter the rats injected with physiological saline are referred to as the control group. The rats were killed 3 days after injection.

Time-effect: Thirty rats were divided randomly into five groups of 5 to 7 rats. Each rat was injected intratracheally with 1 mg of Uf-Ni suspended in 1 ml of physiological saline. Hereinafter the rats injected with physiological saline alone are referred to as the control group. Groups of 5–7 rats were killed at 1, 3, 7, 15 and 30 days after injection, respectively.

Bronchoalveolar lavage

The rats were killed by injecting an overdose of phenobarbital solution (50 mg/ml) into the abdominal cavity, and the lungs and trachea were removed en bloc. The wet lung weight was measured after removing the heart and the mediastinal lymphoid and adipose tissue. The lungs: the body weight ratio (lung index) was calculated. Bronchoalveolar lavage (BAL) was accomplished by 4 × 10 ml saline wash at 37°C, with massage. The recovery of bronchoalveolar lavage fluid (BALF) for each rat was measured, and the BALF recovery rate calculated. The BALF was centrifuged (1,500 rpm, 10 min), and the first tubes were used to measure the activity of the LDH and total concentration of protein. The numbers of total cells, macrophages, neutrophils and lymphocytes were evaluated for BALF pooled from each rat.

Biochemical and cytological evaluation of BALF

The LDH activity in the first tube of BALF was measured by with an LDH C II- test kit (Wako Pure Chemical Industries’ Ltd.) by the lactate matrix method. The concentration of total protein in BALF was assessed by the Lowry method. Cells were obtained by centrifugation from all the BALF, and counted in a hemacytometer by the conventional method, and cell differentiation was done by the Wright-Giemsa method.

Histopathology

Animals for histopathological examination were sacrificed as described above. The lungs were removed and fixed with intratracheal instillation of a 10% neutral buffered formalin solution, embedded in paraffin and sectioned at 5 µm; the sections were stained with hematoxylin and eosin, and examined microscopically.

Statistical analysis

Value were expressed as the means and standard errors. Student’s t-test was adopted for statistical testing of differences between means of the effect indices.

Result

Dose-response

1. Rate of recovery of BALF: The rate of recovery of BALF did not differ significantly among the experimental groups and control groups. Data are not shown.

2. Body weight and wet lung weight: The body weight of rats after instillation with Uf-Ni, 0, 0.1, and 0.5 mg decreased on day 1, and then increased from 2 days after instillation. In rats exposed to 1 mg Uf-Ni, body weight was significantly decreased compared to controls from 2 days after instillation (Fig. 1). In rats instilled with 5 mg Uf-Ni, body weight was significant decreased from 1 day after injection.

The absolute and relative wet lung weights of rats instilled with 0.1, 0.5, 1 and 5 mg Uf-Ni were significantly higher than those of controls at 3 days after injection. There was a dose-related increase in the lung wet weight after instillation of various doses of Uf-Ni (Table 1).

3. Cellular and biochemical constituents in BALF: Table 2 shows the results for cellular and biochemical constituents in BALF after instillation of Uf-Ni and other doses of Uf-Ni. Even instillation with 0.1 mg of Uf-Ni caused severe inflammatory response as measured by increases in the total numbers of cells and neutrophils in BALF. There was also a dose-related increase in total cells and neutrophils after instillation with Uf-Ni.

Uf-Ni also caused a marked increase in LDH activity and total protein in BALF (Table 2). There was also a dose-related increase in LDH activity and total protein in BALF.

Time-effect

1. Recovery of BALF: The recovery of BALF did not differ significantly among the experimental groups and control groups. Data are not shown.

2. Lung wet weight: The absolute and relative wet lung weight of rats instilled with 1 mg Uf-Ni were significantly higher than those of controls at 1, 3, 7, 15 and 30 days after injection (Table 3).

3. Cellular and biochemical constituents in BALF: Table 4 shows the cellular and biochemical constituents in BALF at 1, 3, 7, 15 and 30 days after instillation. Uf-Ni injection initially caused a marked increase in the numbers of total cells, macrophages, neutrophils and lymphocytes in BALF. Total numbers of cells and neutrophils in BALF were decreasing within the experimental period, but at 30 days after injection of Uf-Ni, the total numbers of cells were decreasing within the experimental period, but at 30 days after injection of Uf-Ni, the total numbers of cells
Table 2. Cellular and biochemical parameters in BALF at 3 days after injection of Uf-Ni

<table>
<thead>
<tr>
<th>Dose</th>
<th>Total cells (×10⁶)</th>
<th>Macrophages (%)</th>
<th>Neutrophils (%)</th>
<th>Lymphocytes (%)</th>
<th>LDH activity (Wroblewski U)</th>
<th>Total protein (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.1 ± 0.3</td>
<td>97.2 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>21.6 ± 0.9</td>
<td>107.9 ± 10.3</td>
</tr>
<tr>
<td>0.1</td>
<td>9.2 ± 0.4*</td>
<td>67.3 ± 1.9*</td>
<td>29.5 ± 3.5*</td>
<td>2.7 ± 0.6*</td>
<td>33.6 ± 1.3*</td>
<td>361.3 ± 33.9*</td>
</tr>
<tr>
<td>0.5</td>
<td>20.1 ± 2.2*</td>
<td>57.6 ± 3.0*</td>
<td>36.8 ± 4.7*</td>
<td>5.6 ± 0.7*</td>
<td>90.9 ± 4.8*</td>
<td>379.6 ± 9.5*</td>
</tr>
<tr>
<td>1.0</td>
<td>40.0 ± 4.1*</td>
<td>56.7 ± 3.0*</td>
<td>41.2 ± 2.5*</td>
<td>2.1 ± 0.4*</td>
<td>406.5 ± 16.2*</td>
<td>2397.6 ± 238.0*</td>
</tr>
<tr>
<td>5.0</td>
<td>46.2 ± 5.5*</td>
<td>44.1 ± 1.9*</td>
<td>49.4 ± 2.2*</td>
<td>6.4 ± 0.7*</td>
<td>468.0 ± 50.0*</td>
<td>2510.7 ± 212.7*</td>
</tr>
</tbody>
</table>

Note. Values are the mean ± SE of 5–6 rats. *Significantly different from the control group P<0.01.

Table 3. Lung wet weight and relative lung wet weight of rats after instillation with Uf-Ni at various times

<table>
<thead>
<tr>
<th>Days</th>
<th>Groups</th>
<th>Lung wet weight (g)</th>
<th>Relative lung wet weight (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>1.36 ± 0.04</td>
<td>5.44 ± 0.19</td>
</tr>
<tr>
<td>1</td>
<td>Uf-Ni</td>
<td>2.31 ± 0.17*</td>
<td>9.93 ± 0.74*</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>1.49 ± 0.03</td>
<td>5.91 ± 0.14</td>
</tr>
<tr>
<td>3</td>
<td>Uf-Ni</td>
<td>2.09 ± 0.06*</td>
<td>9.70 ± 0.51*</td>
</tr>
<tr>
<td>7</td>
<td>Control</td>
<td>1.48 ± 0.06</td>
<td>5.08 ± 0.20</td>
</tr>
<tr>
<td>7</td>
<td>Uf-Ni</td>
<td>2.51 ± 0.11*</td>
<td>9.32 ± 0.51*</td>
</tr>
<tr>
<td>15</td>
<td>Control</td>
<td>1.72 ± 0.05</td>
<td>5.30 ± 0.13</td>
</tr>
<tr>
<td>15</td>
<td>Uf-Ni</td>
<td>2.35 ± 0.12*</td>
<td>7.55 ± 0.46*</td>
</tr>
<tr>
<td>30</td>
<td>Control</td>
<td>1.86 ± 0.02</td>
<td>4.77 ± 0.27</td>
</tr>
<tr>
<td>30</td>
<td>Uf-Ni</td>
<td>2.50 ± 0.09*</td>
<td>6.58 ± 0.08*</td>
</tr>
</tbody>
</table>

Note. Values are the mean ± SE of 5–6 rats. *Significantly different from the control group P<0.01.

Fig. 1. Body weight of rats after 1 to 3 days after injection of various doses of particles. Note. Values are the mean ± SE of 5–6 rats. *Significantly different from the control group P<0.01.
to 6). By 30 days after instillation, Uf-Ni had induced extensive and severe inflammatory reactions, with accumulations of macrophages and neutrophils in the alveoli. Alveolar walls were infiltrated by enlarged and large macrophages with large nuclei and prominent nucleoli; neutrophils and hyperplastic type II cells were also present in alveolar walls (Fig. 7).

**Discussion**

Nickel and its compounds are known to cause lung inflammation, fibrosis, emphysema, alveolar proteinosis and tumor\(^{10-14}\). The aim of this study was to determine the toxicity of Uf-Ni, one of the new category of ultrafine particles, on the lungs in terms of dose and time response. We investigated the changes in wet lung weight as an index of inflammation, and inflammatory cellular and biochemical changes in BALF at 3 days after instillation of different doses of Uf-Ni. The results clearly demonstrated that the toxic effect Uf-Ni on lungs is dose-responsive. We also investigated changes in wet lung weight, histopathology, and cellular and biochemical changes in BALF up to 30 days after instillation with 1 mg of Uf-Ni. The effects of Uf-Ni on these indices suggested that Uf-Ni induced severe and persistent lung inflammation and epithelial injury up to 30 days after instillation.

Analysis of the cellular and biochemical profile of the BALF after exposure to pulmonary toxins is a useful method for characterizing the inflammatory response of lungs: it can be used to assess lung injury induced by mineral dusts or metallic compounds\(^ {7,15-17}\). Injection of 0.1 mg Uf-Ni, a low dose, produced massive edema, as

### Table 4. Cellular and biochemical parameters in BALF up to 30 days after injection of Uf-Ni

<table>
<thead>
<tr>
<th>Days</th>
<th>Groups</th>
<th>Total cells (×10⁶)</th>
<th>Macrophages (%)</th>
<th>Neutrophils (%)</th>
<th>Lymphocytes (%)</th>
<th>LDH activity (Wroblewski U)</th>
<th>Total protein (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>6.3 ± 0.4</td>
<td>97.5 ± 0.3</td>
<td>1.5 ± 0.4</td>
<td>1.0 ± 0.2</td>
<td>21.6 ± 0.8</td>
<td>107.9 ± 10.3</td>
</tr>
<tr>
<td>1</td>
<td>Uf-Ni</td>
<td>49.0 ± 3.5*</td>
<td>38.6 ± 0.9*</td>
<td>60.0 ± 31.0*</td>
<td>1.4 ± 0.2*</td>
<td>516.6 ± 29.6*</td>
<td>2787.7 ± 64.6*</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>6.1 ± 0.3</td>
<td>97.6 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>21.3 ± 0.9</td>
<td>107.8 ± 10.3</td>
</tr>
<tr>
<td>3</td>
<td>Uf-Ni</td>
<td>40.0 ± 4.1*</td>
<td>56.7 ± 3.0*</td>
<td>41.2 ± 2.5*</td>
<td>2.1 ± 0.4*</td>
<td>406.3 ± 16.2*</td>
<td>2397.6 ± 238.0*</td>
</tr>
<tr>
<td>7</td>
<td>Control</td>
<td>6.5 ± 0.2</td>
<td>97.7 ± 0.4</td>
<td>1.2 ± 0.4</td>
<td>1.1 ± 0.2</td>
<td>21.5 ± 1.1</td>
<td>111.4 ± 3.7</td>
</tr>
<tr>
<td>7</td>
<td>Uf-Ni</td>
<td>31.0 ± 2.0*</td>
<td>67.0 ± 0.6*</td>
<td>29.8 ± 0.5*</td>
<td>3.2 ± 0.4*</td>
<td>343.5 ± 16.2*</td>
<td>2223.6 ± 83.7*</td>
</tr>
<tr>
<td>15</td>
<td>Control</td>
<td>6.1 ± 0.2</td>
<td>97.7 ± 0.4</td>
<td>1.4 ± 0.4</td>
<td>0.9 ± 0.1</td>
<td>21.0 ± 0.9</td>
<td>112.5 ± 3.1</td>
</tr>
<tr>
<td>15</td>
<td>Uf-Ni</td>
<td>23.0 ± 2.3*</td>
<td>71.0 ± 2.0*</td>
<td>25.5 ± 1.8*</td>
<td>3.5 ± 0.4*</td>
<td>260.5 ± 0.9*</td>
<td>2080.5 ± 36.2*</td>
</tr>
<tr>
<td>30</td>
<td>Control</td>
<td>6.0 ± 0.3</td>
<td>97.6 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>21.4 ± 0.5</td>
<td>115.9 ± 7.60</td>
</tr>
<tr>
<td>30</td>
<td>Uf-Ni</td>
<td>17.0 ± 1.5*</td>
<td>76.0 ± 0.6*</td>
<td>14.2 ± 1.3*</td>
<td>9.8 ± 0.8*</td>
<td>210.2 ± 5.6*</td>
<td>2033.2 ± 62.2*</td>
</tr>
</tbody>
</table>

Note. Values are the mean ± SE of 5–6 rats. *Significantly different from the control group P<0.01.

![Fig. 2. Lung section from rats sacrificed at 1 day after instillation with physiological saline. Normal parenchymal structure are seen (400 × original magnification, HE stain).](image)

![Fig. 3. Lung section from rats sacrificed at 1 day after instillation with 1 mg ultrafine nickel. The alveolar spaces are filled with inflammatory cells. More than 50% are neutrophils. The number of cells in alveolar walls is increased (400 × original magnification, HE stain).](image)
shown by increased absolute and relative lung wet weight, together with increases in leukocyte influx. This was accompanied by cell damage, as measured by LDH and protein in BALF. These effects of Uf-Ni were dose-related, and were most severe at doses of 1 and 5 mg of Uf-Ni.

It is generally accepted that the primary role of neutrophils in the lung is a defensive one, but when they are present in large numbers or activated by phagocytes or inflammatory stimuli, they can release chemotactic factors and factors which stimulate fibroblast proliferation. LDH is a cytoplasmic enzyme that is released extracellularly by damaged cells, and therefore the LDH activity in BALF reflects the degree of damage to lung cells and tissue. From our results on the kinetics of total cells, neutrophils, LDH activity and total protein in BALF, it is clear that Uf-Ni is highly and persistently inflammogenic, even at a low mass dose.

Concomitant histopathological studies tended to confirm the BALF studies showing that Uf-Ni causes severe inflammation. At day 1 after instillation with Uf-Ni, the alveolar spaces were filled with cells, more than 50% of which were neutrophils, and this inflammation persisted, with an increase in macrophages and neutrophils up to 30 days after instillation. Continuing epithelial injury...
was indicated by type II cell hyperplasia in the alveoli. Nickel is an established carcinogen and many clinical observations suggest the involvement of chronic inflammation in the development of tumors. This study does not elucidate the mechanism where by Uf-Ni is able to cause inflammation, but our previous studies have suggested that lung inflammation caused by exposure to Uf-Ni was more serious than that caused by standard nickel, and our previous studies also found that some ultrafine particles have free radical activity and they may underlie the effect reported here. Free radicals from the particle surface can cause transcripts of pro-inflammatory gene products via oxidative stress responsive transcript factors such as NF-kB. This could lead to the inflammatory response caused by Uf-Ni, although a direct toxic effect on lung cells is obviously also a factor in leading to inflammation.

In conclusion, our study confirmed that ultrafine particles are highly and persistently inflammatory in the lungs.

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References