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ScienceDirect
Toxicology Letters 170 (2007) 185–192
Toxicology Letters
www.elsevier.com/locate/toxlet

Gene expression in nanotoxicology research: Analysis by differential display in BALB3T3 fibroblasts exposed to cobalt particles and ions

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Received 27 October 2006; received in revised form 7 March 2007; accepted 8 March 2007
Available online 13 March 2007

Abstract

Broadly defined, nanoscale materials are substances in which at least one critical dimension is less than 100 nm. Nanoscale materials are employed in several industrial applications as well as in biology and medicine. Despite their wide use, very little research has been carried out on the potential toxicity of nanoparticles. For this reason, we report on a molecular approach in nanotoxicology research. Using the differential display technique, we focused our attention on mRNA expression in a BALB3T3 A31-1-1 cell line that was not exposed and exposed for 72 h to 1 μM of cobalt microparticles (Co-μ), nanoparticles (Co-nano), and ions. In the experiments, we obtained 10 differentially expressed sequences. These genes represent candidate biomarkers capable of indicating specific cellular effects after Co-nano exposure. In addition, our results show that treatment with Co-nano somehow activates cellular pathways of defense and repair mechanisms. It is also evident that molecular techniques are valuable tools in nanotoxicology research, where they will certainly find wide use.

Keywords: Nanoparticles; Cobalt; Nanotoxicity; Biomarker; Differential display

1. Introduction

Broadly defined, nanoscale materials are substances in which at least one critical dimension is less than 100 nm. Ultrafine particulate matter is a well-known example of ambient nanoscale particles. Moreover, great attention of the scientific world is being directed to manufactured nanoscale materials of current or projected commercial importance. Nanoscale materials can, in theory, be engineered from any chemical substance; semiconductor nanocrystals, organic dendrimers, carbon fullerenes, and carbon nanotubes represent a few of the many examples (Oberdörster et al., 2005a). Nanoscale materials are already becoming commercially available for industrial applications and consumer use and in the fields of biology and medicine as drug and delivery formulations, for tissue engineering, for destroying tumours by hyperthermia, for probes of DNA structure, and for biosensors (Salata, 2004; Wang et al., 2005; Yang et al., 2006).

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doi:10.1016/j.toxlet.2007.03.005

Despite their wide use, very little research has focused on the potential toxicity of nanoparticles (Donaldson et al., 2004; Oberdörster et al., 2005a). Ultrafine particle inhalation toxicology studies suggest that particle size can impact toxicity due principally to two factors: the large surface area and its reactivity or intrinsic toxicity (Tran et al., 2000; Donaldson and Tran, 2002). If surface chemistry is influenced by the size of the particle, surface properties can be changed by coating nanoscale particles with different materials. This interaction of surface area and particle composition in eliciting biological responses adds an extra dimension of complexity in evaluating potential adverse events that may result from exposure to these materials (Oberdorster et al., 2005b). There are indications in the literature that manufactured nanoscale materials may spread in the body in unpredictable ways, and certain nanoscale materials have been observed to preferentially accumulate in particular organelles. Furthermore, the unique and diverse physicochemical properties of nanoscale materials suggest that their toxicological properties may differ from those of the corresponding bulk materials (Mishra et al., 2005).

Metallic cobalt, as a nanoparticle, is also used in biological and medical applications in different forms, from the simplest, such as cobalt oxide, to complex organic compounds or biopolymers (Wang et al., 2005; Yang et al., 2006). With the aim of finding specific biomarkers of cobalt nanoparticle exposure, this study focuses on the differences in the mRNA expression of BALB3T3 fibroblasts exposed or not exposed to cobalt microparticles (Co-nano), nanoparticles (Co-μ), and ions at the inhibitory concentration of 20% of the control (IC_{20}) for 72 h. Differences in gene expression are shown by differential display (Liang and Pardee, 1992). Differential display is one of the techniques with which genes that are differentially expressed can be identified. An advantage of differential display over array technology is that it does not require any *a priori* knowledge of the sequences of the mRNAs whose differential expression are being searched for. Differential display is still superior to DNA microarray and oligoarray in terms of number of publications, also because array technology continues to struggle with reproducibility problems (Liang, 2006).

2. Materials and methods

2.1. Chemicals

Cobalt chloride ($CoCl_2$, [7791-13-1]) was supplied by Alfa Aesar, Karlsruhe, Germany; cobalt nanoparticles (Co-nano) were supplied by Laboratory of Biomaterials, University of Modena & Reggio Emilia, Modena, Italy and cobalt-microparticles (Co-μ < 2 μm, [7440-48-4]) by Sigma–Aldrich, Milan, Italy. The compounds were physically characterized by scanning electron microscopy (SEM) and dynamic light scattering (DLS) at the Biomedical Material and System Unit of JRC. In freshly prepared stock solutions, Co-nano formed aggregates with a mean size of about 500 nm (ranging from 200 to 950 nm). The mean size of the Co-μ aggregates was about 450 nm (ranging from 400 to 700 nm). The compounds were analyzed for elemental impurities by inductively coupled plasma mass spectrometry (Perkin-Elmer SCIEX apparatus, Ontario, Canada). The results of the analysis of 45 elements (not reported in the present work) indicated that the degree of chemical purity of the compounds was suitable such that any possible artefacts concerning cytotoxic effects induced by elements other than Co as particles in our *in vitro* system could be avoided. All other chemicals were of analytical grade. $CoCl_2$ was dissolved in MilliQ water at a concentration of 10 mM. The freshly prepared mother solution was sterilized using a 0.2-μm filter (Millipore, Italy) and diluted in complete culture medium to obtain a concentration of 1 μM for testing. Co-nano and Co-μ powders were weighed and suspended in MilliQ water at a concentration of 10 mM. The freshly prepared mother solution was ultrasonicated for 15 min and immediately diluted in complete culture medium to reach the concentration of 1 μM.

2.2. Cell culture and media

Mouse immortalized fibroblasts Balb/3T3 clone A31-1-1 were purchased, mycoplasma-free and source certified, from the Istituto Zooprofilattico Sperimentale of Brescia, Italy. Experimental cultures were prepared from deep-frozen stock vials and always kept in a subconfluent state. They were maintained in low-glucose Dulbecco's Modified Eagle medium (DMEM) (Gibco, Invitrogen Corporation, Italy) containing 10% (v/v) of semi synthetic Foetal Clone Serum III (FCIII, Hyclone, Celbio, Milano, Italy), 4.8 mM of L-glutamine, 1% (v/v) of Fungizone liquid (250 μg/ml), 0.6% (v/v) of penicillin/streptomycin, 10,000 U/ml penicillin and 10,000 U/ml streptomycin (Gibco, Invitrogen Corporation, Italy), and at a 1:15 ratio for cell passage using trypsin/EDTA (1×) liquid 0.5 g/l trypsin 1:250 and 0.2 g/l Na EDTA in Hanks' B.S.S. (Gibco, Invitrogen Corporation, Italy). Cell preparations were maintained at standard cell culture conditions 37 °C, 5% CO_2 and 95% humidity for 24 h.

2.3. Cytotoxicity test to determine the IC_{20}

Dose–effect relationships were set by exposing cells to six concentrations of Co-nano, Co-μ, and $CoCl_2$ ranging from 1 to 100 μM for 72 h by a colony-forming efficiency assay (CFE). Briefly, cells were seeded at a density of 200 cells/dish in 4 ml DMEM complete culture medium (60 mm × 15 mm dish, Corning, 6 dishes per determination). After 24 h, the medium was replaced with 4 ml of fresh culture medium containing Co-nano, Co-μ, or $CoCl_2$ at concentrations ranging