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Activation of human eosinophils with palladium nanoparticles (Pd NPs): importance of the actin cytoskeleton in Pd NPs-induced cellular adhesion

P. Chhay, M. Murphy-Marion, Y. Samson, D. Girard*

Laboratoire de recherche en inflammation et physiologie des granulocytes, Université du Québec, INRS-Institut Armand-Frappier, Laval, Quebec, Canada

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ABSTRACT

Palladium (Pd) is known to be released into the environment in the fine and ultrafine (at the nanoscale) airborne particle fractions mainly from automobile catalytic converters leading to an increase human exposure to this noble metal. It was reported that Pd can induce allergic reactions in individuals exposed to it via different ways. Some studies reported an increased number of eosinophils into airways following NP exposure *in vivo* in rodent models of allergies and inflammation. Knowing the importance of eosinophils in allergies, asthma and other lung diseases, it is surprising to observe that the direct effect of Pd at the nanoscale in eosinophils has been poorly documented. The aim of this study was to determine how Pd NPs will affect the biology of human eosinophils. Characterization of Pd NPs by dynamic light scattering indicates the presence of some aggregates when suspended in diverse solutions used here for the different experiments. Pd NPs did not significantly induce cell necrosis and apoptosis in eosinophils (0.5–150 μg/ml) as assessed by trypan blue exclusion assay, flow cytometry after staining with FITC-annexin V and propidium iodide and by morphological observations by optical microscopy. PD NPs, unlike the positive controls, did not induce reactive oxygen species (ROS) but were found to target the actin cytoskeleton, since actin was differently re-located intracellularly when compared to untreated cells as determined by fluorescence microscopy. Clearly, Pd NPs were found to increase adhesion of eosinophils onto human endothelial EA.hy926 cells. Using cytochalasin D, a cell-permeable and potent inhibitor of actin polymerization, this ability to increase adhesion was drastically reversed. Our results indicate that Pd NPs can target the cytoskeleton and increase the adhesion of human eosinophils by an actin-dependent mechanism. These findings show that human eosinophils can be activated by Pd NPs emphasizing the importance of fully investigating how these NPs could alter the biology of human cells involved in allergies, asthma and other lung diseases as well as in various other inflammatory disorders.

1. Introduction

Nanoparticles (NPs) possess an increased surface area and small size over their corresponding bulk materials given them unique properties. Since few years, the use of NPs in several sectors is increasing very rapidly. For examples, the potential applications of NPs in optical, biomedical, and electronic fields are gaining increasing interest (Arora et al., 2012; Chaloupka et al., 2010). Palladium (Pd) is a noble metal that belongs to the platinum group elements (PGEs) that also include platinum, iridium, osmium, rhodium and ruthenium. Although Pd is mainly used in the automobile industry as an active catalyst material in automobile catalytic converters, it also has applications in jewelry, electronics, dentistry, only to name a few (Ravindra et al., 2004). Of note, Pd is a common component of dental alloys and the first case of exacerbation of bronchial asthma caused by Pd allergy was reported several years ago (Yoshida et al., 1999). Pd could be released into the environment in the fine and ultrafine (smaller than 100 nm) airborne particle fractions (Rauch et al., 2002). Because of the presence of Pd at such nanoscale and since inhalation represents one of the major routes of human exposure to Pd NPs, some recent studies aiming at better understanding their impact on health have reported some adverse effects of Pd NPs, including inflammation. For example, Pd NPs were found to reduce the cell viability and to induce apoptosis in primary human bronchial epithelial cells as well as to increase the production of the pro-inflammatory IL-8 chemokine (Wilkinson et al., 2011). While Pd salts were found to inhibit cytokine release from peripheral blood mononuclear cells (PBMCs) of non-atopic women, Pd NPs were found to increase the release of IFN-γ (Boscolo et al., 2010). In one other study, the production of reactive oxygen species (ROS) from human monocytic leukemia THP-1 cells was increased by Pd NPs (Neubauer et al., 2015). In vivo, intravenous administration of Pd NPs was reported to cause significant renal tubular dysfunction in rats (Fontana et al., 2015). In

* Corresponding author at: INRS – Institut Armand – Frappier, 531 boul. des Prairies, Laval, QC, H7V 1B7, Canada.
E-mail address: denis.girard@iaf.inrs.ca (D. Girard).

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one other study, the highest tested concentration of Pd NPs (12 μg/kg) increased the production of several cytokines (IL-1α, IL-4, IL-6, IL-10, IL-12, GM-CSF and INF-γ) measured in the rat serum (Iavicoli et al., 2015). Despite these observations indicating that Pd and/or Pd-NPs possess pro-inflammatory properties and that Pd is known to induce allergies (Iguchi et al., 2016; Kobayashi et al., 2013), no study reported how Pd could alter the biology of eosinophils known to exert an important role in lung diseases and diverse allergies. Such lack of information in the literature could, however, be explained by the fact that nanotoxicology is a relatively recent discipline that has gained increasing attention only in the recent few years (Oberdorster et al., 2005). Of note, an increase number of eosinophils was observed in response to NP exposure in airway rodent models of allergies including cerium dioxide (CeO_2), nickel oxide (NiO), zinc oxide (ZnO), copper oxide (Sevier et al., 2001), titanium dioxide (TiO_2) and silica NPs (Brandenberger et al., 2013; Cho et al., 2010; Larsen et al., 2010; Roy et al., 2013). Despite all of the above observations, in addition to an older study reporting occupational asthma caused by palladium 18 years ago (Daenen et al., 1999), although not at the nanoscale, it is surprising that the direct effect of Pd NP on the biology of human eosinophils, cells greatly involved in lung diseases including asthma, has been neglected. Although inhalation is probably the primary route of human exposure to NPs and that lungs would capture them, NPs possess the capacity to cross membranes and then being distributed throughout the body, including blood circulation.

Recently, using the human eosinophilic cell line AML14.3D10, we reported that ZnO and nanosilver (20 nm) NPs induced apoptosis and cytoskeleton breakdown as evidenced by the cleavage of lamin B1. These NPs were found to increase the production of different analytes including the potent pro-inflammatory CXCL8 (IL-8) chemokine. In contrast, TiO_2, CeO_2, and nanosilver of 70 nm NPs did not alter the apoptotic rate as well as the production of pro-inflammatory cytokines, indicating that distinct NPs do not activate similarly human eosinophils. More recently, using freshly isolated human eosinophils, we demonstrated that, unlike AML-14.3 D10 cells, ZnO NPs delayed eosinophil apoptosis by inhibiting caspases and by preventing caspase-4 and Bcl-xL degradation. In addition, treatment with ZnO NPs did not induce production of reactive oxygen species but increase *de novo* protein synthesis as well as the production of the pro-inflammatory IL-1β and IL-8 cytokines. Taking together, the results of this study establish for the first time that human eosinophils represent potential new direct targets to NPs. More recently, although we performed a study focusing on the ability of TiO_2 NPs to increase the adhesion of human eosinophils onto endothelial cells, several other NPs were found to increase such eosinophil adhesion, including Pd NPs, yet by an unknown mechanism (Murphy-Marion and Girard, 2017). In the present study, in order to increase our knowledge on the mode of action of Pd NPs, we investigated how they affect the biology of both human eosinophilic AML-14.3D10 cells and freshly isolated mature human eosinophils.

2. Materials and Methods

2.1. Chemicals

RPMI-1640, HEPES, penicillin/streptomycin (P/S), *Viscum album* agglutinin-I (VAA-I), phorbol 12-myristate 13-acetate (PMA), sodium pyruvate, Hoechst 33342 and cytochalasin D (CD) were purchased from Sigma-Aldrich, (Saint-Louis, Missouri). Recombinant human GM-CSF and eotaxin, IL-5 was purchased from Peprotech Inc. (Rocky Hill, NJ, USA) and FITC-phalloidin from Invitrogen/Life Technologies Inc. (Burlington, ON).

2.2. Pd NPs

An aqueous solution of Pd NPs of 1.5 mg/ml, stabilized by sodium polyacrylate was purchased from Sciventions Inc. (Toronto, ONT, Canada). According to the manufacturer, the particle size is 1–10 nm (90%) as determined by transmission electron microscopy.

2.3. Characterization of Pd NPs by dynamic light scattering

The size distribution and zeta potential of Pd NPs were determined using a Zetasizer Nano-ZS (model ZEN3600) from Malvern Instruments Inc. (Westborough, MA). Measurements were performed with NPs in suspension in HBSS, RPMI-1640 and RPMI–1640 + 10% human serum according to the tested functions, at a concentration of 100 μg/ml, as detailed under. In addition, measurements were performed with Pd NPs suspended in and RPMI–1640 + 10% human serum + sodium pyruvate, the culture medium for AML14.3D10 cells.

2.4. Human EA.hy926 and AML14.3D10 cell cultures

The human umbilical vein cell line, EA.hy926 (ATCC® CRL-2922™) was purchased from American Type Culture Collection (Manassas, VA), and was grown in RPMI-1640 supplemented with 10% fetal calf serum and antibiotics. Cell viability was systematically evaluated before and after each treatment, and mortality never exceeded 5%. Human eosinophilic AML14.3D10 cells (referred to as 3D10 cells hereafter) were generated and kindly provided by Drs. C. C. Paul and M. Baumann (Wright State University, Dayton, OH) (Baumann and Paul, 1998) and were used as we previously published (Lavastre et al., 2005; Vallieres et al., 2016). In brief, 3D10 cells were cultured at 37 °C in a 5% CO_2 atmosphere in the same buffer as above with addition of 1 M sodium pyruvate. Cells were cultivated at a density between 2×10^5 and 1×10^6/ml, and the medium was changed three times per week. Before performing the different experiments, the cell viability was systematically verified by trypan blue exclusion and was found to routinely be > 98%.

2.5. Eosinophil isolation

Blood donations were obtained from informed and consenting individuals according to our institutionally approved procedures. Granulocytes were isolated from venous blood of healthy volunteers by dextran sedimentation followed by centrifugation over Ficoll-Hypaque, to obtain neutrophils and eosinophils as previously described (Lavastre et al., 2005). When > 5% of eosinophils were detected in granulocyte enriched preparations, as determined by cytology from cytocentrifuged preparations colored by the Hema 3 Stain Set (Biochemical Sciences Inc., Swedesboro, NJ), eosinophils were separated from neutrophils by negative immunomagnetic selection using anti-human CD16-coated magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's recommendations. Purity after eosinophil isolation was routinely > 96% and cell viability was always greater than 98%, as determined by trypan blue exclusion before and after treatment with NPs.

2.6. Cell viability

Human 3D10 cells and freshly isolated human eosinophils (1×10^6 cells/ml of RPMI-10% autologous serum) were incubated at 37 °C in 5% CO_2 in 12-well plates during 24 h in the presence of buffer or increasing concentrations (0–150 μg/ml) of Pd NPs. Cell viability was assessed by trypan blue exclusion assay.

2.7. Detection of intracellular ROS

Human 3D10 or eosinophils (1×10^6 cells/ml) were suspended in HBSS containing 10 μM CM-H_2DCFDA for 15 min at 37 °C as previously published (Simard et al., 2011). Cells were then washed twice before being incubated in the presence of buffer or 50 or 150 μg/ml Pd-NPs for 0–90 min. PMA (0.1 μg/ml) was used as a positive control.