Journal of Bioscience and Bioengineering
VOL. xx No. xx, 1–7, 2018

www.elsevier.com/locate/jbiosc

In vivo tissue distribution and safety of polyacrylic acid-modified titanium peroxide nanoparticles as novel radiosensitizers

Kenta Morita,[1,2] Takahiro Suzuki,[1] Yuya Nishimura,[1] Kazuhisa Matsumoto,[3] Chiya Numako,[3] Kazuyoshi Sato,[4] Masao Nakayama,[5] Ryohei Sasaki,[5] Chiaki Ogino,[1,*] and Akihiko Kondo[1,6]

Department of Chemical Science and Engineering, Graduate School of Engineering, Kobe University, 1-1 Rokkodaicho, Nada-ku, Kobe 657-8501, Japan,[1] Research Facility Center for Science and Technology, Kobe University, 1-1 Rokkodaicho, Nada-ku, Kobe 657-8501, Japan,[2] Graduate School of Science, Chiba University, 1-33 Yayoi, Inage, Chiba 263-8522, Japan,[3] Division of Environmental Engineering Science, Graduate School of Science and Technology, Gunma University, 1-5-1 Tenjincho, Kiryu, Gunma 376-8515, Japan,[4] Division of Radiation Oncology, Kobe University Graduate School of Medicine, 7-5-2 Kusunokicho, Chuou-ku, Kobe 650-0017, Japan,[5] and Organization of Advanced Science and Technology, Kobe University, 1-1 Rokkodaicho, Nada-ku, Kobe 657-8501, Japan[6]

Received 8 September 2017; accepted 16 January 2018
Available online xxx

Polyacrylic acid (PAA)-modified titanium peroxide nanoparticles (PAA-TiO_x NPs) are promising radiosensitizers. PAA-TiO_x NPs were synthesized from commercial TiO_2 nanoparticles that were modified with PAA and functionalized by H_2O_2 treatment. To realize practical clinical uses for PAA-TiO_x NPs, their tissue distribution and acute toxicity were evaluated using healthy mice and mice bearing tumors derived from xenografted MIAPaCa-2 human pancreatic cancer cells. Healthy mice were injected with PAA-TiO_x NPs at 25 mg/kg body weight via the tail vein, and tumor-bearing mice were injected either into the tumor locally or via the tail vein. The concentration of PAA-TiO_x NPs in major organs was determined over time using inductively coupled–plasma atomic emission spectrometry. After 1 h, 12% of the PAA-TiO_x NP dose had accumulated in the tumor, and 2.8% of the dose remained after 1 week. Such high accumulation could be associated with enhanced permeability and retention effects of the tumor, as PAA-TiO_x NPs are composed of inorganic particles and polymers, without tumor-targeting molecules. The liver accumulated the largest proportion of the injected nanoparticles, up to 42% in tumor-bearing mice. Blood biochemical parameters were also investigated after intravenous injection of PAA-TiO_x NPs in healthy mice. PAA-TiO_x NPs invoked a slight change in various liver-related biochemical parameters, but no liver injury was observed over the practical dose range. In the future, PAA-TiO_x NPs should be modified to prevent accumulation in the liver and minimize risk to patients.

[**Key words:** Titanium peroxide; Titanium dioxide; Nanoparticle; Radiosensitizer; Tissue distribution; Acute toxicity; Enhanced permeability and retention effect; Cancer]

Since discovery of the X-ray, radiotherapy has been one of the three major weapons used against cancer, the other two being surgical resection and chemotherapy. Radiosensitizing therapy involves the administration of a radiosensitizer to improve the efficacy of radiotherapy. Numerous researchers have investigated a variety of drugs and other substances as potential radiosensitizers to enhance the therapeutic effect of X-rays (1,2).

Nanoparticles (NPs) consisting of heavy metals and their oxides are a major category of radiosensitizers (3–8). The efficacy of X-rays is enhanced via interactions with the heavy metal atoms of NPs, which results in the emission of secondary electrons, X-rays, and/or Auger electrons that injure target cells. Metals of a high atomic number are generally chosen for use in NPs in order to invoke stronger interactions. Gold is one such radiosensitizing heavy metal, and its use in NPs has been investigated since the dawn of radiotherapy (9,10). A more recently developed type of NP composed of hafnium dioxide, designated NBTXR3, has been evaluated in clinical trials (11,12).

Polyacrylic acid (PAA)-modified titanium peroxide nanoparticles (PAA-TiO_x NPs) are now being developed as novel radiosensitizers. PAA-TiO_x NPs are made from anatase titania nanoparticles (TiO_2 NPs) by surface modification using PAA and H_2O_2. In our previous study, we demonstrated that PAA-TiO_x NPs enhance therapeutic X-ray irradiation *in vivo*. Growth of tumors xenografted into nude mice was effectively suppressed by combining PAA-TiO_x NP injection with X-ray irradiation therapy (13). As titanium is a relatively light atom, its interaction with X-rays results in only limited emission of secondary X-rays and electrons. However, our recent study suggested that PAA-TiO_x NPs continuously release H_2O_2 molecules into the liquid phase of a dispersion, which contributes to their radiosensitizing effect (14).

Reports suggest that titanium metal and TiO_2 are generally non-toxic to animals (15,16). As such, NPs incorporating titanium have been used as white pigments and food additives. However, more recent research has suggested that the bulk form of some substances that had previously been regarded as non-toxic do in fact pose a risk

* Corresponding author. Tel./fax: +81 78 803 6193.
E-mail addresses: kmorita@port.kobe-u.ac.jp (K. Morita), suuutakaa@yahoo.co.jp (T. Suzuki), nyuya@landscape.kobe-u.ac.jp (Y. Nishimura), y99u9i@chiba-u.jp (K. Matsumoto), numako@chiba-u.jp (C. Numako), kazuyoshi-sato@gunma-u.ac.jp (K. Sato), naka2008@med.kobe-u.ac.jp (M. Nakayama), yuunasasaki@hotmail.com (R. Sasaki), ochiaki@port.kobe-u.ac.jp (C. Ogino), akondo@kobe-u.ac.jp (A. Kondo).

1389-1723/$ – see front matter
https://doi.org/10.1016/j.jbiosc.2018.01.012

Please cite this article in press as: Morita, K., et al., *In vivo* tissue distribution and safety of polyacrylic acid-modified titanium peroxide nanoparticles as novel radiosensitizers, J. Biosci. Bioeng., (2018), https://doi.org/10.1016/j.jbiosc.2018.01.012

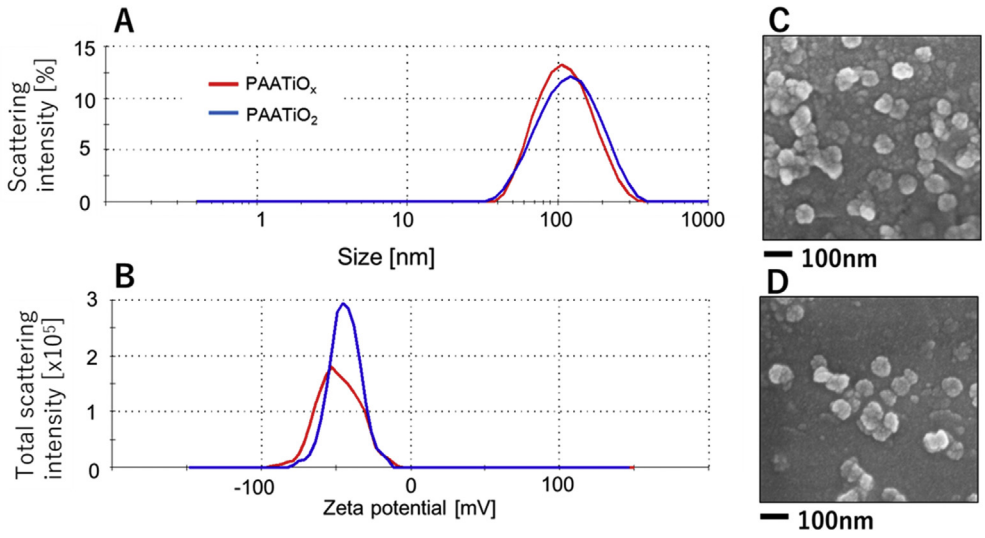

FIG. 1. Characterization of PAA-TiO_x NPs and PAA-TiO_2 NPs. (A) Particle size and (B) zeta potential distribution were measured by DLS. SEM images of (C) PAA-TiO_x NPs and (D) PAA-TiO_2 NPs.

to animal health in medical or dietary use when administered in formulations containing structures of sub-micrometer size (17). Hence, regardless of the common nature of substances such as gold (18), silver (19), carbon (20), silica (21), zinc oxide (22) or titania (23,24), it is important to investigate issues related to tissue distribution and toxicity of all nano-structured materials that might be used in medical applications or consumer products.

Despite the wide use of TiO_2 NPs, few studies have examined their biodistribution and toxicity following intravenous injection. Importantly, PAA-TiO_x NPs, which are synthesized from TiO_2 NPs, have never been assessed as to their level of safety. Thorough investigation of the tissue distribution and toxicity of these NPs is imperative before they can be utilized in radiosensitizing therapy. Thus, in the present study, we determined the tissue distribution and acute toxicity of PAA-TiO_x NPs using healthy mice and tumor-bearing mice.

MATERIALS AND METHODS

Synthesis of PAA-TiO_x NPs and PAA-TiO_2 NPs The TiO_2 nanoparticles (STS-01) used in this study were purchased from Ishihara Sangyo Kaisha, Ltd. (Osaka, Japan). Details of the procedure for synthesizing PAA-TiO_x NPs are provided in a previous report (13). Briefly, PAA-modified titanium dioxide NPs (PAA-TiO_2 NPs) were synthesized from TiO_2 NPs via a hydrothermal method for surface modification with PAA. PAA-TiO_x NPs were then synthesized from PAA-TiO_2 NPs by immersion in 6% H_2O_2 solution.

Analysis of NP size and morphology Synthesized NPs were characterized by scanning electron microscopy (SEM) using a JSM-5610LVS instrument (JEOL, Tokyo, Japan). Colloidal dispersions were dried on aluminum foil, and the samples were coated with platinum-paradium using an auto fine coater (JFC-1600; JEOL) operated at 20 mA for 90 s. The acceleration voltage was 5 kV and the emission current was 10 μA for shooting the photos. Dynamic light scattering (DLS) was performed using a Zetasizer nano ZS (Malvern Instruments Ltd., Worcestershire, UK) to measure the NP diameter and Z-potential.

Cells and animals Balb/cAJcl mice (4 weeks old) were used as a model of healthy animals; Balb/cAJcl-nu/nu mice served as the xenograft model. All mice were obtained from CLEA Japan, Inc. (Tokyo, Japan) and housed in an individually ventilated cage system (2GM140; Tecniplast S.p.A., Buguggiate [VA], Italy) with room humidity and temperature controlled at 55 ± 10% and 23 ± 2 °C, respectively, and with the ventilation speed set at 75 cycles/h. The mice were subjected to a 12-h light/dark cycle and given free access to water and a standard laboratory diet. The animal experiments were approved by the Institutional Animal Care and Use Committee (permission number: 25-11-01) and carried out according to Kobe University Animal Experimentation Regulations.

MIAPaCa-2 human pancreatic cells were purchased from the American Type Culture Collection (Rockville, MD, USA) and maintained in RPMI 1640 medium (Nacalai Tesque, Kyoto, Japan) supplemented with 10% fetal bovine serum (Thermo Fisher Scientific, Waltham, MA, USA), 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B (Nacalai Tesque). Cells were cultured at 37 °C in a humidified incubator with 5% CO_2 atmosphere.

Production of MIAPaCa-2 human pancreatic cancer xenograft mice MIAPaCa-2 cells (2×10^6) were dispersed in Matrigel Basement Membrane Matrix (Corning, NY, USA) and then injected subcutaneously into the hind legs of nude mice. The xenografted mice were employed to administration experiments of nanoparticles 4 weeks after the inoculation.

Administration of PAA-TiO_x NPs The dose of PAA-TiO_x NPs was determined based on a consideration of the detection range of inductively coupled plasma–atomic emission spectrometry (ICP-AES) and the concentration at which PAA-TiO_x NPs exhibited a synergistic effect with X-ray irradiation *in vivo* in a previous study (13). PAA-TiO_x NPs and PAA-TiO_2 NPs were diluted in phosphate-buffered saline (PBS) to 5 mg/ml. Healthy mice were injected with NP dispersion via the tail vein at a dose of 25 mg/kg body weight. In xenografted mice ($n = 7$), NP dispersion was injected directly into the tumor or the tail vein at the same concentration. Control mice ($n = 5$) received an intravenous injection of 100 μl of PBS.

ICP-AES analyses At specified time points after injection (1 h, 1 day, and 1 week), mice were anesthetized with isoflurane and then euthanized. The blood, heart, lungs, liver, spleen, pancreas, kidneys, and tumor (in the case of xenograft mice) were collected and stored in 10% formalin solution (Nacalai Tesque). The organs were minced in alumina crucibles and heated at 1000 °C for 1 h in a 300-Plus electric furnace (Denken-Highdental, Kyoto, Japan). Subsequently, concentrated nitric acid and concentrated sulfuric acid (250 μl each) were added to the crucible with heating at 230 °C for 2 h on a hot plate to digest all residual material. Finally, the digested components were taken up with 10 ml of hydrochloric acid (0.5 M). The concentration of Ti^{4+} ions in the digested organ samples was quantified by ICP-AES using an SPS-3100 instrument (Hitachi High-Tech Science, Tokyo, Japan; calibration: external; nebulizer: conical U-series 2 ml/min [Glass Expansion, Melbourne, Australia]; wavelength: Ti 337.800 nm). Accumulation of PAA-TiO_2 NPs in the organs was calculated as the weight percent (wt%) of the injected NPs in the sample relative to the weight of NPs injected, as all detected Ti^{4+} ions were all considered to have originated from the PAA-TiO_2 NPs.

Biochemical marker test Blood samples were collected from healthy mice at euthanization either 1 day or 1 week after intravenous injection of PAA-TiO_x dispersion, PAA-TiO_2 dispersion, H_2O_2 solution, or PBS in order to examine the biodistribution of the NPs. Blood samples were centrifuged to produce serum, which was then used for analysis of the following biochemical parameters (Oriental Yeast Co., Ltd., Tokyo, Japan; $n = 3$): total protein (TP), albumin (ALB), blood urea nitrogen (BUN), creatinine (CRE), sodium (Na), potassium (K), chloride (Cl), inorganic phosphate (IP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), low-density lipoprotein (LDH), amylase (AMY), total cholesterol (T-CHO), triglycerides (TGs), high-density lipoprotein cholesterol (HDL-C), total bilirubin (T-BIL), and glucose (GLU).

Calculation for delivery efficiency of NPs For a quantitative analysis, delivery efficiency (DE) of NPs was calculated. DE was defined according to following Eqs. 1–3.

$$\text{Trapezoidal area } (T_i) = 0.5(C_i + C_{i-1})(t_i - t_{i-1}) \quad (1)$$

Please cite this article in press as: Morita, K., et al., *In vivo* tissue distribution and safety of polyacrylic acid-modified titanium peroxide nanoparticles as novel radiosensitizers, J. Biosci. Bioeng., (2018), https://doi.org/10.1016/j.jbiosc.2018.01.012