Laboratory of Toxicology and Safety Science[1], Graduate School of Pharmaceutical Sciences, Osaka University; Laboratory of Biopharmaceutical Research[2], National Institute of Biomedical Innovation; Osaka, Japan. Cancer Biology Research Center[3], Sanford Research/USD, Sioux Falls, SD, USA; The Center for Advanced Medical Engineering and Informatics[4], Osaka University, Osaka; Division of Foods[5], National Institute of Health Sciences, Tokyo, Japan

Potential of acute-phase proteins as biomarkers for sub-nano platinum exposure

T. Nagano[1,*], Y. Yoshioka[1,*], K. Higashisaka[1], A. Kunieda[1], K. Hata[1], K. Nagano[2], Y. Abe[3], H. Kamada[2,4], S. Tsunoda[2,4], H. Nabeshi[5], T. Yoshikawa[1], Y. Tsutsumi[1,2,4]

Received April 23, 2012, accepted May 18, 2012

Yasuo Yoshioka, PhD, and Yasuo Tsutsumi, PhD, Laboratory of Toxicology and Safety Science, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan
yasuo@phs.osaka-u.ac.jp, ytsutsumi@phs.osaka-u.ac.jp

These authors contributed equally to this work.

Pharmazie 67: 958–959 (2012)
doi: 10.1691/ph.2012.2626

Recently, nanomaterials (NM) have been used in a range of different fields. However, their safety for human health is not yet sufficiently understood. Therefore, we attempted to establish a NM safety assessment system by focusing on biomarkers that may predict NM-induced adverse biological effects. We previously demonstrated that the acute-phase proteins haptoglobin and serum amyloid A (SAA) have potential as useful biomarkers of silica nanoparticle (nSP) exposure. Here, we investigated the potential of haptoglobin and SAA as biomarkers of sub-nano platinum (snPt) exposure. Serum levels of haptoglobin and SAA were measured in BALB/c mice by enzyme-linked immunosorbent assay. Serum levels of haptoglobin and SAA in snPt-treated mice were significantly higher than those of saline-treated mice. This suggests that haptoglobin and SAA have potential as biomarkers of snPt-induced adverse biological effects. These data provide useful information for the development of safe NM.

Nanomaterials (NM) are substances that have at least one dimension less than 100 nm. Compared with conventional materials of submicron size, NM possess innovative functions such as high electrical conductivity, tensile strength, and tissue permeability (Rutherglen and Burke 2009); they are now widely used in cosmetics, foods, and medicines (Kaur and Agrawal 2007; Cormode et al. 2010). However, the increasing use of NM has resulted in public concern about their potential risks to health. In fact, there are various reports about adverse biological effects induced by NM (Hougaard et al. 2010; Morishige et al. 2010; Yamashita et al. 2011). It is therefore important to develop and promote safe NM because they have the potential to improve the quality of human life. We have tried to establish a NM safety assessment system that uses biomarkers. Biomarkers are expected to be useful not only in the detection of adverse biological responses, but also in the prediction of unknown biological effects induced by NM exposure (Casado et al. 2008). Biomarkers of NM will

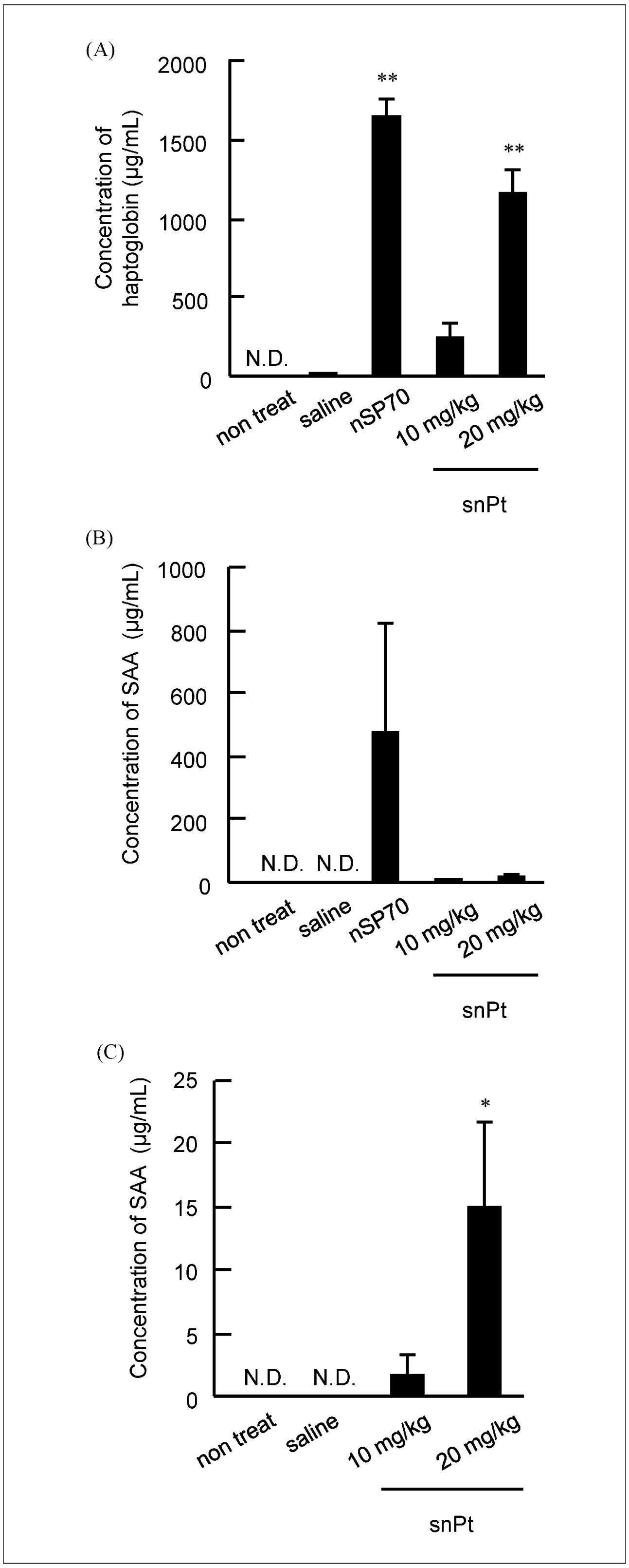

Fig.: The potential of haptoglobin and serum amyloid A (SAA) as biomarkers of snPt. Female BALB/c mice were intravenously injected via the tail vein with sub-nano platinum (snPt), silica nanoparticles (nSP70), or saline, and blood samples were collected at 24 h after treatment. The serum levels of haptoglobin (A) and SAA (B) in each mouse were examined with ELISA. To clear the level of SAA in snPt-treated mice, the result of nSP70-treated mice was removed as shown in (C). Data are presented as mean ± SE. (n = 4–5; $*P < 0.05$, $**P < 0.01$ *versus* value for saline-treated group by ANOVA; N.D., not detected) be invaluable for predicting potential toxicity and establishing strategies for the development, production, and use of safe NM. We previously demonstrated that haptoglobin and serum amyloid A (SAA) have potential as biomarkers for assessing biological responses to silica nanoparticle (nSP) exposure (Higashisaka et al. 2011). Sub-nano platinum (snPt) is already used in cosmetics and foods as an antioxidant agent (Onizawa et al. 2009), and it is expected to have therapeutic applications (Porcel et al. 2010). However, there are some concerns regarding adverse biological effects from snPt exposure (Park et al. 2010). Therefore, there is an urgent need to collect information about the safety of snPt.

Here, we investigated whether haptoglobin and SAA could be used as biomarkers of snPt. We used snPt with a diameter of 0.63 nm, and nSP with a diameter of 70 nm as a positive control. BALB/c mice were administered snPt (10 or 20 mg/kg), nSP (40 mg/kg), or saline via intravenous injection into the tail vein. We decided the dose of snPt by using previous data indicating that haptoglobin and SAA were significantly increased in mice treated with 40 mg/kg nSP (Higashisaka et al. 2011). Serum samples were collected at 24 h after administration and serum levels of haptoglobin and SAA were measured by enzyme-linked immunosorbent assay (ELISA). Serum levels of haptoglobin and SAA in snPt-treated mice were significantly higher than those of saline-treated mice (Fig.). In addition, although the levels of haptoglobin were almost the same in snPt-treated and nSP-treated mice, the levels of SAA were quite different (Fig.). Our results suggest that haptoglobin and SAA are potential biomarkers of snPt and that differences in biological effects are dependent on the type of NM.

Exposure to snPt in daily life can occur through various different routes (Onizawa et al. 2009). For example, snPt contained in food is taken up orally, whereas snPt within the manufacturing environment generally enters the body intranasally. Therefore, the evaluation of biomarkers for oral or intranasal exposure to snPt is also needed.

We are now trying to evaluate why SAA showed different expression in snPt-treated and nSP-treated mice. We expect that this study will provide useful information for the development of biomarkers of NM exposure. We believe that the establishment of a NM safety assessment system based on biomarkers will lead to the development of safe NM.

Experimental

1. Materials

snPt and nSP were purchased from Polytech & Net GmbH (Schwalbach, Germany) and Micromod Partikeltechnologie (Rostock/Warnemünde, Germany), respectively. They were sonicated for 5 min and then vortexed for 1 min prior to use.

2. Animals

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

3. Blood-sample collection

BALB/c mice were administered snPt (10 or 20 mg/kg), nSP (40 mg/kg), or saline via intravenous injection into the tail vein. Blood samples were collected at 24 h after treatment, and serum was harvested by centrifugation at 8000 g for 15 min.

4. Measurement of acute-phase proteins

Serum levels of haptoglobin and SAA were measured with commercial ELISA kits (Life Diagnostics, Inc.; West Chester, PA) according to the manufacturer's instructions.

5. Statistical analyses

All results are expressed as means $\pm$ SE. Differences were compared by using the Bonferroni method after analysis of variance (ANOVA).

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 a the Knowledge Cluster Initiative (MEXT); by Health Labour Sciences Research Grants from the Ministry of Health, Labor and Welfare of Japan (MHLW); by a Global Environment Research Fund from Minister of the Environment; by Food Safety Commission (Cabinet Office); by The Cosmetology Research Foundation; by The Smoking Research Foundation; by The Takeda Science Foundation; by The Nagai Foundation Tokyo; by The Research Foundation for Pharmaceutical Sciences; and by The Japan Food Chemical Research Foundation.

References

Casado B, Iadarola P, Luisetti M, Kussmann M (2008) Proteomics-based diagnosis of chronic obstructive pulmonary disease: the hunt for new markers. Expert Rev Proteomics 5: 693–704.

Cormode DP, Jarzyna PA, Mulder WJ, Fayad ZA (2010) Modified natural nanoparticles as contrast agents for medical imaging. Adv Drug Deliv Rev 62: 329–338.

Higashisaka K, Yoshioka Y, Yamashita K, Morishita Y, Fujimura M, Nabeshi H, Nagano K, Abe Y, Kamada H, Tsunoda S, Yoshikawa T, Itoh N, Tsutsumi Y (2011) Acute phase proteins as biomarkers for predicting the exposure and toxicity of nanomaterials. Biomaterials 32: 3–9.

Hougaard KS, Jackson P, Jensen KA, Sloth JJ, Loschner K, Larsen EH, Birkedal RK, Vibenholt A, Boisen AM, Wallin H, Vogel U (2010) Effects of prenatal exposure to surface-coated nanosized titanium dioxide (UV-Titan). A study in mice. Part Fibre Toxicol 7: 16.

Kaur IP, Agrawal R (2007) Nanotechnology: a new paradigm in cosmeceuticals. Recent Pat Drug Deliv Formul 1: 171–182.

Morishige T, Yoshioka Y, Tanabe A, Yao X, Tsunoda S, Tsutsumi Y, Mukai Y, Okada N, Nakagawa S (2010) 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 392: 160–165.

Onizawa S, Aoshiba K, Kajita M, Miyamoto Y, Nagai A (2009) Platinum nanoparticle antioxidants inhibit pulmonary inflammation in mice exposed to cigarette smoke. Pulm Pharmacol Ther 22: 340–349.

Park EJ, Kim H, Kim Y, Park K (2010) Intratracheal instillation of platinum nanoparticles may induce inflammatory responses in mice. Arch Pharm Res 33: 727–735.

Porcel E, Liehn S, Remita H, Usami N, Kobayashi K, Furusawa Y, Le Sech C, Lacombe S (2010) Platinum nanoparticles: a promising material for future cancer therapy? Nanotechnology 21: 85103.

Rutherglen C, Burke P (2009) Nanoelectromagnetics: circuit and electromagnetic properties of carbon nanotubes. Small 5: 884–906.

Yamashita K, Yoshioka Y, Higashisaka K, Mimura K, Morishita Y, Nozaki M, Yoshida T, Ogura T, Nabeshi H, Nagano K, Abe Y, Kamada H, Monobe Y, Imazawa T, Aoshima H, Shishido K, Kawai Y, Mayumi T, Tsunoda S, Itoh N, Yoshikawa T, Yanagihara I, Saito S, Tsutsumi Y (2011) Silica and titanium dioxide nanoparticles cause pregnancy complications in mice. Nat Nanotechnol 6: 321–328.