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Research Article

Observation of Internal Distribution Behavior of Micro/Nano-Sized Ceramics and Metal Particles in Mice

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Abstract The internal distribution behavior of micro/nano-sized ceramics and metal particles administered into the tail vein of mice was determined using magnetic resonance imaging, scanning X-ray analytical microscopy and inductively coupled plasma-atomic emission spectroscopy. After administration through the tail vein of mice, the particles circulated by blood flow reached and then remained temporarily in certain organs. In this study, we determined that the distribution behaviors in body depend upon the chemical species and the size of the particles.

Keywords biodistribution; micro/nano-sized ceramics particles; magnetic resonance imaging; inductively coupled plasma-atomic emission spectroscope

1 Introduction

Recently, nanoparticle research has been pursued intensively in both university and industry setting [7, 11]. Nano-sized materials show unique properties depending on their size. In particular, metal and metal oxide nanoparticles have been widely investigated because of their potential for many applications. For example, magnetite nanoparticles have been investigated for medical applications such as hyperthermic oncology [5], and gold nanoparticles with surface plasmon resonance (SPR) have been intensively used as bio-sensors. On the other hand, micro-/nano-sized materials have received considerable attention in view of their possible biocompatibility and/or nanotoxicity because of developments in nanotechnology [6, 8]. When the size of particles reaches the micro/nano level, some of them have shown toxicity in vitro even though they are considered biocompatible at the macro level. In a previous study, we determined that even biocompatible materials such as Ti and TiO_2 cause inflammation with the decrease of particle size [10]. Therefore, it is very important to understand the biodistribution of ceramic nanoparticles in a body. In this study, we report our direct observation of the biodistribution of ceramics and metal nanoparticles in mice.

2 Materials and methods

Male mice (Jcl:ICR), 8 to 12 weeks old, were used. Micro/nano-sized ceramics or metal particles (ITO (10 nm), Ba-ferrite (150 nm), TiO_2 (1) (0.5 μm), TiO_2 (2), Pt and Pd (1 μm, respectively)) were used without any purification. These particles dispersed into normal saline, respectively. For magnetic resonance imaging (MRI), 5 mg/mL of magnetic particle dispersion (0.3 mL) was injected into the tail veins of mice under pentobarbital anesthesia. MRI data were obtained using a 7-T horizontal bore spectrometer (Varian Inc., Palo Alto, CA, USA). Radiofrequency pulses were transmitted and MRI signals were received using a 4-cm volume coil. Spin-echo images were acquired with a data matrix of 256 $\times$ 128, field of view of 80 $\times$ 40 mm, slice thickness of 1 mm, echo time of 20 ms and repetition time of 5000 ms [1]. To determine the biodistribution, we dispersed these particles into saline and adjusted the concentration to 10 mg/mL and then 0.6 mL of the dispersion solution was injected into the tail veins of mice. Their organs were excised at several post injection times (from 0 h to 4 weeks). After administration of ceramic or metal particles, the organs (lung, liver, kidney, spleen, etc.) of mice were excised, and the presence of particles was determined using scanning X-ray analytical microscopy (XSAM). The concentrations of injected

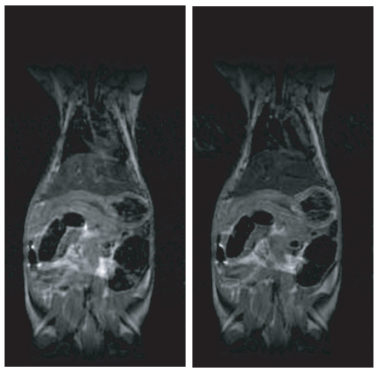

Figure 1: In vivo rodent MR images. The left panel shows the baseline image in the liver and kidney prior to Ba-ferrite injection, and the right panel shows an image of the same cross section 1 hour after administration of nanomagnetite, with a clear decrease in signal intensity in both organs.

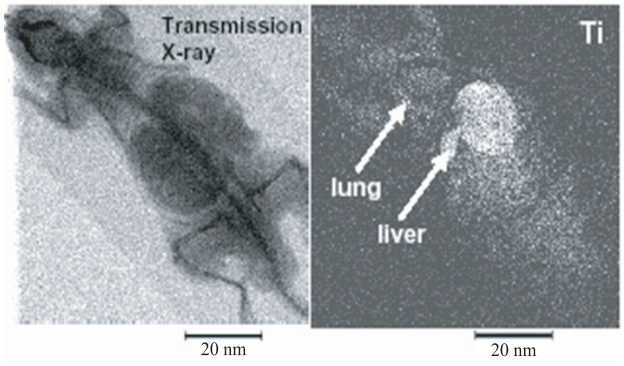

Figure 2: Elemental distribution images (Ti) of mice administered TiO_2 (1) particles at 1 week post-injection.

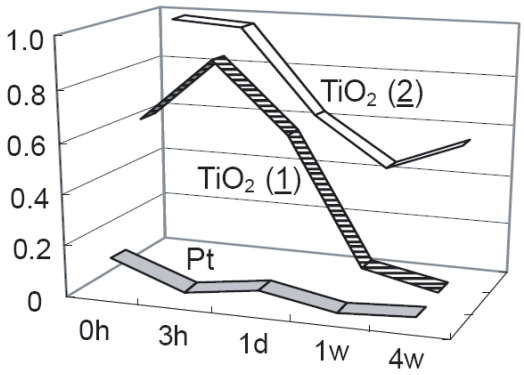

Figure 3: Time dependence of biodistribution of administered micro/nano particles in the lung.

materials were quantitated with inductively coupled plasma-atomic emission spectroscopy (ICP-AES P-4010, Hitachi, Tokyo, Japan) and the concentrations in each organ were estimated. Organs were excised at several post injection times (from 0 h to 4 weeks). To evaluate the concentration of microparticles in the organs, we calcined a part of each organ at 800 °C for 2 h in air and dissolved the samples in nitrohydro chloric acid. All operations on animals were in accord with institutional animal use and care regulations of Hokkaido University.

3 Results and discussion

Figure 1 shows whole-body MR images of a mouse before and 1 h after injection of the Ba-ferrite particles through the tail vein. The contrast of MR signal decreased in the liver, kidney and spleen after Ba-ferrite injection. Paramagnetic particles of Ba-ferrite, magnetite and some magnetic coordinated compounds were expected to act as a contrast agent for MR imaging, which affected the relaxation time to enhance MR images. The result indicates that the magnetic particles reached the sampled organs and then changed the contrast. These observations suggest that the administered ceramics particles reached the organs and then were temporarily trapped there. This result is in good agreement with those of a previous study of nano-magnetite particles [1].

In the case of micro/nano-sized ceramics particles without paramagnetic property, the distribution of administered particles in a body can be determined using XSAM measurement. Figure 2 indicates the transmission X-ray and the elemental distribution images (Ti) of administered titanium oxide (ϕ = 0.5 μm) in a whole mouse determined with XSAM (Figure 2). Fluorescence X-rays from Ti in the lung and liver were observed. The fluorescence intensity from the lung was decreased with post-injection time, while the intensity from the liver was increased.

Though the biodistribution of administered particles in a body varied with the materials, particles were mainly detected in the liver, spleen and lung [2]. The total amount of determined particles decreased with the post-injection time. Previously, we have reported that administered ITO nano-particles in mice were temporarily trapped in the lung and then quickly removed from the lung [4]. In addition, the distribution changed depending on the size of the particle. This result suggests that the biodistribution depends upon not only the materials but also the size of the particles.

Time-dependence of the biodistribution of administered micro/nano-particles was observed using energy-dispersed X-ray spectroscopy. As shown in Figure 3, the time profile was drastically changed depending upon the effects of elements and particle size. For example, Pt particles in the lung showed lower intensity over time, compared to that in the initial period and retained the tendency. On the other hand, TiO_2 particles were temporarily retained in the lung and then re-transported to other organs. The kinetics depended upon the particle size.

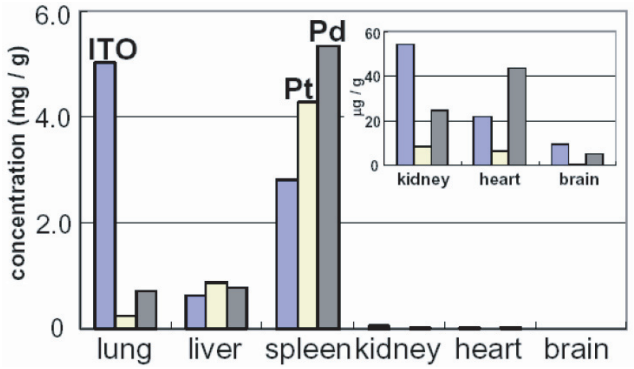

Figure 4: Concentrations of administered ITO, Pd and Pt particles in organs at 1 day post-injection (n = 3).

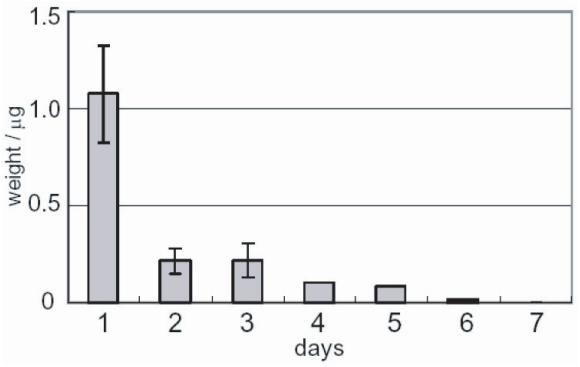

Figure 5: Time dependence of the concentration of excreted Pt from mice via urea (n = 3).

To determine the amount of the micro/nano-particles in the organs, we estimated the concentration of the administered particles with ICP-AES. As shown in Figure 4, the administered ceramics or metal particles were condensed in the lung, spleen and liver at 1 day post-injection. Most of these particles were observed in the spleen. This tendency is similar to that reported in a previous study [2,3]. Several inorganic particles quickly arrived in the spleen after injection. Interestingly, more ITO particles were detected in the lung than in the spleen during this post-injection time. Such a difference is sometimes observed between ceramics and metal particles. For example, TiO_2 particles retained in the lung for a couple of days are then moved to other organs. The profile of the particle movement depends on the particle size. On the other hand, relatively small amounts of Pt particles were observed in the lung from the initial time to after 4 weeks (shown in Figure 3). Several metal particles show a biodistribution tendency to reach the lung after injection and then quickly move on to other organs, especially the liver and spleen. Therefore, the different internal diffusion behavior could depend on the chemical composition and the surface properties of the particles. To investigate the excretion process, we analyzed the urea of mice administered Pt or ITO particles. Collected urea samples were calcined and then dissolved in nitrohydro chloric acid and the concentration was estimated with ICP-AES. As shown in Figure 5, the concentration of Pt in urea was drastically decreased with the post-injection time. This tendency is similar to that of small molecules or hydrophobic carbon nanotubes [9].

In the case of ITO-administered mice, In or Sn elements were observed as a lower limit of detection. Also, we analyzed feces of mice administered Pt particles using X-ray energy-dispersed spectroscopy. However, the intensity of fluorescence of Pt elements was negligible (shown in Figure 6). These results suggest that the administered inorganic particles were excreted only in a small amount; in other words, most of the particles were retained in the body

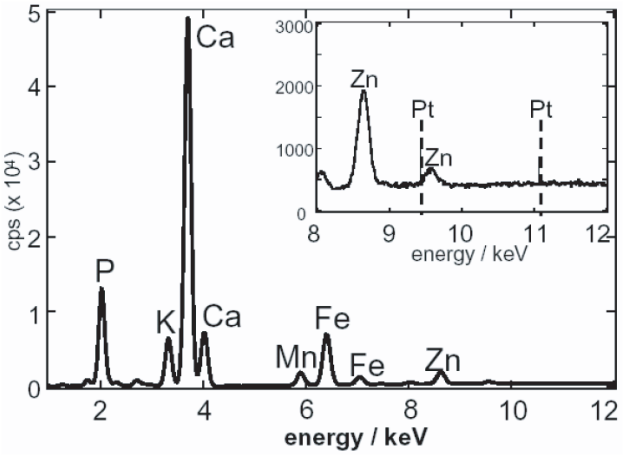

Figure 6: Time dependence of the concentration of administered Pt in feces.

even 4 weeks after their introduction. Though the details are not yet clear, it is assumed that these particles may spread throughout the whole body with a lower limit of detection.

4 Conclusions

In this study, we observed the biodistribution of ceramics and metal particles. The distribution varied depending on the chemical composition and the particle size. In addition, only small amounts of the inorganic particles were excreted via urea and feces. It is assumed that these particles may spread throughout the whole body with a lower limit of detection.

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References

[1] S. Abe, I. Kida, M. Esaki, T. Akasaka, M. Uo, T. Hosono, et al., *Biodistribution imaging of magnetic particles in mice: X-ray scanning analytical microscopy and magnetic resonance imaging*, Biomed Mater Eng, 19 (2009), pp. 213–220.

[2] S. Abe, C. Koyama, T. Akasaka, M. Uo, Y. Kuboki, and F. Watari, *Internal distribution of several inorganic microparticles in mice*, Key Eng Mater, 396-398 (2009), pp. 539–542.

[3] S. Abe, C. Koyama, M. Uo, T. Akasaka, Y. Kuboki, and F. Watari, *Time-dependence and visualization of TiO_2 and Pt particle biodistribution in mice*, J Nanosci Nanotech, 9 (2009), pp. 4988–4991.

[4] S. Abe, T. Yonezawa, T. Akasaka, M. Uo, F. Uchida, and F. Watari, *Observation of biodistribution of indium-tin oxide nanoparticles in mice*, Nano Biomedicine, 1 (2009), pp. 51–55.

[5] T. Atsumi, B. Jeyadevan, Y. Sato, and K. Tohji, *Heating efficiency of magnetite particles exposed to AC magnetic field*, J Magn Magn Mater, 310 (2006), pp. 2841–2843.

[6] H. Karlsson, J. Gustafsson, P. Cronholm, and L. Möller, *Size-dependent toxicity of metal oxide particles-A comparison between nano- and micrometer size*, Toxicol Lett, 188 (2009), pp. 112–118.

[7] N. Toshima and T. Yonezawa, *Bimetallic nanoparticles—novel materials for chemical and physical applications*, New J Chem, 22 (1998), pp. 1179–1201.

[8] J. Veranth, E. Kaser, M. Veranth, M. Koch, and G. Yost, *Cytokine responses of human lung cells (BEAS-2B) treated with micron-sized and nanoparticles of metal oxides compared to soil dusts*, Part Fibre Toxicol, 4 (2007), pp. 1–18.

[9] H. Wang, J. Wang, X. Deng, H. Sun, Z. Shi, Z. Gu, et al., *Biodistribution of carbon single-wall carbon nanotubes in mice*, J Nanosci Nanotech, 4 (2004), pp. 1019–1024.

[10] F. Watari, N. Takashi, A. Yokoyama, M. Uo, T. Akasaka, Y. Sato, et al., *Material nanosizing effect on living organisms: non-specific, biointeractive, physical size effects*, J R Soc Interface, 6 (2009), pp. S371–S388.

[11] T. Yonezawa, S. Takeoka, H. Kishi, K. Ida, and M. Tomonari, *The preparation of copper fine particle paste and its application as the inner electrode material of a multilayered ceramic capacitor*, Nanotechnology, 19 (2008), pp. 145706.01–145706.05.