applications as well [23,24]. Therefore, the localization and biological effects of intranasally administered nanosilica particles must be elucidated. We expect that further studies of the relationship between localization and biological effects will provide useful information for the development of safer, effective NMs.

Conclusions

We have shown that nanosilica particles with diameters of 30, 70, and 100 nm intranasally administered to mice were absorbed into the bloodstream and distributed into certain organs, such as the liver. The obtained results suggest that the activation of an intrinsic cascade pathway induced by nanosilica particles with diameters of 30 and 70 nm, both of which activated coagulation factor XII and platelets, could result in abnormal activation of the coagulation system. We expect that the findings of this study will contribute to the ongoing development of NMs that are safe for use in humans and animals.

Methods

Silica particles

Amorphous nanosilica particles with diameters of 30, 70, and 100 nm, as well as microscale silica particles with diameters of 300 and 1000 nm (Micromod Partikeltechnologie, Rostock/Warnemünde, Germany, designated nSP30, nSP70, nSP100, mSP300, and mSP1000, respectively) were used in this study. The particle numbers of the silica particles were 3.5×10^{13}, 2.8×10^{12}, 9.5×10^{11}, 3.5×10^{10}, and 9.5×10^{8} particles/mg for nSP30, nSP70, nSP100, mSP300, and mSP1000, respectively. Each type of silica particles was sonicated for 5 min and vortexed for 1 min before use.

Animals

BALB/c mice (female, 6–8 weeks) were purchased from Japan SLC, Inc. (Shizuoka, Japan). Mice were housed in a ventilated animal room maintained at 20 ± 2°C with a 12-h light/12-h dark cycle. Mice had free access to water and alfalfa-free forage (FR-2, Funabashi Farm, Funabashi, Japan). All of the animal experimental procedures in this study were performed in accordance with the National Institute of Biomedical Innovation and Osaka University Guidelines for the Welfare of Animals.

Transmission electron microscopy analysis

Five BALB/c mice were intranasally exposed to a 20 μL aliquot (10 μL per nostril) of nSP30, nSP70, nSP100, mSP300, or mSP1000 at a concentration of 500 μg/mouse for 7 days. Twenty-four hours after the final intranasal administration, the nasal cavity, lung, and liver from two mice were excised and fixed in 2.5% glutaraldehyde for 2 h. Then, small pieces of tissue sample were washed with phosphate buffer 3 times and postfixed in sodium cacodylate-buffered 1.5% osmium tetroxide for 60 min at 4°C, block-stained in 0.5% uranyl acetate, dehydrated by dipping each sample through a series of ethanol solutions containing increasing concentration of ethanol, and embedded in Epon resin (TAAB). Ultrathin sections were stained with uranyl acetate and lead citrate. The stained samples were subsequently observed under an electron microscope (H-7650, Hitachi, Tokyo, Japan).

Histopathological examination

Twenty-four hours after the final intranasal administration of each type of silica particles, the nasal cavity, lung, and liver from three mice were excised and fixed immediately in 4% paraformaldehyde. These tissues were embedded in paraffin blocks and then sliced, and the slices were placed on glass slides. After hematoxylin–eosin staining, the slides were observed, and cell aggregation in the nasal cavity, microglial aggregation in the brain, and cell aggregation and rarefaction in the liver were classified into one of five grades (0: none, 1: very slight, 2: mild, 3: moderate, 4: advanced).

Blood biomarker assay

Twenty-four hours after the final intranasal administration of each type of silica particles, blood samples were collected from the heart using plastic syringes (Terumo, Tokyo, Japan) containing 5 IU/mL heparin sodium. Plasma was harvested by centrifuging the blood at 1750 × *g* for 15 min. The levels of ALT, ALB and BUN were determined in the plasma using a biochemical autoanalyzer (Fuji dri-Chem 7000, Fujifilm, Tokyo, Japan).

Hematology analysis

Twenty-four hours after the final intranasal administration of each type of amorphous silica particles, blood samples were collected from the heart using plastic syringes (Terumo) containing 0.1 mM EDTA. Whole blood samples were analyzed with a VetScan HMII Hematology System (Abaxis, Sunnyvale, CA, USA) to determine the number of white blood cells, lymphocytes, monocytes, and platelets.

Measurement of bleeding time and coagulation tests

Twenty-four hours after the final intranasal administration of each type of silica particles, the bleeding time for each mouse was measured by Duke's method [43]. Briefly, an ear of each mouse was cut with a knife, and the blood generated at the site of the cut was absorbed with filter paper every 30 seconds until bleeding ceased. To examine the coagulation tests in each mouse, a blood sample was collected from the heart of each mouse subjected to bleeding-time tests using plastic syringes (Terumo) containing 1:9 (v/v) of 3.8% sodium citrate. Plasma was harvested by

centrifuging the blood at 1750 × *g* for 15 min. APTT and PT levels were determined at 37°C in a Clotek dry-block bath system (Hyland Division, Travenol Laboratories, Inc. Costa Mesa, CA, USA) with APTT and PT reagents (Sysmex, Kobe, Japan), respectively.

In vitro activation tests of coagulation factor XII

One hundred microliters of human health plasma (Scipac, Kent, UK) and 100 μL of various sized silica particles (3.13 mg/mL) were mixed for 1 min at room temperature with an enzyme reaction solution (50 mM Tris–HCl, 0.15 M NaCl, 1 mM $CaCl_2$ and 0.1 mg/mL bovine serum albumin, pH 8.0) containing 2 mM *t*-butyloxycarbonyl-L-glutaminyl-glycyl-L-arginine-4-methyl-courmaryl-7-amide (Peptide Institute, Inc. Ibaraki, Japan) in dimethyl sulfoxide. The initial rate of reaction was calculated by measuring the fluorescence intensity (380 nm excitation, 440 nm emission) every 5 min. The initial rate is given by Initial rate = (relative fluorescent unit of each measurement time – blank) / measurement time.

Detection of TF, sCD40L, and vWF levels in plasma

TF, sCD40L, and vWF levels in plasma were determined using enzyme-linked immunosorbent assay (ELISA) kits (TF: Mouse Tissue Factor ELISA kit (Cusabio, Newark, DE, USA); sCD40L: Mouse sCD40L ELISA (eBioscience, San Diego, CA, USA); vWF: Mouse von Willebrand Factor ELISA kit (Cusabio, Newark, DE, USA)).

Statistical analysis

Differences among each group were compared by using Williams's or Dunnett's method after analysis of variance (ANOVA).

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

TY and YY designed the study. TY, ST, TH, MU, and KI performed the experiments. TY and YY collected and analyzed the data. TY and YY wrote the manuscript. KN, YA, HK, ST, HN, KH, and TY provided technical support and conceptual advice. YT supervised the project. All authors discussed the results and commented on the manuscript. All authors read and approved the final manuscript.

Acknowledgements

This study was supported, in part, by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT) and from the Japan Society for the Promotion of Science (JSPS); and by the Knowledge Cluster Initiative (MEXT); by Health Labour Sciences Research Grants from the Ministry of Health, Labour and Welfare of Japan (MHLW); by a Global Environment Research Fund from the Ministry of the Environment; by Food Safety Commission (Cabinet Office); by The Cosmetology Research Foundation; by The Smoking Research Foundation; by The Research Foundation for Pharmaceutical Sciences; and by The Takeda Science Foundation.

Author details

[1]Laboratory of Toxicology and Safety Science, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan. [2]Laboratory of Biopharmaceutical Research, National Institute of Biomedical Innovation, 7-6-8 Saitoasagi, Ibaraki, Osaka 567-0085, Japan. [3]Cancer Biology Research Center, Sanford Research/USD, 2301 E. 60th Street N, Sioux Falls SD 57104, USA. [4]The Center for Advanced Medical Engineering and Informatics, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan. [5]Division of Foods, National Institute of Health Sciences, 1-18-1, Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan.

Received: 14 February 2013 Accepted: 14 August 2013
Published: 20 August 2013

References

1. Sozer N, Kokini JL: **Nanotechnology and its applications in the food sector.** *Trends Biotechnol* 2009, **27**:82–89.
2. McNeil SE: **Unique benefits of nanotechnology to drug delivery and diagnostics.** *Methods Mol Biol* 2011, **697**:3–8.
3. Sonkaria S, Ahn SH, Khare V: **Nanotechnology and its impact on food and nutrition: a review.** *Recent Pat Food Nutr Agric* 2012, **4**:8–18.
4. Knopp D, Tang D, Niessner R: **Review: bioanalytical applications of biomolecule-functionalized nanometer-sized doped silica particles.** *Anal Chim Acta* 2009, **647**:14–30.
5. Maynard AD, Aitken RJ, Butz T, Colvin V, Donaldson K, Oberdorster G, Philbert MA, Ryan J, Seaton A, Stone V, *et al*: **Safe handling of nanotechnology.** *Nature* 2006, **444**:267–269.
6. Kuhlbusch TA, Asbach C, Fissan H, Gohler D, Stintz M: **Nanoparticle exposure at nanotechnology workplaces: a review.** *Part Fibre Toxicol* 2011, **8**:22.
7. Donaldson K, Poland CA: **Inhaled nanoparticles and lung cancer - what we can learn from conventional particle toxicology.** *Swiss Med Wkly* 2012, **142**:w13547.
8. Poland CA, Duffin R, Kinloch I, Maynard A, Wallace WA, Seaton A, Stone V, Brown S, Macnee W, Donaldson K: **Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study.** *Nat Nanotechnol* 2008, **3**:423–428.
9. Hougaard KS, Jackson P, Jensen KA, Sloth JJ, Loschner K, Larsen EH, Birkedal RK, Vibenholt A, Boisen AM, Wallin H, *et al*: **Effects of prenatal exposure to surface-coated nanosized titanium dioxide (UV-Titan): a study in mice.** *Part Fibre Toxicol* 2010, **7**:16.
10. Morishige T, Yoshioka Y, Tanabe A, Yao X, Tsunoda S, Tsutsumi Y, Mukai Y, Okada N, Nakagawa S: **Titanium dioxide induces different levels of IL-1beta production dependent on its particle characteristics through caspase-1 activation mediated by reactive oxygen species and cathepsin B.** *Biochem Biophys Res Commun* 2010, **392**:160–165.
11. Nabeshi H, Yoshikawa T, Matsuyama K, Nakazato Y, Matsuo K, Arimori A, Isobe M, Tochigi S, Kondoh S, Hirai T, *et al*: **Systemic distribution, nuclear entry and cytotoxicity of amorphous nanosilica following topical application.** *Biomaterials* 2011, **32**:2713–2724.
12. Yamashita K, Yoshioka Y, Higashisaka K, Mimura K, Morishita Y, Nozaki M, Yoshida T, Ogura T, Nabeshi H, Nagano K, *et al*: **Silica and titanium dioxide nanoparticles cause pregnancy complications in mice.** *Nat Nanotechnol* 2011, **6**:321–328.
13. Yoshida T, Yoshioka Y, Fujimura M, Yamashita K, Higashisaka K, Morishita Y, Kayamuro H, Nabeshi H, Nagano K, Abe Y, *et al*: **Promotion of allergic immune responses by intranasally-administrated nanosilica particles in mice.** *Nanoscale Res Lett* 2011, **6**:195.
14. Hirai T, Yoshikawa T, Nabeshi H, Yoshida T, Tochigi S, Ichihashi K, Uji M, Akase T, Nagano K, Abe Y, *et al*: **Amorphous silica nanoparticles size-dependently aggravate atopic dermatitis-like skin lesions following an intradermal injection.** *Part Fibre Toxicol* 2012, **9**:3.
15. Nabeshi H, Yoshikawa T, Matsuyama K, Nakazato Y, Arimori A, Isobe M, Tochigi S, Kondoh S, Hirai T, Akase T, *et al*: **Amorphous nanosilicas induce consumptive coagulopathy after systemic exposure.** *Nanotechnology* 2012, **23**:045101.
16. Morishige T, Yoshioka Y, Inakura H, Tanabe A, Narimatsu S, Yao X, Monobe Y, Imazawa T, Tsunoda S, Tsutsumi Y, *et al*: **Suppression of nanosilica particle-induced inflammation by surface modification of the particles.** *Arch Toxicol* 2012, **86**:1297–1307.
17. Norris LA: **Blood coagulation.** *Best Pract Res Clin Obstet Gynaecol* 2003, **17**:369–383.
18. Zhuo R, Miller R, Bussard KM, Siedlecki CA, Vogler EA: **Procoagulant stimulus processing by the intrinsic pathway of blood plasma coagulation.** *Biomaterials* 2005, **26**:2965–2973.

19. Muller F, Renne T: **Platelet polyphosphates: the nexus of primary and secondary hemostasis.** *Scand J Clin Lab Invest* 2011, **71**:82–86.
20. Blann AD: **Plasma von Willebrand factor, thrombosis, and the endothelium: the first 30 years.** *Thromb Haemost* 2006, **95**:49–55.
21. Pamukcu B, Lip GY, Snezhitskiy V, Shantsila E: **The CD40-CD40L system in cardiovascular disease.** *Ann Med* 2011, **43**:331–340.
22. Merget R, Bauer T, Kupper HU, Philippou S, Bauer HD, Breitstadt R, Bruening T: **Health hazards due to the inhalation of amorphous silica.** *Arch Toxicol* 2002, **75**:625–634.
23. Moghimi SM, Hunter AC, Murray JC: **Nanomedicine: current status and future prospects.** *FASEB J* 2005, **19**:311–330.
24. Slowing II, Vivero-Escoto JL, Wu CW, Lin VS: **Mesoporous silica nanoparticles as controlled release drug delivery and gene transfection carriers.** *Adv Drug Deliv Rev* 2008, **60**:1278–1288.
25. Warheit DB, Carakostas MC, Kelly DP, Hartsky MA: **Four-week inhalation toxicity study with Ludox colloidal silica in rats: pulmonary cellular responses.** *Fundam Appl Toxicol* 1991, **16**:590–601.
26. Lee KP, Kelly DP: **The pulmonary response and clearance of Ludox colloidal silica after a 4-week inhalation exposure in rats.** *Fundam Appl Toxicol* 1992, **19**:399–410.
27. Sonaje K, Lin KJ, Tseng MT, Wey SP, Su FY, Chuang EY, Hsu CW, Chen CT, Sung HW: **Effects of chitosan-nanoparticle-mediated tight junction opening on the oral absorption of endotoxins.** *Biomaterials* 2011, **32**:8712–8721.
28. Vllasaliu D, Exposito-Harris R, Heras A, Casettari L, Garnett M, Illum L, Stolnik S: **Tight junction modulation by chitosan nanoparticles: comparison with chitosan solution.** *Int J Pharm* 2010, **400**:183–193.
29. Wang J, Chen C, Liu Y, Jiao F, Li W, Lao F, Li Y, Li B, Ge C, Zhou G, *et al*: **Potential neurological lesion after nasal instillation of TiO(2) nanoparticles in the anatase and rutile crystal phases.** *Toxicol Lett* 2008, **183**:72–80.
30. Wang J, Liu Y, Jiao F, Lao F, Li W, Gu Y, Li Y, Ge C, Zhou G, Li B, *et al*: **Time-dependent translocation and potential impairment on central nervous system by intranasally instilled TiO(2) nanoparticles.** *Toxicology* 2008, **254**:82–90.
31. Liu Y, Gao Y, Zhang L, Wang T, Wang J, Jiao F, Li W, Liu Y, Li Y, Li B, *et al*: **Potential health impact on mice after nasal instillation of nano-sized copper particles and their translocation in mice.** *J Nanosci Nanotechnol* 2009, **9**:6335–6343.
32. Kim SF, Daniel JW, Francois LP, Anwen MKH, Hui QL, John VH, Barbé CJ: **Biodegradability of sol–gel silica microparticles for drug delivery.** *Journal of Sol-gel Science and Technology* 2009, **49**:12–18.
33. Zhai W, He C, Wu L, Zhou Y, Chen H, Chang J, Zhang H: **Degradation of hollow mesoporous silica nanoparticles in human umbilical vein endothelial cells.** *J Biomed Mater Res B Appl Biomater* 2012, **100**:1397–1403.
34. He Q, Shi J, Zhu M, Chen Y, Chen F: **The three-stage *in vitro* degradation behavior of mesoporous silica in simulated body fluid.** *Microporous and Mesoporous Materials* 2010, **131**:314–320.
35. Bihari P, Holzer M, Praetner M, Fent J, Lerchenberger M, Reichel CA, Rehberg M, Lakatos S, Krombach F: **Single-walled carbon nanotubes activate platelets and accelerate thrombus formation in the microcirculation.** *Toxicology* 2010, **269**:148–154.
36. Burke AR, Singh RN, Carroll DL, Owen JD, Kock ND, D'Agostino R Jr, Torti FM, Torti SV: **Determinants of the thrombogenic potential of multiwalled carbon nanotubes.** *Biomaterials* 2011, **32**:5970–5978.
37. Meng J, Cheng X, Liu J, Zhang W, Li X, Kong H, Xu H: **Effects of long and short carboxylated or aminated multiwalled carbon nanotubes on blood coagulation.** *PLoS One* 2012, **7**:e38995.
38. Nemmar A, Melghit K, Ali BH: **The acute proinflammatory and prothrombotic effects of pulmonary exposure to rutile TiO2 nanorods in rats.** *Exp Biol Med (Maywood)* 2008, **233**:610–619.
39. Colman RW: **Surface-mediated defense reactions. The plasma contact activation system.** *J Clin Invest* 1984, **73**:1249–1253.
40. Colman RW, Schmaier AH: **Contact system: a vascular biology modulator with anticoagulant, profibrinolytic, antiadhesive, and proinflammatory attributes.** *Blood* 1997, **90**:3819–3843.
41. Tavano R, Segat D, Reddi E, Kos J, Rojnik M, Kocbek P, Iratni S, Scheglmann D, Colucci M, Echevarria IM, Selvestrel F, Mancin F, Papini E: **Procoagulant properties of bare and highly PEGylated vinyl-modified silica nanoparticles.** *Nanomedicine (Lond)* 2010, **5**:881–896.
42. Barik TK, Sahu B, Swain V: **Nanosilica-from medicine to pest control.** *Parasitol Res* 2008, **103**:253–258.
43. Angelkort B, Zilkens KW, Wenzel E: **Bleeding time (Duke) as a clinical function test of the primary phase of hemostasis.** *Med Welt* 1976, **27**:2302–2304.

doi:10.1186/1743-8977-10-41
Cite this article as: Yoshida *et al.*: Intranasal exposure to amorphous nanosilica particles could activate intrinsic coagulation cascade and platelets in mice. *Particle and Fibre Toxicology* 2013 **10**:41.

Liver-specific microRNAs as biomarkers of nanomaterial-induced liver damage

IOP PUBLISHING NANOTECHNOLOGY
Nanotechnology 24 (2013) 405102 (7pp) doi:10.1088/0957-4484/24/40/405102

Liver-specific microRNAs as biomarkers of nanomaterial-induced liver damage

Takashi Nagano[1,6], Kazuma Higashisaka[1,6], Akiyoshi Kunieda[1], Yuki Iwahara[1], Kota Tanaka[1], Kazuya Nagano[2], Yasuhiro Abe[3], Haruhiko Kamada[2,4], Shin-ichi Tsunoda[2,4], Hiromi Nabeshi[5], Tomoaki Yoshikawa[1], Yasuo Yoshioka[1,7] and Yasuo Tsutsumi[1,2,4,7]

[1] Laboratory of Toxicology and Safety Science, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan
[2] Laboratory of Biopharmaceutical Research, National Institute of Biomedical Innovation, 7-6-8, Saito-Asagi, Ibaraki, Osaka 567-0085, Japan
[3] Cancer Biology Research Center, Sanford Research/USD, Sioux Falls, SD, USA
[4] The Center for Advanced Medical Engineering and Informatics, Osaka University, 1-6, Yamadaoka, Suita, Osaka 565-0871, Japan
[5] Division of Foods, National Institute of Health Sciences, Tokyo, Japan

E-mail: yasuo@phs.osaka-u.ac.jp and ytsutsumi@phs.osaka-u.ac.jp

Received 9 May 2013, in final form 23 August 2013
Published 12 September 2013
Online at stacks.iop.org/Nano/24/405102

Abstract
Although nanomaterials are being used in various fields, their safety is not yet sufficiently understood. We have been attempting to establish a nanomaterials safety-assessment system by using biomarkers to predict nanomaterial-induced adverse biological effects. Here, we focused on microRNAs (miRNAs) because of their tissue-specific expression and high degree of stability in the blood. We previously showed that high intravenous doses of silica nanoparticles of 70 nm diameter (nSP70) induced liver damage in mice. In this study, we compared the effectiveness of serum levels of liver-specific or -enriched miRNAs (miR-122, miR-192, and miR-194) with that of conventional hepatic biomarkers (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)) as biomarkers for nSP70. After mice had been treated with nSP70, their serum miRNAs levels were measured by using quantitative RT-PCR. Serum levels of miR-122 in nSP70-treated mice were the highest among the three miRNAs. The sensitivity of miR-122 for liver damage was at least as good as those of ALT and AST. Like ALT and AST, miR-122 may be a useful biomarker of nSP70. We believe that these findings will help in the establishment of a nanomaterials safety-assessment system.

1. Introduction

Nanomaterials are defined as substances that have at least one dimension less than 100 nm long. They are now widely used in cosmetics, foods, and medicines because they possess innovative functions such as high electrical conductivity, tensile strength, and tissue permeability [1, 2]. However, with the development of nanomaterials has come concern that, unlike the case with microsized materials, their unique characteristics may induce unexpected biological responses [3–5]. For example, recent reports have shown that carbon nanotubes can induce mesothelioma-like lesions in mice, similar to those induced by asbestos [6]. In addition, our group has shown that silica nanoparticles with a diameter of 70 nm (nSP70) can induce severe liver damage and pregnancy complications in mice, and reactive oxygen species (ROS) generation and DNA damage *in vitro*, whereas microsized silica particles do not have these effects [7–10]. Nevertheless, because nanomaterials have the potential to improve our quality of life, it is important that we develop and promote the use of safe forms. Furthermore, the development of

[6] These authors contributed equally to the work.
[7] Address for correspondence: Laboratory of Toxicology and Safety Science, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan.

biomarkers of the biological effects of nanomaterials would be invaluable for establishing a nanomaterials safety-assessment system and strategies for the development, production, and use of safe forms of nanomaterials. We have already explored biomarkers, with a focus on proteins, and have shown that acute-phase proteins such as haptoglobin, serum amyloid A, and C-reactive protein could be useful biomarkers of the biological effects of silica or platinum nanoparticles [11–13]. However, the use of proteins alone is not sufficient for predicting the adverse biological effects of nanomaterials, as we need to evaluate biomarkers consisting of other biological molecules.

microRNAs (miRNAs) are highly conserved and small (18- to 25-nucleotide) RNAs that play pivotal roles in gene expression—specifically at the post-transcriptional level—in plants and animals. In humans, miRNAs are involved in the regulation of development, cell differentiation, proliferation, and apoptosis [14, 15], because they regulate as many as one-third of all messenger RNAs (mRNAs) [16, 17]. Furthermore, recent reports have demonstrated that miRNAs would serve as useful biomarkers for the non-invasive, tissue-specific evaluation of diseases and drug-induced tissue damage [18, 19]. This is because they show tissue-specific expression [20, 21] and exist in a stable form in the blood, saliva, and urine [22–24].

From this perspective, we focused on miRNAs as biomarkers for predicting the biological effects of nanomaterials. Although there have been some analyses of the changes in miRNA expression following exposure to nanomaterials [25, 26], there have been very few attempts to use miRNAs as biomarkers of nanomaterials. We therefore need to collect systematic information on miRNAs as biomarkers so as to predict nanomaterials-induced biological effects and thus establish a nanomaterials safety-assessment system.

In this study, as a first trial, we attempted to investigate whether liver-specific or -enriched miRNAs (miR-122, miR-192, and miR-194) could be used as reliable biomarkers for liver damage induced by nSP70.

2. Materials and methods

2.1. Materials

Silica particles were purchased from micromod Partikeltechnologie (Rostock/Warnemünde, Germany). Silica particles with diameters of 70, 300, and 1000 nm (nSP70, nSP300, and mSP1000, respectively) were used. In addition, we used nSP70 modified with surface functional groups-a carboxyl group (nSP70-C) or an amino group (nSP70-N). The particles were sonicated for 5 min and then vortexed for 1 min before use. Lipopolysaccharide (LPS) and D-galactosamine hydrochloride (D-GalN) were purchased from Sigma-Aldrich (Tokyo, Japan).

2.2. Animals

Female BALB/c mice were purchased from Nippon SLC, Inc. (Shizuoka, Japan) and used at 6–7 weeks of age. All of the animal experimental procedures were performed in accordance with the Osaka University and National Institute of Biomedical Innovation guidelines for the welfare of animals.

2.3. Blood sample collection

BALB/c mice ($n = 5$ or 6 per group) were treated in various experiments with nSP70 at 10, 20, or 40 mg kg^{-1} or with nSP70-C, nSP70-N, nSP300, or mSP1000 at 40 mg kg^{-1}, via injection into the tail vein. As positive control of induction of liver damage, BALB/c mice ($n = 5$ or 6 per group) were intraperitoneally treated with LPS at 10 μg kg^{-1} and D-GalN at 700 mg kg^{-1}. Blood samples were collected at varying times (4, 8, 24, or 72 h after treatment) in several experiments. Sera were harvested by blood centrifugation at 8000g for 15 min.

2.4. Biochemical analysis

Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured with a DRI-CHEM 7000 biochemical analyzer (Fujifilm Corp., Tokyo, Japan).

2.5. Isolation of total RNA from serum

We extracted total RNA, including small RNA, from serum samples by using a miRNeasy Mini Kit (QIAGEN, Tokyo, Japan) in accordance with the manufacturer's instructions. In brief, 250 μl of QIAzol lysis reagent was added to 50 μl of serum. After the addition of 50 μl of chloroform, the samples were mixed completely and centrifuged. Then, 100 μl of supernatant was transferred to a new tube and mixed with 150 μl of 100% ethanol. The sample was then applied directly to a RNeasy Mini spin column (QIAGEN) and the combined RNA to column was cleaned with wash buffers to remove impurities. Total RNA was eluted in 30 μl of RNase-free water.

2.6. Real-time quantitative reverse-transcription PCR (qRT-PCR)

Serum levels of miR-122, miR-192, and miR-194 were measured by using TaqMan RT-PCR. Each miRNA in 5 μl of purified total RNA was reverse transcribed with a miRNA-specific stem-loop reverse transcriptase primer (Life Technologies, Tokyo, Japan). TaqMan RT-PCR was performed on a StepOne Plus real-time PCR system (Life Technologies, Tokyo, Japan). Generally, typical internal controls for miRNA expression, such as U6 RNA and 5S rRNA, are degraded in serum samples [27]. Therefore, we equalized all of the conditions and then normalized the levels of each miRNA by serum volume. The expression level of each miRNA is shown as a relative fold-change. Relative expression was compared between the silica-particle-treated group and the untreated group.

2.7. Statistical analyses

All results are expressed as means ± SEM differences were compared by using the Student's t-test or Bonferroni test after ANOVA (analysis of variance).

3. Results

3.1. Physicochemical properties of silica nanoparticles

Silica nanoparticles are common nanomaterials that are being used in cosmetics, foods, and medicines [28, 29]. Considering that further use of silica nanoparticles is expected, it is important to assess their safety. We used silica particles with diameters of 70 nm (nSP70), 300 nm (nSP300), and 1000 nm (mSP1000), as well as nSP70 with carboxyl (nSP70-C) or amino (nSP70-N) surface functional groups. In our previous study, we confirmed by transmission electron microscopy that all silica particles of the above-listed sizes and types were smooth-surfaced spheres [7–10]. We have already shown that the hydrodynamic diameters of nSP70, nSP70-C, nSP70-N, nSP300, and mSP1000 are 64.6, 69.6, 71.8, 322, and 1140 nm, respectively, and their zeta potentials (overall surface potentials) are −52.7, −76.3, −29.0, −62.1, and −67.0 mV, respectively [7–10]. The carboxyl and amino surface modifications therefore altered the surface charge of the nSP70 particles. In addition, we have already shown that the size-distribution spectrum of each set of silica particles had a single peak, and the measured hydrodynamic diameter corresponded almost precisely to the primary particle size of each set of silica particles [7–10]. These results indicated that the silica particles used in this study were well dispersed in solution.

3.2. Liver-specific or -enriched miRNAs as biomarkers of silica nanoparticles

To evaluate the usefulness of microRNAs (miRNAs) as biomarkers for nanomaterials, we focused on a model of liver damage induced by nSP70. We have previously shown that treatment of mice with high-dose nSP70 intravenously via the tail vein induced lethal toxicity and severe liver damage. Therefore, we assessed the usefulness of the liver-specific or -enriched miRNAs miR-122, miR-192, and miR-194 as biomarkers of liver damage. miR-122 and miR-192 are the two miRNAs expressed most abundantly in the human liver [30], and hepatic miR-194 is highly expressed in hepatic epithelial cells, including hepatocytes, which are parenchymal cells and account for more than 80% of liver cells [31]. Therefore, to collect systematic information on whether miRNAs have the potential to be biomarkers of nanomaterials, we selected those three major miRNAs in liver. Mice were given nSP70, nSP300, or mSP1000 via the tail vein. A dose rate of 40 mg kg^{-1} was chosen, because in the case of nSP70 this dose rate significantly increases the levels of ALT and AST [10]. As positive control, mice were intraperitoneally treated with LPS and D-GalN. At 8 h after treatment, we evaluated the serum level of each miRNA by using qRT-PCR and those of ALT and AST (i.e. the conventional hepatic biomarkers) by using biochemical analysis. The levels of miR-122 (figure 1(A)), ALT (figure 1(B)), and AST (figure 1(C)) in LPS/D-GalN-treated mice were significantly increased compared to those of vesicle-treated mice. The levels of miR-122 (figure 1(A)) and miR-192 (figure 1(D)) in nSP70-treated mice were significantly greater than those in saline-, nSP300- or mSP1000-treated mice and untreated mice. In contrast, the serum levels of miR-194 did not differ significantly among any groups (figure 1(E)). In addition, the levels of ALT (figure 1(B)) and AST (figure 1(C)) in nSP70-treated mice were significantly greater than those in saline-, nSP300- or mSP1000-treated mice and untreated mice. Thus, miR-122 and miR-192 may be useful as biomarkers for evaluating liver damage induced by nSP70. We focused on miR-122 in the following assessment, because it had the highest rate of expression among the three miRNAs.

3.3. Sensitivity and time-dependency of miR-122 expression

To more precisely assess the serum levels of miR-122 as a biomarker, we examined the sensitivity and time-dependency of miR-122 expression after treatment with nSP70. Mice were intravenously treated with nSP70 at 10, 20, or 40 mg kg^{-1}. Blood samples were collected 8 h after treatment and the levels of miR-122, ALT, and AST were measured. The serum levels of miR-122 (figure 2(A)), ALT (figure 2(B)), and AST (figure 2(C)) showed dose-dependent patterns of expression. In mice treated with 40 mg kg^{-1} nSP70 and 20 mg kg^{-1} in the case of AST, the levels of all three markers were significantly greater than in untreated or saline-treated mice. Thus, as was the case with ALT and AST, the increase in serum miR-122 levels was dependent on the dose of nSP70. Next, to assess the time-dependency of miR-122 expression, we examined the serum levels of miR-122 (figure 2(D)), ALT (figure 2(E)), and AST (figure 2(F)) at 4, 8, 24, and 72 h after intravenous injection of nSP70 at 40 mg kg^{-1}. The serum levels of miR-122 in nSP70-treated mice were significantly higher than those in saline-treated mice at every time point, as was the case with ALT and AST. These results suggested that the pattern of release of miR-122 into the blood was the same as those of ALT and AST.

3.4. miR-122 response after treatment of mice with surface-modified nSP70

Our group has previously demonstrated that modification of nSP70 with surface functional groups such as a carboxyl group (nSP70-C) or amino group (nSP70-N) can reduce the toxic effects of nSP70 (e.g., pregnancy complications in mice and ROS generation and DNA damage *in vitro*) [8, 9]. Here, we tested the use of miR-122 as a biomarker to determine whether the safety of nSP70 could be improved by adding functional groups. BALB/c mice were treated intravenously with nSP70, nSP70-C, or nSP70-N at 40 mg kg^{-1}. At 8 h after treatment, we measured the levels of miR-122 (figure 3(A)), ALT (figure 3(B)), and AST (figure 3(C)). The serum levels of miR-122 in nSP70-C- or nSP70-N-treated mice were significantly lower than that in nSP70-treated mice and were almost the same as those in untreated or saline-treated mice. The serum levels of ALT after treatment with nSP70-C or nSP70-N showed a trend similar to those of miR-122. In contrast, although the level of AST in nSP70-N-treated mice was significantly lower than that in nSP70-treated mice, the

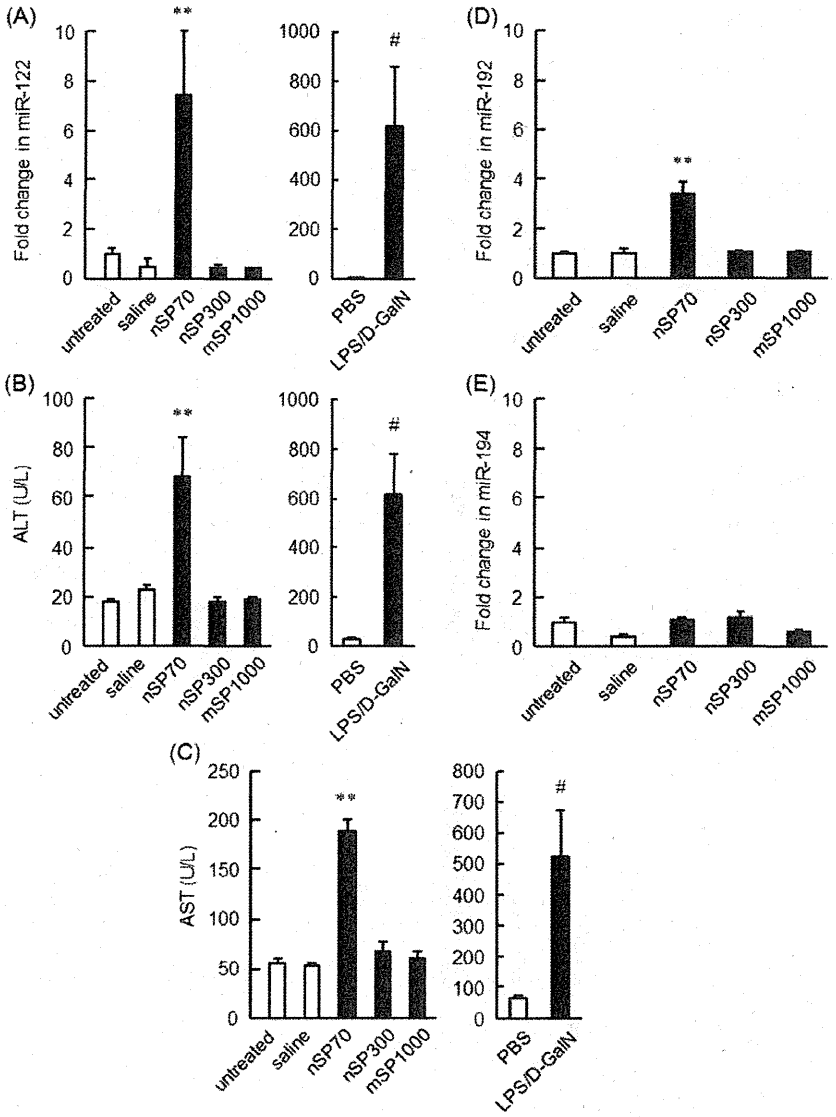


Figure 1. Usefulness of liver-specific microRNAs (miR-122, miR-192, and miR-194) as biomarkers for the development of safe silica nanoparticles. BALB/c mice were intravenously treated with nSP70, nSP300, or mSP1000 at 40 mg kg^{-1}. In addition, as positive control, mice were intraperitoneally treated with LPS and D-GalN. After 8 h, the serum levels of each microRNA, (A) miR-122, (D) miR-192, and (E) miR-194, were examined by real-time quantitative reverse-transcription PCR. Serum levels of (B) alanine aminotransferase (ALT) and (C) aspartate aminotransferase (AST) were measured by biochemical analysis. Data are presented as means ± SEM ($n = 6$; $^{**}P < 0.01$ versus value for saline-, nSP300-, mSP1000-treated group and untreated group by ANOVA; $^{\#}P < 0.05$ versus value for PBS-treated group by Student's *t*-test).

AST level in nSP70-C-treated mice was significantly greater than that in untreated or saline-treated mice. These results suggest that serum miR-122 may be useful as a biomarker for assessing improvement of the safety of nSP70 through surface modification.

4. Discussion

Our group showed previously that nSP70 can induce severe liver damage in mice [10]. Here, we examined whether liver-specific or -enriched miRNAs (miR-122, miR-192, and miR-194) were potentially useful as biomarkers of the liver damage induced by nSP70. Although miR-192 and miR-194 are thought to be involved in controlling proliferation and metastasis in liver cancer, the details of their functions are less well understood than in the case of miR-122 [31]. miR-122 is a key regulator of cholesterol and fatty-acid metabolism in the liver [32]. Therefore, it is likely that changes in the levels of miR-122 will predict not only liver damage but also the biological effects of that damage, such as abnormalities in fat metabolism. In addition, miR-122 has also been shown to be biomarkers for drug-induced liver injury in mice, rats, and human [33–35].

First, we showed that serum levels of miR-122 and miR-192 in nSP70-treated mice were elevated 8 h after treatment (figures 1(A) and (D)), as were those of ALT and AST (figures 1(B) and (C)), and that the levels of miR-122

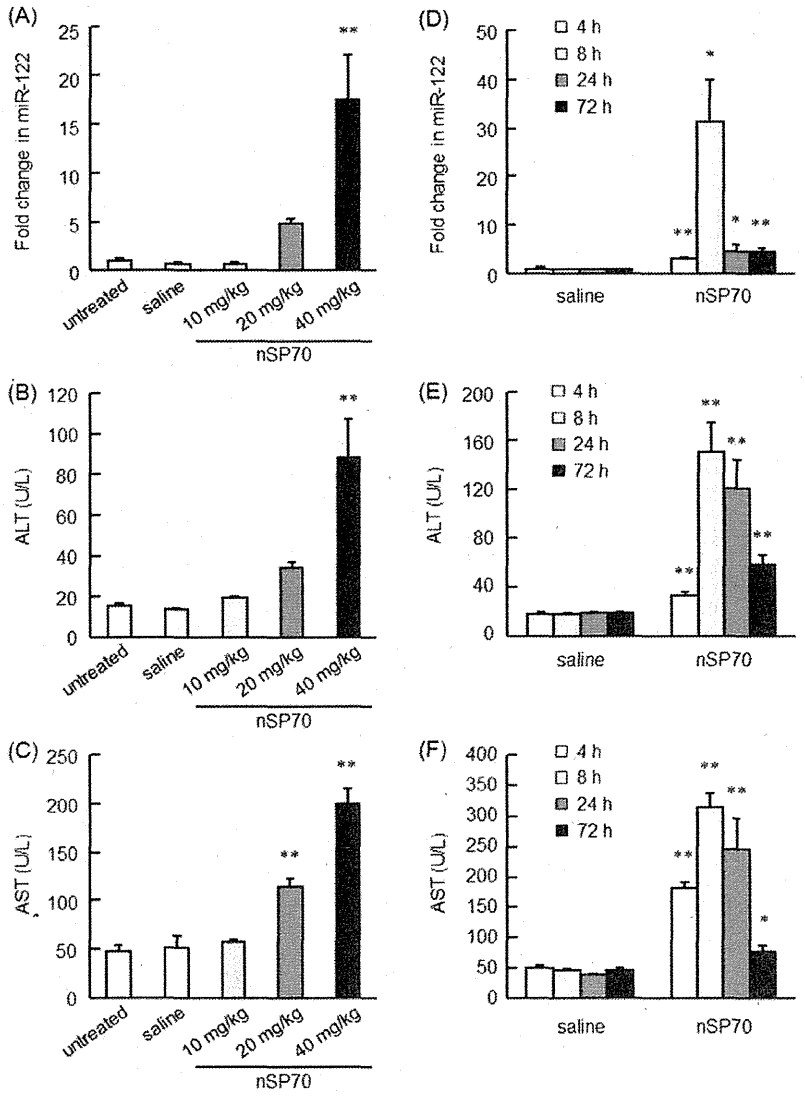


Figure 2. Serum levels of miR-122 after treatment with nSP70. BALB/c mice were intravenously treated with nSP70 at 10, 20, or 40 mg kg^{-1}. At 8 h after treatment with nSP70, the levels of (A) miR-122, (B) alanine aminotransferase (ALT), and (C) aspartate aminotransferase (AST) were analyzed. In addition, at 4, 8, 24, and 72 h after treatment with nSP70 at 40 mg kg^{-1}, the levels of (D) miR-122, (E) ALT, and (F) AST were examined. Data are presented as means ± SEM ($n = 5$ or 6; $^{*}P < 0.05$, $^{**}P < 0.01$ versus value for saline-treated group and untreated group by ANOVA).

were much higher than those of miR-192. We considered that this was because miR-122 accounts for 70% of all liver miRNA. By contrast, the serum levels of miR-194 in nSP70-treated mice did not differ significantly from those in untreated or saline-treated mice (figure 1(E)). The expression of miR-194 may change markedly with time; because we analyzed this expression only at 8 h after treatment, further time-course studies are needed before this miRNA can be discarded as a biomarker.

The serum levels of ALT and AST in mice treated with nSP70 at 40 mg kg^{-1} were beyond the physiological range, at about 4 times the values in untreated or saline-treated mice. In contrast, the serum level of miR-122 in nSP70-treated mice was about 15 times that in untreated or saline-treated mice (figures 2(A)–(C)). This suggests that miR-122 may be a more sensitive biomarker than the currently used ALT and AST in detecting liver damage induced by nSP70. In addition, as shown in figures 2(A)–(C), we evaluated the dose-dependent expression of miR-122 only at 8 h after treatment with nSP70, and we showed that the pattern of expression of miR-122 was almost the same as that of ALT and AST. In another study, injection of acetaminophen did not cause serum ALT elevation at 1 h, although obvious increases in miR-122 and miR-192 levels were already observable [36]. Therefore, evaluation at time points earlier than 8 h after nSP70 injection with lower doses could reveal the possibility that miR-122 is a more sensitive predictor of nSP70-induced liver damage than ALT and AST.

As shown in figures 2(D)–(F), the serum levels of ALT and AST in mice treated with nSP70 at 24 h showed significantly greater than those in saline-treated mice, while for miRNA-122, it dropped at 24 h to the same level as

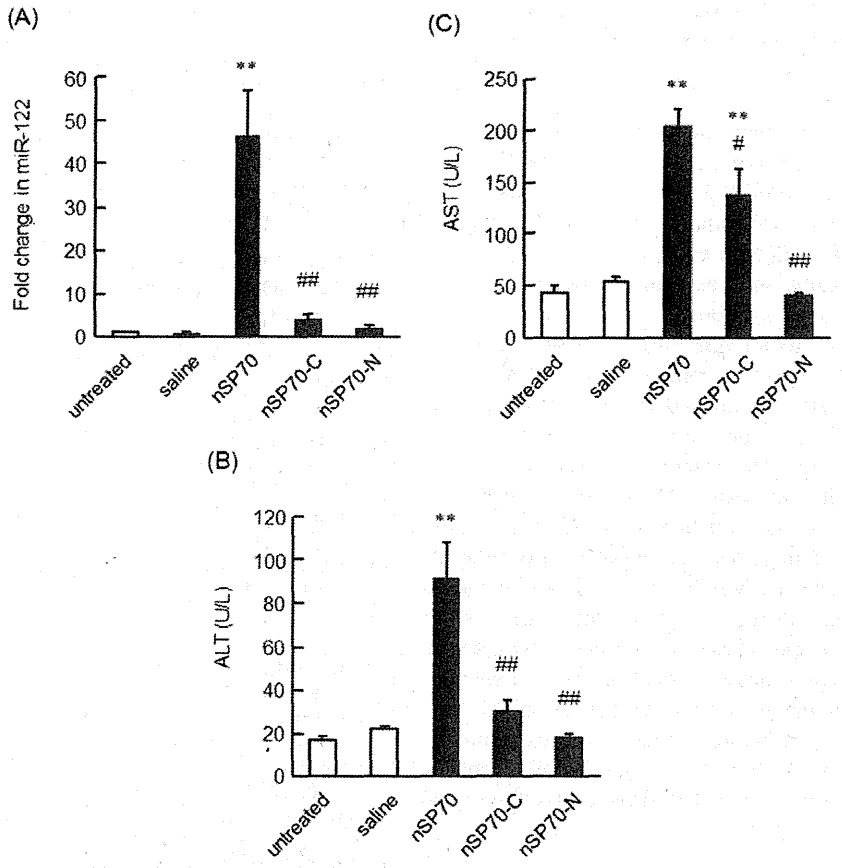


Figure 3. Responses of serum levels of miR-122 to treatment with surface-modified nSP70. BALB/c mice were intravenously treated with nSP70, nSP70-C, or nSP70-N at 40 mg kg^{-1}. Serum levels of (A) miR-122, (B) alanine aminotransferase (ALT), and (C) aspartate aminotransferase (AST) were measured 8 h after treatment. Data are presented as means ± SEM ($n = 5$ or 6; $^{**}P < 0.01$ versus value for saline-treated group and untreated group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus value for nSP70-treated group by ANOVA).

72 h. In general, miRNAs are considered to be stable in the blood [37]. However, Yamaura *et al* showed that although miR-122 was stable in human plasma, it was unstable in rat plasma [38]. Therefore, although detailed information on the stability of mouse miR-122 is unavailable, it is possible that RNase action in the blood leads to a rapid decrease in miR-122 levels. Currently, there are two hypothetical pathways by which miRNAs can enter the circulation [39]. One is by direct leakage from cells, and the other is by release from cells via microvesicles. It is thought that direct secretion of miRNAs occurs in tissue damage or cell apoptosis [39]. We previously demonstrated that nSP70 administered via the tail vein was distributed mainly to the liver and induced cytotoxicity in primary hepatocytes isolated from nSP70-treated mice [7]. Taking these results together, it is conceivable that most miR-122 is leaked into the blood and degraded by RNase between 8 and 24 h after nSP70 treatment. On the other hand, the level of miR-122 in nSP70-treated mice showed significant increments compared to that of saline-treated mice over the time course of the experiment. Therefore, its change in expression over the full time course of the experiment was still useful for evaluating nSP70-induced liver damage. For these reasons, we consider that miR-122 is as useful as ALT and AST in evaluating nSP70-induced liver damage. However, because we cannot explain this in detail, we are now trying to investigate the mechanism involved.

Here, we evaluated the time course of miR-122 expression over only a short period of time after nSP70 treatment. However, considering that we are exposed to nanomaterials on a daily basis, there is a need to investigate the biological effects of long-term exposure to them. Recent reports have shown that long-term exposure to titanium dioxide nanomaterials in mice induces ROS production in the lung [40]. A nanomaterials safety-assessment system is required for the evaluation of not only acute effects but also chronic effects. Therefore, we intend to analyze nanomaterial-induced chronic biological effects by evaluating the long-term course of miRNA expression.

Although the levels of miR-122, ALT, and AST were lower in mice treated with modified nSP70 than in those treated with unmodified nSP70 (figure 3), the AST level in nSP70-C-treated mice was higher than that in untreated mice. It is possible that nSP70-C and nSP70-N is safer than nSP70, because we previously demonstrated that differences in the surface charge state or kind of functional group of nanomaterials lead to different biological effects and cell responses [41–43]. On the other hand, AST is abundant not only in the liver but also in the skeletal muscle, heart, and kidney. Taking these results together, we consider that the

increase in AST in nSP70-C-treated mice was attributable to damage to tissues other than the liver. We are now analyzing in detail the effects of nanomaterials by focusing on other tissues.

This study is just the first step in the development of a miRNA-based safety-assessment system for nanomaterials. We have shown here that miR-122 is almost as useful as ALT and AST as a biomarker for nanomaterials. Recent reports have shown that many miRNAs exist not only in blood but also in other body fluids such as urine or saliva [22, 23]. In light of these reports, we consider that miR-122 might have the potential to predict nSP70-induced liver damage non-invasively from analyses of urine or saliva. In addition, many tissue-specific miRNAs other than miR-122 have been reported [44]. Therefore, to develop more specific or more sensitive biomarkers than the established system, there is a strong need for comprehensive analyses of the miRNAs associated with various tissues, such as brain and pancreas, for which we do not have enough useful biomarkers. Considering that miRNAs regulate the expression of the mRNAs involved in protein translation, identifying the changes that occur in miRNA expression upon exposure to nanomaterials will enable us to predict the biological effects of these materials. We are now trying comprehensively to explore those miRNAs that show changes in expression levels upon exposure to nanomaterials. We hope that these studies will help to establish a system for evaluating the safety and usefulness of nanomaterials.

5. Conclusions

We have revealed here that miR-122 and miR-192 may be useful biomarkers of liver damage induced by nSP70. miR-122, in particular, may be comparable to ALT and AST as a biomarker for this purpose.

Acknowledgments

This study was supported, in part, by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT) and from the Japan Society for the Promotion of Science (JSPS); and by the Knowledge Cluster Initiative (MEXT); by Health Labour Sciences Research Grants from the Ministry of Health, Labour and Welfare of Japan (MHLW); by a Global Environment Research Fund from the Ministry of the Environment; by Food Safety Commission, Japan; by the Cosmetology Research Foundation; by the Smoking Research Foundation; by the Research Foundation for Pharmaceutical Sciences; by the Japan Food Chemical Research Foundation; and by the Takeda Science Foundation.

References

[1] Kaur I P and Agrawal R 2007 *Recent Pat. Drug Deliv. Formul.* **1** 171–82
[2] Cormode D P, Jarzyna P A, Mulder W J and Fayad Z A 2010 *Adv. Drug Deliv. Rev.* **62** 329–38
[3] Nel A, Xia T, Madler L and Li N 2006 *Science* **311** 622–7
[4] Donaldson K, Murphy F A, Duffin R and Poland C A 2010 *Part. Fibre Toxicol.* **7** 5
[5] Shvedova A A, Kagan V E and Fadeel B 2010 *Annu. Rev. Pharmacol. Toxicol.* **50** 63–88
[6] Poland C A *et al* 2008 *Nature Nanotechnol.* **3** 423–8
[7] Nabeshi H *et al* 2011 *Biomaterials* **32** 2713–24
[8] Yamashita K *et al* 2011 *Nature Nanotechnol.* **6** 321–8
[9] Nabeshi H *et al* 2011 *Part. Fibre Toxicol.* **8** 1
[10] Nabeshi H *et al* 2012 *Nanotechnology* **23** 045101
[11] Higashisaka K *et al* 2011 *Biomaterials* **32** 3–9
[12] Higashisaka K *et al* 2012 *Nanoscale Res. Lett.* **7** 555
[13] Nagano T *et al* 2012 *Pharmazie* **67** 958–9
[14] Schickel R, Boyerinas B, Park S M and Peter M E 2008 *Oncogene* **27** 5959–74
[15] Cordes K R and Srivastava D 2009 *Circ. Res.* **104** 724–32
[16] Esquela-Kerscher A and Slack F J 2006 *Nature Rev. Cancer* **6** 259–69
[17] Pauley K M, Cha S and Chan E K 2009 *J. Autoimmun.* **32** 189–94
[18] Kosaka N, Iguchi H and Ochiya T 2010 *Cancer Sci.* **101** 2087–92
[19] Brase J C, Wuttig D, Kuner R and Sultmann H 2010 *Mol. Cancer* **9** 306
[20] Lagos-Quintana M, Rauhut R, Yalcin A, Meyer J, Lendeckel W and Tuschl T 2002 *Curr. Biol.* **12** 735–9
[21] Aboobaker A A, Tomancak P, Patel N, Rubin G M and Lai E C 2005 *Proc. Natl Acad. Sci. USA* **102** 18017–22
[22] Michael A, Bajracharya S D, Yuen P S, Zhou H, Star R A, Illei G G and Alevizos I 2010 *Oral Dis.* **16** 34–8
[23] Hanke M, Hoefig K, Merz H, Feller A C, Kausch I, Jocham D, Warnecke J M and Sczakiel G 2010 *Urol. Oncol.* **28** 655–61
[24] Yamada Y *et al* 2011 *Cancer Sci.* **102** 522–9
[25] Halappanavar S, Jackson P, Williams A, Jensen K A, Hougaard K S, Vogel U, Yauk C L and Wallin H 2011 *Environ. Mol. Mutagen.* **52** 425–39
[26] Burklew C E, Ashlock J, Winfrey W B and Zhang B 2012 *PLoS One* **7** e34783
[27] Cortez M A and Calin G A 2009 *Exp. Opin. Biol. Ther.* **9** 703–11
[28] Knopp D, Tang D and Niessner R 2009 *Anal. Chim. Acta* **647** 14–30
[29] Peters R *et al* 2012 *ACS Nano* **6** 2441–51
[30] Hou J *et al* 2011 *Cancer Cell* **19** 232–43
[31] Meng Z, Fu X, Chen X, Zeng S, Tian Y, Jove R, Xu R and Huang W 2010 *Hepatology* **52** 2148–57
[32] Rotllan N and Fernandez-Hernando C 2012 *Cholesterol* **2012** 847849
[33] Wang K, Zhang S, Marzolf B, Troisch P, Brightman A, Hu Z, Hood L E and Galas D J 2009 *Proc. Natl Acad. Sci. USA* **106** 4402–7
[34] Starkey Lewis P J *et al* 2011 *Hepatology* **54** 1767–76
[35] Starckx S *et al* 2013 *Toxicol. Pathol.* **41** 795–804
[36] Shi Q, Yang X and Mendrick D L 2013 *Biomark. Med.* **7** 307–15
[37] Gilad S *et al* 2008 *PLoS One* **3** e3148
[38] Yamaura Y, Nakajima M, Takagi S, Fukami T, Tsuneyama K and Yokoi T 2012 *PLoS One* **7** e30250
[39] Zen K and Zhang C Y 2012 *Med. Res. Rev.* **32** 326–48
[40] Sun Q *et al* 2012 *J. Biomed. Mater. Res.* A **100** 2554–62
[41] Nabeshi H *et al* 2011 *Nanoscale Res. Lett.* **6** 93
[42] Morishige T *et al* 2012 *Arch. Toxicol.* **86** 1297–307
[43] Yoshida T *et al* 2012 *Biochem. Biophys. Res. Commun.* **427** 748–52
[44] Laterza O F *et al* 2009 *Clin. Chem.* **55** 1977–83

Yamagishi et al. Nanoscale Research Letters 2013, 8:395
http://www.nanoscalereslett.com/content/8/1/395

Nanoscale Research Letters
a SpringerOpen Journal

NANO EXPRESS Open Access

Acute and chronic nephrotoxicity of platinum nanoparticles in mice

Yoshiaki Yamagishi[1], Akihiro Watari[1*], Yuya Hayata[1], Xiangru Li[1], Masuo Kondoh[1], Yasuo Yoshioka[2], Yasuo Tsutsumi[2] and Kiyohito Yagi[1]

Abstract

Platinum nanoparticles are being utilized in various industrial applications, including in catalysis, cosmetics, and dietary supplements. Although reducing the size of the nanoparticles improves the physicochemical properties and provides useful performance characteristics, the safety of the material remains a major concern. The aim of the present study was to evaluate the biological effects of platinum particles less than 1 nm in size (snPt1). In mice administered with a single intravenous dose of snPt1, histological analysis revealed necrosis of tubular epithelial cells and urinary casts in the kidney, without obvious toxic effects in the lung, spleen, and heart. These mice exhibited dose-dependent elevation of blood urea nitrogen, an indicator of kidney damage. Direct application of snPt1 to *in vitro* cultures of renal cells induced significant cytotoxicity. In mice administered for 4 weeks with twice-weekly intraperitoneal snPt1, histological analysis of the kidney revealed urinary casts, tubular atrophy, and inflammatory cell accumulation. Notably, these toxic effects were not observed in mice injected with 8-nm platinum particles, either by single- or multiple-dose administration. Our findings suggest that exposure to platinum particles of less than 1 nm in size may induce nephrotoxicity and disrupt some kidney functions. However, this toxicity may be reduced by increasing the nanoparticle size.

Keywords: Nanosized materials; Platinum particles; Kidney; Nephrotoxicity; Safety evaluation

Background

Nanomaterials have been developed and used as innovative materials in a wide range of industrial fields, including electronics, medicine, food, clothing, and cosmetics; these reagents are expected to provide significant benefits to humans. Nanomaterials are defined as substances that have at least one dimension size below 100 nm. The reduced size provides novel physicochemical properties, including increased thermal electrical conductivity, durability, and strength [1-3]. Although these characteristics may yield improved performance and novel functions, several reports have suggested that various types of nanomaterials, such as carbon nanotubes, titanium dioxide, fullerenes, quantum dots, and silica, exhibit harmful biological effects [4-12]. Additionally, some reports have shown that the characteristics of nanoparticles (e.g., size and surface features) can affect their biological and pathological actions [10,13-16]. Therefore, evaluation of the potential health risks attributable to nanomaterials is indispensable for the safe handling and use of these materials. However, little information is available regarding the safety evaluation of materials less than 1 nm in size.

Platinum nanoparticles have been utilized in a number of manufacturing applications, including catalysis, cosmetics manufacturing, and the processing of dietary supplements. As products using platinum nanoparticles become more familiar in our daily lives, the chances of exposure to platinum nanoparticles are increasing, as are concerns about unanticipated harmful biological effects of these materials [17,18]. In fact, there are some reports that platinum nanoparticles can induce inflammation in mice or impair the integrity of DNA [19,20]. On the other hand, platinum nanoparticles have antioxidant activity and inhibit pulmonary inflammation (e.g., as caused by exposure to cigarette smoke) [21-23]. These reports indicate that the biological effects of platinum nanoparticles remain poorly defined; the biological safety of sub-nanosized platinum particles (those of less

* Correspondence: akihiro@phs.osaka-u.ac.jp
[1]Laboratories of Bio-Functional Molecular Chemistry, Graduate School of Pharmaceutical Sciences, Osaka University, Suita, Osaka 565-0871, Japan
Full list of author information is available at the end of the article

than 1 nm in size; snPt1) remains unknown. Recently, we reported that snPt1 can induce hepatotoxicity [24]. However, the biological effects of snPt1 on other organs remain unclear. In this study, we evaluated the effect of snPt1 on tissues after single- and multi-dose administration in mice. In addition, we investigated the relationship between platinum particle size and biological response by also testing platinum particles of 8 nm in size (snPt8).

Methods

Platinum particles

Platinum particles with nominal mean diameters of less than 1 nm (snPt1) and 8 nm (snPt8) were purchased from Polytech & Net GmbH (Rostock, Germany). The particle sizes were confirmed using a Zetasizer Nano-ZS (Malvern Instruments, Malvern, UK). The particles were stocked as 5 mg/ml aqueous suspensions. The stock solutions were suspended using a vortex mixer before use. Other reagents used in this study were of research grade.

Animals

BALB/c and C57BL/6 male mice were obtained from Shimizu Laboratory Supplies Co., Ltd. (Kyoto, Japan) and were housed in an environmentally controlled room at 23°C ± 1.5°C with a 12-h light/12-h dark cycle. Mice had *ad libitum* access to water and commercial chow (Type MF, Oriental Yeast, Tokyo, Japan). BALB/c mice were injected intravenously with snPt1 or snPt8 at 5 to 20 mg/kg body weight. C57BL/6 mice were injected intraperitoneally with snPt1 or snPt8 at 10 mg/kg body weight, or with an equivalent volume of vehicle (water). At 24 h after the injection of the vehicle or test article, the kidney and liver were collected. For testing the chronic effects of platinum particles, C57BL/6 mice were injected intraperitoneally with snPt1 or snPt8 at 10 mg/kg body weight, or with an equivalent volume of vehicle (water). Intraperitoneal doses were administered as twice-weekly injections for 4 weeks. At 72 h after the last injection of vehicle or test article, the kidney and liver were collected. All experimental protocols conformed to the ethical guidelines of the Graduate School of Pharmaceutical Sciences at Osaka University.

Histological analysis

For animals dosed intravenously with snPt1 or snPt8, the kidney, spleen, lung, heart, and liver were removed at 24 h post-injection and fixed with 4% paraformaldehyde. For

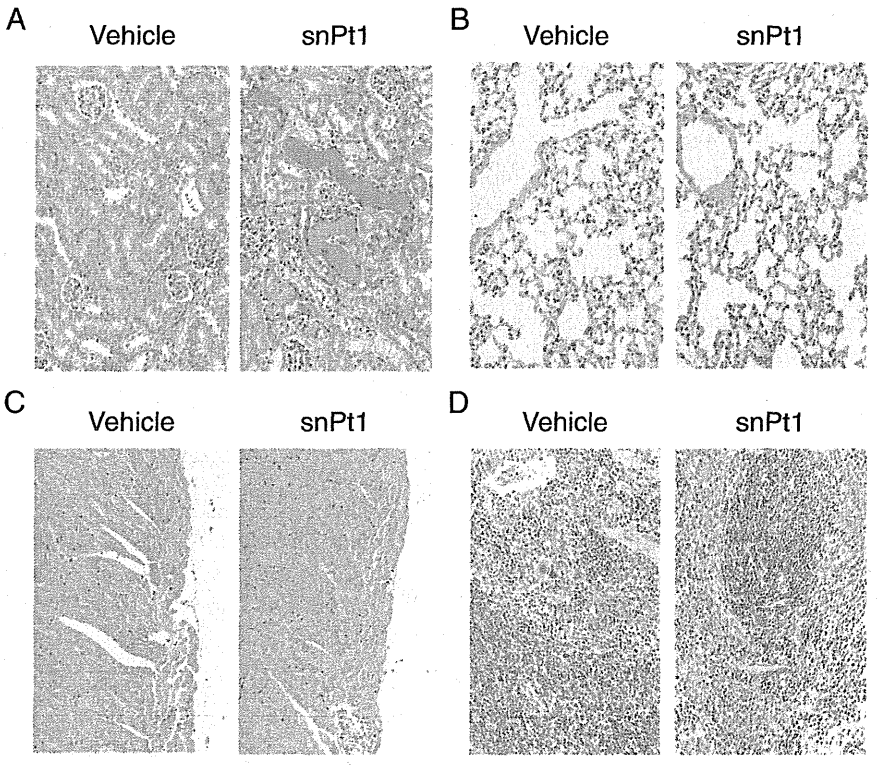


Figure 1 Histological analysis of the organs in snPt1-treated mice. Vehicle (water) or snPt1 (15 mg/kg) was administered intravenously to mice. At 24 h after administration, the kidney **(A)**, lung **(B)**, heart **(C)**, and spleen **(D)** were collected and fixed with 4% paraformaldehyde. Tissue sections were stained with hematoxylin and eosin and observed microscopically.

animals dosed intraperitoneally with snPt1 or snPt8, the kidney and liver were removed at 24 h (for single administration) or 72 h (for multiple administration) post-injection and fixed with 4% paraformaldehyde. Thin tissue sections were stained with hematoxylin and eosin for histological observation.

Biochemical assay

Serum blood urea nitrogen (BUN) was measured using a commercially available colorimetric assay kit (Wako Pure Chemical, Osaka, Japan) according to the manufacturer's protocol. In brief, collected serum (10 μl) was combined with 1 ml color A reagent (including urease) and incubated at 37°C for 15 min. Following the addition of 1 ml Color B reagent, the samples were incubated at 37°C for 10 min. Absorbance of samples was measured at a wavelength of 570 nm.

Statistical analysis

Data are presented as mean ± SEM. Statistical analysis was performed by Student's *t* test. $P < 0.05$ was considered significant.

Results and discussion

To investigate acute biological effects of snPt1, we administered 15 mg/kg of snPt1 to BALB/c mice by intravenous injection and performed histological analysis in the kidney, lung, heart, liver, and spleen at 24 h post-injection. As shown in Figure 1, necrosis of tubular epithelial cells and urinary casts were observed in the kidney by hematoxylin-eosin staining, whereas no apparent tissue abnormality was observed in the lung, heart, and spleen. Consistent with previous results [24], the liver showed vacuole degeneration after the administration of snPt1 (data not shown). These observations indicate that snPt1 induced acute tissue injury in the kidney and liver following intravenous administration. Next, we examined a serum biochemical marker of kidney function, BUN, to confirm the kidney tissue toxicity. Consistent with the histological analysis, intravenous dosing with snPt1 elevated serum BUN level at doses over 15 mg/kg (Figure 2A). The serum BUN level increased 24 h later and returned to normal level after 48 h (Figure 2B). When we directly added snPt1 at concentrations of 10, 20, 40, and 60 μg/ml to *in vitro* cultures of Madin-Darby canine kidney (MDCK) cells, severe cytotoxicity was observed in a dose-dependent manner (Additional file 1: Figure S1). These results indicate that snPt1 (at doses of greater than or equal to 15 mg/kg) induced toxicity in both the kidney and liver, but not in the lung, heart, or spleen, after a single intravenous administration.

Previously, we and other groups reported that the biological effects of nanoparticles differed with material size [10,11,25,26]. Therefore, we examined whether platinum particles with a diameter of 8 nm (snPt8) and snPt1 produce different effects in kidney. As shown in Figure 3A, snPt1 administration resulted in dose-dependent increases in serum BUN levels, whereas snPt8 (at the same

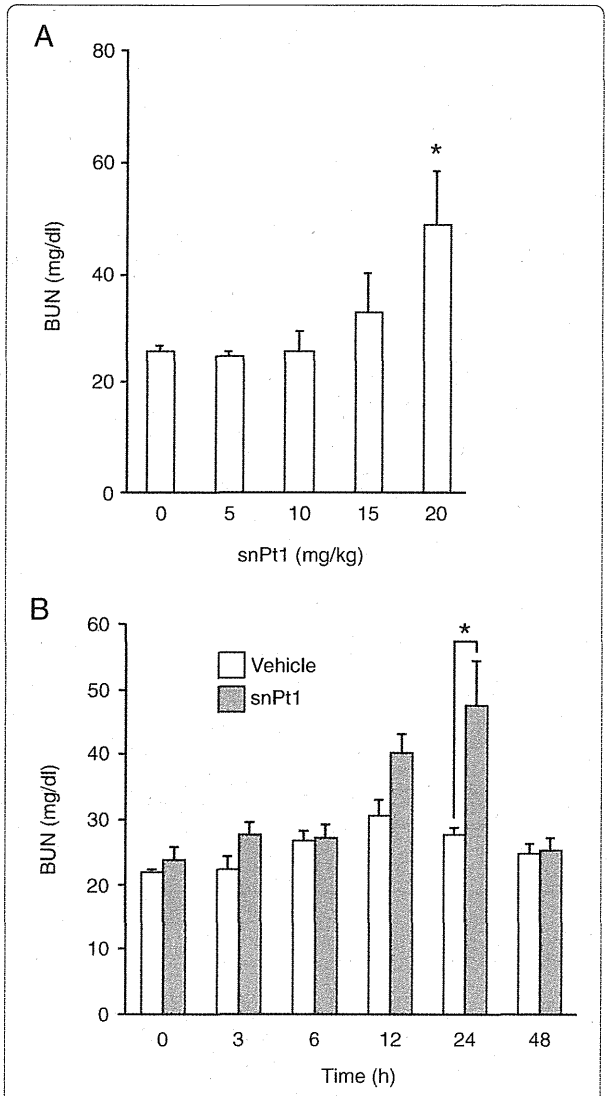
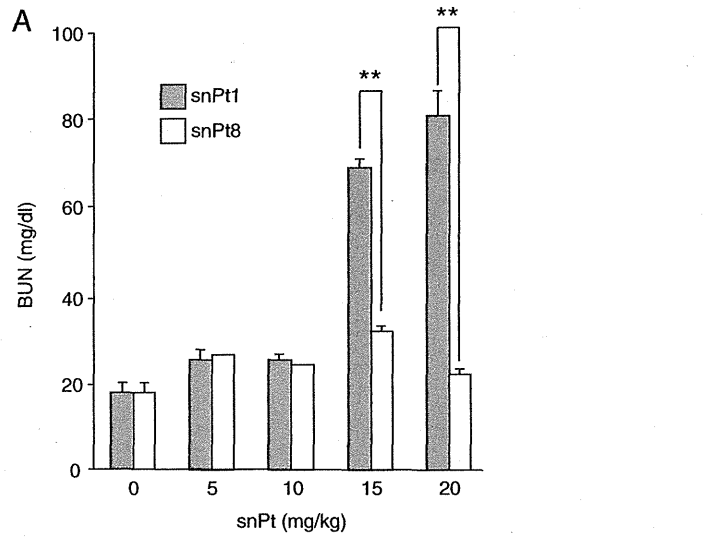
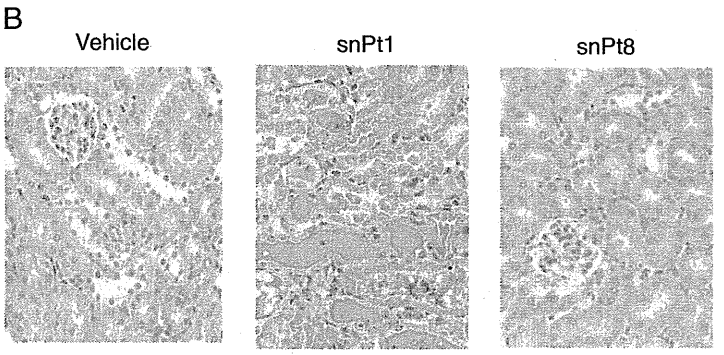


Figure 2 Biochemical analysis in snPt1-treated mice. (A) Dose dependency of snPt1-induced kidney injury. snPt1 was administrated intravenously at 5, 10, 15, or 20 mg/kg. At 24 h after administration, blood was recovered, and serum was collected and used for measurement of BUN, as described in the 'Methods' section. Data are mean ± SEM ($n = 6$ to 10). Single asterisk (*) connotes a significant difference when compared with the vehicle-treated group ($P < 0.05$). **(B)** Time-dependent changes in a biological marker of kidney injury. snPt1 was administered intravenously to mice at 20 mg/kg. Blood was recovered at 0, 3, 6, 12, 24, and 48 h after administration. Serum was collected and used for measurement of BUN, as described in the 'Methods' section. Data are mean ± SEM ($n = 8$ to 10). Single asterisk (*) connotes significant difference when compared with the vehicle-treated group ($P < 0.05$).





C

	Vehicle	snPt1	snPt8
mg/kg	0	20	20
Urinary cast	0	3	0
Necrosis of tubular epithelial cell	0	3	0
Tubular atrophy	0	0	0
Inflammatory cell infiltration	0	0	0

Grade 0:none 1:slight, 2:mild, 3:moderate, 4:severe

Figure 3 Effect of particle size of platinum on kidney injury. (A) snPt1 or snPt8 was injected intravenously into mice at the indicated doses. Blood was recovered at 24 h after injection. Serum BUN levels were measured. Data are mean ± SEM (n = 5). Double asterisk (**) connotes significant difference between the snPt1- and snPt8-treated groups ($P < 0.01$). **(B)** Histological analysis of kidney tissues in acute snPt1- or snPt8-treated mice. Vehicle or test article (snPt1 or snPt8 at 20 mg/kg) was administered intravenously to mice as a single dose. At 24 h after administration, the kidneys were collected and fixed with 4% paraformaldehyde. Tissue sections were stained with hematoxylin and eosin and observed under a microscope. **(C)** Acute kidney injury score in mice treated with vehicle, snPt1, or snPt8. Grade 0: none, 1: slight, 2: mild, 3: moderate, 4: severe.

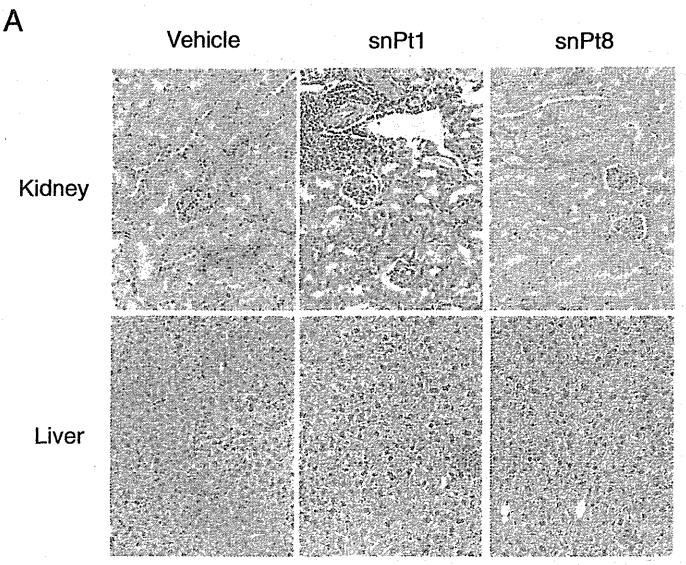
dose levels) did not. Histological analysis showed that intravenous administration (at 20 mg/kg) of snPt1, but not that of snPt8, induced renal injury (Figure 3B,C). These tissue injuries also were observed following the injection in C57BL/6 mice (data not shown), demonstrating that the toxicity was not mouse strain-specific. Furthermore, renal cytotoxicity was not observed in snPt8-treated MDCK cells (Additional file 1: Figure S1),

confirming the size dependence of the nanoparticle renal cytotoxicity. The hepatotoxicity of the platinum particles also was reduced by altering particle size [24]. These findings indicate that the snPt1-induced nephrotoxicity is not observed following treatment with the same dose level of snPt8.

Finally, we used histological analysis to investigate the effects on C57BL/6 mice of chronic exposure to snPt1 and snPt8. snPt1 and snPt8 (both at 10 mg/kg) were injected intraperitoneally into mice twice per week for 4 weeks; repeat administration via the tail vein was precluded due to tissue necrosis of the mouse tail upon multiple intravenous administrations. In the multiple intraperitoneal administrations, necrosis at the injection site was not observed. Single intraperitoneal administration of 10 mg/kg snPt1 (but not that of snPt8) induced necrosis of tubular epithelial cells and urinary casts in the kidney, similar to the results seen with intravenous administration (Additional file 2: Figure S2A,B). Chronic intraperitoneal administration of snPt1 at 10 mg/kg induced urinary casts, tubular atrophy, and inflammatory cell accumulation in the kidney, whereas the liver did not show tissue injury (Figure 4A,B). On the other hand, chronic exposure to snPt8 (at the same dose level) did not show apparent histological effects in the kidney (Figure 4A,B). These findings suggest that chronic exposure to 10 mg/kg snPt1, but not to snPt8, induced severe kidney injury. Notably, this chronic exposure to snPt1 induced additional (cumulative) kidney injury beyond that seen with acute exposure.

Following exposure, nanoparticles are transported into the blood and reach the systemic circulation, from which the nanoparticles distribute and accumulate in several organs such as the lung, liver, spleen, kidneys, brain, and heart [27-30]. Because the kidney is able to remove molecules from the circulation, renal excretion is an expected

A



B

	Vehicle	snPt1	snPt8
mg/kg	0	10	10
Urinary cast	0	3	0
Necrosis of tubular epithelial cell	1	1	1
Tubular atrophy	0	3	0
Inflammatory cell infiltration	0	3	0

Grade 0:none 1:slight, 2:mild, 3:moderate, 4:severe

Figure 4 Histological analysis of kidney tissues in multi-dose snPt1- or snPt8-treated mice. (A) Vehicle or test article (snPt1 or snPt8 at 10 mg/kg) was administered intraperitoneally to mice as twice-weekly doses for 4 weeks. At 72 h after last administration, the kidney and liver were collected and fixed with 4% paraformaldehyde. Tissue sections were stained with hematoxylin and eosin and observed under a microscope. **(B)** Chronic kidney injury scores in mice treated with vehicle, snPt1, or snPt8. Grade 0: none, 1: slight, 2: mild, 3: moderate, 4: severe.

route for elimination of nanoparticles. In fact, functionalized single-wall carbon nanotubes (SWCNT), following injection into mice, are rapidly excreted by the kidney [31]. The hepatobiliary system also is an important route for the elimination of foreign substances and particles [32]. Because these organs play pivotal roles in eliminating foreign substances, various nanomaterials are accumulated there and lead to tissue injury. As one example, our previous work showed that snPt1-treated mice exhibited acute hepatotoxicity [24]. In the present study, we investigated the biological effects of snPt1 after intravenous or intraperitoneal administration in mice and demonstrated that snPt1 induced nephrotoxicity and impaired renal function, as evidenced by BUN levels. In contrast, we could not find apparent toxic effects on the heart, lung, or spleen after the single intravenous administration of snPt1, although the disposition of these nanoparticles will need to be assessed further.

The underlying mechanism of snPt1-induced tissue injury still remains unclear. Cisplatin, which is a platinating agent used as part of the anti-cancer regimen for various types of cancers [33,34], exerts its antitumor activity by binding preferentially to the nucleophilic positions on guanine and adenine of DNA, resulting in the formation of intra- and inter-strand crosslinks. Eventually, the crosslinks lead to DNA-strand breaks and killing of cancer cells [35]. However, cisplatin usage is limited due to nephrotoxicity, leading to lesions in the epithelial tubules [36,37]. Cisplatin also causes toxicity in the liver and blood [38]. These observations suggest that the toxic effects of cisplatin resemble those of snPt1. A previous study reported that platinum nanoparticles entered human lung fibroblasts (cell line IMR-90) and human glioblastoma cells (U251) and induced cytotoxicity thorough intracellular reactive oxygen species (ROS) production and DNA damage following p53 activation and upregulation of p21, which leads to growth arrest and apoptosis [39]. Our observation of snPt1-induced cytotoxicity in cell culture suggests that snPt1 may be internalized by renal cells, with concomitant induction of ROS production or DNA damage. However, alternative toxic effects (such as cytotoxicity of inflammatory cytokines on renal cells by accumulation of inflammatory cells in the kidney) might emerge during chronic exposure to snPt1.

At equivalent dose levels, platinum particles of 8 nm in size did not induce apparent toxic effects in renal tissues by acute or chronic administration. This result suggests that selection of specific size ranges for the platinum particles might overcome the undesirable side effects. Current studies have shown that organic cation transporter 2 (OCT2) is highly expressed in kidney and plays an important role in the nephrotoxicity of cisplatin [40,41]. Identification of the snPt1 transporter may help to clarify the mechanism of snPt1-induced nephrotoxicity.

Conclusions

In the present study, we investigated the biological safety of platinum nanoparticles in mice and found that platinum particles of less than 1 nm induced kidney injury, although the injurious effects were reduced by increasing the nanoparticle size. For future nanoparticle applications, it will be critical to further understand the bioactivity and kinetics of materials less than 1 nm in size. Accumulation of toxicity profiles will aid in the creation of the safe and efficacious nanomaterials and contribute to the advancement of the field.

Additional files

Additional file 1: Figure S1. Cytotoxicity of snPt1 in renal cells. MDCK cells were treated with vehicle, snPt1, or snPt8 at 0, 10, 20, 40, or 60 μg/ml. After 24 h exposure, morphology of the cells was photographed. Higher magnification images are shown in the insets.

Additional file 2: Figure S2. (A) Histological analysis of kidney tissues in intraperitoneally administered mice. Vehicle or test article (snPt1 or snPt8 at 10 mg/kg) was administered intraperitoneally to mice as a single dose. At 24 h after administration, kidneys were collected and fixed with 4% paraformaldehyde. Tissue sections were stained with hematoxylin and eosin and observed under a microscope. (B) Acute kidney injury score in mice treated intraperitoneally with vehicle, snPt1, or snPt8. Grade 0: none, 1: slight, 2: mild, 3: moderate, 4: severe.

Abbreviations

snPt1: platinum particles less than 1 nm in size; snPt8: platinum particles of 8 nm in size; BUN: blood urea nitrogen; MDCK: Madin-Darby canine kidney; ROS: reactive oxygen species.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AW, MK, and KY designed this study. YY (Yoshioka) and YT prepared samples. YY (Yamagishi), YH, and XL performed the experiments. AW and KY wrote this manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors thank all members of our laboratory for useful comments. This study was partly supported by a grant from the Ministry of Health, Labour, and Welfare of Japan.

Author details

[1]Laboratories of Bio-Functional Molecular Chemistry, Graduate School of Pharmaceutical Sciences, Osaka University, Suita, Osaka 565-0871, Japan. [2]Laboratories of Toxicology and Safety Science, Graduate School of Pharmaceutical Sciences, Osaka University, Suita, Osaka 565-0871, Japan.

Received: 30 May 2013 Accepted: 1 September 2013
Published: 23 September 2013

References

1. Ariga K, Hu X, Mandal S, Hill JP: **By what means should nanoscaled materials be constructed: molecule, medium, or human?** *Nanoscale* 2010, 2:198–214.
2. Service RF: **U.S. nanotechnology. Health and safety research slated for sizable gains.** *Science* 2007, 315:926.
3. Patra CR, Bhattacharya R, Mukhopadhyay D, Mukherjee P: **Fabrication of gold nanoparticles for targeted therapy in pancreatic cancer.** *Adv Drug Deliv Rev* 2010, 62:346–361.
4. Aiso S, Yamazaki K, Umeda Y, Asakura M, Kasai T, Takaya M, Toya T, Koda S, Nagano K, Arito H, Fukushima S: **Pulmonary toxicity of intratracheally**

instilled multiwall carbon nanotubes in male Fischer 344 rats. *Ind Health* 2010, **48**:783–795.

5. Murray AR, Kisin E, Leonard SS, Young SH, Kommineni C, Kagan VE, Castranova V, Shvedova AA: **Oxidative stress and inflammatory response in dermal toxicity of single-walled carbon nanotubes.** *Toxicology* 2009, **257**:161–171.
6. Yamashita K, Yoshioka Y, Higashisaka K, Morishita Y, Yoshida T, Fujimura M, Kayamuro H, Nabeshi H, Yamashita T, Nagano K, Abe Y, Kamada H, Kawai Y, Mayumi T, Yoshikawa T, Itoh N, Tsunoda S, Tsutsumi Y: **Carbon nanotubes elicit DNA damage and inflammatory response relative to their size and shape.** *Inflammation* 2010, **33**:276–280.
7. Warheit DB, Reed KL, Sayes CM: **A role for nanoparticle surface reactivity in facilitating pulmonary toxicity and development of a base set of hazard assays as a component of nanoparticle risk management.** *Inhal Toxicol* 2009, **21**(Suppl 1):61–67.
8. Chen J, Dong X, Zhao J, Tang G: ***In vivo* acute toxicity of titanium dioxide nanoparticles to mice after intraperitioneal injection.** *J Appl Toxicol: JAT* 2009, **29**:330–337.
9. Geys J, Nemmar A, Verbeken E, Smolders E, Ratoi M, Hoylaerts MF, Nemery B, Hoet PH: **Acute toxicity and prothrombotic effects of quantum dots: impact of surface charge.** *Environ Health Perspect* 2008, **116**:1607–1613.
10. Nishimori H, Kondoh M, Isoda K, Tsunoda S, Tsutsumi Y, Yagi K: **Silica nanoparticles as hepatotoxicants.** *Eur J Pharm Biopharm: official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik eV* 2009, **72**:496–501.
11. Nishimori H, Kondoh M, Isoda K, Tsunoda S, Tsutsumi Y, Yagi K: **Histological analysis of 70-nm silica particles-induced chronic toxicity in mice.** *Eur J Pharm Biopharm: official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik eV* 2009, **72**:626–629.
12. Park EJ, Kim H, Kim Y, Yi J, Choi K, Park K: **Carbon fullerenes (C60s) can induce inflammatory responses in the lung of mice.** *Toxicol Appl Pharmacol* 2010, **244**:226–233.
13. Nabeshi H, Yoshikawa T, Arimori A, Yoshida T, Tochigi S, Hirai T, Akase T, Nagano K, Abe Y, Kamada H, Tsunoda S, Itoh N, Yoshioka Y, Tsutsumi Y: **Effect of surface properties of silica nanoparticles on their cytotoxicity and cellular distribution in murine macrophages.** *Nanoscale Research Letters* 2011, **6**:93.
14. Hauck TS, Ghazani AA, Chan WC: **Assessing the effect of surface chemistry on gold nanorod uptake, toxicity, and gene expression in mammalian cells.** *Small* 2008, **4**:153–159.
15. Mayer A, Vadon M, Rinner B, Novak A, Wintersteiger R, Frohlich E: **The role of nanoparticle size in hemocompatibility.** *Toxicology* 2009, **258**:139–147.
16. Nafee N, Schneider M, Schaefer UF, Lehr CM: **Relevance of the colloidal stability of chitosan/PLGA nanoparticles on their cytotoxicity profile.** *Int J Pharm* 2009, **381**:130–139.
17. Horie M, Kato H, Endoh S, Fujita K, Nishio K, Komaba LK, Fukui H, Nakamura A, Miyauchi A, Nakazato T, Kinugasa S, Yoshida Y, Hagihara Y, Morimoto Y, Iwahashi H: **Evaluation of cellular influences of platinum nanoparticles by stable medium dispersion.** *Metallomics: Integrated Biometal Science* 2011, **3**:1244–1252.
18. Gehrke H, Pelka J, Hartinger CG, Blank H, Bleimund F, Schneider R, Gerthsen D, Brase S, Crone M, Turk M, Marko D: **Platinum nanoparticles and their cellular uptake and DNA platination at non-cytotoxic concentrations.** *Arch Toxicol* 2011, **85**:799–812.
19. Park EJ, Kim H, Kim Y, Park K: **Intratracheal instillation of platinum nanoparticles may induce inflammatory responses in mice.** *Arch Pharm Res* 2010, **33**:727–735.
20. Pelka J, Gehrke H, Esselen M, Turk M, Crone M, Brase S, Muller T, Blank H, Send W, Zibat V, Brenner P, Schneider R, Gerthsen D, Marko D: **Cellular uptake of platinum nanoparticles in human colon carcinoma cells and their impact on cellular redox systems and DNA integrity.** *Chem Res Toxicol* 2009, **22**:649–659.
21. Onizawa S, Aoshiba K, Kajita M, Miyamoto Y, Nagai A: **Platinum nanoparticle antioxidants inhibit pulmonary inflammation in mice exposed to cigarette smoke.** *Pulm Pharmacol Therapeut* 2009, **22**:340–349.
22. Watanabe A, Kajita M, Kim J, Kanayama A, Takahashi K, Mashino T, Miyamoto Y: ***In vitro* free radical scavenging activity of platinum nanoparticles.** *Nanotechnology* 2009, **20**:455105.
23. Kajita M, Hikosaka K, Iitsuka M, Kanayama A, Toshima N, Miyamoto Y: **Platinum nanoparticle is a useful scavenger of superoxide anion and hydrogen peroxide.** *Free Radic Res* 2007, **41**:615–626.
24. Yamagishi Y, Watari A, Hayata Y, Li X, Kondoh M, Tsutsumi Y, Yagi K: **Hepatotoxicity of sub-nanosized platinum particles in mice.** *Pharmazie* 2013, **68**:178–182.
25. Oberdorster G: **Safety assessment for nanotechnology and nanomedicine: concepts of nanotoxicology.** *J Intern Med* 2010, **267**:89–105.
26. Jiang J, Oberdorster G, Elder A, Gelein R, Mercer P, Biswas P: **Does nanoparticle activity depend upon size and crystal phase?** *Nanotoxicology* 2008, **2**:33–42.
27. Zhu MT, Feng WY, Wang B, Wang TC, Gu YQ, Wang M, Wang Y, Ouyang H, Zhao YL, Chai ZF: **Comparative study of pulmonary responses to nano- and submicron-sized ferric oxide in rats.** *Toxicology* 2008, **247**:102–111.
28. Furuyama A, Kanno S, Kobayashi T, Hirano S: **Extrapulmonary translocation of intratracheally instilled fine and ultrafine particles via direct and alveolar macrophage-associated routes.** *Arch Toxicol* 2009, **83**:429–437.
29. Oberdorster G, Sharp Z, Atudorei V, Elder A, Gelein R, Kreyling W, Cox C: **Translocation of inhaled ultrafine particles to the brain.** *Inhal Toxicol* 2004, **16**:437–445.
30. Ai J, Biazar E, Jafarpour M, Montazeri M, Majdi A, Aminifard S, Zafari M, Akbari HR, Rad HG: **Nanotoxicology and nanoparticle safety in biomedical designs.** *Int J Nanomedicine* 2011, **6**:1117–1127.
31. Ruggiero A, Villa CH, Holland JP, Sprinkle SR, May C, Lewis JS, Scheinberg DA, McDevitt MR: **Imaging and treating tumor vasculature with targeted radiolabeled carbon nanotubes.** *Int J Nanomedicine* 2010, **5**:783–802.
32. Longmire M, Choyke PL, Kobayashi H: **Clearance properties of nano-sized particles and molecules as imaging agents: considerations and caveats.** *Nanomedicine (Lond)* 2008, **3**:703–717.
33. Daugaard G: **Cisplatin nephrotoxicity: experimental and clinical studies.** *Dan Med Bull* 1990, **37**:1–12.
34. Brabec V, Kasparkova J: **Modifications of DNA by platinum complexes. Relation to resistance of tumors to platinum antitumor drugs.** *Drug Resist Updat* 2005, **8**:131–146.
35. Wang D, Lippard SJ: **Cellular processing of platinum anticancer drugs.** *Nat Rev Drug Discov* 2005, **4**:307–320.
36. Dobyan DC, Levi J, Jacobs C, Kosek J, Weiner MW: **Mechanism of cis-platinum nephrotoxicity: II. Morphologic observations.** *J Pharmacol Exp Therapeut* 1980, **213**:551–556.
37. Miller RP, Tadagavadi RK, Ramesh G, Reeves WB: **Mechanisms of cisplatin nephrotoxicity.** *Toxins* 2010, **2**:2490–2518.
38. Litterst CL, Gram TE, Dedrick RL, Leroy AF, Guarino AM: **Distribution and disposition of platinum following intravenous administration of cis-diamminedichloroplatinum(II) (NSC 119875) to dogs.** *Cancer Res* 1976, **36**:2340–2344.
39. Asharani PV, Xinyi N, Hande MP, Valiyaveettil S: **DNA damage and p53-mediated growth arrest in human cells treated with platinum nanoparticles.** *Nanomedicine (Lond)* 2010, **5**:51–64.
40. Tanihara Y, Masuda S, Katsura T, Inui K: **Protective effect of concomitant administration of imatinib on cisplatin-induced nephrotoxicity focusing on renal organic cation transporter OCT2.** *Biochem Pharmacol* 2009, **78**:1263–1271.
41. Yonezawa A, Inui K: **Organic cation transporter OCT/SLC22A and H (+)/organic cation antiporter MATE/SLC47A are key molecules for nephrotoxicity of platinum agents.** *Biochem Pharmacol* 2011, **81**:563–568.

doi:10.1186/1556-276X-8-395
Cite this article as: Yamagishi *et al.*: Acute and chronic nephrotoxicity of platinum nanoparticles in mice. *Nanoscale Research Letters* 2013 **8**:395.

アンチエイジング等
高機能化粧品開発を考える
Technology

経皮吸収性に着目したナノマテリアルの安全性評価研究

大阪大学大学院 薬学研究科 毒性学分野[1]，大阪大学 臨床医工学融合研究教育センター[2]，
医薬基盤研究所 バイオ創薬プロジェクト[3]，医薬基盤研究所 抗体スクリーニングプロジェクト[4]
東阪和馬[1]，吉岡靖雄[1,2]，堤 康央[1,2,3,4]
KAZUMA HIGASHISAKA, YASUO YOSHIOKA, YASUO TSUTSUMI

1 ナノマテリアルの実用化

20世紀以降，科学技術の発展を基盤として産業化／工業化が進展することで，大量生産，大量消費の社会を生み出し，われわれ人類の生活水準は格段に向上してきた。そして，21世紀における現在でも科学技術の発展はとどまることなく，人類の生活の質の向上に向けて，さまざまなテクノロジーが開発されている。特に近年では，革新的なナノテクノロジーの進展も相まって，ナノマテリアルの開発・実用化が世界的に進んでいる。欧州委員会が発表する定義によると，「ナノマテリアル」とは，非結合状態，または強凝集体（アグリゲート）または弱凝集体（アグロメレート）であり，個数濃度のサイズ分布で50%以上の粒子について1つ以上の外径が1nmから100nmのサイズ範囲である粒子を含む，自然の，または偶然にできた，または製造された材料を意味する[1]。例えば，カーボンナノチューブやフラーレンといった新素材や，ナノシリカやナノ酸化チタンなどの従来から使用されてきたサブミクロンサイズ（数百nm～数十μm）以上の素材を，直径100nm以下に微小化したものが開発されている。

ナノマテリアルは，その粒子径の微小化に伴い比表面積が劇的に増大した結果，従来までのサブミクロンサイズ以上の素材に比べて，電気的・磁気的・光学的特性や組織浸透性などが飛躍的に向上する。そのため，これら特有の機能を活かし，すでに香粧品・医薬品・食品・電子部品など，さまざまな産業分野の製品に実用化されている。例えば，化粧品のファンデーションや紫外線遮蔽剤として利用されている非晶質ナノシリカ（以下，ナノシリカと表記）や酸化チタンは，粒子径を微小化することによって使用感や紫外線遮蔽能を向上させることが可能である。そのため，サンスクリーン，美白剤，アンチエイジング剤などの目的により，粒子径の微小化，および経皮適用製品への応用が一層進んでいる。

さらに近年では，ナノサイズのものばかりではなく，抗体や分子と同程度の大きさ（10nm以下）にまで制御されたナノマテリアル（われわれは，これら素材をサブナノマテリアルとして定義，提唱している）が開発されている。これらサブナノマテリアルは，いまだ定義そのものがあいまいではあるものの，ナノマテリアルとも異なる体内・細胞内動態を示すなど，ナノマテリアルと分子の特性を併せ持つ可能性が示されており，新たな素材として期待されている。すなわち，ナノマテリアルは高度な生活水準を誇る現代人にとって，もはや必要不可欠な存在となっており，今後も分野を問わずあらゆる領域で，その使用拡大やさらなる有効活用が期待されている。

2 ナノマテリアルの安全性評価の必要性

近年，われわれの薬・食・環境に対する安全への懸念や健康への関心が年々高まりつつある。例えば，小麦加水分解物含有石鹸「茶のしずく石鹸」の事例にみられるように，皮膚における小麦抗原の非意図的な曝露により，重篤な食物アレルギーを発症することが先般世間を賑わせた[2]。これは，ナノマテリアルの安全性評価においても，ナノマテリアルの実用化に伴う意図的な経皮曝露機会の増加や，今後ますますその開発・実用化が進展することを鑑みると，現実の曝露経路を加味した検討が必要不可欠であることを示唆するものといえる。

一般的に，経皮曝露する化学物質の安全性を評価するうえで重要な点は，体内吸収性を精査することである。体内に吸収される物質は，全身のあらゆる組織へ移行する可能性があるため，曝露局所のみではなく，全身を対象として生体影響を評価する必要がある。しかしながら，これまで経皮曝露を介した微粒子影響が語られることは非常に少なかった。これは，分子量500ダルトン以上の物質を透過させないともいわれる強固な皮膚バリア[3]を，サブミクロンサイズ以上の粒子が突破することは困難であろうとの認識によるものである。

一方で，ナノマテリアルが開発され，微粒子が皮膚を透過して体内に侵入することはないという従来の概念は覆されつつある。例えば，Rancan らは，粒子径の微小化に伴い，ナノシリカが経皮吸収され，体内に移行することを報告している[4]。また，凝集性の高さから，皮膚を介して体内に侵入することはないといわれてきたナノ酸化チタンにおいても，その物性によっては皮膚を介して体内に吸収される可能性が示されている[5,6]。このように，ナノマテリアルが体内に移行し得るという事実は，ナノマテリアルの体内での生体影響・安全性を精査する必要性を示している（図1）。すなわち，ナノマテリアルが有する，粒子径・表面電荷などの物性や，体内吸収性・組織分布といった曝露実態・動態（蓄積性を含めたADME情報）と，ADME情報に基づいた毒性解析，いわゆるADMET解析を実施し，物性-体内・細胞内動態-安全性の連関を定量的に評価，理解することで，ナノマテリアルのリスク解析に資する情報を集積することが求められる。

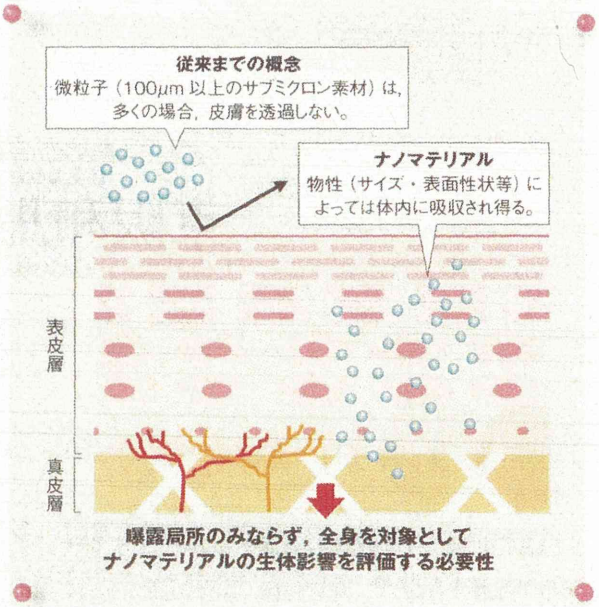


図1　安全なナノマテリアルの開発に向けた安全性評価の必要性

このような観点からわれわれは，ヒトの健康確保を第一義的な目的とし，ナノマテリアルの安全性情報の収集を目的としたNano-Safety Science（ナノ安全科学）研究と，安全なものはその利用促進に，一方で安全性に懸念があるものに関しては単に危険性を訴え規制するのではなく，安全性を