Toxicology in Vitro 42 (2017) 191–199

Contents lists available at ScienceDirect

Toxicology in Vitro

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

In vitro evaluation of the potential toxic effects of palladium nanoparticles on fibroblasts and lung epithelial cells

I. Iavicoli[a,*], M. Farina[b], L. Fontana[c], D. Lucchetti[b], V. Leso[c], C. Fanali[b], V. Cufino[b], A. Boninsegna[b], K. Leopold[d], R. Schindl[d], D. Brucker[d], A. Sgambato[b]

[a] Section of Occupational Medicine, Department of Public Health, University of Naples Federico II, Via S. Pansini 5, 80131 Naples, Italy
[b] Institute of General Pathology, Catholic University of the Sacred Heart, Largo Francesco Vito 1, 00168 Rome, Italy
[c] Institute of Public Health, Catholic University of the Sacred Heart, Largo Francesco Vito 1, 00168 Rome, Italy
[d] Institute of Analytical and Bioanalytical Chemistry, University of Ulm, Albert Einstein- Str. 11, 89081 Ulm, Germany

ARTICLE INFO

Keywords:
Palladium nanoparticles
Cell growth
Cell cycle progression
Apoptosis
DNA damage
Reactive oxygen species production

ABSTRACT

Palladium nanoparticles have been increasingly used in catalytic processes, wastewater treatment, electronics, and biomedicine. However, recent evidence proved that these nanoparticles are able to induce adverse effects both in *in vitro* and *in vivo* models. Nevertheless, molecular mechanisms underlying the toxic effects are still poorly understood. Therefore, this study aimed to investigate the potential toxicological mechanisms of palladium nanoparticles assessing their effects on normal diploid rat fibroblast and lung carcinoma human epithelial cell lines. Several endpoints such as cell growth, cell cycle progression, DNA damage, induction of apoptosis, reactive oxygen species production and expression of cell cycle regulatory proteins were evaluated. Results showed that palladium nanoparticles inhibited cell growth in a dose- and time-dependent manner in both cell lines, although with a more evident action on fibroblasts. Interestingly, inhibition of cell growth was not associated with the induction of apoptosis. Cell cycle progression was arrested in the G0/G1 phase and DNA damage was evident in both cell lines even if only a slight increase in the intracellular reactive oxygen species levels was detected. These findings provide valuable insight into understanding the molecular mechanisms responsible of palladium nanoparticles toxicity whose identification is essential to define an adequate risk assessment process.

1. Introduction

The rapid development of the nanotechnology industry has led to an increase in the production and application of engineered nanoparticles (NPs). The recent widespread use of different types of NPs is primarily due to their particular physico-chemical properties (*i.e.* small size, large surface area and high reactivity) making their use extremely attractive and beneficial in many industrial applications and consumer products (Iavicoli et al., 2014). However, these unique NP characteristics raised also public and scientific concerns regarding the greater biological reactivity and different toxicological profiles they may provide to particles in the nano-size range compared to their macro-scale counterparts, which may enhance their impact on the environment and human health (Borm et al., 2006; Nel et al., 2006; Pujalté et al., 2011). However, although there is an evident need to reach a deeper and more comprehensive understanding of NP toxicity in order to ensure a safe handling and use of these substances, the current knowledge on the health and safety aspects of NPs is still in a formative and developing phase (Djurišić et al., 2015; Iavicoli et al., 2011, 2013; Sajid et al., 2015). In particular, the comprehension of the relationship between the physical and chemical properties of NPs and the induction of toxic biological responses is still largely incomplete.

In this context, palladium (Pd)-NPs are characterized by invaluable catalytic, mechanical and optical properties which offered the opportunity for their employment in a number of industrial applications. These include catalytic reactions, *i.e.* dehalogenation, reduction, hydrogenation and C—C bond forming for the processing of environmental pollutants or automotive emission control, as well as their usage for the composition of electrical equipment, or as sensors for various analytical detections (Dumas and Couvreur, 2015; Hennebel et al., 2012). This inevitably resulted in increasing levels of Pd-NPs introduced into occupational and general living environments. Furthermore, the surface abrasion and deterioration of automotive catalytic converters can cause a significant release of the metal into the environment also in the nanoscale dimension due to the way in which Pd is impregnated into the cordierite substrate of the catalysts (Fontana et al., 2015). Therefore,

* Corresponding author.
E-mail address: ivo.iavicoli@unina.it (I. Iavicoli).

http://dx.doi.org/10.1016/j.tiv.2017.04.024
Received 1 December 2016; Received in revised form 24 April 2017; Accepted 25 April 2017
Available online 01 May 2017

the likely increase in exposure to nanometer-sized Pd particles has generated growing concerns regarding the possible adverse effects that Pd-NPs might have on human health.

In this scenario, some *in vitro* studies showed that the exposure to Pd-NPs could be responsible for the induction of different toxic effects on various cellular models. The assessment of cell viability and reactive oxygen species (ROS) production in human cell lines, CaCo-2 colon adenocarcinoma cells and HaCaT keratinocyte cells, treated with Pd-NPs, revealed only minor effects on cell viability and no significant ROS production (Hildebrand et al., 2010). On the other hand, Wilkinson et al. (2011) showed that the exposure of primary bronchial epithelial cells (PBEC) and lung carcinoma epithelial cells (A549) to Pd-NPs resulted in concentration-dependent cytotoxicity, induction of apoptosis and alterations in the secretion of biomarkers such as IL-8 and PGE2. The influence of Pd-NPs on the release and expression of cytokines was also investigated in peripheral blood mononuclear cells (PBMC) of non-atopic and Pd-sensitized women demonstrating their ability to exert important immunomodulatory effects (Boscolo et al., 2010; Reale et al., 2011). Furthermore, the addition of Pd-NPs to normal human PBMC induced a significant accumulation of cells in the G0/G1-phase and a significant reduction in GS- and G2/M-phases associated with a moderate, but not significant increase of intracellular ROS (Petrarca et al., 2014). Concerning *in vivo* results, our recent investigations, conducted on female Wistar rats, proved the ability of these NPs to significantly affect the immune system of treated animals enhancing the serum levels of several cytokines secreted by different Th lymphocyte subsets (Iavicoli et al., 2015) as well as to exert a nephrotoxic effect characterized by a significant renal tubular dysfunction (Fontana et al., 2015).

In this preliminary phase of knowledge, it appears evident the need to verify and confirm findings obtained by these previous nanotoxicological studies in order to reach more definite conclusions concerning the hazardous properties of Pd-NPs with the most advanced perspective to understand the molecular mechanisms underlying their toxicological profile. Therefore, this study aimed to evaluate potential toxicological effects and possible modes of action of Pd-NPs acutely and subacutely applied to different *in vitro* cell culture models, a normal diploid rat fibroblast cell line and a human lung carcinoma epithelial cell line. In particular, the impact of Pd-NPs on cell growth, cell cycle progression, induction of apoptosis, DNA damage, ROS production and expression of cell cycle regulatory proteins was investigated.

2. Materials and methods

2.1. Cell culture

The Rat-1 rat embryo fibroblasts were cultured in Modified Eagle's Medium (MEM) supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C, as previously reported (Iavicoli et al., 2012). The A549 lung carcinoma epithelial cell line (American type culture collection, Rockville, MD, USA) were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C.

2.2. Preparation and characterization of uncoated palladium nanoparticle hydrosol

Aqueous suspensions of Pd-NPs were prepared by chemical reduction of Pd(II). For this purpose, 500 µl of a commercially available Pd stock standard solution (1000 mg/l, $Pd(NO_3)_2$ in 0.5 mol/l HNO_3, Merck, Darmstadt, Germany) were added to 100 ml of a reducing solution and the mixture was shaken thoroughly. The reducing solution was freshly prepared by dissolving 11 mg of sodium borohydride (p.a., purity ≥ 96%, Merck, Darmstadt, Germany) in 10 ml of ultrapure water (obtained from a Milli-Q system, Millipore, Billerica, USA; resistivity 18.2 MΩ/cm), and subsequent dilution to 100 ml in ultrapure water. The immediate formation of Pd-NPs is indicated by a color change of the mixture from transparent to dark grey, however, in order to achieve complete reaction, the mixture was kept at room temperature in the dark for 12 h. The molar excess of reductant over Pd of a factor of 60 guaranties complete reduction of Pd(II) to Pd(0). Pd concentration in this hydrosol was 4.71 ± 0.05 mg Pd/l, as determined by continuum source - graphite furnace atomic absorption spectrometry (CS-GFAAS; contrAA 600, Analytik Jena, Jena, Germany; used spectral line at 244.791 nm) using aqueous calibration applying adequate dilutions of a Pd stock standard solution (1000 mg/l, $Pd(NO_3)_2$ in 0.5 mol/l HNO_3, traceable to Standard Reference Materials from the National Institute of Standards and Technology, Merck, Darmstadt, Germany) in 0.5 mol/l HNO_3. The absence of any relevant metal contamination in the Pd-NP hydrosol was confirmed by total reflection X-ray spectrometry (S2 Picofox, Bruker AXS GmbH, Karlsruhe, Germany; values found are: c (K) = 34 µg/l; Cu = 0.8 µg/l; Zn = 0.3 µg/l; Fe ≤ 0.1 µg/l). Transmission electron microscopy (TEM; Zeiss EM 10, Carl Zeiss Microscopy GmbH, Jena, Germany; operating voltage 80 kV) was applied for size characterization of Pd-NPs. Particle size distribution was determined from measurement of 500 individual particles depicted by TEM images using ImageJ software (National Institutes of Health, Bethesda, MD) to be 10 ± 6 nm (Fig. 1). During TEM sample preparation, *i.e.* the drying process of the Pd-NPs on the copper grids of the TEM sample carriers, chain-like aggregates of particles are formed as can be seen. This cannot be avoided, however, it does not reflect the situation in NP suspension were such aggregates of several hundreds

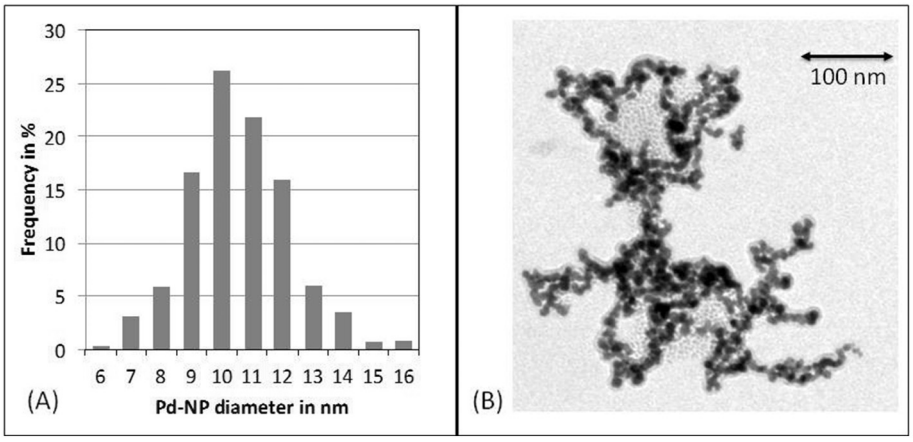

Fig. 1. A - Size distribution histogram of Pd-NPs. Mean size was 10 ± 6 nm; B - TEM images of Pd-NPs. The mean size was obtained from evaluation and measurement of maximum particle length of 500 individual NPs depicted by TEM images.