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Original Article

Safety evaluation of titanium dioxide nanoparticles by their absorption and elimination profiles

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ABSTRACT — If titanium dioxide nanoparticles are inert and non-biodegradable, they must be evaluated similarly to fullerenes, carbon nanotubes and asbestos. We surveyed the titanium level in typical raw food materials, and then intravenously injected titanium dioxide nanoparticles (primary particle diameter: 15 nm; secondary particle size: 220 nm) in mice and determined their tissue distribution and elimination. As a result, an unexpectedly high titanium concentration was observed in several foods. It was also detected in blood and tissues of healthy mice without administration of titanium dioxide nanoparticles. Then, forced *i.v.* injection of the nanoparticles was performed in mice. The titanim level was significantly increased in blood and tissues, but no increase was found in the brain after *i.v.* injection. Most titanium was concentrated in the liver after injection, but the liver level decreased over time (ca. 30% decrease in 1 month). These data show that titanium must be eliminated from the body, and suggest that we should reconsider an evaluation method for toxicity of titanium dioxide nanoparticles.

Key words: Titanium dioxide, Nanoparticle, Absorption, Elimination

INTRODUCTION

Many nanomaterials have been prepared and evaluated for their functions and physical and chemical properties. In particular, biocompatible materials, ultrafine microstructures, and molecularly recognized and signaling materials have been broadly studied as leading-edge and advanced materials; fullerenes and carbon nanotubes are typical examples. In addition, titanium dioxide and zinc oxide nanoparticles are used not only in several materials but also in humans in UV-care cosmetics. These nanomaterials may be categorized as non-biodegradable, and liposomes and nano- and micro-emulsions are found in labile nanoparticles. Are these nanomaterials safe for humans (Maynard *et al.*, 2006; Behling, 2007; Scientific Committee on Consumer Products, 2007; Scientific Committee on Emerging and Newly-Identified Health Risks, 2007)?

Titanium and titanium dioxide are believed to be highly inert and safe, even when they are absorbed via the GI tract and skin. If they are inert and non-biodegradable, we should evaluate the titanium dioxide nanoparticles similarly to fullerenes, carbon nanotubes (Singh *et al.*, 2006) and asbestos. It was found from our preliminary survey and experiments, however, that titanium is naturally contained in the body as well as in vegetables and soil, indicating that we may ingest titanium compounds daily despite no detailed information on the chemical structure of such compounds. In addition, size is very important for nonbiodegradable nanoparticles. Titanium dioxide particles bigger than 100 nm have been used in several foods and toothpastes (Lomer *et al.*, 2005); thus our strategy to estimate the safety of titanium oxide nanoparticles must be modified.

In the present study, therefore, we surveyed the titanium level in typical raw food materials, and then intravenously injected titanium dioxide nanoparticles (primary particle size, ca. 15 nm; secondary particle size, ca. 220 nm: see below in detail) into mice, and determined the tissue distribution and elimination kinetics of titanium.

MATERIALS AND METHODS

Surveillance and determination method of titanium concentration in typical foodstuffs

Typical cooking ingredients were selected, and their titanium concentration was measured using an ICP-MS (Agilent 7500ce, Agilent Technologies, Inc., Santa Clara,

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CA, USA). Titanium concentration in blood and tissue samples as well as mouse diets (Oriental Yeast Co., ltd., Tokyo, Japan) was also determined using ICP-MS. In addition, a bibliographical search was done for the titanium concentration in foods, soil and others.

Preparation of titanium dioxide nanoparticles dispersed in saline

Titanium dioxide nanoparticles (MT-150AW, particle diameter of 15 nm, as explained below) were obtained from Tayca (Osaka, Japan). Highly dispersed titanium dioxide nanoparticles, HD-MT-150AW, were prepared by the following patented method for dense skins of hydrated amorphous silica bound to a core. MT-150AW (100 g) was mixed and well dispersed in 900 g of water, and then the pH of the mixture was adjusted to pH 9.0 by adding NaOH solution and the mixture heated to 80°C. Next, 200 g/l (as SiO_2) liquid glass (sodium silicate) (215 ml) and 10% H_2SO_4 (180 ml) were added to the mixed solution (ca. 1,000 g) and vigorously stirred for 2 hr (Iler, 1959). The final pH of the resultant mixture was adjusted to 8.0 to 8.5. Stirring was continued for another 30 min and then the pH of the solution was adjusted to 7.0 by addition of NaOH solution. Finally, the reaction mixture was filtered and 2,000 g of purified water was added to rinse. The solution was dried at 120°C for 12 hr and jet-milled to obtain HD-MT-150AW.

Titanium dioxide nanoparticles dispersed in physiological saline, DIS-HD-MT-150AW, were prepared by sonication of HD-MT-150AW. A mixture of 6 g of HD-MT-150AW and 54 g of physiological saline purchased from Sigma-Aldrich (St. Louis, MO, USA) was sonicated using a sterile ultrasonic grinding device (Ultrasonic Generator Model US-300, Nihonseiki Kaisha, Ltd., Tokyo, Japan) for 3 min under cooling conditions to obtain DIS-HD-MT-150AW.

Determination of physical and chemical properties of titanium dioxide nanoparticles

The silica content in HD-MT-150AW was analyzed using fluorescent X-ray spectroscopy (3270E, Rigaku Corp., Tokyo, Japan), and the crystal form of the nanoparticles was determined using X-ray diffraction (X'Pert Pro, PANalytical, Ea Almelo, Netherlands). The total number of bacteria in DIS-HD-MT-150AW was determined by a plate counting method.

The primary particle-size distribution of HD-MT-150A was analyzed using image processing software (MacView Ver. 3, Mountech Co., Ltd., Tokyo, Japan). The secondary particle-size distribution of DIS-HD-MT-150AW was determined using a dynamic light scattering particle-size analyzer (Microtrac 9340-UPA, Nikkiso Co., Ltd., Tokyo, Japan).

Intravenous administration of titanium dioxide nanoparticles

Male ddY mice weighing about 30 g were used in all animal experiments. These experiments were done under the guidelines of Life Science Research Center, Josai University. Saline suspension (50 μl) of titanium dioxide nanoparticles (DIS-HD-MT-150AW) (36,250 μg/ml, 1,813 μg/animal) was intravenously injected into the tail vein of mice under anesthesia by *i.c.* injection of sodium pentobarbital. The blood, brain, lung, heart, liver, spleen and kidney were excised 5 min, 72 hr and 1 month after injection. Tissue and blood were dissolved using Soluene-350 (Perkin-Elmer, Waltham, MA, USA) to determine the titanium concentration by ICP-MS, as explained above.

Morphological evaluation of excised tissues

Slices of the liver were observed by electron microscope (JEM2000EX, JEOL Ltd., Tokyo, Japan) to evaluate the intra- and inter-cellular presentation of titanium dioxide nanoparticles.

RESULTS AND DISCUSSION

Survey of titanium concentration in typical food materials

As described in the Introduction, titanium is naturally contained in several vegetables and soil, although there is no detailed information on the chemical structure of the compounds. The titanium level in several foodstuffs was therefore determined and a brief survey was carried out on several food materials; Table 1 summarizes these surveys and the experimental results. An unexpectedly high titanium concentration was observed in several food materials. In particular, the titanium concentration in soybeans (3.24 μg/g) and shrimp (2.52 μg/g) was high. Itoh *et al.* (2005) reported that the titanium concentration in several soils was over 3,300 μg/g, which may be related to the relatively high concentration in vegetables (*i.e.*, 20-30 μg/g for Chinese cabbage).

Preparation and characterization of titanium oxide nanoparticles

The preparation and physical properties (size) are very important for the safety and toxicity of titanium dioxide nanoparticles. Titanium dioxide nanoparticles dispersed in physiological saline, DIS-HD-MT-150AW, were prepared using the following two steps: preparation of highly dispersed titanium dioxide nanoparticles, HD-MT-150AW

Table 1. Titanium level in several foodstuffs and others

Food/Soil	Production area	Conc. (μg/g)	Food/Soil	Production area	Conc. (μg/g)
beef	Japan	0.17	lettuce [a]		41 ± 15
beef	Australia	0.26	Boston lettuce [a]		10 ± 14
pork	Japan	0.26	Japanese parsley [a]		53 ± 12
pork	USA	0.44	Japanese radish (leaf) [a]		37 ± 33
chicken	Japan	0.14	Welsh onion [a]		20 ± 13
egg	Japan	1.70	tomato [a]		39.1 ± 9.2
salmon	Norway	0.37	paprika [a]		14 ± 16
shrimp	India	2.52	sprout [a]		38 ± 16
onion	Japan	0.33	AIN 93G [b]	Josai Univ.	6.29
potato	Japan	0.12	α cornstarch		0.18
paprika	Netherlands	0.19	β cornstarch		0.18
carrot	USA	0.59	casein		1.60
corn	USA	0.60	cellulose		< 0.1
flour	mainly USA	0.31	soft water	USA	< 0.05
soybeans	Japan	3.24	water	Thailand	< 0.05
rice	Japan	0.91	soft water	France	< 0.05
orange	USA	0.65	mid-hard water	France	< 0.05
lemon	USA	1.64	hard water	France	< 0.05
grapefruit	South Africa	0.39	urban water	Thailand	< 0.05
banana	Philippines	< 0.1	soft water	Japan	< 0.05
Japanese parsley [a]	Japan	101 ± 19	soft water	Japan	< 0.05
Japanese parsley [a]	Japan	10 ± 13	water [a]	Japan	13 ± 17 (ng/g)
Chinese cabbage [a]	Japan	29.5 ± 14	water [a]	Japan	3.2 ± 8.0 (ng/g)
Chinese cabbage [a]	Japan	20 ± 13	soil [a]	Japan	4980 ± 220
bok-choy [a]		35.0 ± 8.8	soil [a]	Japan	3380 ± 180
Chinese cabbage [a]		22 ± 19	soil [a]	Japan	5540 ± 230
Cabbage [a]		52.7 ± 11	soil [a]	Japan	5870 ± 260
Macrophyll [a]		12.0 ± 6.0			

[a] Itoh *et al*. (2005), [b] from Prof. Wada (Josai Univ.)

from MT-150AW, and dispersion of HD-MT-150AW in saline by sonication to obtain DIS-HD-MT-150AW.

The surface of HD-MT-150AW was coated with silica. The isoelectric point of titanium dioxide is about 5-7, so titanium dioxide nanoparticles (MT-150AW) dispersed in physiological saline can be easily agglomerated. In other words, the surface modification of MT-150AW to HD-MT-150AW by silica was effective to avoid agglomeration in neutral saline, because the isoelectric point of silica is about 2-3.

The silica content of HD-MT-150AW analyzed by fluorescent X-ray spectroscopy was 27.5 wt%. X-ray diffraction patterns of HD-MT-150AW showed that the crystal form of titanium dioxide was rutile (data not shown). No bacteria were detected in DIS-HD-MT-150AW, as determined by the plate counting method.

Fig. 2 shows transmission electron microscope images of MT-150AW and HD-MT-150AW. Fig. 1 illustrates the grading curve of HD-MT-150AW, showing that it had a relatively narrow primary particle-size distribution and a mean particle diameter of 15 nm.

Fig. 3 shows the grading curve of the particles in DIS-HD-MT-150AW. The secondary particle-size distribution was wide, with an average particle size of 0.22 μm (220

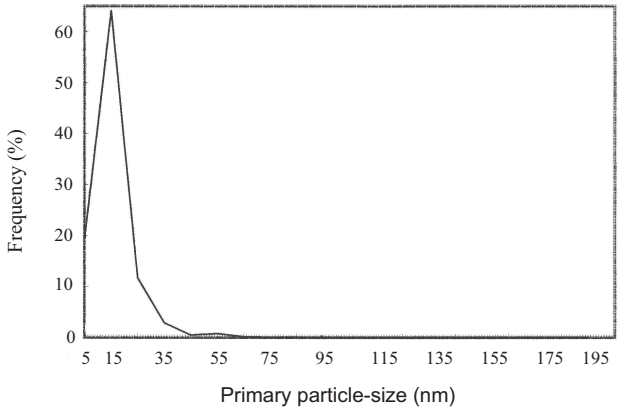

Fig. 1. Primary particle-size distribution of HD-MT-150AW analyzed by image processing software

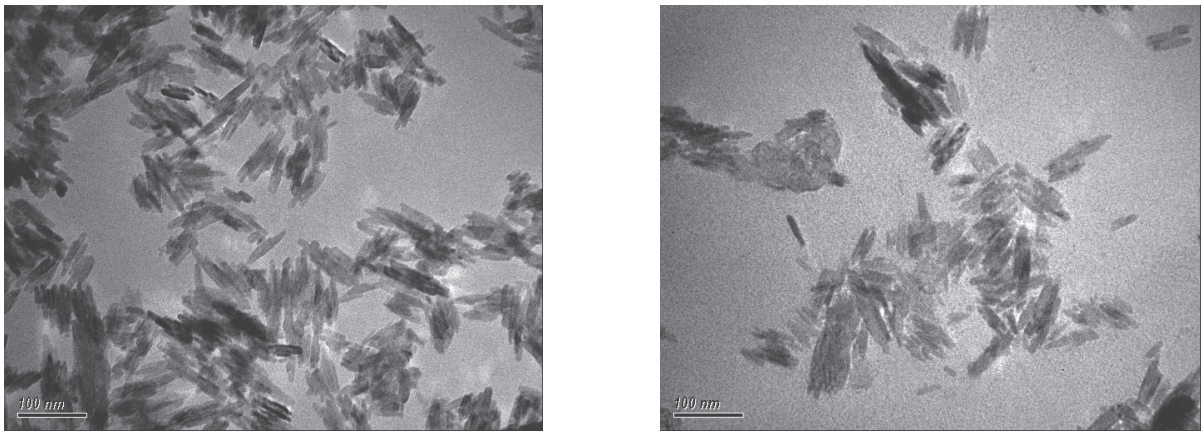

Fig. 2. Transmission electron microscope image of MT-150AW and HD-MT-150AW
Left side: MT-150AW, Right side: HD-MT-150AW .

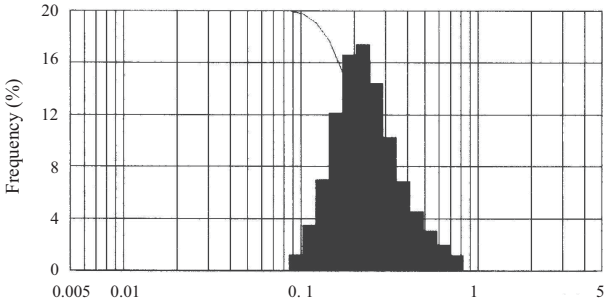

Fig. 3. Grading curve of particles in DIS-HD-MT-150AW analyzed by dynamic light scattering particle-size analyzer

nm). Fig. 3 shows that DIS-HD-MT-150AW contains only a small number of particles with a particle diameter below 100 nm. Fig. 4 shows a transmission electron microscope image of DIS-HD-MT-150AW particles. DIS-HD-MT-150AW clearly contained the primary particles of HD-MT-150AW.

Tissue distribution of titanium after *i.v.* injection of titanium dioxide nanoparticles

Titanium was detected in the blood and tissue of healthy mice without the administration of titanium dioxide nanoparticles, and the titanium levels are shown in Figs. 5 and 6 (see blank column for control). For example, titanium concentrations in the blood and liver were 4 and 15.4 μg/g tissue, respectively. Then, the titanium level was determined in the mouse diets and was found to be 17.5 μg/ml. Since the experimental animals consume about 5 g solid feed per day, the amount of titanium ingested was calculated to be 90 μg/day. Thus, most titanium in mice must come from ingested titanium materials.

Forced *i.v.* injection of the nanoparticles was then performed in mice. The titanium level was significantly increased in blood and tissue, but no increase was found in the brain after *i.v.* injection. No increase in the brain level may ignore the severe toxicity reported by Long *et al.* (2007). Most titanium was concentrated in the liver after injection, followed by the kidney as shown in Figs. 5 and 6. Interestingly, the liver level decreased over time (ca 30% decrease in 1 month), suggesting that titanium must be eliminated from the body. Fig. 7 shows the total recovery of titanium from the tissues. The titanium lev-

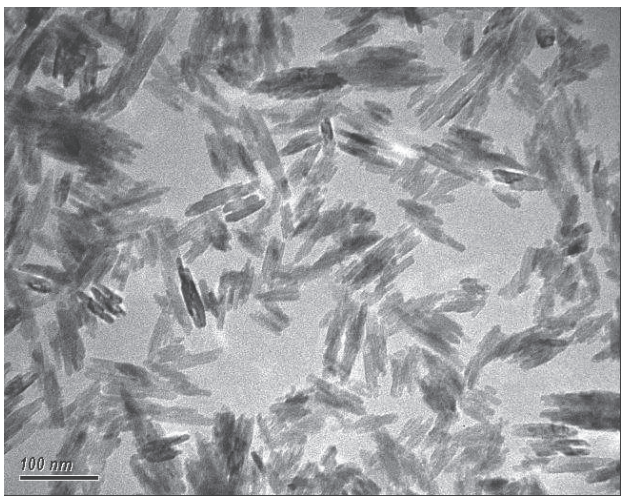

Fig. 4. Transmission electron microscope image of DIS-HD-MT-150AW

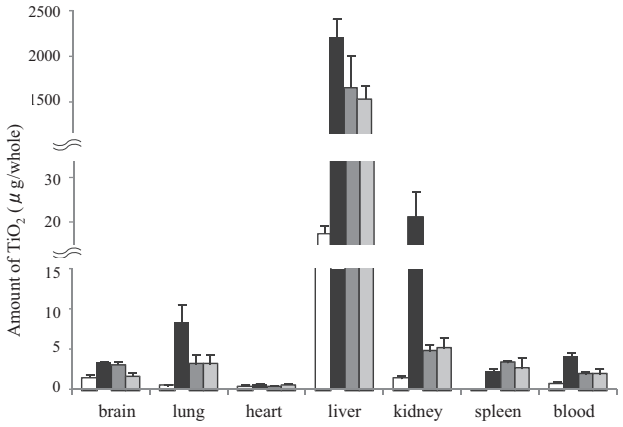

Fig. 5. Amount of TiO_2 in tissue and blood after *i.v.* injection
□ : before injection (control), ■, ▥ and ▤: 5 min, 72 hr and 1 month after injection. Each column represents the mean ± S.E. of 3 to 5 experiments.

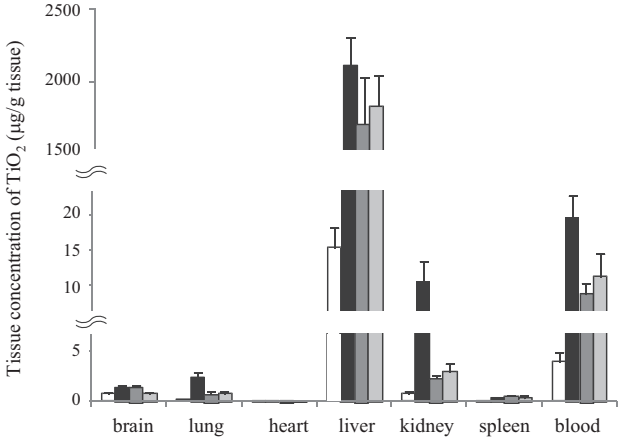

Fig. 6. Tissue concentration of TiO_2 after *i.v.* injection
Each column is the same as in Fig. 5.

el in mice apparently decreased over time after injection. Recently, a similar *i.v.* injection study of titanium dioxide nanoparticles was performed in rats by Fabian *et al.* (2008), where titanium was mostly detected in the liver and decreased over time. Jani *et al.* (1994) showed the distribution of orally administered titanium dioxide particles (100-500 nm) in the liver and spleen. Dental implants made of titanium were found to dissolve in biological tissues and were not toxic (Mu *et al.*, 2002; Hanawa, 2005). Their and our results suggest that titanium dioxide nanoparticles should be considered as a biodegradable or easily eliminated compound, not like asbestos and carbon nanotubes.

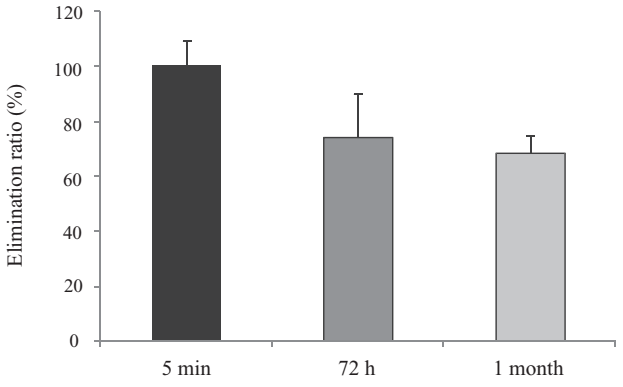

Fig. 7. Elimination ratio of TiO_2
Each column represents the mean ± S.E. of 5 experiments.

Finally, morphological evaluation was performed in mouse liver after administration of titanium dioxide nanoparticles, which were observed in hepatic cells. Titanium dioxide nanoparticles may be dissolved by macrophagic activity in the liver (Olmedo *et al.*, 2007). Detailed information will be presented in a separate paper.

Titanium was naturally contained in mice, especially in the liver, as well as in vegetables and soil. The titanium level gradually decreased after forced administration (*i.v.* injection) of titanium dioxide nanoparticles into mice. Thus, our strategy to estimate the safety of titanium dioxide nanoparticles in humans must be modified, although further experiments are necessary (Behling, 2007).

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