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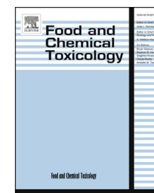

Size-related cytotoxicological aspects of polyvinylpyrrolidone-capped platinum nanoparticles

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The nanotechnological concept is based on size-dependent properties of particles in the 1–100 nm range. Nevertheless, the connection between their size and effect is still not clear. Thus, we focused on reductive colloidal synthesis, characterization and biological testing of Pt nanoparticles (PtNPs) capped with biocompatible polymer polyvinylpyrrolidone (PVP). Synthesized PtNPs were of 3 different primary sizes (approx. ~10; ~14 and > 20 nm) and demonstrated exceptional haemocompatibility. *In vitro* treatment of three different types of malignant cells (prostate – LNCaP, breast – MDA-MB-231 and neuroblastoma – GI-ME-N) revealed that even marginal differences in PtNPs diameter resulted in changes in their cytotoxicity. The highest cytotoxicity was observed using the smallest PtNPs-10, where $24IC_{50}$ was lower (3.1–6.2 μg/mL) than for cisplatin (8.1–19.8 μg/mL). In contrast to MDA-MB-231 and LNCaP cells, in GI-ME-N cells PtNPs caused noticeable changes in their cellular structure without influencing their viability. Post-exposure analyses revealed that PtNPs-29 and PtNPs-40 were capable of forming considerably higher amount of reactive oxygen species with consequent stimulation of expression of metallothionein (MT1/2 and MT3), at both mRNA and protein level. Overall, our pilot study demonstrates that in the nanoscaled world even the smallest differences can have crucial biological effect.

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1. Introduction

Platinum-based drugs are employed for the treatment of numerous solid malignancies including breast, ovarian, head and neck, colorectal, bladder and neuroblastomas. They have been in clinical use since 1971 when the first studies demonstrated their efficacy (Kelland, 2007). However, due to their non-selectivity for cancer cells, they are highly toxic for non-malignant tissue as well, leading to many severe side effects, such as acute sensory neurotoxicity accompanied by neuromuscular signs or risk of infertility and ototoxicity (Grolleau et al., 2001). Due to these severe side effects, development of new platinum-based anticancer agents with high efficiency and low or negligible side effects is warranted for cancer chemotherapy.

For these purposes, platinum nano-formulations can be employed to increase selectivity for tumour tissue while lowering side effects. One promising way is to encapsulate platinum cytostatics into a carrier, which protects cargo against undesired interactions with non-target tissues (Hauert and Bhatia, 2014). In that way, lipoplatin (liposomal cisplatin) evades immune surveillance, circulates for long periods in bodily fluids and extravasates through the compromised endothelium of the tumour vasculature. Clinical phases demonstrate significant reduction in oto- and nephrotoxicity (Stathopoulos et al., 2010). Another way is to develop new drugs based on inorganic platinum nanoparticles (PtNPs), formed from platinum core that can be functionalized by multiple bioactive groups (Pajic et al., 2016). The novel properties of nanoparticles, *i.e.*,

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particles having at least one dimension between 1 and 100 nm, are attributed to their small size, increased specific surface area and concomitant surface display of their constituent atoms. While many nanomaterials are commonly used, their interactions with biomolecules as well as their health effects are largely unknown (Englert, 2007). Therefore, it is not surprising that PtNPs are subject of substantial research with potential applications in a wide variety of areas. From these, PtNPs have been shown to possess the capacity to enter cells (Gehrke et al., 2011). In addition, it has been proved that they can induce DNA damage, increase the cellular glutathione amount (Pelka et al., 2009) or activate p53 due to genotoxic stress (Asharani et al., 2010). Furthermore, Porcel et al. demonstrated that PtNPs can be also exploited to increase sensitivity of cancer cells to fast ion radiation utilized for hadron anticancer therapy (Porcel et al., 2010). However it must be noted that the effects of PtNPs are significantly influenced by their surface modification. It has to be further mentioned that PtNPs are also present in environment. Notably, >99% of the emitted Pt is present in its metallic form Pt^0 with up to 36% of these particles in a size of less than 3 μm and 6% even smaller than 0.3 μm (Schmid et al., 2007). Nevertheless, the effects of Pt in nanostructured dimensions are still mostly unknown.

Hence, in the present study, we focused on synthesis of PtNPs using various qualities of polyvinylpyrrolidones (PVP) as capping agents, which resulted in Pt^0 core-based nanoparticles of different sizes. Further, we aimed to study the properties of PtNPs using a homogenous set of experimental data describing the link between the size of PtNPs and their toxicity. Cytotoxicity was investigated in three cell types representing three types of malignant diseases with high incidence (prostate, breast and neuroblastoma). Particularly, we investigated the influence of PtNPs on proliferation, haemocompatibility, migration, genotoxicity, generation of intracellular oxidative stress and expression of metallothioneins (MTs) at mRNA and protein levels.

2. Material and methods

2.1. Chemicals

Listed chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) in ACS purity, unless noted otherwise.

2.2. Reductive colloidal synthesis of PtNPs capped with PVP

0.07 g of $PtCl_4$ (Mr = 336.89) with addition of 33 μL of 37% HCl (*v*/*v*) was dissolved in 10 mL of water. 0.14 g of PVP of different molecular weights [MW (10 k, 29 k and 40 k)] was dissolved in 40 mL of water. Then, 5 mL of $H_2[PtCl_6]$ solution was added while stirring for 1 h. Finally, 50 mg of $Na[BH_4]$ was added and the solution was stirred for 2 h and filled to 50 mL with water. Prepared PtNPs were characterized and stored in dark at 4 °C. Prior the experiments, PtNPs solutions were normalized according to the content of platinum using atomic absorption spectrometer 280Z with Zeeman background correction (Agilent Technologies, Santa Clara, CA, USA).

2.3. Transmission electron microscopy (TEM)

For documentation of structure, MIRA2 LMU (Tescan, Brno, Czech Republic) fitted with In-Beam SE detector was used. An accelerating voltage of 5 kV gave satisfactory results regarding maximum throughput. TEM analyses were performed using ~4 μL of the sample deposited onto 400-mesh copper grids coated with a continuous carbon layer. Dried grids were imaged by Tecnai F20 TEM (FEI, Eindhoven, Netherlands) at 120 kV.

2.4. Quasielastic dynamic light scattering (DLS) and zeta potential analyses

Particle size and ζ-potential were evaluated using a particle size analyzer (Zetasizer Nano ZS90, Malvern instruments, Malvern, UK). For measurements, PtNPs were prepared in Ringer's solution (6.5 g NaCl, 0.42 g KCl, 0.25 g $CaCl_2$ and 0.2 g of sodium bicarbonate dissolved in 1 L of water), which is an isotonic solution resembling bodily fluids. Prior measurements, samples were incubated at 25 °C for 15 min.

2.5. Attenuated total reflectance Fourier transform-infrared spectroscopy (ATR-FT-IR)

FT-IR spectra were collected using a Nicolet iS10 FT-IR spectrometer with diamond ATR attachment (Thermo Electron Inc., San Jose, USA). The sample solution was supplied dropwise (5 μL) on the diamond crystal of the ATR cell and the thin film was measured after spontaneous evaporation of the solvent. Spectra were recorded at 25 °C from 4000 to 650 cm^{-1} at a resolution of 2 cm^{-1}. Each spectrum was acquired by merging 64 interferograms.

2.6. Evaluation of colloidal stability of PtNPs

PtNPs dispersed in the Ringer's solution were placed in the stationary rack and kept at 25 °C. To demonstrate their colloidal stability, photographic documentation of PtNPs sedimentation was performed for 7 days.

2.7. Cell lines and culture conditions

Five different human cell lines were used in this study: *i*) LNCaP – human cell line established from a lymph node metastatic lesion of prostatic adenocarcinoma, *ii*) MDA-MB-231–human cell line established from a pleural effusion of a 51-year-old woman with metastatic breast cancer, *iii*) GI-ME-N – human cell line established from a bone marrow metastasis of a 42-month-old boy with stage IV neuroblastoma after 6 months of chemotherapy, *iv*) PNT1A - human cell line established by immortalization of normal adult prostatic epithelial cells by transfection with a plasmid containing SV40 genome with a defective replication origin and *v*) HaCaT - spontaneously transformed aneuploidy immortal, non-tumorigenic keratinocyte cell line derived from adult human skin. All cell lines used in this study were purchased from Health Protection Agency Culture Collections (Salisbury, UK).

LNCaP, MDA-MB-231, PNT1A and HaCaT were cultured in RPMI-1640 with 10% foetal bovine serum (FBS), GI-ME-N were cultured in RPMI-1640, GlutaMAX™ with 10% FBS. Media were supplemented with penicillin (100 U/mL) and streptomycin (0.1 mg/mL) and the cells were maintained at 37 °C in a humidified incubator Galaxy® 170 R (Eppendorf, Hamburg, Germany).

2.8. Viability and proliferation tests

The viability was assessed using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Briefly, the suspension of 5000 cells in 50 μL medium was added to each well of microtiter plates, followed by incubation for 24 h at 37 °C with 5% CO_2 to ensure cell growth. To determine the effects on cell viability, PtNPs or cisplatin (CDDP) within concentration range of 0.01–50 μg/mL were applied. Treatment was carried out for 24 h. Then, 10 μL of MTT [5 mg/mL in phosphate buffered saline (PBS)] was added to the cells and the mixture was incubated for 4 h at 37 °C. After that, MTT-containing medium was replaced by 100 μL of 99.9% dimethyl sulfoxide (DMSO) and after 5 min incubation the absorbance of the

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