Influence of platinum nanoparticles on human primary keratinocytes

Goralczyk Anna[1], Szmyd Radoslaw[1], Cierniak Agnieszka[1], Skalniak Lukasz[1], Lipert Barbara[1], Filon Francesca Larese[2], Crosera Matteo[2], Borowczyk Julia[3], Laczna Eliza[3], Drukala Justyna[3], Klein Andrzej[1], Jura Jolanta[1].

1 *Department of General Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow*
2 *University of Trieste, UCO Medicina del Lavoro, Trieste*
3 *Department of Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow*

Introduction

Nanoparticles (NPs) have diverse applications in industry, medical devices, therapeutic agents and cosmetics. Nanoparticles are defined as structures that have at least one dimension of nanometer scale. The unique physicochemical properties of engineered nanoparticles are attributable to their small size, large surface area, chemical composition, surface reactivity, charge, shape and media interactions. Although impressive from the perspective of material science, the novel properties of NPs could lead to adverse biological effects with the potential to create toxicity.

Because of their strong catalytic activity, platinum nanoparticles (PtNPs) are commonly used in converters of automobiles causing emission of PtNPs to the environment. In recent years PtNPs have attracted even more attention due to the fact that they have been shown to quench reactive oxygen species. They are added to diet supplements or cosmetics due to their anti-oxidative and anti-aging properties. While the commercialization of platinum nanoparticles expands rapidly, their impact on health and environment is still not well understood.

In this study, the cellular influences induced by 4-5 nm polyvinylpyrrolidone-coated PtNPs on human primary keratinocytes was investigated. Cytotoxicity, genotoxicity, morphology, metabolic activity and changes in the expression of proteins were studied in PtNPs- treated cells.

Aim of study

This study was undertaken to examine the potential toxicity effects of 4-5 nm PVP-coated platinum nanoparticles on Normal Human Epidermal Keratinocytes (NHEK).

Methods

NHEK cells from three individuals were obtained from Lonza (Switzerland).

The following study was carried out:

1. Verification of cell viability by MTT assay
2. Study of cell metabolic activity by measurement of ATP content.
3. Determination of proliferation rate by BrdU assay.
4. Verification of cells migration by time-lapse microscopy.
5. Determination of mitochondria damage by measurement of caspase 9 activity.
5. Determination of apoptosis induction by measurement of caspase 3/7 activity
7. Verification of cell cycle arrest.
6. Measurement of DNA damage using comet assay.
8. Protein activation analysis by Western Blot technique.

Results

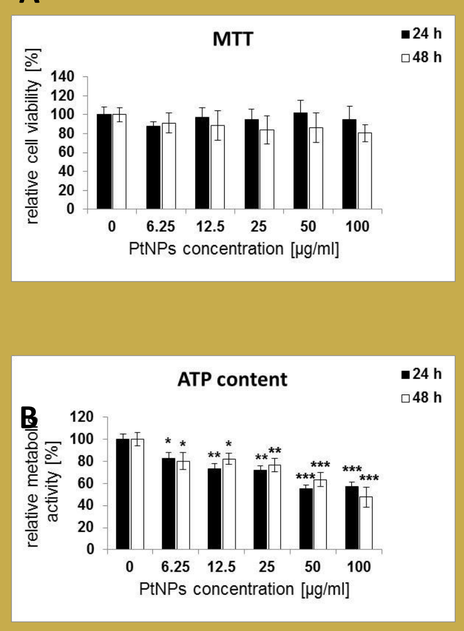


Fig. 1. Cell viability of NHEK cells exposed to PtNPs. NHEK cells (plated at the density of $1{,}25\times10^4$ cells/cm^2) were treated with different concentration of PtNPs (6,25-100 µg/ml) for 24 h and 48 h. Untreated cells served as a control. Cells were incubated with tetrazolium MTT for 3h. The decrease in absorbance was measured at 570 nm, with 650 nm as reference wavelength **(A)**. Cell viability was also analysed by the measurement of intracellular ATP content **(B)**. For statistics Student's t-test was performed (*p<0.05; **p<0.01; ***p<0.001). This graphs represent the mean ±SD of three independent experiments.

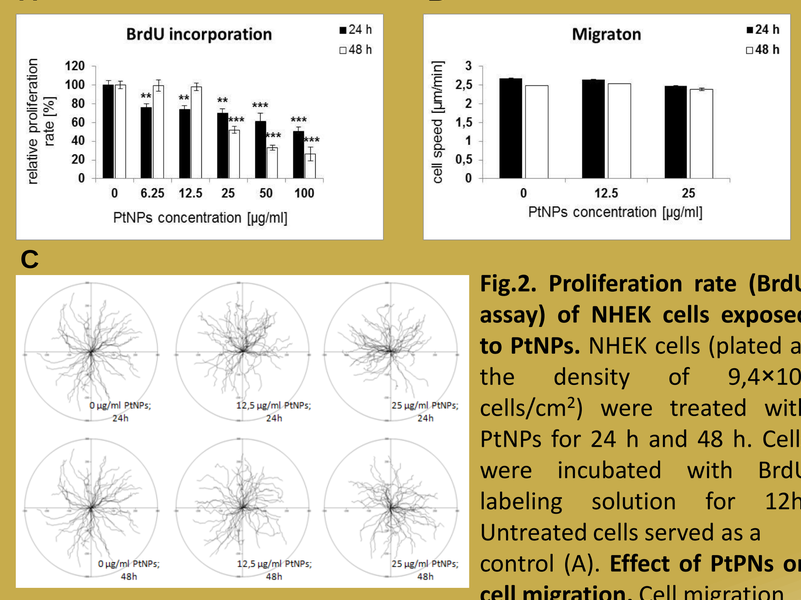


Fig.2. Proliferation rate (BrdU assay) of NHEK cells exposed to PtNPs. NHEK cells (plated at the density of $9{,}4\times10^3$ cells/cm^2) were treated with PtNPs for 24 h and 48 h. Cells were incubated with BrdU labeling solution for 12h. Untreated cells served as a control (A). **Effect of PtPNs on cell migration.** Cell migration was examined with a Leica DMI6000B microscope equipped with LAS AF software. NHEKs were plated in 6 wells culture plates at the density of $6.4\text{x}10^3$cells/cm^2 and treated with PtNPs. Cells movements were recorded 24h and 48h after PtNPs addition for 90 minutes with time-lapse 1.5 min. (B). The tracks of individual cells were analyzed to determinate the total length of the cell trajectory (TLCT) (C). Student's t-test was carried out for statistics (*p<0.05; **p<0.01; ***p<0.001).

Results

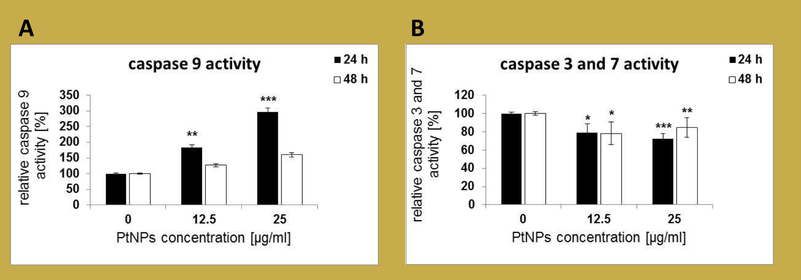


Fig.3. Mitochondria damage and apoptotic cell death measured by the determination of caspase 9, 3 and 7 activity. The luminescent assay for caspase 9 (A), 3 and 7 (B) activity was performed using 3 µg of total protein isolated from NHEK cells after incubation with different concentration of PtNPs for 48h. For statistics Student's t-test was performed (*p<0.05; **p<0.01; ***p<0.001). This graph represents the mean ±SD of three independent experiments.

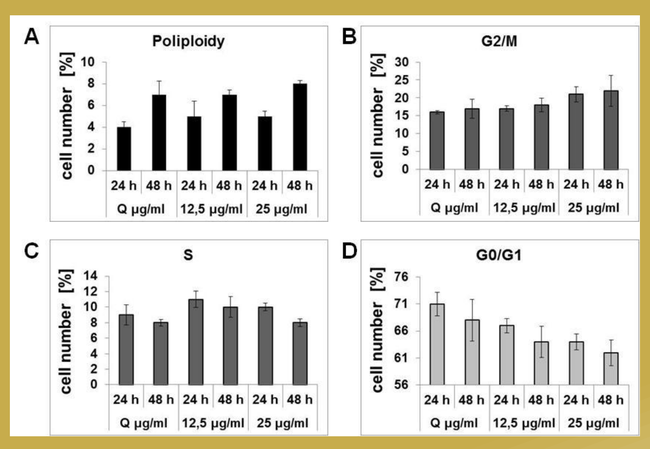


Fig.4. Cell cycle analysis of PtNPs- treated NHEK cells. The impact of different doses of PtNPs on cell cycle was examined in NHEK cells by flow cytometry . NHEKs cells were plated in 6 wells culture plates at the density of $6.4\text{x}10^3$cells/cm^2 and treated with 12.5 and 25 µg/ml PtNPs. DNA content was evaluated by flow cytometry (LSR II, Becton Dickinson).

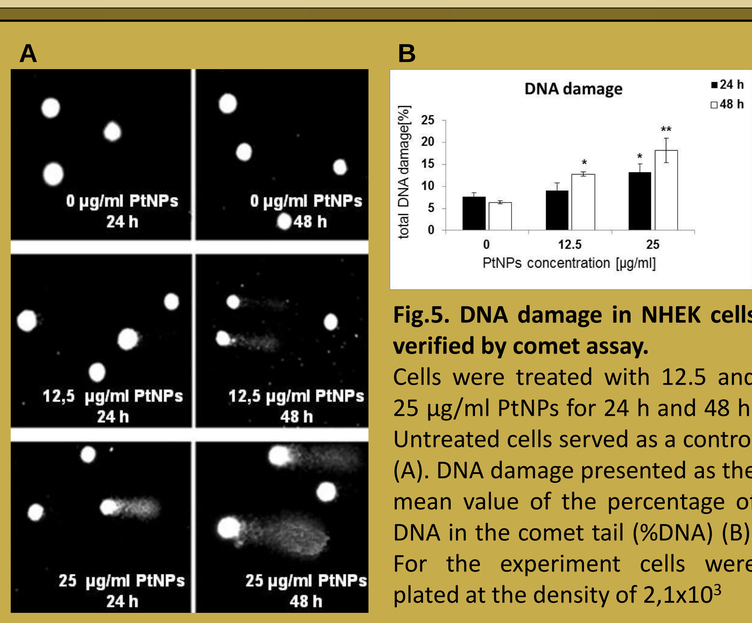


Fig.5. DNA damage in NHEK cells verified by comet assay. Cells were treated with 12.5 and 25 µg/ml PtNPs for 24 h and 48 h. Untreated cells served as a control (A). DNA damage presented as the mean value of the percentage of DNA in the comet tail (%DNA) (B). For the experiment cells were plated at the density of $2{,}1\text{x}10^3$ cells/cm^2. Statistically significant differences were calculated by RIR Tukey test (*p<0.05; **p<0.01; ***p<0.001). This graph represents the mean ±SD of three independent experiments.

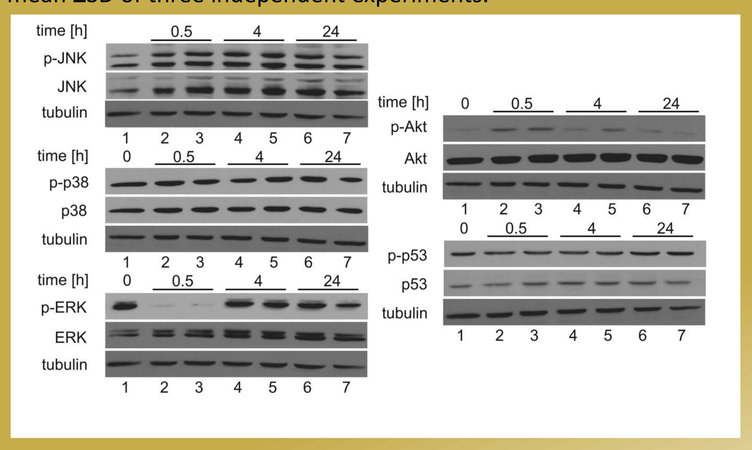


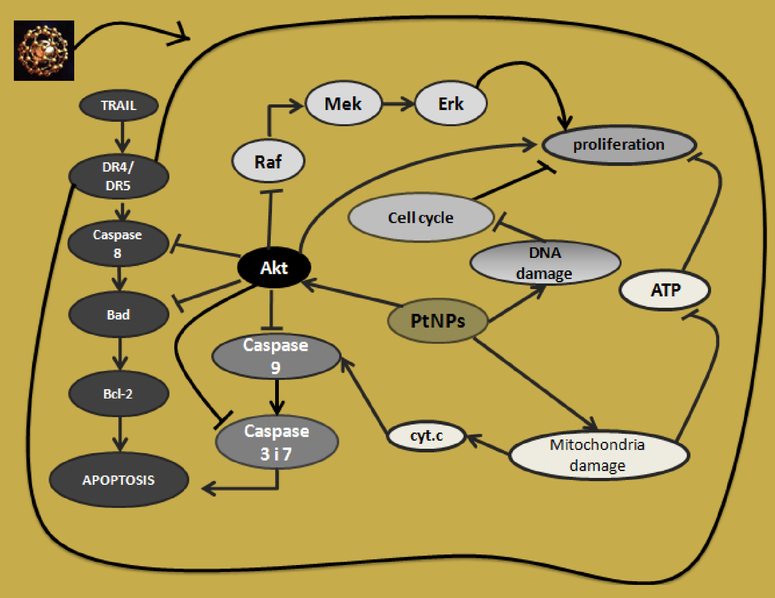
Fig.6. The activation of MAP kinases, Akt and p53 verified by Western Blot analysis. NHEKs were cultured in 60 mm tissue culture dishes at density of 9.5×10^3 cells/cm^2. The cells were exposed to 12.5 µg/ml (lines 2, 4 and 6) or 25 µg/ml (lines 3, 5 and 7) PtNPs for the indicated periods of time. Untreated cells were used as a control (line 1). Tubulin was used as an internal control to monitor for equal loading. These blots are representative of three independent experiments.

Conclusions

NHEK exposed to PtNPs displayed:

1. No changes in cell viability,
2. Decrease in ATP content,
3. Inhibition of cell proliferation,
4. No changes in cell migratory activity,
5. Activation of caspase 9 and inactivation of caspase 3/7
6. Cell cycle arrest in G2/M phase
7. Increase in DNA damage
8. Activation of Akt kinase and inactivation of ERK1/2

Molecular mechanizm of PtNPs action in the cell



The studies show, that despite the positive effects, nanoparticles may present possible danger, concerning activation of genotoxic processes, although no cytotoxic effects were observed. This data might contribute to better understanding of effects triggered by PtNPs on cells and may provide valuable information to develop safety regulations for the use of nanoparticles in various applications.

Literature

1.Gehrke H, Pelka J, Hartinger C, Blank H, Bleimund F, Schneider R, Gerthsen D, Bräse S, Crone M, Türk M, Marko D. ***Platinum nanoparticles and their cellular uptake and DNA platination at non-cytotoxic concentration.***
2. Zhong C, Maye M, Luo J, Kariuki N. ***Nanoparticles in catalysis.***
3. Asharani P, Xinyi N, Prakash Hande M, Valiyaveettil S. ***DNA damage and p53-mediated growth arrest in human cells treated with platinum nanoparticle.***
4. Pelka J, Gehrke H, Esselen M, Tuirk M, Crone M, Brause S, Muller T, Blank H. ***Cellular uptake of platinum nanoparticles in human colon carcinoma cells and their impact on cellular redox systems and DNA integrity.***

The study was supported by Ministry of Science and Higher Education: 776/N-COST/2010/0 and by European Union: Cost action BM 0903