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Human brain endothelial barrier cells are distinctly less vulnerable to silver nanoparticles toxicity than human blood vessel cells A cell-specific mechanism of the brain barrier?

Paulina Sokołowska, PhD[a,*], Kamila Białkowska, MSc[a], Małgorzata Siatkowska, PhD[a], Marcin Rosowski, PhD[a], Magdalena Kucińska, PhD[b], Piotr Komorowski, PhD[a,b], Krzysztof Makowski, PhD[c], Bogdan Walkowiak, PhD[a,b,c,d]

[a]*Molecular and Nanostructural Biophysics Laboratory, Bionanopark Ldt., Lodz, Poland*
[b]*Nanomaterial Structural Research Laboratory, Bionanopark Ldt., Lodz, Poland*
[c]*Industrial Biotechnology Laboratory, Bionanopark Ldt., Lodz, Poland*
[d]*Department of Biophysics, Institute of Materials Science, Lodz University of Technology, Poland*

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Abstract

The blood–brain barrier (BBB) constitutes a distinctive and tightly regulated interface between the brain and the peripheral circulation. The objective of studies was to compare responses of human endothelial cells representing the model of blood vessels – EA.hy926 and HUVEC cells and the model of the brain endothelial barrier – HBEC5i cells to silver nanoparticles (SNPs). A contact of SNPs with endothelial cells resulted in a formation of SNP agglomerates. Consequently, the SNPs uptake by endothelial cells affected cell viability and membrane integrity however observed responses were different. Brain endothelial barrier HBEC5i cells were much less vulnerable to SNPs toxicity comparing to EA.hy926 and HUVEC cells. It can be ascribed to the presence of specialized cellular components of the brain barrier, protecting HBEC5i cells against toxic SNPs. Fundamental understanding of SNPs inducing the BBB dysfunction may initiate engineering novel SNPs which are safe for the BBB and thereby safe for the brain.

Key words: Brain endothelial barrier; BBB; Blood vessels; Endothelial cells; Silver nanoparticles

Silver nanoparticles (SNPs) are most frequently applied materials in consumer products and medical applications.[1] The recent studies have demonstrated a potent antitumor activity of SNPs.[2–4] However, the use of SNPs in anticancer therapies is still limited due to the lack of profound knowledge regarding the pharmacological and toxicological profiles of these NPs. The studies on distribution of SNPs demonstrated that particles were transported mainly to the liver and the spleen but they were also found in brain, heart, lungs, kidneys and testes. Most organs were able to eliminate SNPs over time with the exception of a brain and testes.[5,6] SNPs were detected in the brain even up to 28 days after last administration.[5] SNPs may disrupt the blood–brain barrier (BBB) integrity and reach the brain inducing neuronal cell death.[7] Continuous accumulation of SNPs even at very low concentrations may result in neuronal degeneration and necrosis as SNPs were proved to be highly neurotoxic.[7–16] Fundamental understanding of phenomena how SNPs induce the BBB dysfunction and the loss of brain protection could enable us to develop nanomedical treatment and help to ensure that nanoparticles, which are not intended to reach the brain, do not cause adverse effects.

The objective of the studies was to compare the responses of human endothelial cells representing two different research models to commercially available silver nanoparticles. The permanent cell line EA.hy926 and the primary human umbilical vein endothelial cells (HUVEC) were used for studies on

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*Corresponding author: Molecular and Nanostructural Biophysics Laboratory, Bionanopark Ldt., 93-465 Lodz, Poland.

E-mail address: p.sokolowska@bionanopark.pl (P. Sokołowska).

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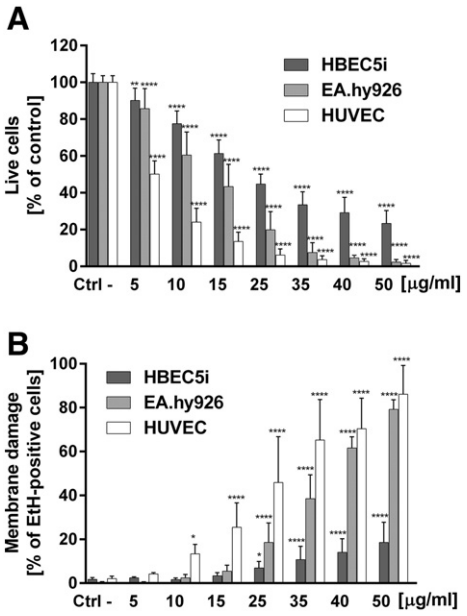

Figure 1. Analysis of **(A)** cell viability and **(B)** membrane damage of HBEC5i, EA.hy926 and HUVEC cells evoked by the 24-h exposition to SNPs. Data are presented as mean ± SD. Statistical significance *vs*. control is indicated when appropriate (*$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$). For analysis of statistical significance between cell types see supplementary materials, Tables 1 and 2.

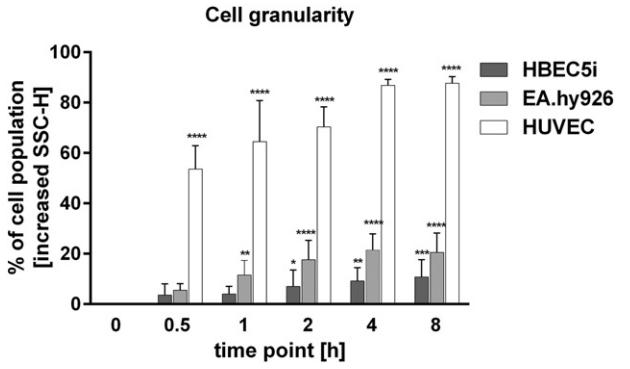

Figure 2. Analysis of cell granularity of HBEC5i, EA.hy926 and HUVEC cells modulated by SNPs. Data are presented as mean ± SD. Time point "0" represents cells SNPs-untreated. Statistical significance *vs*. control is indicated when appropriate (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$). For analysis of statistical significance between cell types see supplementary materials, Table 3.

functions of blood vessels, whereas HBEC5i cell line represents a model of the human brain endothelial barrier. Brain microvascular cells constitute distinctive and tightly regulated interface between the brain and the peripheral circulation and play a crucial role in the maintenance of the strict environment required for normal brain function.[17] Therefore, it can be hypothesized that responses of human endothelial blood vessel cells and brain microvascular cells to the SNPs' exposure may vary due to the particular function of the brain endothelial barrier.

Methods

SNPs were from Sigma–Aldrich (Warsaw, Poland). Cell viability was assessed as a function of intracellular esterase activity and membrane integrity. Analysis was performed with the use of the automated, fluorescent microscope InCell Analyzer 2000 (GE Healthcare, Little Chalfont, UK). Interactions of nanoparticles with cells were investigated with the use of flow cytometer FACS Aria III (BD Biosciences, Erembodegen, Belgium) and scanning electron microscope, Quanta 250 (FEI, Eindhoren, Netherlands). For further details see supplementary materials.

Results

HBEC5i, EA.hy926 and HUVEC endothelial cells were exposed simultaneously to different concentrations of SNPs for 24 h. SNPs potently and in a dose-dependent manner diminished cell viability of all endothelial cells, but brain microvascular HBEC5i cells were significantly less vulnerable to the cytotoxic effect of SNPs comparing to blood vessel cells EA.hy926 and particularly primary HUVEC cells (Figure 1, *A*). Together with cell viability, the effect of SNPs on cell membrane integrity was evaluated, showing a concentration-dependent membrane damage of studied cell types, but HBEC5i cells were less susceptible to SNPs toxicity comparing to EA.hy926 and HUVEC cells (Figure 1, *B*). Primary endothelial HUVEC cells were the most vulnerable to the cytotoxic effect of SNPs with a significant decrease in live cells and a high percentage of cell membrane damage with low SNP concentrations (Figure 1, *A* and *B*).

Accumulation of SNPs inside cells and surface interactions between cells and SNPs can be evaluated using forward (FSC) and side (SSC) scattered light analysis by flow cytometry. In the current study endothelial cells were exposed to SNPs and the interactions of nanoparticles with cells were evaluated at indicated time points (Figure 2). Results demonstrated significant changes in the cell structure/granularity represented by increase in SSC parameter, whereas FSC parameter (ascribed to the cell size) remained unchanged. The data clearly showed a time-dependent accumulation of SNPs by endothelial cells of all cell types within the first 30 min of incubation followed by a plateau after 4 h of incubation with SNPs (Figure 2). Accumulation efficiencies depended on a cell line. At each time point, populations of HBEC5i cells showing increased granularity were dramatically lower than populations of HUVEC cells and, to a lesser degree, EA.hy926 cells (Figures 2 and 3, *A, B, C*).

The scanning electron microscope (SEM) images showed that SNPs formed agglomerates of different sizes which were easily observed and associated with cellular plasma membrane after a 30-min exposition of cells to SNPs (Figure 4, *A*, *B* and *C*). Comparison of images taken with the use of SE or BSE detectors showed numerous irregular-shaped cellular protrusions filled with SNP agglomerates, formed as a step in the process of SNPs uptake (Figure 4, *D, E*). Cells showing membrane damage that resulted from the interaction with SNP agglomerates were also observed (Figure 4, *F*).