Biological effects of nano-nickel in rat lungs after administration by inhalation and by intratracheal instillation.

A Ogami¹ Y Morimoto¹ M Murakami¹ T Myojo¹ T Oyabu² and I Tanaka²

¹ Department of Occupational Pneumology,
² Department of Environmental Health Engineering,
Institute of Industrial Ecological Sciences, University of Occupational and Environmental Health, Japan, 1-1 Iseigaoka Yahatanishiku Kitakyushu, Japan 807-8555

E-mail: gamisan@med.uoeh-u.ac.jp

Abstract. We examined the biological effects of the nickel oxide (NiO) nanoparticles by inhalation and instillation study. Wistar male rats were exposed to NiO nanoparticles (nNiOs) for 4 weeks (6 hrs/day). The nNiOs was black-colored NiO (99.8%) and average particle size (APS) was 20 nm. The geometric mean diameter of the particles in the chamber and the daily average exposure concentration were 139 ± 12 nm and 1.0 ± 0.5 × 10⁵ particles/cc, respectively. The deposited amount of nNiOs in the rat lungs at 4 days after the inhalation exposure ended was 29 ± 4 μg. Although nNiOs exposure group showed temporal significant increase in the number of total cells in bronchoalveolar lavage fluid (BALF) at 4 days after the exposure end, the difference were not seen at one month after an exposure end compared with control group. The histopathological change was not severe just after the inhalation nor throughout the observation time. Elemental mappings of nickel showed that nickel particles were located in agglomeration at the pulmonary macrophages.

1. Introduction

Nanoparticles have specific optical and electric properties. The production of highly functional materials by utilizing such properties has already begun; however, the effects of nanoparticles on humans have not been elucidated. Concerns about the biological effects of these nanoparticles being produced for use in industrial products have arisen since epidemiologic data have shown a correlation between airborne nanoparticles, as typified by PM₂.₅ derived from the combustion of fossil fuel, and cardiovascular diseases[1]. There is a need for inhalation exposure tests that simulate human exposure to nanoparticles to observe their health effects experimentally. In general, nanoparticles have a tendency to agglomerate; therefore, it is necessary to prevent the agglomeration of the nanoparticles while determining the particle size during the inhalation exposure. Nickel compounds are classified as carcinogenic substances (Group 1) in the International Agency for Research on Cancer (IARC) categories. Nickel oxide is a nickel compound with extremely poor solubility that is considered to have high biopersistence in the lung[2]. With its physiological and chemical characteristic usefulness, NiO is preferable referential particle for evaluation of the pulmonary effect of other nanoparticles. In
this study, we evaluated histopathological changes in the lungs of rats following inhalation exposure to nanoparticles. The inhalation experiment was conducted after determining that the generated nanoparticles were dispersed stably in the chamber.

2. Material and Methods

2.1. Nickel oxide particles
The NiO nanoparticles (nNiOs) were black-colored NiO (99.8%) and average particle size (APS) was 20 nm (Nanostructured & Amorphous Materials Inc.).

2.2. Animals
Wistar rats (males, 9 weeks old, 30 animals in each group) had been fed standard diet, and were maintained in an inhalation chamber. All of the experimental procedures were approved by the Experimentation Committee of the University of Occupational and Environmental Health, Japan.

2.3. Inhalation procedure
The generation and inhalation system of nNiOs is described elsewhere[3]. Briefly, the generation system was composed of an ultrasonic nebulizer and diffusion dryers. The particles were generated in the gas phase by spraying suspension. Thirty rats were exposed by inhalation to nNiOs for 4 weeks (6 hrs/day). Control rats were exposed to fresh air only. Ten rats from each group were randomly sacrificed at 4 days, 1 month and 3 months after the 4 weeks exposure. The geometric mean diameter of the particles in the chamber and the daily average exposure concentration were 139 ± 12 nm and 1.0 ± 0.5 × 10^5 particles/cc, respectively (Fig. 1). The deposited amount of nNiOs in the rat lungs at 4 days after the inhalation was 29 ± 4 μg.

![Figure 1. nNiOs particle concentration in chamber after nebulization.](image)

2.4. Bronchioalveolar lavage fluid (BALF) and Histopathological examination
The left lung was clamped and 50 ml of bronchoalveolar lavage fluid (BALF) was collected from the right lung. The number of inflammatory cells in the collected BALF was determined by an automatic cell counter (Celltac MEK 5204 Nihon Koden, Tokyo, JAPAN) and the neutrophil fraction was counted from the cytospin sample stained by Diff-Quik kit (International Reagent Co., Kobe, Japan). After extracting the BALF, the left lung was inflated and fixed by intratracheal infusion with 4 % paraformaldehyde at 25 cm H_2O pressure for one night and then embedded in paraffin. The paraffin sections of 3μm thickness were stained with Hematoxylin and Eosin. After each specimen was HE-stained, 6 random digital images, focusing mainly on the alveoli and excluding large vessels and...
trachea, were taken per lung section with a digital camera (DS-5M, Nikon Instech Co. Ltd., Kanagawa, Japan) under light microscopy. A 300-point grid was placed over each image on the computer screen and we examined pulmonary inflammation in each using the point counting method (PCM) [4].

2.5. Ni mapping of lung tissue by intratracheal instillation
One mg of nNiOs was suspended in each 0.4 ml of physiological saline aliquots, sonicated thoroughly, and intratracheally instilled into the animals (Wistar rats, males, 10 weeks old). At 3 days after instillation, the left lung was inflated and fixed as same procedures as the inhalation study. The paraffin sections of 3μm thickness were stained with HE. The elemental mappings of Ni in the HE-stained lung tissue or pulmonary macrophages on the cytospin samples were analyzed directly by scanning electron microscopy and Energy Dispersive X-Ray Micro Analyzer (HORIBA).

3. Results

3.1. BALF findings and Histopathological findings
Figure 2 shows the representative image of rat lung sections with HE stain at 4 days after nNiOs inhalation. The histopathological change was not severe throughout the observation time.

![Figure 2. Representative image of rat lung sections with HE stain at 4 days after nNiOs inhalation. (x100)](image)

Although nNiOs showed temporal significant increase in the number of total cells in bronchoalveolar lavage fluid (BALF) at 4 days after the exposure end (p< 0.01), the difference were not seen at one month after an exposure end compared with control group (Fig.3). The inflammation areas by PCM are also shown in Figure 3. In nNiOs, inflammation areas significantly increased at 4 days after inhalation period ended. No significant differences in inflammation areas were found at 1 month and 3 months.

3.2. Nickel mapping on lung tissue by intratracheal instillation
Elemental mappings of nickel showed that nickel particles were located in agglomeration at the mild inflammatory lesion or pulmonary macrophages.
4. Discussion

This study was designed to evaluate the biological effects of the nickel oxide (NiO) nanoparticles in an inhalation study. Using the exposure system in this study, we confirmed stable exposure of NiO and the deposited amount of NiO nanoparticles in the rat lungs at 4 days after the inhalation was $29 \pm 4 \mu g$. We also calculated that the biological half-time of NiO nanoparticle from the lungs was 62 days ($r^2 = 0.90$) [3].

The distribution and location of inhaled nanoparticles are important for evaluation of clearance. We tried to visualize the location of particles in the alveoli after entering the airway. Using the scanning electron microscopy and Energy Dispersive X-Ray Micro Analyzer, we are able to map the nickel elements directly on the lung tissue. Although single nanoparticle was unable to detect for its sensitivity, the mapping of Ni element on the lung tissue after intratracheal instillation of nNiOs showed the most of the particles were located in the alveolar macrophage as an aggregated form.

Figure 3. Total cell count in BALF (left) and pulmonary inflammation (right) after inhalation of nNiOs.

Figure 4. Nickel mapping image on the lung tissue after intratracheal instillation of nNiOs. Black dots are nickel-positive sites by scanning electron microscopy and Energy Dispersive X-Ray Micro Analyzer.
Histopathological findings in the nNiOs group and evaluation by point counting method (PCM) showed slight temporal inflammatory features and the temporal increase pattern of inflammation rate in the lung at 4 days. This temporal inflammatory trend was also observed in the increase of total cell numbers in bronchoalveolar lavage fluid (BALF) after inhalation. These inflammatory responses were very low compared with the other inhalation studies on particles with micrometer size. This minimum or mild histopathologic change observed in the lungs was consistent with the findings that initial deposited amounts of nanoparticles in the lungs were small and the clearance from the lungs was not impaired. The slight inflammation seen in this study in spite of the small deposition and normal clearance may be due to the toxicity of NiO.

In conclusion, we exposed rats to NiO nanoparticles by inhalation at a stable particle size and concentration by using an ultrasonic nebulizer for 4 wk. Although NiO exposure group showed temporal significant increase in the number of total cells in bronchoalveolar lavage fluid (BALF) at 4 days after the exposure end, the difference were not seen at one month after an exposure end compared with control group. The histopathological change was not severe just after the inhalation nor throughout the observation time. Elemental mappings of nickel following the intratracheal instillation showed that nickel particles were located in agglomeration at the pulmonary macrophages.

Acknowledgments
This study was supported by a Grant of the New Energy and Industrial Technology Development Organization (NEDO) of Japan.

References
[1] Brunekreef B and Forsberg B 2005 Epidemiological evidence of effects of coarse airborne particles on health  *Eur Respir J* 26 309-318
[2] NTP Toxicology and Carcinogenesis Studies of Nickel Oxide (CAS No. 1313-99-1) in F344 Rats and B6C3F1 Mice (Inhalation Studies), 1996.
[3] Oyabu T, Yamato H, Ogami A, Morimoto Y, Akiyama I, Ishimatsu S, Hori H, and Tanaka I 2006 The effect of lung burden on biopersistence and pulmonary effects in rats exposed to potassium octatitanate whiskers by intratracheal instillation  *J Occup Health* 48 44-48
[4] Ogami A, Morimoto Y, Yamato H, Oyabu T, Kajiwara T, and Tanaka I 2004 Patterns of histopathological change determined by the point counting method and its application for the hazard assessment of respirable dust  *Inhal Toxicol* 16 793-800