



Leading Opinion

Acute phase proteins as biomarkers for predicting the exposure and toxicity of nanomaterials[☆]

Kazuma Higashisaka^{a,b,1}, Yasuo Yoshioka^{a,b,c,*,1}, Kohei Yamashita^{a,b}, Yuki Morishita^{a,b}, Maho Fujimura^{a,b}, Hiromi Nabeshi^a, Kazuya Nagano^b, Yasuhiro Abe^b, Haruhiko Kamada^{b,c}, Shin-ichi Tsunoda^{b,c,d}, Tomoaki Yoshikawa^{a,b}, Norio Itoh^a, Yasuo Tsutsumi^{a,b,c,**}

^a Department of Toxicology and Safety Science, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan

^b Laboratory of Pharmaceutical Proteomics, National Institute of Biomedical Innovation, 7-6-8, Saito-Asagi, Ibaraki, Osaka 567-0085, Japan

^c The Center for Advanced Medical Engineering and Informatics, Osaka University, 1-6, Yamadaoka, Suita, Osaka 565-0871, Japan

^d Department of Biomedical Innovation, Graduate School of Pharmaceutical Sciences, Osaka University, 7-6-8, Saito-Asagi, Ibaraki, Osaka 567-0085, Japan

ARTICLE INFO

Article history:

Received 8 July 2010

Accepted 31 August 2010

Available online 22 September 2010

Keywords:

Nanoparticle

Plasma proteins

Silica

Surface modification

ABSTRACT

Recently, nanomaterials have become an integral part of our daily lives. However, there is increasing concern about the potential risk to human health. Here, we attempted to identify biomarkers for predicting the exposure and toxicity of nanomaterials by using a proteomics based approach. We evaluated the changes of protein expression in plasma after treatment with silica nanoparticles. Our analyses identified haptoglobin, one of the acute phase proteins, as a candidate biomarker. The results of ELISA showed that the level of haptoglobin was significantly elevated in plasma of mice exposed to silica nanoparticles with a diameter of 70 nm (nSP70) compared to normal mice and those exposed to silica particles with a diameter of 1000 nm. Furthermore, the other acute phase proteins, C-reactive protein (CRP) and serum amyloid A (SAA) were also elevated in plasma of nSP70 treated mice. In addition, the level of these acute phase proteins was elevated in the plasma of mice after intranasal treatment with nSP30. Our results suggest that haptoglobin, CRP and SAA are highly sensitive biomarkers for assessing the risk of exposure to silica nanoparticles. We believe this study will contribute to the development of global risk assessment techniques for nanomaterials.

© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

With the recent development of nanotechnology, nanomaterials such as silica nanoparticles are beginning to be used on a global scale. In comparison to conventional materials with submicron size, nanomaterials display unique properties such as high levels of

electrical conductivity, tensile strength and chemical reactivity [1]. Nanomaterials have already been used in various fields such as electronic engineering, cosmetics and medicine [2,3]. Because nanotechnology is emerging as a leading industrial sector, humans will be increasingly exposed to a wide range of synthetic nanomaterials with diverse properties.

The increasing use of nanomaterials has raised public concerns about the potential risks to human health [4–6]. For example, it is reported that carbon nanotubes induce mesothelioma-like lesions in mice in a similar way to crocidolite asbestos [7]. Other reports showed that exposure to titanium dioxide particles induce inflammatory responses and lung injury in mice [8,9]. In addition, our group showed that silica nanoparticles with a diameter of 70 nm can penetrate mouse skin and enter the circulatory system (unpublished data). Furthermore our group demonstrated that silica nanoparticles induce severe liver damage after systemic administration [10–12]. However, current knowledge of the potential risk of nanomaterials is considered insufficient. Indeed, concerns about the potential dangers of nanomaterials have led the World Health Organization and the Organization for Economic

[☆] *Editor's Note:* This paper is one of a newly instituted series of scientific articles that provide evidence-based scientific opinions on topical and important issues in biomaterials science. They have some features of an invited editorial but are based on scientific facts, and some features of a review paper, without attempting to be comprehensive. These papers have been commissioned by the Editor-in-Chief and reviewed for factual, scientific content by referees.

* Corresponding author. The Center for Advanced Medical Engineering and Informatics, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan. Tel.: +81 6 6879 8230; fax: +81 6 6879 8234.

** Corresponding author. Department of Toxicology and Safety Science, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6, Yamadaoka, Suita, Osaka 565-0871, Japan. Tel.: +81 6 6879 8230; fax: +81 6 6879 8234.

E-mail addresses: yasuo@phs.osaka-u.ac.jp (Y. Yoshioka), ytsutsumi@phs.osaka-u.ac.jp (Y. Tsutsumi).

¹ These authors contributed equally to the work.

Co-operation and Development to call for an urgent and detailed evaluation of their safety. Therefore, it is extremely important to progress these safety evaluations in order to facilitate the development of nanomaterials that are harmless to humans, because nanomaterials have the potential to improve the quality of human life. In particular, it is hoped that a risk assessment system can be developed to estimate or predict the safety and toxicity of nanomaterials.

Molecular biomarkers, obtained from biological samples such as blood, urine and tissue, constitute an objective indicator for correlating against various physiological conditions or variation of disease state [13,14]. By using biomarkers, we are able to predict not only the present disease and clinical condition but the risk of acquiring disease in the future. Nowadays, biomarkers that act as predictors of cancer have already been developed and are commonly used in clinical practice [14]. Furthermore, such an approach is capable of predicting adverse effects of drugs and medicines [15,16]. By contrast, studies of biomarkers for nanomaterials have barely advanced. These biomarkers would represent the unity of local and systemic physiological responses induced as a result of the exposure. Therefore, biomarkers for nanomaterials will be invaluable for predicting their potential toxicity and establishing strategies for the safe development of nanomaterials production and use.

Here we attempted to develop potential biomarkers of nanomaterials using a proteomics analysis with the aim of developing safe forms of nanomaterials.

2. Materials and methods

2.1. Materials

Silica particles were purchased from Micromod Partikeltechnologie (Rostock/Warnemünde, Germany). The silica particles with diameters of 30, 70, 300 and 1000 nm (nSP30, nSP70, nSP300 and mSP1000, respectively), and nSP70 with surface functional groups such as carboxyl group and amino group (nSP70-C and nSP70-N, respectively) were used in this study. The silica particles were sonicated for 5 min and vortexed for 1 min prior to use.

2.2. Animals

Female BALB/c mice were purchased from Nippon SLC, Inc (Shizuoka, Japan) and used at 6–8 weeks of age. All of the animal experimental procedures in this study were performed in accordance with the National Institute of Biomedical Innovation guidelines for the welfare of animals.

2.3. Blood sample collection

For administration of silica particles through an intravenous route, BALB/c mice were treated with nSP70, nSP300, mSP1000, nSP70-C, nSP70-N or saline at 0.8 mg/mouse. At various times (6 h, 24 h, 3 day and 7 day) after treatment of these silica particles, blood samples were collected. For administration of silica particles through an intranasal route, BALB/c mice were treated with nSP30, nSP70 or saline intranasally at 0.5 mg/mouse. Blood samples were collected 24 h after the treatment of these silica nanoparticles.

2.4. Analysis of biomarkers for nanomaterials using a proteomics approach

BALB/c mice were treated with 0.8 mg/mouse nSP70 or saline intravenously. After 24 h, blood samples were collected and plasma was harvested by centrifuging blood at 12000 rpm for 15 min. Proteo prep (Sigma–Aldrich; Saint Louis, MO) was used to remove albumin and immunoglobulins from the plasma according to the manufacturer's instructions. Plasma samples were then analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by Coomassie Brilliant Blue staining. Plasma diluted into aliquots corresponding to 10 µg protein were mixed with an equal volume of Laemmli sample buffer (BIO-RAD, Tokyo, Japan) containing 5% 2-mercaptoethanol and boiled for 5 min prior to electrophoresis. Electrophoresis was performed at 15 mA for 10 min (stacking) followed by separation (600 V, 40 mA, 100 W) for approximately 45 min, using Precision Plus Protein Kaleidoscope molecular weight markers (BIO-RAD) as standards.

2.5. Identification of candidate proteins as biomarkers

Bands of interest were excised from the gel and then destained with 50% acetonitrile (ACN)/25 mM NH_4HCO_3 for 10 min, dehydrated with 100% ACN for 10 min, and then dried using a centrifugal concentrator. Next, 8 µl of 20 µl/ml trypsin solution (Promega, Madison, WI) diluted 5-fold in 50 mM NH_4HCO_3 was added to each gel piece and then incubated overnight at 37 °C. We used three solutions to extract the resulting peptide mixtures from the gel pieces. First, 50 µl of 50% (v/v) ACN in 0.1% aqueous trifluoroacetic acid (TFA) was added to the gel pieces, which were then sonicated for 30 min. Next, we collected the solution and added 80% (v/v) ACN in 0.1% TFA. Finally, 100% ACN was added for the last extraction. The peptide solution were dried and resuspended in 10 µl of 0.1% formic acid. The resulting peptide mixture was then analyzed by nano-flow liquid chromatography/tandem mass spectrometry (LC/MS; maXis, Bruker Daltonik GmbH, Bremen, Germany).

2.6. Measurement of acute phase proteins

Plasma levels of haptoglobin, C-reactive protein (CRP) and serum amyloid A (SAA) were measured by commercial enzyme-linked immunosorbent assay (ELISA) kits (Life Diagnostics, Inc.; West Chester, PA), according to the manufacturer's instructions.

2.7. Statistical analyses

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

3. Results

3.1. Identification of biomarkers of nanomaterials

We used silica particles as a model nanomaterial because it is one of the most common nanomaterials to have been developed. Silica particles are increasingly being used as additives in cosmetics and foods [17,18]. It is predicted that the global market for silica particles will soon grow to \$2 billion and a ton of silica particles is currently produced worldwide every year. Here, we used silica particles with a diameter of 30, 70, 300 and 1000 nm (nSP30, nSP70, nSP300 and mSP1000, respectively). The mean secondary particle diameters of the silica particles measured by Zetasizer were 33, 79, 326 and 945 nm, respectively (data not shown). The silica particles were confirmed to be well dispersed smooth-surfaced spheres by transmission electron microscopy (data not shown).

Initially, we attempted to identify protein biomarkers in mice by analyzing changes in the level of each plasma protein following treatment with silica nanoparticles using a proteomics approach. BALB/c mice were intravenously treated with nSP70 (0.8 mg/mouse) or saline and then plasma samples were collected 24 h later. Because albumin and immunoglobulins are known to account for the majority of plasma proteins, they were removed from the samples prior to analysis so that variation in the level of other proteins could be more closely monitored. The change of protein levels in plasma after treatment with nSP70 was assessed by SDS-PAGE analysis (Fig. 1). The intensity of a band of molecular mass 37 kDa was more intense in the plasma of nSP70 treated mice than that of saline treated control mice (Fig. 1). The band was excised and analyzed by LC/MS in order to identify the corresponding protein. This analysis identified the induced band after treatment with nSP70 as haptoglobin, one of the acute phase proteins.

3.2. The level of haptoglobin after treatment with silica particles

To assess the change of haptoglobin level in plasma after administration of silica particles, BALB/c mice were intravenously treated with nSP70, nSP300 or mSP1000 at 0.8 mg/mouse. We did not use nSP30 in the experiment, because nSP30 induced the toxic side effects after intravenous treatment at this dose. We confirmed that nSP70, nSP300 or mSP1000 at this dose did not induce any

Download English Version:

<https://daneshyari.com/en/article/8394>

Download Persian Version:

<https://daneshyari.com/article/8394>

[Daneshyari.com](https://daneshyari.com)