





Neuron and Glial Cells Exposed to Cerium Dioxide Nanoparticles: Results from MTT and γ H2AX Assays [†]

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Abstract: Cerium dioxide nanoparticles (CeO₂NP) show antioxidant enzyme-like properties and reactive oxygen species (ROS) scavenging activity, making them a promising material for potential therapeutic applications in neurodegenerative diseases. The objective of this work was to assess the biological behavior of CeO₂NP in human SH-SY5Y neuronal and A172 glial cells by means of the MTT assay and the γ H2AX assay. Despite the significant dose- and time-dependent NP internalization by both cell lines, nanoceria generally presented scarce cytotoxicity or genotoxicity, essentially restricted to the highest NP doses and longest exposure times. In conclusion, a high biocompatibility of CeO₂NP was observed under the conditions tested.

Keywords: cerium dioxide nanoparticles; cellular uptake; cytotoxicity; genotoxicity; neurotoxicity; nanoceria biocompatibility; nanomedicine

1. Introduction

Cerium dioxide nanoparticles (CeO₂NP) show antioxidant enzyme mimetic properties and oxygen free radical scavenging activity in biological systems. These unique structure-dependent features make them a promising material for potential biomedical applications, namely as an antitumor agent for regenerative therapy, gene therapy, or targeted drug delivery. However, their cellular uptake, action mechanism, and potential adverse effects are not totally understood yet [1].

Specifically, many central nervous system (CNS) diseases are characterized by the accumulation of reactive oxygen species (ROS), which induce severe damage to the brain tissues and irreversible neurodegeneration. CeO₂NP has been raised as a novel potential agent in the treatment of neurodegenerative diseases, due mostly to their remarkable property to reduce oxidative stress in damaged cells through their ROS scavenging ability,

the wide range of free radicals they can scavenge, and their self-regenerating redox cycle [2]. Moreover, recent studies in animal models showed that CeO₂NP can cross the blood-brain barrier (BBB) due to their nanoscale diameters [3].

On this basis, the main objective of this work was to assess whether CeO₂NP could induce adverse effects at the cellular and/or genetic level to verify their suitability for their application in the diagnosis and treatment of nervous system diseases. To achieve this aim, the possible alterations in SH-SY5Y neuronal and A172 glial cell viability and induction of DNA double-strand breaks were determined in the presence of a wide dose range of CeO₂NP (1–100 µg/mL) by means of the MTT assay and the γH2AX assay, respectively. In the first stage, the physicochemical characterization of the CeO₂NP and their ability to be taken up by the cells were assessed.

2. Materials and Methods

Cerium (IV) oxide nanopowder (CAS No. 1306-38-3) was obtained from Sigma-Aldrich Co.; according to the supplier, their primary particle size was <25 nm. The average hydrodynamic diameter and zeta potential in both cell culture media were analyzed by dynamic light scattering (DLS) and mixed mode measurement phase analysis light scattering (M3-PALS), respectively, using a Zetasizer Nano-ZS (Model ZEN 3600; Malvern Instruments Ltd., Malvern, UK).

CeO₂NP internalization by both cell types was evaluated by flow cytometry, using a FACSCalibur cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) [4]. The potential antiproliferative effect of CeO₂NP was evaluated by MTT assay, following Mosmann (1983) [5] with some methodological modifications to avoid interference of the NP with the standard protocol [6,7], using a SPECTROstar Nano microplate reader (BMG Labtech, Ortenberg, Germany). To evaluate the potential CeO₂NP genotoxicity, the γH2AX assay was carried out by flow cytometry in a FACSCalibur cytometer (Becton Dickinson) [8]. For all these experiments, both cell types were incubated with a dose range of nanoceria (1–100 µg/mL) for 3, 24, and 48 h. Negative controls used were cell culture media, and positive controls were 200 µg/mL TiO₂NP for internalization, 1% Triton X-100 for the MTT assay, and 1 µg/mL bleomycin for the γH2AX assay.

Statistical analyses were performed using the IBM SPSS Statistics package for Windows (version 27.0). Differences among groups were analyzed by Kruskal–Wallis test and the Mann–Whitney *U*-test for two-by-two comparisons, and associations between two variables were assessed by Pearson's correlation. All experiments were run at least in triplicate. Experimental data were expressed as mean ± standard error, and a *p*-value lower than 0.05 was considered significant.

3. Results and Discussion

Table 1 shows the main physicochemical properties of CeO₂NP dispersed in both cell culture media at the highest dose used for toxicity tests. Results revealed that CeO₂NP were stable and did not agglomerate in any culture medium since their hydrodynamic size remained with minimal variations, and the zeta potential values showed a stable negative surface charge at all time points tested.

Table 1. Physicochemical description of cerium dioxide nanoparticles (100 µg/mL).

CeO ₂ NP	Time Point	Dispersed in A172 Cell Culture Medium	Dispersed in SH-SY5Y Cell Culture Medium
Hydrodynamic diameter (nm) DLS	0 h	140.1 ± 3.0	148.1 ± 19.8
	3 h	142.0 ± 3.6	134.4 ± 4.9
	24 h	156.2 ± 7.7	139.5 ± 3.7
	48 h	166.7 ± 6.5	139.3 ± 3.5
	0 h	−11.8 ± 0.7	−11.3 ± 0.9
Zeta potential (mV) M3-PALS	3 h	−12.1 ± 0.7	−11.5 ± 0.5
	24 h	−12.6 ± 0.7	−11.3 ± 1.1
	48 h	−12.4 ± 0.7	−11.7 ± 0.7

DLS—dynamic light scattering; M3-PALS—mixed mode measurement-phase analysis light scattering.

Flow cytometric analysis of CeO₂NP internalization by both cell types showed efficient and similar dose- and time-dependent NP uptake, although intensity was slightly higher in neuronal cells than in glial cells (Figure 1). The results obtained agree with other previous studies, employing different methodologies, that showed dose-dependent CeO₂NP uptake in other human cell types [9–11].

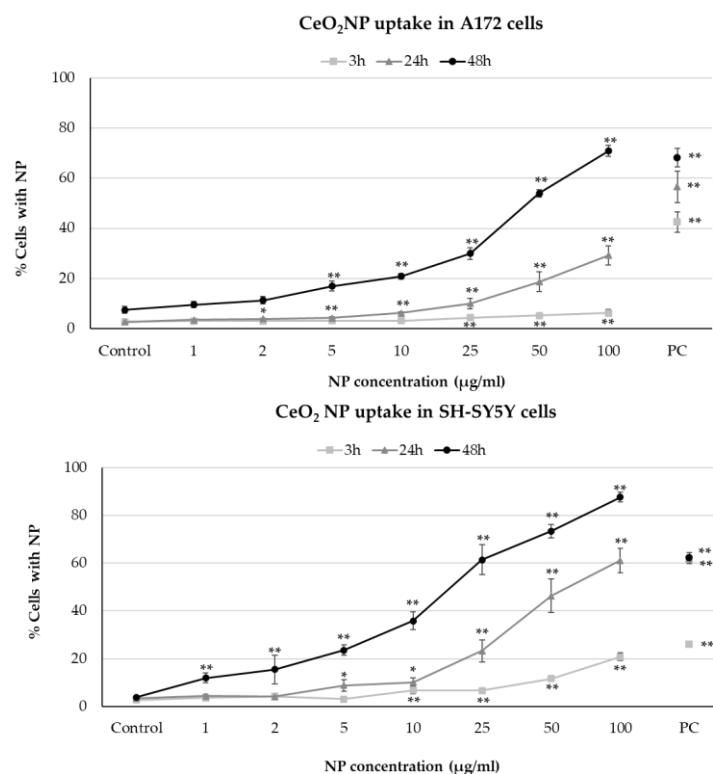


Figure 1. Flow cytometry analysis of CeO₂NP cellular uptake in A172 glial cells (**top**) and SH-SY5Y neuronal cells (**bottom**), exposed for 3, 24, and 48 h. * $p < 0.05$; ** $p < 0.01$, significant differences regarding the corresponding control. PC, positive control (200 μg/mL TiO₂NP).

After cell exposure to CeO₂NP, viability was assessed by employing a modified version of the MTT assay (Figure 2). In general, no cytotoxicity was observed at the shortest exposure times (3 and 24 h) for glial cells, since cell viability was not reduced by more than 20%. At 48 h, a dose-dependent relationship was obtained, with significantly decreasing viability at concentrations over 25 μg/mL. In contrast, CeO₂NP revealed low cytotoxicity at all times and doses tested in SH-SY5Y cells, maintaining viability values above 80% in general; hence, these NPs did not induce cytotoxicity in this cell line. Our results agree with those obtained in some previous studies that revealed cytotoxicity or drastic decreases in cell viability only at high CeO₂NP doses (>50 μg/mL) and/or large exposure periods (>24 h) [9–11].

Results obtained from H2AX phosphorylation analysis showed dose- and time-dependent increases in the percentage of glioblastoma cells with γH2AX, significant only at the highest concentration after 3 h of exposure and at all doses tested after 24 h (Figure 3). A similar effect, but slightly more intense (with significant increases at all tested conditions), was observed in neuroblastoma cells.

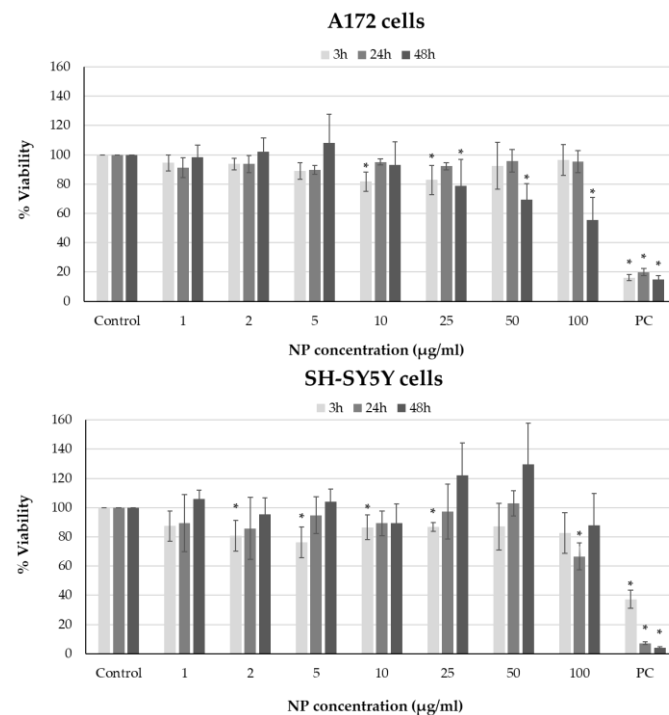


Figure 2. Cell viability evaluation of A172 glioblastoma cells (**top**) and SH-SY5Y neuroblastoma cells (**bottom**) exposed to CeO₂NP for 3, 24, and 48 h. * $p < 0.01$, significant differences with respect to the corresponding control. PC, positive control (1% Triton X-100).

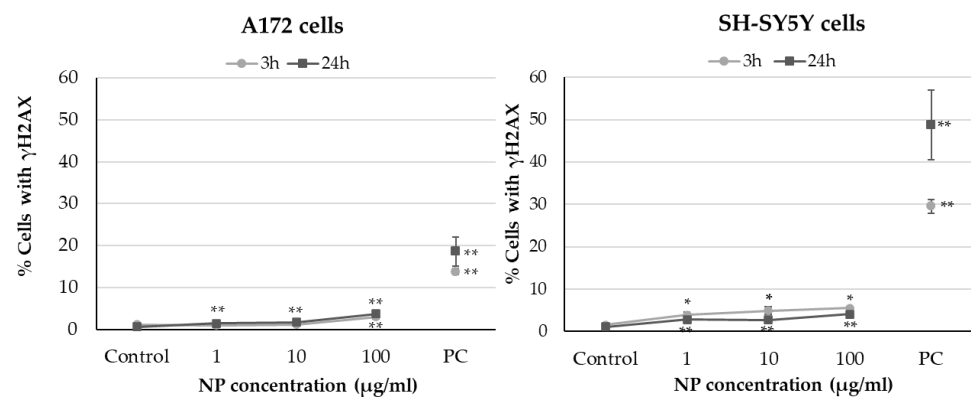


Figure 3. H2AX histone phosphorylation analysis after treatment of A172 cells (**left**) and SH-SY5Y cells (**right**) with CeO₂NP for 3 and 24 h. * $p < 0.05$; ** $p < 0.01$, significantly different regarding the corresponding control. PC, positive control (1 µg/mL bleomycin).

Despite the significant increases in phosphorylated H2AX observed in our study, the values obtained were always lower than 6%, indicating a scarce genotoxic potential of CeO₂NP in both cell types tested. The genotoxic effects found in different human cell types exposed to these NPs have been controversial. Franchi et al. [12] reported no significant increases in phosphorylated H2AX in fibroblasts exposed to low CeO₂NP concentrations (10 µg/mL) for 24 h. Some previous works showed that exposure of human cells to nanoceria doses from 10 to 200 µg/mL did not induce significant primary DNA damage (evaluated by the comet assay) [10,11]. On the contrary, other studies employing the γH2AX test or comet assay revealed that NP doses as low as 6 µg/mL had a higher genotoxic potential even after 3 h of exposure [13,14].

4. Conclusions

The results obtained showed significant dose- and time-dependent NP internalization by both cell lines. Low CeO₂NP-induced cytotoxicity was observed in neuronal cells at all times and doses tested. Notable cytotoxicity in glial cells was restricted to 48 h of treatment at concentrations over 25 µg/mL. Genotoxicity obtained in glial and neuronal cells treated with CeO₂NP was limited since levels of γH2AX were always lower than 6%.

In general, it is possible to consider a high biocompatibility of CeO₂NP under the conditions tested, except for glioblastoma cells exposed for 48 h to medium concentrations. These results provide a better understanding of the interaction of CeO₂NP with cellular systems and their possible adverse effects, specifically at the level of the nervous system.

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