materials proceedings

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Proceeding Paper

Cytotoxic Effects of Zinc Oxide Nanoparticles on Human Glial Cells †

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† Presented at the 4th International Online Conference on Nanomaterials, 5–19 May 2023; Available online: https://iocn2023.sciforum.net.

Abstract: Despite being one of the most studied nanomaterials, much remains unknown about the mechanism of action of zinc oxide (ZnO) nanoparticles (NP). The aim of this work was to evaluate the effects on cell viability caused by the exposure of glial cells to ZnO NP, and the role of Zn^{2+} in the observed effects. The impact of ZnO NP or Zn^{2+} ions on cell viability was assessed by MTT assay. The exposure to ZnO NP induced a significant decrease in cell viability. The presence of Zn^{2+} ions released from ZnO NP was not entirely responsible for the observed cytotoxic effects.

Keywords: zinc oxide nanoparticles; glial cells; Zn^{2+} ions; viability; cellular uptake

Citation: Valdiglesias, V.; Touzani, A.; Ramos-Pan, L.; Alba-González, A.; Folgueira, M.; Moreda-Piñeiro, J.; Méndez, J.; Pásaro, E.; Fernández-Bertólez, N.; Laffon, B. Cytotoxic Effects of Zinc Oxide Nanoparticles on Human Glial Cells. *Mater. Proc.* **2023**, *14*, 23. https://doi.org/10.3390/IOCN2023-14509

Academic Editor: José Luis Arias Mediano

Published: 5 May 2023

1. Introduction

One of the most widely used nanomaterials at present is zinc oxide (ZnO) nanoparticles (NP), with multiple applications in cosmetics, the technological industry and, recently, also in biomedicine [1–4]. This widespread use means that humans are increasingly being exposed to these NP, raising concerns about how this could affect health. Due to the well-demonstrated ability of these NP to easily reach the brain [3,5], their effects on the nervous system deserve particular attention. Despite being one of the most studied NP from a toxicological point of view, much remains unknown about how they may affect specific cell types, such as glial cells, and about their mechanism of action. On this basis, the aim of this work was to evaluate the effects on cell viability caused by the exposure of human glioblastoma A172 cells to ZnO NP, and the role of Zn^{2+} in the observed effects.

2. Materials and Methods

A stock suspension of ZnO NP (CAS No. 1314-13-2, Sigma–Aldrich Co. (Madrid, Spain), final concentration 100 µg/mL) was prepared, prior to treatments in A172 cell culture medium, and ultrasonicated (Sonoplus mini 20, Bandelin, Berlin, Germany) as previously described [6]. Average hydrodynamic size and zeta potential of particles were

determined 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). The Zn^{2+} content in the cell culture medium after NP treatments was analysed by flame atomic absorption spectroscopy (FAAS) (PerkinElmer Model 2380 atomic absorption spectrometer, PerkinElmer Instruments, Waltham, MA, USA).

A flow cytometry approach (FACSCalibur, Becton Dickinson, Franklin Lakes, NJ, USA) was employed to assess the potential of the ZnO NP to enter the cells [7], whereas NP effects on viability were evaluated by MTT assay using a SPECTROstar Nano (BMG Labtech, Ortenberg, Germany) microplate reader [8]. For these experiments, cells were incubated at different ZnO NP or $ZnSO_4$ concentrations, or the control solutions (cell culture medium), for different exposures times.

Differences among groups were analysed by Kruskal–Wallis test, with Mann–Whitney *U*-test for two-by-two comparisons and by employing SPSS for Windows statistical package (version 20.0, IBM, New York, NY, USA). The associations between two variables were analysed by Pearson's correlation. All experiments were run at least in triplicate. Experimental data were expressed as mean ± standard error and a p-value of <0.05 was considered significant.

3. Results and Discussion

3.1. Nanoparticle Characterization and Cellular Uptake

The ZnO NP employed in the present study were less than 100 nm spherical NP (data provided by the manufacturer). The results obtained from the analysis of hydrodynamic size and zeta potential are collected in Table 1. Despite the variable range obtained for zeta potential, the dispersion of ZnO NP resulted as being quite stable, with slight variations in the hydrodynamic size, ranging between 273.97 nm and 315.01 nm.

Table 1. Physical-chemical characterization of ZnO NP.

		Hydrodynamic Diameter (nm) [a]	Zeta Potential (mV) [a]
Time (h)	0	302.09 ± 0.84	−1.73 ± 4.05
	3	315.01 ± 3.16	2.79 ± 3.01
	24	269.16 ± 1.36	−20.25 ± 2.03
	48	273.97 ± 5.91	−13.46 ± 2.36

[a] Mean ± standard deviation.

Table 2 shows the results obtained from the flow cytometry analysis of cellular uptake of ZnO NP at different concentrations and exposure times.

Table 2. ZnO NP uptake by glial A172 cells (% of cells with NP, mean ± standard deviation).

ZnO NP Concentration (μg/mL)	Exposure Time		
	3 h	24 h	48 h
Control	1.06 ± 0.18	1.13 ± 0.16	0.76 ± 0.06
0.1	1.12 ± 1.16	2.04 ± 0.24 **	1.98 ± 0.29 **
0.5	1.21 ± 0.18	4.41 ± 1.20 **	3.18 ± 0.47 **
2.5	1.21 ± 0.12	4.99 ± 1.28 **	2.15 ± 0.39 **
10	1.03 ± 0.13	7.06 ± 1.80 **	4.11 ± 0.61 **
25	1.05 ± 0.21	6.79 ± 1.53 **	6.42 ± 0.68 **
50	1.71 ± 0.31	9.34 ± 1.06 **	11.75 ± 0.88 **
100	5.28 ± 0.55 **	7.92 ± 0.69 **	28.06 ± 2.41 **
Positive control	42.42 ± 4.06 **	64.11 ± 2.25 **	71.88 ± 1.21 **

** $p < 0.01$, significant difference with regard to the corresponding control. Positive control: TiO_2 NP (200 μg/mL).

According to flow cytometry results, NP were effectively internalized by the cells at all exposure times in a dose-dependent manner, although uptake rate was notably much lower at 3 h. These results agree with some previous studies that observed a similar dose- and time-dependent cellular internalization of ZnO NP, evaluated by diverse methods, in other different cell types [9–13]. Nevertheless, current uptake results should be confirmed by means of a suitable microscopy technique.

3.2. Zn^{2+} Ion Release

Results obtained from the FAAS experiments to quantify the release of zinc ions from the ZnO NP are shown in Figure 1.

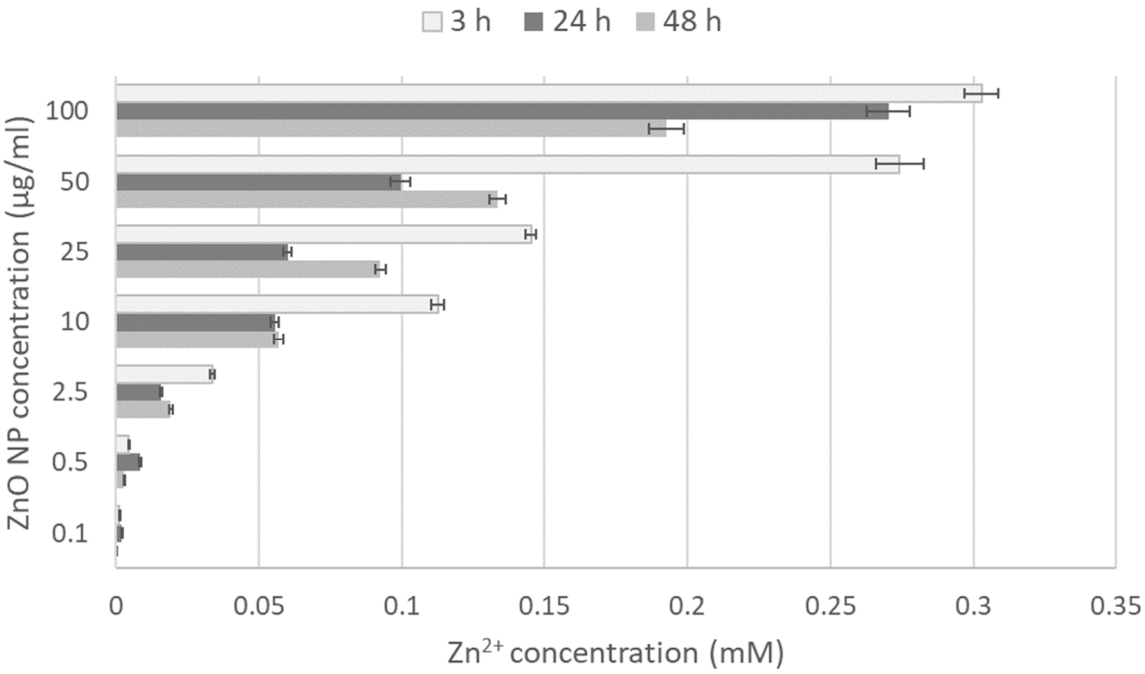

Figure 1. Analysis of Zn^{2+} ions released from ZnO NP to the cell culture medium.

Increased levels of dissolved Zn^{2+} ions from the ZnO NP surface were observed (from 2.5 µg/mL on) at all treatment periods (3, 24 and 48 h), but notably higher at the shortest time. The release of Zn^{2+} ions from ZnO NP is well documented in the literature, and this demonstrates that the solubility of these NP can be highly variable and dependent on both the NP physicochemical properties and the matrix in which they are suspended [14–17].

3.3. ZnO NP Effects on Glial Cells

After discarding any potential interference of the NP with MTT assay, the effects of ZnO NP on viability of glial A172 cells were evaluated. The results from these experiments are shown in Figure 2. The effects on cellular viability were moderate after 3 h of exposure, reaching 60% viability only at the highest concentrations. However, after 24 and 48 h of treatments, the decreases in viability were much marked, reaching values close to 90% mortality from 50 or 25 µg/mL on, respectively. Similarly, several authors previously described a dose-dependent decrease in cell viability of various cell types exposed to ZnO NP (reviewed in [18]).

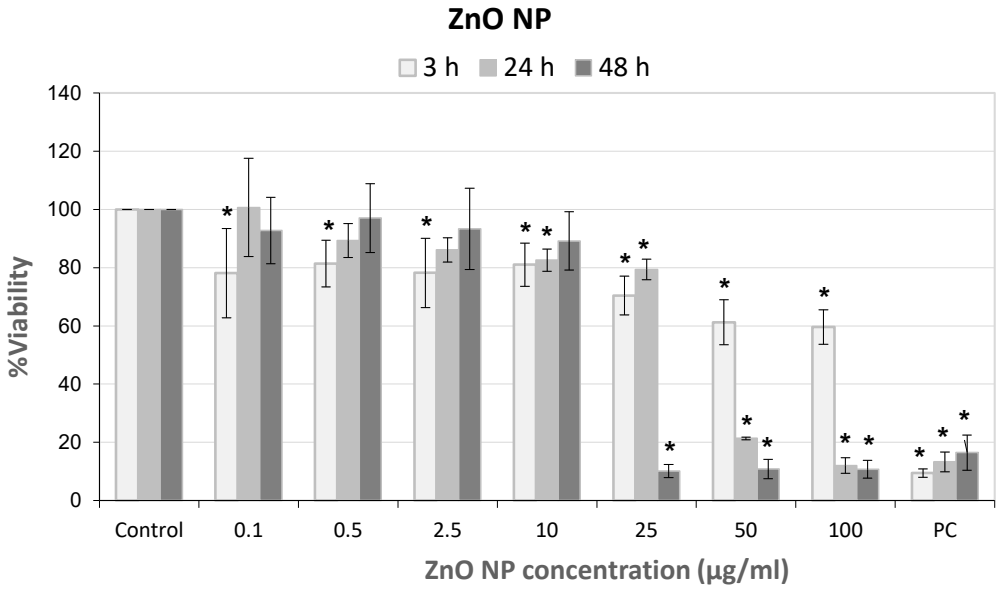

Figure 2. Cytotoxicity of ZnO NP in A172 glial cells at different exposure times. Bars represent mean standard error. * $p < 0.05$, significant difference with regard to the corresponding control. PC: Positive control: triton X-100 (1%).

3.4. Role of Zn^{2+} Ions Released from ZnO NP

In order to test the role of the Zn^{2+} released from ZnO NP in the cytotoxic effects observed, as previously pointed out [19,20], in the present work, A172 cells were treated with zinc sulfate ($ZnSO_4$) at several concentrations chosen according to FAAS analysis. The results obtained from these analyses showed that a significant decrease in cell viability was just observed at Zn^{2+} ion concentrations greater than 0.2 and 0.3 mM for 24 and 48 h, respectively (Figure 3). According to this, the release of Zn^{2+} ions from ZnO NP does not explain, at least completely, their effects on glial cell viability, and hence other different action mechanisms, e.g., the production of ROS or induction of oxidative stress, as previously reported for these NP [21–23], should be considered in attempting to explain the observed effects.

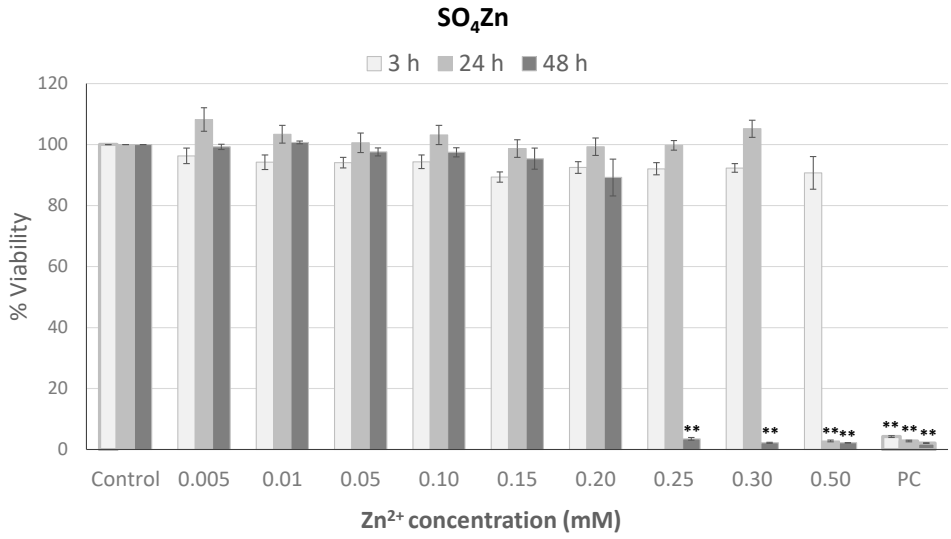

Figure 3. Cytotoxicity of $ZnSO_4$ in A172 glial cells at different exposure times. Bars represent mean standard error. ** $p < 0.01$, significant difference with regard to the corresponding control. PC: Positive control: triton X-100 (1%).

4. Conclusions

ZnO NP were effectively internalized by A172 cells and the exposure induced a significant decrease in cell viability, depending on the dose and treatment duration. The ability of ZnO NP to release Zn^{2+} ions into the medium in a concentration-dependent manner was confirmed; however, their presence was not entirely responsible for the observed cytotoxic effects. These results increase the knowledge about the toxic potential of ZnO NP in human glial cells and the involvement of released Zn^{2+} ions in the observed effects.

Author Contributions: Conceptualization, V.V., N.F.-B. and B.L.; methodology, A.T., L.R.-P., A.A.-G., J.M.-P., N.F.-B.; formal analysis, M.F., J.M., B.L.; writing—original draft preparation, V.V., E.P.; writing—review and editing, J.M., B.L., M.F.; supervision, V.V., N.F.-B. and B.L.; funding acquisition, E.P., V.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Ministry of Science and Innovation: MCIN/AEI/10.13039/501100011033 (Grant PID2020-114908GA-I00), Xunta de Galicia (ED431B 2022/16 and ED481A 2019/003), CICA-Disrupting Project 2021SEM-B2, and Ministry of Education, Culture and Sport [BEAGAL18/00142 to V.V.].

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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