materials proceedings

MDPI

Proceeding Paper

Cytotoxic Effects of Zinc Oxide Nanoparticles on Human Glial Cells †

Vanessa Valdiglesias [1,2], Assia Touzani [1,2], Lucía Ramos-Pan [1,2], Anabel Alba-González [3], Mónica Folgueira [3], Jorge Moreda-Piñeiro [4], Josefina Méndez [1], Eduardo Pásaro [2,5], Natalia Fernández-Bertolez [1,2] and Blanca Laffon [2,5,*]

[1] Universidade da Coruña, Grupo DICOMOSA, Centro Interdisciplinar de Química e Bioloxía—CICA, Departamento de Psicología, Facultad de Ciencias de la Educación, Campus Elviña s/n, 15071, A Coruña, Spain; vanessa.valdiglesias@udc.es (V.V.); assia.touzani@udc.es (A.T.); josefina.mendez@udc.es (L.R.-P.); email1@email.com (J.M.); natalia.fernandezb@udc.es (N.F.-B.)
[2] Instituto de Investigación Biomédica de A Coruña (INIBIC). Oza, 15071 A Coruña, Spain; eduardo.pasaro@udc.es
[3] Universidade da Coruña, Grupo NEUROVER, Centro Interdisciplinar de Química e Bioloxía—CICA, Rúa As Carballeiras, 15071 A Coruña, Spain; anabel.albag@udc.es (A.A.-G.); m.folgueira@udc.es (M.F.)
[4] Universidade da Coruña, Grupo Química Analítica Aplicada (QANAP), Instituto Universitario Medio Ambiente (IUMA), Departamento de Química, Facultad de Ciencias, Campus A Zapateira s/n, 15071 A Coruña, Spain; jorge.moreda@udc.es
[5] Universidade da Coruña, Grupo NanoToxGen, Centro Interdisciplinar de Química e Bioloxía—CICA, Departamento de Biología, Facultad de Ciencias, Campus A Zapateira s/n, 15071 A Coruña, Spain
* Correspondence: blanca.laffon@udc.es
† 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 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. Exposure to ZnO NP induced a significant decrease in cell viability. The presence 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-Bertolez, N.; Laffon, B. Cytotoxic Effects of Zinc Oxide Nanoparticles on Human Glial Cells. *Mater. Proc.* **2023**, *5*, x. https://doi.org/10.3390/xxxxx
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 cosmetic, 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 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 their mechanism of action. On this basis, the aim of this work was to evaluate the effects on cell viability caused by 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., final concentration 100 μg/mL) was prepared prior treatments in A172 cell culture medium and ultrasonicated (Branson Sonifier, USA), 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.). 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).

A flow cytometry approach (FACSCalibur, Becton Dickinson) 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) 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, by employing SPSS for Windows statistical package (version 20.0). 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). Results obtained from the analysis of hydrodynamic size and zeta potential are collected in Table 1. Despite the variable range obtained for zeta potential, dispersion of ZnO NP resulted 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.

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.

Table 2. ZnO NP uptake by glial A172 cells (% of cells with NP).

ZnO NP Concentration	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 (200μg/mL) [a] *Mean ± standard deviation.*

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.

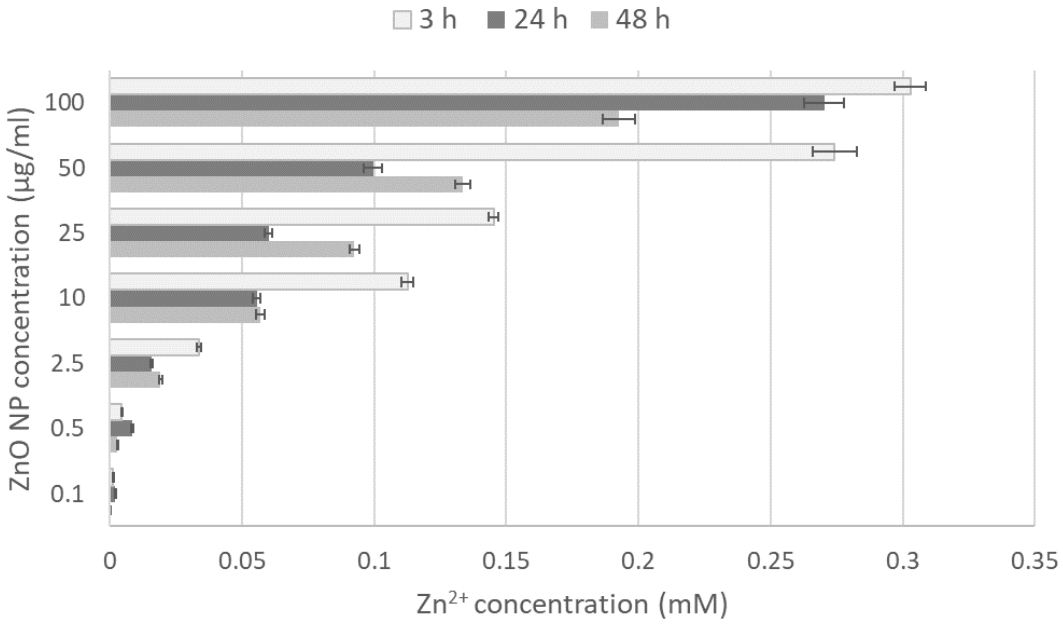

Figure 1. Analysis of Zn^{+2} ions released from ZnO NP to the cell culture medium.

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 demonstrated 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. Results from these experiments are shown in Figure 2. 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]).

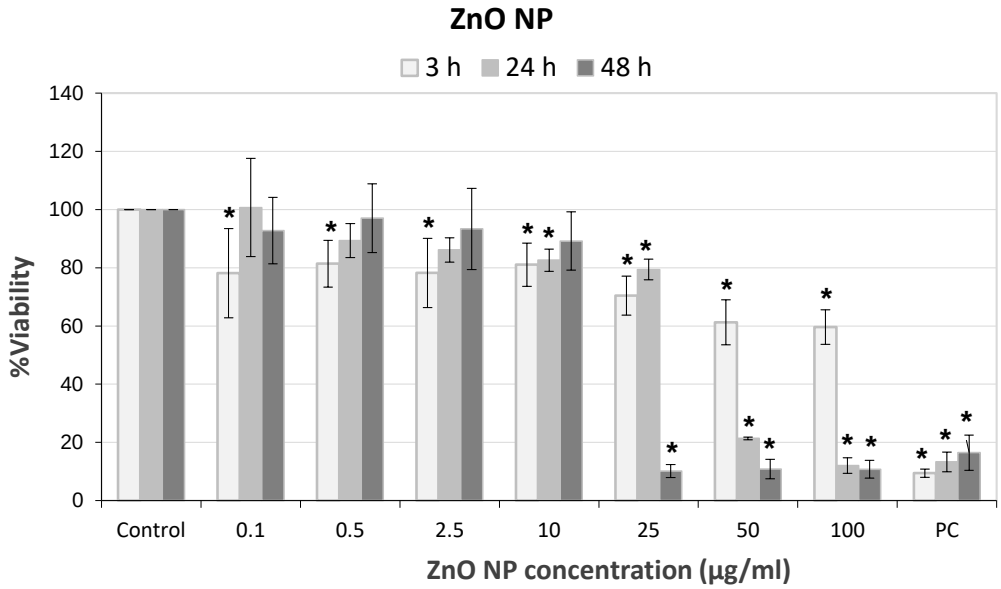

Figure 2. Cytotoxicity of ZnO NP in A172 glial cells at different exposure times. Bars represent mean standard error. * $p < 0.05$, significant difference with regard to the corresponding control. PC: Positive control: triton X-100 (1%).

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 accordingly to FAAS analysis. 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 of ZnO NP does not explain, at least completely, their effects on glial cells viability, and hence other different action mechanism, e.g., production of ROS or induction of oxidative stress, previously reported for these NP [21–23] should be considered to explain the observed effects.

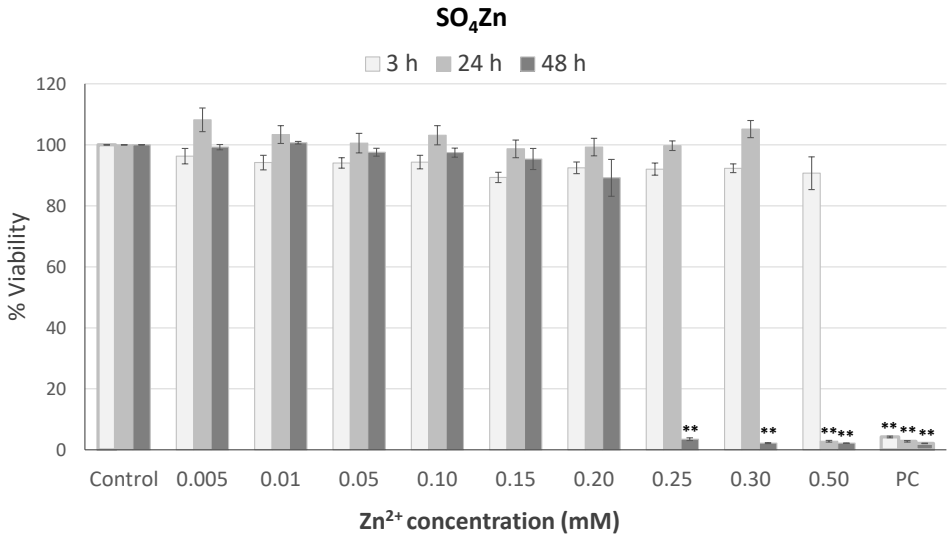

Figure 3. Cytotoxicity of $ZnSO_4$ in A172 glial cells at different exposure times. Bars represent mean standard error. ** $p < 0.01$, significant difference with regard to the corresponding control. PC: Positive control: triton X-100 (1%).

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.

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

References

1. Du, J.; Tang, J.; Xu, S.; Ge, J.; Dong, Y.; Li, H.; Jin, M. ZnO nanoparticles: Recent advances in ecotoxicity and risk assessment. *Drug Chem. Toxicol*. **2020**, *43*, 322–333.
2. Shi, L.E.; Li, Z.H.; Zheng, W.; Zhao, Y.F.; Jin, Y.F.; Tang, Z.X. Food Additives & Contaminants: Part A Synthesis, antibacterial activity, antibacterial mechanism and food applications of ZnO nanoparticles: A review. *Food Addit. Contam. Part A* **2014**, *31*, 173–186.
3. Singh, S. Zinc oxide nanoparticles impacts: Cytotoxicity, genotoxicity, developmental toxicity, and neurotoxicity. *Toxicol. Mech. Methods* **2019**, *29*, 300–311.
4. Smaoui, S.; Chérif, I.; Hlima, H.B.; Khan, M.U.; Rebezov, M.; Thiruvengadam, M.; Sarkar, T.; Shariati, M.A.; Lorenzo, J.M. Zinc oxide nanoparticles in meat packaging: A systematic review of recent literature. *Food Packag. Shelf Life* **2023**, *36*, 101045.
5. Guo, Z.; Zhang, P.; Chakraborty, S.; Chetwynd, A.J.; Monikh, F.A.; Stark, C.; Ali-Boucetta, H.; Wilson, S.; Lynch, I.; Valsami-Jones, E. Biotransformation modulates the penetration of metallic nanomaterials across an artificial blood–brain barrier model. *Proc. Natl. Acad. Sci. USA* **2021**, *118*, e2105245118.
6. Valdiglesias, V.; Costa, C.; Kiliç, G.; Costa, S.; Pásaro, E.; Laffon, B.; Teixeira, J.P.; Paulo, J. Neuronal cytotoxicity and genotoxicity induced by zinc oxide nanoparticles. *Environ. Int*. **2013**, *55*, 92–100.
7. Fernández-Bertólez, N.; Costa, C.; Brandão, F.; Duarte, J.A.; Teixeira, J.P.; Pásaro, E.; Valdiglesias, V.; Laffon, B. Evaluation of cytotoxicity and genotoxicity induced by oleic acid-coated iron oxide nanoparticles in human astrocytes. *Env. Mol. Mutagen* **2019**, *60*, 816–829.
8. Valdiglesias, V.; Costa, C.; Sharma, V.; Kiliç, G.; Pásaro, E.; Teixeira, J.P.; Dhawan, A.; Laffon, B. Comparative study on effects of two different types of titanium dioxide nanoparticles on human neuronal cells. *Food Chem. Toxicol*. **2013**, *57*, 352–361.
9. Sharma, A.K.; Singh, V.; Gera, R.; Purohit, M.P.; Ghosh, D. Zinc oxide nanoparticle induces microglial death by NADPH-oxidase-independent reactive oxygen species as well as energy depletion. *Mol. Neurobiol*. **2017**, *54*, 6273–6286.
10. Sruthi, S.; Mohanan, P.V. Investigation on cellular interactions of astrocytes with zinc oxide nanoparticles using rat C6 cell lines. *Colloids Surf. B Biointerfaces* **2015**, *133*, 1–11.
11. Heim, J.; Felder, E.; Tahir, M.N.; Kaltbeitzel, A.; Heinrich, U.R.; Brochhausen, C.; Mailänder, V.; Tremel, W.; Brieger, J. Genotoxic effects of zinc oxide nanoparticles. *Nanoscale* **2015**, *7*, 8931–8938.
12. Zhang, J.; Qin, X.; Wang, B.; Xu, G.; Qin, Z.; Wang, J.; Wu, L.; Ju, X.; Bose, D.D.; Qiu, F.; et al. Zinc oxide nanoparticles harness autophagy to induce cell death in lung epithelial cells. *Cell Death Dis*. **2017**, *8*, e2954.
13. Mittag, A.; Hoera, C.; Kämpfe, A.; Westermann, M.; Kuckelkorn, J.; Schneider, T.; Glei, M. Cellular uptake and toxicological effects of differently sized zinc oxide nanoparticles in intestinal cells. *Toxics* **2021**, *9*, 96.
14. Eixenberger, J.E.; Anders, C.B.; Hermann, R.J.; Brown, R.J.; Reddy, K.M.; Punnoose, A.; Wingett, D.G. Rapid dissolution of ZnO nanoparticles induced by biological buffers significantly impacts cytotoxicity. *Chem. Res. Toxicol*. **2017**, *30*, 1641–1651.
15. Liang, Z.; Zhang, F.; Zhang, H. In Vitro response of immune cells on metal oxide nanoparticles with different solubility. *J. Nanosci. Nanotechnol*. **2016**, *16*, 5546–5552.
16. Sharma, V.; Anderson, D.; Dhawan, A. oxide nanoparticles induce oxidative DNA damage and ROS-triggered mitochondria mediated apoptosis in human liver cells (HepG2). *Apoptosis Zinc* **2012**, *17*, 852–870.

17. Yan, Y.; Wang, G.; Huang, J.; Zhang, Y.; Cheng, X.; Chuai, M.; Brand-Saberi, B.; Chen, G.; Jiang, X.; Yang, X. Zinc oxide nanoparticles exposure-induced oxidative stress restricts cranial neural crest development during chicken embryogenesis. *Ecotoxicol. Environ. Saf.* **2020**, *194*, 110415.
18. Pandurangan, M.; Kim, D.H. In vitro toxicity of zinc oxide nanoparticles: A review. *J. Nanoparticle Res*. **2015**, *17*, 158.
19. Bozym, R.A.; Chimienti, F.; Giblin, L.J.; Gross, G.W.; Korichneva, I.; Li, Y.; Libert, S.; Maret, W.; Parviz, M.; Frederickson, C.J.; et al. Free zinc ions outside a narrow concentration range are toxic to a variety of cells in vitro. *Exp. Biol. Med*. **2010**, *235*, 741–750.
20. Holmes, A.M.; Mackenzie, L.; Roberts, M.S. Disposition and measured toxicity of zinc oxide nanoparticles and zinc ions against keratinocytes in cell culture and viable human epidermis. *Nanotoxicology* **2020**, *14*, 263–274.
21. Nair, S.; Sasidharan, A.; Divya Rani, V.V.; Menon, D.; Nair, S.; Manzoor, K.; Raina, S. Role of size scale of ZnO nanoparticles and microparticles on toxicity toward bacteria and osteoblast cancer cells. *J. Mater. Sci. Mater. Med*. **2009**, *20*, 235–241.
22. Patel, P.; Kansara, K.; Senapati, V.A.; Shanker, R.; Dhawan, A.; Kumar, A. Cell cycle dependent cellular uptake of zinc oxide nanoparticles in human epidermal cells. *Mutagenesis* **2016**, *31*, 481–490.
23. Wong, S.W.Y.; Leung, P.T.Y.; Djurišić, A.B.; Leung, K.M.Y. Toxicities of nano zinc oxide to five marine organisms: Influences of aggregate size and ion solubility, in: Analytical and Bioanalytical Chemistry. *Anal. Bioanal. Chem*. **2010**, *396*, 609–618.