**Biodistribution of gold nanoparticles in mice and investigation of their possible translocation by nerve uptake around the alveolus**

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**ABSTRACT** — The effect of nanoparticles in the environment on our health is a cause of concern. The greatest concern with respect to the biological effect of nanoparticles is that they remain in the body and invade tissues, overcoming the protective mechanisms of the body. It is generally believed that nanoparticles invading a living body move into the blood and are carried by the bloodstream to all organs. However, some studies have shown that the inhaled nanoparticles directly translocate to the central nervous system by nerve uptake. Here quantification of the amount of migration of nanoparticles to organs in short time spans (1, 3, and 6 hr) was attempted by animal experiments. Furthermore, the possibility of migration of nanoparticles through the nerves that project around the alveolus, including the nodose ganglion and dorsal root ganglion (DRG), was investigated. Gold (Au) nanoparticles (15 nm) were administered to mice by intratracheal instillation and tail vein injection. After tail vein injection, most nanoparticles were distributed in the liver. After intratracheal instillation, approximately 80% of detected nanoparticles remained in the lungs at 1 hr and were believed to be translocated to digestive organs, including the stomach and intestine, at 3 and 6 hr. With respect to quantification in ganglia, the levels in most samples were lower than the limit of quantification of inductively coupled plasma mass spectrometry (ICP-MS). However, Au nanoparticles were detected in DRG in only some samples of intratracheal instillation. Therefore, this suggests the possibility of translocation of nanoparticles to DRG via nerves.

**Key words:** Nanoparticles, Gold, Biodistribution, ICP-MS, Nerve uptake

**INTRODUCTION**

Nanotechnology has increasingly developed in recent years, and much further use of nanomaterials in various fields is expected. However, the effect of nanoparticles in the environment on our health is a cause of concern. Although the mass of nanoparticles is much smaller than that of fine particles, the number of particles and surface area per mass is markedly greater. Therefore, it is of concern that a living body may be affected by the small size, concentration, surface area, and some physicochemical characteristics unique to nanoparticles. The greatest concern with respect to the biological effect of nanoparticles is that they remain in the body and invade the tissues, overcoming the protective mechanisms of the body. Geiser et al. (2005) exposed pulmonary macrophages to fluorescent polystyrene microspheres (1, 0.2, and 0.078 μm) and assessed particle uptake by confocal laser scanning microscopy under the condition of with and without inhibitor. They showed that treatment with phagocytosis inhibitor inhibits the uptake of 1 μm particles, but not uptake of the smaller particles.

Several *in vivo* studies have reported on the toxicity caused by nanoparticles that invade the living body. Wang et al. (2007) investigated the acute toxicity of 25, 80 nm and fine TiO₂ particles after a single oral exposure. From the changes of biochemical parameters, they demonstrated that TiO₂ particles induced the significant lesions of liver and kidneys in female mice, and from the histopathological examination, the obvious hepatic injury and renal lesion were observed. Chen et al. (2006) investigated the toxicity of copper nanoparticles with microcopper particles and cupric ions after oral administration. LD₅₀ determined for 23.5 nm, 17 μm copper particles and cupric ions are 413, > 5000 and 110 mg/kg body weight, respectively. Pathological changes and grave injuries on
kidney, liver and spleen were observed in mice exposed to 23.5 nm nano-copper particles. Khandoga et al. (2004) show that ultrafine carbon particles (14 nm) induce platelet accumulation in the hepatic microvasculature of healthy mice after intra-arterial administration.

It is generally thought that nanoparticles invading the living body move into the blood and are mainly carried by the bloodstream to the all organs of the body (Hagens et al., 2007). However, some studies have shown that the inhaled nanoparticles directly translocate to the central nervous system by nerve uptake. In one study, rats were exposed to manganese oxide UFPs (30 nm) with either both nostrils patent or the right nostril occluded (Elder et al., 2006). It was concluded that the olfactory neuronal pathway is efficient for translocating inhaled manganese oxide as solid ultrafine particles to the central nervous system. In another study, mice were exposed to diesel exhaust particles and metal concentrations in the olfactory system via nerves located around the alveolus. There- deposit in the alveolus are transferred to the central nerv- ous system by nerve uptake. In one study, rats were exposed to manganese oxide UFPs (30 nm) with either both nostrils patent or the right nostril occluded (Elder et al., 2006). It was concluded that the olfactory neuronal pathway is efficient for translocating inhaled manganese oxide as solid ultrafine particles to the central nervous system. In another study, mice were exposed to diesel exhaust particles and metal concentrations in the olfactory system via nerves located around the alveolus. Therefore, in this study, quantification of the amount of migration of nanoparticles to organs throughout the body after lung deposition was attempted.

Nanosized particles, particularly those approximately 20 nm in size, reach the deeper parts of the lung and get deposited (ICRP, 1994). It is possible that particles that deposit in the alveoli are transferred to the central nervous system via nerves located around the alveolus. Therefore, in this study, quantification of the amount of migration of nanoparticles to organs throughout the body after lung deposition was attempted.

In the test guidelines of the Organization for Economic Cooperation and Development (OECD, 2009), which is accepted internationally as a hazard assessment method, inhalation exposure test is defined as a test method for determining the toxicity of inhalable test substance. However, in the inhalation exposure, it is difficult to evaluate the exposure amount correctly and it takes much time to expose a detectable amount of particles in case of nanoparticles.

Therefore, in this study, as a method to follow the behavior of the particles in a short time after lung deposition, nanoparticle suspension was administered by intratracheal instillation, and migration amount of the particles was evaluated. Furthermore, it was also evaluated that migrating of particles after blood invasion by tail vein administration. In addition, the possibility of migration of the particles through nerves projecting around the alveolus, such as the nodose ganglion and dorsal root ganglion (DRG), was studied.

**MATERIALS AND METHODS**

**Reagents**

Gold (Au) nanoparticles (BBI Solutions, Cardiff, UK) were used as the administered particles. The particle size was 15 nm and the concentration was \(1.4 \times 10^{12}\) particles/mL. \(\text{HNO}_3\) for analysis of poisonous metal grade (Wako Pure Chemical Industries, Osaka, Japan) was used for the preparation of the samples for inductively coupled plasma mass spectrometry (ICP-MS) analysis. Indium standard solution (Wako Pure Chemical Industries) was used as the internal standard.

**Animals**

Seven- to nine-week-old male C57BL/6 mice (20.1-25.3 g) were purchased from Shimizu Laboratory Supplies Co., Ltd (Kyoto, Japan). The mice were provided a commercial diet and water *ad libitum* under controlled conditions (temperature: 22.5 ± 2°C; humidity: 55 ± 5%; lighting: 12-hr light/dark cycle).

**Experimental design**

The mice were divided into the following groups: intravenous injection group \((n = 6\) for each time span), intratracheal instillation group \((n = 5-6\) for each time span), and control groups for both administration methods \((n = 3\) for each time span). The time spans until dissection following each administration were 1, 3, and 6 hr. The mice in the exposure groups were administered 50 \(\mu\)L of Au nanoparticle suspensions, and those in control group were treated with 50 \(\mu\)L of normal saline. In intratracheal instillation, the mice were anesthetized with Nembutal (300-350 \(\mu\)L, 5 mg/mL) before administration and before dissection. After collecting approximately 300 \(\mu\)L of blood by cardiac puncture, perfusion was performed with 0.2% heparin-containing normal saline for 10 min at a flow rate of 3 mL/min.

The following organs were isolated: brain, buccal cavity, esophagus, trachea, lymph nodes, heart, lung, thymus, nodose ganglion, liver, kidney, spleen, stomach, stomach contents, large intestine, large intestinal contents, small intestine, small intestinal contents, vertebra, DRG (isolated from the 4th to 8th dorsal vertebra), testis, femur bone (right foot only), and femur muscles (right foot only). In intratracheal instillation, the lung was washed with phosphate-buffered saline, and the fluid was collected as bronchoalveolar lavage fluid (BALF). The isolated organs were dried at 105°C for 6 hr, and their dry weights were measured.
ICP-MS analysis

Au in the organs was determined by an X series II ICP-MS (Thermo Scientific, Waltham, MA, USA). For pretreatment, a microwave digestion system Model MARS 6 (CEM Corporation, Matthews, NC, USA) was used for microwave dissolution. Each isolated organ and 100 μL of blood were digested with 10 mL of 60% HNO₃ for 15 min at 200°C, and the solutions were diluted to 1 M HNO₃ with ultrapure water. Indium was added as an internal standard. This solution was filtered through a 0.45-μm membrane filter (Advantec, Tokyo, Japan).

The Au nanoparticle stock solution was also digested by the same method; this was used as the standard solution for ICP-MS analysis. To determine the lower limit of quantification, the lowest concentration standard solution in the dilution series was measured 9 times. A calibration curve for Au was prepared by measuring the samples of other concentrations of the dilution series. The sample measurement was repeated 3 times, and the average value was employed.

Statistical analysis

Migration rate to each organ was shown as mean value. Migration rate at each time of liver and vertebra was shown by the means ± standard deviations (S.D.). For statistical analysis, Student’s t-test was used for comparison between each groups. p value less than 0.05 were considered significant.

RESULTS

For ICP-MS analysis, the lower limit of quantification of Au nanoparticle concentration was 2.8 × 10⁴ particles/mL. The isolated organs were diluted 130 times with ultrapure water after dissolution in nitric acid so that if a concentration of 3.6 × 10⁶ particles/mL was present in an organ, it was quantifiable by ICP-MS.

Au concentration in each isolated organ was measured by ICP-MS. In almost all samples of the control groups, the concentration of Au in the organs was lower than the limit of quantification.

For both the intratracheal instillation group and the tail vein injection group, the sum of the number of Au nanoparticles that could be detected in all isolated organs from 1 mouse are plotted in Fig. 1 (n = 5-6). In this experiment, all mice were administered 50 μL of an Au nanoparticle suspension; however, there was a large variation in the sum of the number of particles that could be detected. For simplicity, the particle distribution rate in each organ (P) was defined using Eq. 1, and the distribution of nanoparticles in each organ was expressed using this value.

\[
P (\%) = \frac{N_{\text{organ}}}{N_{\text{total}}} \times 100
\]  
(Eq. 1)

where \(N_{\text{organ}}\) is the number of nanoparticles in an isolated organ and \(N_{\text{total}}\) is the sum of \(N_{\text{organ}}\) except for blood, DRG, lymph nodes, and nodose ganglion.

Figures 2 and 3 show the results of P for tail vein
injection and intratracheal instillation, respectively. Following tail vein injection, approximately 95% of nanoparticles were observed to be distributed in the liver and approximately 3% were distributed in the spleen, and the distribution rate in other organs were less than 1% in all time spans (Fig. 2). Following intratracheal instillation, approximately 80% of nanoparticles remained in the lungs in 1-hr group. In the 3- and 6-hr groups, it reduced to approximately 30%-40%; however, these values increased in the stomach, intestine, and liver (Fig. 3).

Figure 4 shows the values of \( P \) in the liver following intratracheal instillation. The average value increased with time after administration; however, there was no significant difference for the large dispersion in each group.

Figure 5 shows the values of \( P \) in the vertebra following tail vein injection and intratracheal instillation. There was little difference in the average value of \( P \) between each time span in both administration methods. On comparison of the administration methods, the average values for intratracheal administration was slightly higher at any time span.

Tables 1 and 2 show the results of the number of nanoparticles detected in the blood, lymph nodes, nodose ganglion, and DRG. The number of samples in which particles could be detected is shown in parentheses after the number of particles.

**DISCUSSION**

Following tail vein injection, nanoparticles in the blood could be quantified in 4 out of 6 mice in the 1-hr group, whereas in the 3-hr group, they were lower than the lower limit of quantification in all 6 mice (Table 1). Therefore, it is believed that following tail vein injection, most of the administered particles were transferred to various organs, particularly to the liver, through the blood within approximately 1 hr. In existing research, most nanoparticles administered intravenously were removed from blood. Lankveld et al. (2010) administered the three types of silver nanoparticles (20, 80, 110 nm) into the tail vein of rats and investigated the particle number concentration in the blood until 1 hr after administration. As a result, the number of particles in the blood was rapidly decreased until 10 min after administration. Xie et al. (2010) was examined biodistribution of silica nanoparticles (20, 80 nm) labeled with 125I at 1, 3, 7, 10 and 30 days after administration. Nanoparticles contained in blood at day 1 was 0.06% of injected dose and they reduced after that. In intravenous administration, most of administered nanoparticles were accumulated in liver and spleen (Choi et al., 2010; Lankveld et al., 2010).

Furthermore, following intratracheal instillation, approximately 0.5% of particles were observed to be distributed in the liver at 1 hr after administration. For clearance of nanoparticles from lungs, there are several studies.

Kreyling et al. (2013) administered gold nanoparticles (1.4, 2.8, 5, 18, 80, 200 nm) intratracheally to rats and investigated the clearance rate of from the lung 24 hr after administration. Most of administered particles were remained in the lungs, and the migration rate is about 0.1% in particles larger than 5 nm. The Semmler-Behnke et al. (2008) also administered 1.4 and 18 nm of gold nanoparticles intratracheally. Residual rates were 91.5% and 99.8%, respectively, at 24 hr after administration. Oyabu et al. (2007) exposed oxide nickel nanoparticles (139 nm) to rats by inhalation for 4 weeks. The amount of nickel in the lungs were measured with ICP-MS at 4 days, one month, 3 months after the end of the exposure. As a result, biological half-life was 62 days.

In both methods of administration, the distribution rate of particles in the vertebra was similar in the 1-hr group, (approximately 0.6%) (Fig. 5). If the particles had been transferred to the vertebra via the blood, the distribution rate in the vertebra following intratracheal instillation should have been much lower than that following tail vein injection. In addition, at each time span following intratracheal instillation, the distribution rate in the blood was lower than the lower limit of quantification for most of the samples. Therefore, the transfer of the particles to the vertebra was believed not to be via blood.

Apart from the blood pathway, the lymph and nerve pathways are believed to be involved in biodistribution. In both administration methods, the amount of Au nanoparticles in lymph nodes at 1 hr could be quantified in only 1 mouse (Tables 1 and 2). In a previous study, 15-nm silver particles were administered to rats for 6 hr by inhalation, and quantification of silver nanoparticles in various organs, including lung-associated lymph nodes (tracheobronchial and mediastinal lymph nodes) was attempted by ICP-MS analysis. Silver nanoparticles were not detected in lung-associated lymph nodes from 30 min to 2 hr after exposure (Takenaka et al., 2001). Therefore, this suggested that blood and lymph were not the major pathways of nanoparticle translocation to the vertebra at 1 hr after intratracheal instillation. Au nanoparticles above the lower limit of quantification were detected in DRG from 1 mouse in the 1-hr group after intratracheal instillation (Table 2). If the Au nanoparticles detected in the vertebra were not translocated via blood or lymph, it is possible that the Au nanoparticles detected in DRG were transferred via nerves. This is believed to be due to retrograde
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Fig. 2. Distribution of nanoparticles detected in isolated organs following tail vein injection. Data are expressed as mean values of $P$.

Fig. 3. Distribution of nanoparticles detected in isolated organs following intratracheal instillation. Data are expressed as mean values of $P$. 
transport resulting in the accumulation of particles in the cell bodies of DRG.

At the cellular level, there are some reports which showed the toxicity of gold nanoparticles. Coradeghini et al. (2013) exposed cells to gold nanoparticles (5 and 15 nm) for 72 hr and revealed cytotoxic effects only for 5 nm at concentration larger than 50 M if measured by colony forming efficiency (CFE). Even if nanoparticles migrate to the central nervous system via nerve uptake after inhalation, the migration rate is very small from the results of this paper, and the risk is thought to be very low. However, since the transition mechanism is not clear, it is necessary to consider using particles that have different physical properties.

Table 1. Number of nanoparticles detected in the blood, lymph nodes, nodose ganglion, and DRG following tail vein injection. The number of samples in which the count was above the lower limit of quantification is shown in parentheses.

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Blood (100 μL)</th>
<th>Lymph nodes</th>
<th>Nodose ganglion</th>
<th>DRG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hr (n = 6)</td>
<td>0.4 – 1.0 × 10⁸ (4)</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>3 hr (n = 6)</td>
<td>N.D.</td>
<td>3.7 – 4.1 × 10⁶ (2)</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>6 hr (n = 6)</td>
<td>N.D.</td>
<td>3.8 – 4.5 × 10⁶ (2)</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

Table 2. Number of nanoparticles detected in the blood, lymph nodes, nodose ganglion, and DRG following intratracheal instillation. The number of samples in which the count was above the lower limit of quantification is shown in parentheses.

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Blood (100 μL)</th>
<th>Lymph nodes</th>
<th>Nodose ganglion</th>
<th>DRG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hr (n = 5)</td>
<td>3.1 × 10⁸ (1)</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>3 hr (n = 6)</td>
<td>2.8 × 10⁷ (1)</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>6 hr (n = 6)</td>
<td>6.0 × 10⁶ (1)</td>
<td>3.8 × 10⁶ (1)</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

Fig. 4. Values of P in the liver at each time span. Data are presented as mean ± S.D. Non-significant changes were observed between each time span.

Fig. 5. Values of P in the vertebra at each time span. Data are presented as mean ± S.D. Non-significant changes were observed between each time span or each administration method.
Conflict of interest---- The authors declare that there is no conflict of interest.

REFERENCES


