Chemico-Biological Interactions 236 (2015) 82–89

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Dysfunction of methionine sulfoxide reductases to repair damaged proteins by nickel nanoparticles

Po-Hao Feng [a,b,1], Ya-Li Huang [c,d,1], Kai-Jen Chuang [c,d], Kuan-Yuan Chen [a,b], Kang-Yun Lee [a,b], Shu-Chuan Ho [a,e], Mauo-Ying Bien [e,f], You-Lan Yang [e], Hsiao-Chi Chuang [a,e,*], On behalf of the Taiwan CardioPulmonary Research (T-CPR) Group

[a] Division of Pulmonary Medicine, Department of Internal Medicine, Shuang Ho Hospital, Taipei Medical University, Taipei, Taiwan
[b] Department of Internal Medicine, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan
[c] Department of Public Health, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan
[d] School of Public Health, College of Public Health and Nutrition, Taipei Medical University, Taipei, Taiwan
[e] School of Respiratory Therapy, College of Medicine, Taipei Medical University, Taipei, Taiwan
[f] Division of Pulmonary Medicine, Department of Internal Medicine, Taipei Medical University Hospital, Taipei, Taiwan

ARTICLE INFO

Article history:
Received 22 December 2014
Received in revised form 21 April 2015
Accepted 6 May 2015
Available online 13 May 2015

Keywords:
Autophagy
Benzo(a)pyrene diolepoxide protein adduct
Extracellular signal-regulated kinase
Methionine oxidation
Methionine sulfoxide reductases
Nickel nanoparticle

ABSTRACT

Background: Protein oxidation is considered to be one of the main causes of cell death, and methionine is one of the primary targets of reactive oxygen species (ROS). However, the mechanisms by which nickel nanoparticles (NiNPs) cause oxidative damage to proteins remain unclear.
Objectives: The objective of this study is to investigate the effects of NiNPs on the methionine sulfoxide reductases (MSR) protein repairing system.
Methods: Two physically similar nickel-based nanoparticles, NiNPs and carbon-coated NiNP (C-NiNPs; control particles), were exposed to human epithelial A549 cells. Cell viability, benzo(a)pyrene diolepoxide (BPDE) protein adducts, methionine oxidation, MSRA and B3, microtubule-associated protein 1A/1B-light chain 3 (LC3) and extracellular signal-regulated kinase (ERK) phosphorylation were investigated.
Results: Exposure to NiNPs led to a dose-dependent reduction in cell viability and increased BPDE protein adduct production and methionine oxidation. The methionine repairing enzymatic MSRA and MSRB3 production were suppressed in response to NiNP exposure, suggesting the oxidation of methionine to MetO by NiNP was not reversed back to methionine. Additionally, LC3, an autophagy marker, was down-regulated by NiNPs. Both NiNP and C-NiNP caused ERK phosphorylation. LC3 was positively correlated with MSRA ($r = 0.929$, $p < 0.05$) and MSRB3 ($r = 0.893$, $p < 0.05$).
Conclusions: MSR was made aberrant by NiNP, which could lead to the dysfunction of autophagy and ERK phosphorylation. The toxicological consequences may be dependent on the chemical characteristics of the nanoparticles.

1. Introduction

Increasing engineered nanodevices and nanostructures are being developed in material science, medicine and electronics; however, the potential risk associated with the manufacture, use and disposal of nanoscaled materials has not been fully characterised. The most recognised paradigms of nanotoxicity are oxidative stress and inflammation, but the underlying mechanisms remain unclear [1]. Nickel nanoparticles (NiNPs), for example, have been widely used in industries, such as catalysts, sensors and electronic applications. Numerous studies have shown that NiNPs alter oxidative balance and cell fate in cells [2] and animals [3]. Notably, respiratory disordered due to NiNP exposure have been reported based on human evidence [4]. The crosslinking evidence from cells to humans suggests that the investigation into the biological

Abbreviations: Bcl-2, B-cell lymphoma 2; BPDE, benzo(a)pyrene diolepoxide; C-NiNPs, carbon-coated nickel nanoparticles; EDX, energy-dispersive X-ray; p-ERK1/2, extracellular signal-regulated kinase phosphorylation; ERK, extracellular signal-regulated kinase; FE-SEM, field emission-scanning electron microscope; MSRA, methionine sulfoxide reductases A; MSRB, methionine sulfoxide reductases B; MetO, methionine sulfoxide; LC3, microtubule-associated protein 1A/1B-light chain 3; NiNPs, nickel nanoparticles; RNS, reactive nitrogen species; ROS, reactive oxygen species; SRB, sulphorhodamine B.

* Corresponding author at: Taiwan CardioPulmonary Research (T-CPR) Group, School of Respiratory Therapy, College of Medicine, Taipei Medical University, Taipei, Taiwan. Tel.: +886 2 27361661x3515; fax: +886 2 27391143.

E-mail addresses: 13199@s.tmu.edu.tw (P.-H. Feng), ylhuang@tmu.edu.tw (Y.-L. Huang), kjc@tmu.edu.tw (K.-J. Chuang), a112378633@gmail.com (K.-Y. Chen), kangyunlee68@gmail.com (K.-Y. Lee), shu-chuan@tmu.edu.tw (S.-C. Ho), mybien@tmu.edu.tw (M.-Y. Bien), ylyang@tmu.edu.tw (Y.-L. Yang), r92841005@ntu.edu.tw (H.-C. Chuang).

[1] These authors contributed equally to the study.

http://dx.doi.org/10.1016/j.cbi.2015.05.003
0009-2797/

mechanisms underlying NiNP-induced adverse human health effects is an emerging task.

Protein oxidation occurs when reactive oxygen species (ROS) and reactive nitrogen species (RNS) interact with both the amino acid side chains and peptidic backbone [5]. Sulphur-containing amino acids and aromatic amino acids in proteins are the most susceptible to be attacked [5]. Sulphur-containing residues, such as methionine and cysteine, are responsible for more than 70% of the ROS trapping activity in proteins [6,7]. ROS oxidises methionine to methionine sulfoxide (MetO; oxidised methionine), forming two enantiomers, *S*-MetO and *R*-MetO. *S*-MetO and *R*-MetO can be catalytically reversed by the peptide MetO enzymes, methionine sulfoxide reductases A (MSRA) and B (MSRB), respectively, allowing the recovery of the protein function in some cases [8]. In eukaryotes, for example, MSRA is observed in the cytosol and mitochondria [8]. MSRB are designated as MSRB1 presented in the nucleus and the cytosol, MSRB2 localised in the mitochondria and MSRB3 found in the endoplasmic reticulum and the mitochondria [9]. The MSR system is an important repair mechanism for oxidised proteins, which catalyses MetO back to methionine; however, the effects of NiNPs on MSR function remain unclear.

Exposure to nanomaterials can trigger cellular pathways that are broadly classified as death and survival signals. Cell death is an essential orchestrated process, which is divided into three main pathways: apoptosis, autophagy and necrosis [10]. Protein oxidation results in aberrant proteins and in the loss of their functions, and these proteins can be degraded by autophagy. Extracellular signal-regulated kinase (ERK) 1/2 plays an important role in the regulation of cell survival. ERK1/2 inhibits apoptosis by preventing p53 activity and by regulating B-cell lymphoma 2 (Bcl-2) family proteins (also a regulator for autophagy). Previous reports have suggested that the dysfunction of autophagy is an important mechanism in response to nanomaterial toxicity [11]; however, the upstream protein repairing mechanism after NiNP exposure remains unclear. The objective of this study is to investigate the function of the MSR system and the alteration in cell death regulation by autophagy and ERK1/2 phosphorylation (p-ERK1/2) after exposure to NiNP.

2. Materials and methods

2.1. Particle preparation and characterisation

NiNPs and carbon-coated NiNPs (C-NiNPs; served as control particles) obtained from the Nanostructured & Amorphous Materials, Inc. (Houston, TX, USA) were used in this study. The average size was 20 nm in diameter determined by aerodynamic particle sizer (APS). The purity (wt%) and specific surface area (m^2/g) were 99.9 and 40–60, respectively. An Inspect™ field emission-scanning electron microscope (FE-SEM; JEOL 2100, Jeol, Japan) and an energy-dispersive X-ray (EDX) microanalysis were used in this study. The preparation for FE-SEM and EDX analyses has been previously reported [12]. Briefly, the samples were fixed on 13-mm aluminium SEM stubs. The samples were coated with platinum (Pt) using a sputter coater. The FE-SEM was used to image the samples operating at an accelerating voltage of 15 kV and a 2.5 spot size. The elements of the samples were examined using the EDX Genesis Microanalysis System. NiNPs and C-NiNPs were freshly prepared at 20, 50 and 150 μg/ml in cell medium (with 10% foetal bovine serum) by vortexing for 4 h at room temperature. The hydrodynamic diameters were determined using dynamic light scattering (DLS; Malvern Zetasizer Nano ZS, UK). The zeta potential values were determined using a Zetasizer (Nano Z; Malvern, UK). The amount of endotoxin in the NiNPs and C-NiNPs was determined using the Pierce LAL Chromogenic Endotoxin Quantitation Kit (Rockford, IL, USA).

2.2. Cell culture

Human alveolar epithelial A549 cells were obtained from the American Type Culture Collection. The cells were cultured in RPMI containing 10% foetal bovine serum, penicillin and streptomycin and incubated in air at 37 °C, 95% humidity and 5% CO_2.

2.3. Cell treatment

Cells were seeded onto 24-well surface-treated culture plates and incubated for 24 h at the density of 1×10^5 cells/well (BD Biosciences, UK). The RPMI medium was removed following the addition of the NiNP and C-NiNP samples prepared in serum-free RPMI medium at a mass concentration of 0, 20, 50 and 150 μg/ml. The cells were then incubated at 37 °C for 4 h in a humidified atmosphere with 5% CO_2. The concentrations of NiNPs and C-NiNPs were chosen to produce cell death.

2.4. Cell viability

The sulphorhodamine B (SRB) colorimetric assay was used to determine the cell viability according a previous method [13]. Briefly, the cells were fixed with 10% (wt/vol) trichloroacetic acid and stained for 30 min. The protein-bound dye was dissolved in 10 mM Tris base solution after removing the excess dye and measured for OD at 510 nm using a microplate reader. The cell viability was presented after adjusting the control.

2.5. Benzo(a)pyrene diolepoxide protein adducts, MSRA, MSRB3, microtubule-associated protein 1A/1B-light chain 3 and p-ERK1/2

The cells were collected from the plates and stored at −20 °C overnight in 1× PBS after NiNP and C-NiNP exposure. After two freeze–thaw cycles to break up the cell membranes, the cell lysates were centrifuged at 5000×*g* at 2 °C. The supernatants were collected for BPDE, MSRA, MSRB3, LC3 and phosphorylated ERK1/2 (p-ERK1/2) determination. An enzyme-linked immunosorbent assay (ELISA) was used to determine benzo(a)pyrene diolepoxide (BPDE) protein adducts (OxiSelect™, Cell Biolabs, San Diego, CA, USA), MSRA (Uscn Life Science, Wuhan, Hubei, China), MSRB3 (Uscn Life Science, Wuhan, Hubei, China), microtubule-associated protein 1A/1B-light chain 3 (LC3; CUSABIO, Wuhan, Hubei, China) and p-ERK1/2 (ERK1/2 pThr202/Tyr204 + Total PhosphoTracer ELISA Kit; Abcam, Cambridge, MA, USA) according to the manufacturer's instructions.

2.6. Determination of methionine oxidation

The methionine oxidation interacted with particles has been reported previously [14,15]. Briefly, a 1 mg/ml solution of recombinant bovine serum albumin was prepared with sterile PBS and then vortexed with 150 μg/ml NiNP and C-NiNP for 2 h. Samples were diluted with 6.5 mM dithiothreitol and then alkylated using 10 mM iodoacetamide. A Q-Exactive MS (Thermo Fisher Scientific, Bremen, Germany) coupled to an UltiMate 3000 RSLC system was used to determine the tryptic peptides after digestion. A liquid chromatography equipped with a C18 column (Acclaim PepMap RSLC, 75 μm × 150 mm, 2 μm, Dionex) was used to separate the peptide. Full MS scans were performed with an *m*/*z* range of 300–2000, and the ten most intense ions from the MS scan were subjected to fragmentation to yield MS/MS spectra. The raw data were processed into peak lists by Proteome Discoverer v1.4 for Mascot database searches (http://www.matrixscience.com). The