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Dose-dependent clearance kinetics of intratracheally administered titanium dioxide nanoparticles in rat lung

Naohide Shinohara [a,*], Yutaka Oshima [b], Toshio Kobayashi [b], Nobuya Imatanaka [c], Makoto Nakai [b], Takayuki Ichinose [d], Takeshi Sasaki [e], Guihua Zhang [a], Hiroko Fukui [a], Masashi Gamo [a]

[a] National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki 305-8569, Japan
[b] Chemicals Evaluation and Research Institute (CERI), Hita, Oita 877-0061, Japan
[c] Chemicals Evaluation and Research Institute (CERI), Bunkyo, Tokyo 112-0004, Japan
[d] Toray Research Center, Inc., Otsu, Shiga 520-8567, Japan
[e] National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki 305-8565, Japan

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ABSTRACT

AEROSIL® P25 titanium dioxide (TiO_2) nanoparticles dispersed in 0.2% disodium phosphate solution were intratracheally administered to male F344 rats at doses of 0 (control), 0.375, 0.75, 1.5, 3.0, and 6.0 mg/kg. The rats were sacrificed under anesthesia at 1 day, 3 days, 7 days, 4 weeks, 13 weeks, and 26 weeks after administration. Ti levels in various pulmonary and extrapulmonary organs were determined using sensitive inductively coupled plasma sector field mass spectrometry. One day after administration, the lungs contained 62–83% of TiO_2 administered dose. Twenty-six weeks after administration, the lungs retained 6.6–8.9% of the TiO_2 administered at the 0.375, 0.75, and 1.5 mg/kg doses, and 13% and 31% of the TiO_2 administered at the 3.0 and 6.0 mg/kg doses, respectively. The pulmonary clearance rate constants from compartment 1, k_1, were estimated using a 2-compartment model and were found to be higher for the 0.375 and 0.75 mg/kg doses of TiO_2 (0.030/day for both) than for TiO_2 doses of 1.5–6.0 mg/kg (0.014–0.022/day). The translocation rate constants from compartment 1 to 2, k_{12}, were estimated to be 0.015 and 0.018/day for the 0.375 and 0.75 mg/kg doses, and 0.0025–0.0092/day for doses of 1.5–6.0 mg/kg. The pulmonary clearance rate constants from compartment 2, k_2, were estimated to be 0.0086 and 0.0093/day for doses of 0.375 and 0.75 mg/kg, and 0–0.00082/day for 1.5–6.0 mg/kg doses. Translocation of TiO_2 from the lungs to the thoracic lymph nodes increased in a time- and dose-dependent manner, accounting for 0.10–3.4% of the administered dose at 26 weeks. The measured thoracic lymph node burdens were a much better fit to the thoracic lymph node burdens estimated assuming translocation from compartment 1 to the thoracic lymph nodes, rather than those estimated assuming translocation from compartment 2 to the thoracic lymph nodes. The translocation rate constants from the lungs to the thoracic lymph nodes, $k_{Lung \rightarrow Lym}$, were 0.000037–0.00081/day, and these also increased with increasing doses of TiO_2. Although a small amount of TiO_2 had translocated to the liver by 3 days after the administration (0.0023–0.012% of the highest dose administered, 6.0 mg/kg), translocation to the other extrapulmonary organs was not detected.

1. Introduction

The safety of nanomaterials has been the focus of worldwide concern because of the lack of information available regarding their potential risks for workers and the general population. Therefore, the toxicity of nanomaterials has been tested internationally. Nano-sized titanium dioxide (TiO_2) particles (primary particle size <100 nm), one of the most typical industrial nanomaterials, have been utilized in sunscreen, cosmetics, and photo catalysis since the 1980s. Global demand for TiO_2 nanomaterials was estimated at 2100–2500 tons per year in 2008 (Fuji Chimera Research Institute, Inc., 2009). Since TiO_2 is water-insoluble and inert, it is generally regarded as having low toxicity in humans and is even used as an additive in food products. However, nano-sized particles may be more toxic or show a more widespread organ distribution than micron-sized particles

* Corresponding author. Tel.: +81 29 861 8415; fax: +81 29 861 8030.
E-mail address: n-shinohara@aist.go.jp (N. Shinohara).

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(Donaldson et al., 2001, 2004; Oberdörster et al., 2005; De Jong et al., 2008).

In order to evaluate the toxicity of TiO_2 nanoparticles, toxicokinetic data are beneficial. Since it is well established that pulmonary clearance of particles is inhibited following the administration of higher doses, a phenomenon known as overload (Morrow, 1992), it is important to evaluate the dose-dependency of pulmonary clearance when considering inhalation toxicity. In addition, after inhalation or intratracheal administration of TiO_2 nanoparticles, Ti have been detected in the lungs and lung-associated lymph nodes, while Ti levels in other organs such as the liver, spleen, kidneys, and brain were below the detection limit (Bermudez et al., 2004; Ma-Hock et al., 2009; van Ravenzwaay et al., 2009; Oyabu et al., 2013; Sager et al., 2008).

One-compartment models have been often used for the evaluation of pulmonary clearance (Bermudez et al., 2004; Oyabu et al., 2013). First order clearance rate constants for highly persistent substances often decrease as the observation period increases. Therefore, first order clearance rate constants estimated by using a 1-compatment model over different observation periods cannot be compared with each other. In addition, a 1-compartment model does not fit the measured burden closely. A two-compartment model was reported to provide a better fit to the measured burden and can be applied to evaluate both faster and slower clearances (Shinohara et al., 2010). However, there are no studies evaluating the clearance of TiO_2 nanoparticles from the lung using a 2-compartment model.

The present study aimed to elucidate dose-dependent pulmonary clearance kinetics and dose-dependent translocation kinetics to extrapulmonary organs of TiO_2 nanoparticle. In this study, we administered TiO_2 nanoparticles intratracheally to rats at 5 doses and investigated their pulmonary clearance and translocation from the lung to extrapulmonary organs over 26 weeks. We determined the TiO_2 burden in the lungs after sampling of bronchoalveolar lavage fluid (BALF), BALF, and trachea, as well as the thoracic lymph nodes (right and left posterior mediastinal lymph nodes, parathymic lymph nodes), liver, spleen, and kidneys using a highly sensitive inductively coupled plasma sector field mass spectroscopy (ICP-SFMS; double-focusing ICP-MS). The pulmonary clearance rate constants estimated using a classical 2-compartment model were compared over a range of doses. AEROSIL® P25 TiO_2 nanoparticles, which have often been employed for toxicity testing of TiO_2 nanoparticles and have been shown to induce lung inflammation (Rehn et al., 2003; Sager et al., 2008; Warheit et al., 2007) were used in the present study.

2. Material and methods

2.1. Preparation of TiO_2 suspension

AEROSIL® P25 TiO_2 nanoparticles (Evonik Industries, Germany), consisting of approximately 80% anatase and 20% rutile forms of TiO_2, were used in the present study. These spherical 21 nm particles had a specific surface of $50 \pm 15\ m^2/g$, and >99.5% purity (Catalog value; Nippon Aerosil Co., Ltd.).

P25 TiO_2 nanoparticles (2 g) were sonicated in 50 mL of 0.2% disodium phosphate solution (DSP) (food additive grade, Wako Pure Chemical Industries, Ltd. , Japan) for 3 h in an ultrasonic bath (5510J-MT; Branson Ultrasonics Co., USA) and then centrifuged at $1000 \times g$ for 30 min at 20 °C (CF16RXII and T15A41; Hitachi Koki Co., Ltd. , Japan). Supernatant (30 mL) was collected as stock suspension. The concentration of the stock suspension was determined by weight (AUW220D; Shimadzu Co., Japan) after drying in a thermostatic chamber (ON-300S; Asone Co., Japan). Suspensions of 0.375, 0.75, 1.5, 3.0, and 6.0 mg/mL were prepared for administration by diluting the stock suspension with 0.2% DSP. The size distribution and ζ potential of the TiO_2 nanoparticles in the administered suspension were determined by dynamic light scattering (DLS) (Zetasizer nano-ZS; Malvern Instruments Ltd., UK). The specific surface area of TiO_2 nanoparticles in administered suspension was determined using the BET-method after washing with pure water and drying in a thermostatic chamber.

2.2. Experimental procedure

All animal were treated in accordance with the guideline for the animal experiment of our laboratory which referred to the guidelines of Ministry of the Environment, Japan, Ministry of Health, Labour and Welfare, Japan, Ministry of Agriculture, Forestry and Fisheries, Japan, Ministry of Education, Culture, Sports, Science and Technology, Japan. The present experiment was approved by the Animal Care and Use Committee, Chemicals Evaluation and Research Institute, Japan, and by the Institutional Animal Care and Use Committee, National Institute of Advanced Industrial Science and Technology.

Male F344/DuCrlCrlj rats were obtained from Charles River Laboratories Japan, Inc. (Kanagawa, Japan). The animals were 12 weeks old with mean body weight of 246 g (range, 215–273 g) at the start of the study. Rats were anesthetized by isoflurane inhalation and treated by intratracheal administration of five concentrations of TiO_2 nanoparticles (0.375, 0.75, 1.5, 3.0, and 6.0 mg/mL) and negative control (0.2% DSP) at 1 mL/kg body weight using MicroSprayer® Aerosolizer (Model IA-1B-R for Rat; Penn-Century, Inc., USA).

Five rats in each group were euthanized and dissected at 1 day, 3 days, 7 days, 4 weeks, 13 weeks, and 26 weeks after TiO_2 nanoparticle administration. The animals were euthanized by exsanguination from the abdominal aorta under intraperitoneal pentobarbital anesthesia (50 mg/kg body weight). Thereafter, the trachea was cannulated with a disposable feeding needle, which was then tied in place. The lungs were lavaged with 7 mL of physiological saline freely flowing from 30 cm above the rat and this fluid was collected in a tube placed 30 cm below the rat. This lavage was performed twice and >90% of the 14 mL of lavage fluid was recovered. After BALF sampling, the lungs, trachea, right and left posterior mediastinal lymph nodes, parathymic lymph nodes, liver, kidneys, and spleen of each animal were dissected, rinsed with saline, and weighed.

2.3. Analysis

The Ti contents in the lungs after BALF sampling, BALF, trachea, right and left posterior mediastinal lymph nodes, parathymic lymph nodes, and liver of every animal were analyzed. The Ti contents in the kidneys and spleen of the negative control and highest dose groups were analyzed. In addition, the Ti contents in the stock suspension, drinking water, and food were also analyzed.

The lungs after BALF sampling, kidneys, and spleen were homogenized with 2 mL of ultrapure water (Milli-Q Advantage A10 Ultrapure Water Purification System, Merck Millipore, USA), and the liver was homogenized with 10 mL of ultrapure water. An electric homogenizer (PT10-35 Kinematica AG and NS-50; Microtec Co. Ltd., Japan) was used and the resulting homogenates were stored at < -30 °C until analysis.

All samples were treated with acid prior to determination of Ti levels. Nitric acid (HNO_3; 68%, 0.5 mL) and hydrogen peroxide (H_2O_2; 35%, 0.2 mL) were added to 0.1 mL of BALF, HNO_3 (1 mL), and sulfuric acid (H_2SO_4; 98%, 0.2 mL) were added to 1 g of homogenized tissues, HNO_3 (0.5 mL) and H_2SO_4 (0.1 mL) were added to whole lymph node samples, HNO_3 (1 mL) and H_2O_2 (0.3 mL) were added to 0.02 g of animal feed, and H_2SO_4 (0.5 mL) and hydrofluoric acid (HF; 38%, 0.5 mL) were added to 20 μL and 100 μL for high and low concentrations of the administered TiO_2 suspension, respectively. Drinking water was diluted 10-fold with 10% HNO_3 solution,