Research Article

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Zinc oxide nanoparticles: potential novel applications in cellular physiology, pathology, neurosciences and cancer research

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Abstract: With the rapid development of nanotechnology during the past several years, attention has been focused on metallic nanomaterials, due to their specific physical and chemical characteristics. Zinc oxide nanoparticles (ZnO NPs) have numerous potential applications in industry, as a part of various consumer products, but also in medical research. Anticancer properties of ZnO NPs have been suggested in cell cultures, however, the precise mechanism responsible for their activity in these conditions remains elusive. Cytotoxicity and genotoxicity of ZnO NPs are also unclear. Apart from cancer research, ZnO NPs are today widely researched in almost all areas of fundamental medicine. In this short review, we discuss recently published articles on ZnO NPs applications in cellular physiology, pathology, neurosciences and oncology.

Keywords: Zinc; Nanomaterial; Toxicity; Gene; Signaling

1 Introduction

Nanotechnology is today a rapidly growing discipline closely related to many aspects of physics, chemistry, biology and medicine. During the last two decades, many

new nanomaterials have been discovered, some of which have numerous potential applications in various medical fields, either as agents for research purposes, or potential addition to conventional diagnostic or therapeutic methods [1–6]. In recent years, particular attention has been given to inorganic metallic nanomaterials and their oxides, because of the distinctive and sometimes unique biological properties [7–10]. Biodistribution, biotransformation and elimination of metals significantly differs when their diameter is less than 100 nanometers (a definition of metallic nanoparticle). Passage through biological barriers, specific interactions with cell enzymes and nuclear chromatin, as well as interference in specific cell signaling pathways is what makes metallic nanomaterials unique, compared to other chemical substances and compounds [11–16].

Recently, many researchers have pointed out possible applications of zinc oxide nanoparticles (ZnO NPs) in different medical and non-medical fields [17]. Zinc oxide as a conventional inorganic compound is generally used as an additive to numerous consumer products such as batteries, transistors, paints, lubricants and pigments. In medicine, it is commonly applied in treatments relating to skin disorders such as acne, eczema, and other skin inflammations. Nanoparticles of ZnO are today present in many sunscreen products because of their specific interaction with light, in terms of reduced light scatter.

Apart from these uses, there are many indications that ZnO nanoparticles may have potentially valuable applications in physiology and cell biology research. They may modulate activity and effects of several important biochemical signaling pathways and consequently alter functions on cellular and molecular levels [18, 19]. In the central nervous system, ZnO NPs may have effects on synaptic plasticity, neuronal apoptosis, and even cognitive psychic functioning, at least in animal experimental models (ref?). Genotoxicity, cytotoxicity and neurotoxicity of ZnO NPs has not yet been fully investigated, although there are concerns regarding their potential detrimental health effects. Therefore, any novel knowledge on cellular and subcellular interactions of ZnO NPs, may have certain public

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health impact, especially having in mind their widespread use in industry and significant presence in general population through various consumer products.

In this review, we focus on the research published within the last ten years of medical and biological issues relating to use of ZnO NPs. We cover important research articles in the fields of experimental physiology, pathology and neurosciences, as well as recent research on ZnO NP anticancer properties. Potential cytotoxicity and genotoxicity concerns are also mentioned and discussed.

2 Modulation of intracellular signaling pathways by zinc oxide nanoparticles

Zinc oxide nanoparticles may interfere in various signaling pathways, both in physiological and pathological conditions [19–21]. This may lead numerous cellular responses, including production and release of inflammatory chemical mediators, changes in intercellular communication and programmed cell death (apoptosis). Exposure of cells to ZnO NPs may also lead to more discrete functional and morphological alterations, such as changes in protein expression in the cell membrane, or the rate of expression of certain genes.

In immune system cells such as macrophages, Toll-like receptor 6 (TLR6) signaling may be involved in ZnO NP-induced inflammatory and functional responses. As described by Roy et al. (2014a), ZnO NPs cause activation and maturation markers CD1d, MHC-II, CD86 and CD71 to be more expressed in these cells [20]. Other enzymes such as tumor necrosis factor receptor-associated factor and interleukin-1 receptor associated kinase are also affected. Mitogen-activated protein kinase (MAPK) pathways may also be involved in generation of inflammatory response.

Toll-like receptors may also be responsible for adjuvant effects of ZnO NPs during inflammatory responses. In such cases, TLR 2, 4 and 6 receptors may be increased, which may be related to the increase of several enzymes such as TNFR-associated factor 6 (TRAF 6), IL-1 receptor associated kinase 1 (IRAK 1) and myeloid differentiation primary response protein-88 (MyD88), as described in the recent study in 2014 [21]. This is not necessarily the only pro-inflammatory pathway affected by ZnO NPs since it was found that Src (family of protein tyrosine kinases) signaling was also impacted, possibly via mediators IP3 (Inositol 1,4,5-tris-phosphate), p-Syk (*Phospho-Syk*), p-PLC- γ

(variant of Phosphoinositide phospholipase C) and even cyclic AMP.

Mitochondria-related signaling pathway may also be activated as the result of ZnO NP exposure. As shown by Wang and associates in 2018, this may take place in murine photoreceptor cells which enter apoptosis as the result. Several important chemical changes may occur in photoreceptor cells prior to apoptosis such as the reduction of the level of intracellular adenosine triphosphate, or the increase in generation of reactive oxygen species. Also, collapse of the mitochondrial membrane potential, and activation of Bax and Caspase 3, as well as the reduction of Bcl-2 expression may significantly contribute to cell death in these circumstances [19].

In relation to programmed cell death, Bcl-2 and other above-mentioned proteins are not the only ones that are being increased/decreased after cells are treated with ZnO NPs. It is thought that upregulation of p53 also plays a significant role as recently shown by Bai et al. (2017) in a study on human ovarian cancer cells Tumor protein p53 (tumor suppressor p53 or phosphoprotein p53) is a key protective protein that prevents malignant transformation of damaged cells. Signaling pathways in which p53 is engaged activate DNA repair mechanisms, regulate the cell cycle (in terms of its arrest or restart), and, when necessary start apoptosis process. It is suggested that by upregulating this and related pathways, ZnO NPs influence both apoptosis and autophagy, at least in human ovarian cancer cells [22].

In human umbilical vein endothelial cells, ZnO NPs may alter intercellular adhesion molecule-1 (ICAM-1) pathway. Li et al. (2012) demonstrated the regulatory roles of several different proteins/mediators in this signaling. For example, ZnO NPs might influence the levels of c-Jun N-terminal kinase (JNK), mixed lineage kinase 3 (MLK3), and Ras-related C3 botulinum toxin substrate 1 (Rac1)/cell division control protein 42 homolog (Cdc42) [18]. Changes in ICAM-1 signaling are generally important in vascular inflammation and immune system interaction with blood vessels, so these results provide potential important contribution to our understanding of ZnO NP toxicity.

Finally, it should be noted that modulatory effects of ZnO NPs on many intracellular signaling pathways have not yet been fully investigated, and many issues on this matter remain unsolved. This is especially the case with G protein-coupled receptor-triggered signaling cascades which are responsible for numerous gene expression changes and membrane/organelle function. Nevertheless, the mere fact that ZnO NPs may interfere in intracellular signal transduction gives rise to various concerns regarding potential cytotoxicity and general safety of this compound.

3 Zinc oxide nanoparticles and cancer

Some authors today consider zinc oxide nanoparticles to be a potentially promising anticancer agent. Most of its anticancer activity is present in *in vitro* conditions, with very limited data on other experimental models. Selectivity of ZnO NPs toward malignant cells has indeed been suggested (ref), however, these data require confirmation by future studies. It is also thought that ZnO NPs might have a certain potential to be used as drug carriers for specific drug delivery to cancer tissue.

It seems that some ZnO NPs possess certain potential to selectively target cancer cells which may be used for the future design of complex conjugates with chemotherapy medications [23]. For example, in 2009, Ostrovsky and colleagues demonstrated that administration of ZnO NPs led to cell death in breast and prostate cancer cell lines, but not in normal (non-malignant) breast/prostate cells [24]. In addition, the authors observed cytotoxic phenomena on human glioma cells (*i.e.* lines A172 and LN18), there were no such effects exerted by nanoparticles on normal brain astrocytes. In 2012, Akhtar and colleagues performed similar research on human bronchial epithelial cell line BEAS-2B, human lung adenocarcinoma A549, and human hepatocellular carcinoma HepG2. It was shown that ZnO NPs selectively cause apoptosis in these malignant cells, while normal astrocytes and hepatocytes remained intact [25]. The ability of ZnO nanoparticle to selectively damage cancer cells can be increased in different ways. Thurber *et al.* (2012) indicated that by incorporating iron ions (iron doping), the cytotoxic effects of ZnO NPs can be significantly augmented. Using flow cytometry, the authors showed that in Jurkat leukemic cancer cells, viability was substantially reduced after cationic ZnO NPs were doped with iron ions. Conversely, no such effect (in that extent) was observed on normal, non-immortalized human lymphocytes [26].

Probably one of the most important mechanisms by which ZnO NPs exhibit cytotoxic effects in cancer cells is through the generation of reactive oxygen species (ROS). Reactive oxygen species include superoxide anion, hydroxyl radical, peroxides and singlet oxygen. These are normally produced in all cells during metabolic processes, but their synthesis may be increased as the result of various (toxic) chemical, physical and biological factors. They may induce DNA damage, trigger inflammatory responses, immune activation, as well as programmed cell death and necrosis [27]. For example, in a recent study, Wang *et al.* (2015) demonstrated that ZnO NPs may augment intracellular ROS levels in a human pulmonary ade-

nocarcinoma cell line. The treated cells exhibited significant depletion of reduced glutathione, which was followed by programmed cell death, as shown by a caspase-3 activity assay and fluorescence microscopy methods [28]. However, it is possible that not all mechanisms of ROS production are involved during the interaction between ZnO NPs and cell signaling. In a recently published work, Sharma *et al.* (2017) showed that ZnO NPs may cause cell death in microglia via generation of NADPH-oxidase-independent ROS. This was concluded because NADPH oxidase inhibitors, diphenyleneiodonium chloride and apocynin were not able to decrease ROS production [29]. In the future, it remains to be seen to what extent ZnO NPs exhibit ROS-related toxicity, and whether this phenomenon is present in all cells, or only in a limited number. Also, it is unclear whether the ROS production is present in *in vivo* conditions, where the cell is surrounded by a specific microenvironment.

One should be very cautious when interpreting the results of cytotoxic effects of ZnO NPs on malignant cells. Although the anticancer effects of these nanoparticles were suggested in different cancer cell lines, data collected by such studies is by no means conclusive enough to recognize ZnO NPs as anticancer medications. Single experiments on a limited number of cell lines *in vitro*, although providing useful findings, do not necessarily imply anticancer effects in a living tissue. Second, there are numerous studies in which authors demonstrated toxic effects, in terms of ROS generation and/or apoptosis on normal nonmalignant cells [10, 30, 31]. In the future, additional research will be required on animal experimental models, in order to demonstrate selectivity of ZnO NPs towards malignant cells. By these means, hepatotoxicity, nephrotoxicity and immunotoxicity in physiological and pathological conditions can also be tested

4 Zinc oxide nanoparticles in neurosciences

Zinc oxide nanoparticles have certain potential in neuroscience research, and recently there have been many efforts to test and primarily prove their adverse effects on neurons and effects on the central nervous system in general. ZnO NPs may have a certain detrimental role in brain development in animal experimental models (ref). Also, these nanoparticles may negatively impact viability of normal cells of eye retina (ref). There are also some limited data and indications that ZnO NPs may influence some

cognitive psychic functions in animals, such as learning, memory (ref).

It seems that ZnO NPs can participate in regulation of various neuroendocrine mechanisms and neuronal factors during development. As suggested by Liu *et al.* (2016), they may alter some aspects of neuron development in the ovary and influence the functioning of ovaries at puberty through effects on neuroendocrine cells. Also, ZnO NPs may impact neuronal factor protein and gene expression in this environment [32]. Prenatal exposure to ZnO NPs may induce changes in neuronal ultrastructural features, which leads to functional changes in learning and memory during adulthood. In a recent work in 2017, it was shown that when pregnant Sprague-Dawley rats (a common animal model in research) were exposed to ZnO NPs, distinctive histopathological changes were present in offspring brains, which were manifested as abnormal neuronal morphology and reduced ability to solve cognitive tests such as the Morris water maze [33].

Effects of ZnO NPs on psychic functions were previously investigated by Amara and the colleagues in 2015. Rats were administrated nanoparticles, of average size 20 – 30 nanometers, intraperitoneally. The study tested their effects on anxiety-related behavior with application of plus-maze test. The anxiety index did not significantly change in exposed animals when compared to controls. However, the authors did find certain biochemical changes in brain tissue, such as the reduction in iron and calcium concentrations [34]. In another study conducted by the same research team, it was shown that ZnO NPs do not significantly alter spatial working memory and exploratory behavior in adult rats. Also, it appears that ZnO NPs do not have substantial effects on neurotransmitters such as dopamine, norepinephrine, and serotonin [35]. As shown from the text above, the overall impact ZnO NPs on animal behavioral parameters remains unclear, and additional research in this field is necessary.

There are also indications that ZnO NPs may induce cell damage in retinal tissue. In 2013, Guo and associates performed a study on a culture of rat retinal ganglion cells (RGC-5). The nanoparticles exerted both cytotoxic and genotoxic effects on ganglion cells, which was related to the changes in levels of proteins caspase-9, caspase-12 and bcl-2. Phenomena such as cell cycle arrest and ROS overproduction were also observed in this cell line [31]. Apart from these effects, ZnO NPs can alter activity and expression of plasma membrane calcium ATPase in the same experimental model [30]. The changes in calcium ATPase are followed by alterations in intracellular calcium homeostasis, which may lead to cell death. In a recently published study, Chen *et al.* (2017) demonstrated that ZnO

NPs have certain cytotoxic effects on murine photoreceptor cells. This is probably related to the blocking of potassium channels, as well as the decrease of expression of Na^+/K^+ -ATPase [36].

It is possible that the one of the major molecular mechanisms of ZnO NP neurotoxicity is based on ROS generation, similarly as with cytotoxicity in cancer cells. Perhaps the property of metallic nanoparticles in general is to exhibit neurotoxicity through this mechanism [10]. Tian *et al.* (2015) reported increased levels of oxidative stress in brain after C57BL/6J mice were exposed to the nanoparticles [37]. Sruthi and Mohanan (2015) investigated the interaction between astrocytes and ZnO NPs, and found the evidence of strong oxidative stress relatively early after the treatment [38]. Recent research from 2018 also indicated that oxidative stress plays important role in brain damage induced by the nanoparticles. Substantial evidence for the presence of oxidative stress in cerebellum were presented, along with other morphological and inflammatory changes [39].

It should be noted, however, that there are studies with results that disagree with the ones mentioned above and rather they indicate the potential protective effects of ZnO NPs on brain tissue. One example is a study conducted by Afifi and Abdelazim (2015) which showed that ZnO NPs, along with silver nanoparticles, ameliorate oxidative stress in diabetic animals. The study was performed on fifty male albino rats, which often serve as experimental models for diabetes. The authors indicated that ZnO NPs have positive effects on antioxidant enzymes superoxide dismutase, glutathione peroxidase, catalase and glutathione reductase by increasing their activity and mRNA expression levels [40].

5 Concluding remarks

Zinc oxide nanoparticles have potentially numerous valuable applications not only in industry and engineering, but also in medical research areas. Anticancer effects of ZnO NPs have been described, however, additional research needs to be conducted in order to make definite conclusions on their applicability in oncology. It has been suggested that ZnO NPs may induce generation of ROS in both malignant and non-malignant cells. There are also some indications that ZnO NPs may exhibit significant neurotoxicity in animal experimental models and alter both brain morphological features, and psychic functioning. Since ZnO NPs are today commonly used in various research fields, and integral part of numerous consumer products,

any new knowledge on their toxic effects might have a significant public health impact. In the future, it remains to be seen to what extent ZnO NPs influence biochemical, physiological and other processes in the human organism.

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References

- [1] K.H. Bae, H.J. Chung, and T.G. Park, *Mol Cells*, 31 (2011) 295-302.
- [2] J.A. Barreto, W. O'Malley, M. Kubeil, B. Graham, H. Stephan, and L. Spiccia, *Adv Mater*, 23 (2011) H18-40.
- [3] A. Bhardwaj, A. Bhardwaj, A. Misuriya, S. Maroli, S. Manjula, and A.K. Singh, *J Int Oral Health*, 6 (2014) 121-126.
- [4] Y. Huang, S. He, W. Cao, K. Cai, and X.J. Liang, *Nanoscale*, 4 (2012) 6135-6149.
- [5] S. Marin, G.M. Vlasceanu, R.E. Tiplea, I.R. Bucur, M. Lemnaru, M.M. Marin, et al., *Curr Top Med Chem*, 15 (2015) 1596-1604.
- [6] J.R. Morones, J.L. Elechiguerra, A. Camacho, K. Holt, J.B. Kouri, J.T. Ramirez, et al., *Nanotechnology*, 16 (2005) 2346-2353.
- [7] S. Chernousova and M. Eppe, *Angew Chem Int Ed Engl*, 52 (2013) 1636-1653.
- [8] A.R. Ferhan, L. Guo, X. Zhou, P. Chen, S. Hong, and D.H. Kim, *Anal Chem*, 85 (2013) 4094-4099.
- [9] K. Hola, Z. Markova, G. Zoppellaro, J. Tucek, and R. Zboril, *Biotechnol Adv*, 33 (2015) 1162-1176.
- [10] B. Song, Y. Zhang, J. Liu, X. Feng, T. Zhou, and L. Shao, *Nanoscale Res Lett*, 11 (2016) 291.
- [11] D. Nikolovski, M. Jeremic, J. Paunovic, D. Vucevic, T. Radosavljevic, S. Radojevic-Skodric, et al., *Rev Adv Mater Sci*, 53 (2018) 74-78.
- [12] J. Paunovic, D. Vucevic, T. Radosavljevic, S. Pantic, D. Nikolovski, and I. Pantic, *Rev Adv Mater Sci*, 49 (2017) 123-128.
- [13] D. Nikolovski, S. Dugalic, and I. Pantic, *J Microsc*, 268 (2017) 45-52.
- [14] I. Pantic, *Sci Prog*, 94 (2011) 97-107.
- [15] I. Pantic, *Rev Adv Mater Sci*, 37 (2014) 15-19.
- [16] I. Pantic, *Rev Adv Mater Sci*, 26 (2010) 67-73.
- [17] P.K. Mishra, H. Mishra, A. Ekielski, S. Talegaonkar, and B. Vaidya, *Drug Discov Today*, 22 (2017) 1825-1834.
- [18] C.H. Li, P.L. Liao, M.K. Shyu, C.W. Liu, C.C. Kao, S.H. Huang, et al., *Toxicol Sci*, 126 (2012) 162-172.
- [19] L. Wang, C. Chen, L. Guo, Q. Li, H. Ding, H. Bi, et al., *Artif Cells Nanomed Biotechnol*, 28 (2018) 1-12.
- [20] R. Roy, S.K. Singh, M. Das, A. Tripathi, and P.D. Dwivedi, *Immunology*, 142 (2014) 453-464.
- [21] R. Roy, D. Kumar, A. Sharma, P. Gupta, B.P. Chaudhari, A. Tripathi, et al., *Toxicol Lett*, 230 (2014) 421-433.
- [22] D.P. Bai, X.F. Zhang, G.L. Zhang, Y.F. Huang, and S. Gurunathan, *Int J Nanomedicine*, 12 (2017) 6521-6535.
- [23] J.W. Rasmussen, E. Martinez, P. Louka and D.G. Wingett, *Expert Opin Drug Deliv*, 7 (2010) 1063-1077.
- [24] S. Ostrovsky, G. Kazimirsky, A. Gedanken and C. Brodie, *Nano Res*, 2 (2009) 882- 890.
- [25] M.J. Akhtar, M. Ahamed, S. Kumar, M.M. Khan, J. Ahmad, and S.A. Alrokayan, *Int J Nanomedicine*, 7 (2012) 845-857.
- [26] A. Thurber, D.G. Wingett, J.W. Rasmussen, J. Layne, L. Johnson, D.A. Tenne, et al., *Nanotoxicology*, 6 (2012) 440-452.
- [27] G. Bisht and S. Rayamajhi, *Nanobiomedicine*, 3 (2016) 9.
- [28] C. Wang, X. Hu, Y. Gao, and Y. Ji, *Biomed Res Int*, 2015 (2015) 423287.
- [29] A.K. Sharma, V. Singh, R. Gera, M.P. Purohit, and D. Ghosh, *Mol Neurobiol*, 54 (2017) 6273-6286.
- [30] D. Guo, H. Bi, D. Wang, and Q. Wu, *Int J Biochem Cell Biol*, 45 (2013) 1849-1859.
- [31] D. Guo, H. Bi, Q. Wu, D. Wang, and Y. Cui, *J Nanosci Nanotechnol*, 13 (2013) 3769-3777.
- [32] X.Q. Liu, H.F. Zhang, W.D. Zhang, P.F. Zhang, Y.N. Hao, R. Song, et al., *Toxicol Lett*, 256 (2016) 19-32.
- [33] F. Xiaoli, W. Junrong, L. Xuan, Z. Yanli, W. Limin, L. Jia, et al., *Nanomedicine (Lond)*, 12 (2017) 777-795.
- [34] S. Amara, I.B. Slama, K. Omri, J. El Ghoul, L. El Mir, K.B. Rhouma, et al., *Toxicol Ind Health*, 31 (2015) 1202-1209.
- [35] S. Amara, I. Ben-Slama, I. Mrad, N. Rihane, M. Jeljeli, L. El-Mir, et al., *Nanotoxicology*, 8 Suppl 1 (2014) 208-215.
- [36] C. Chen, W. Bu, H. Ding, Q. Li, D. Wang, H. Bi and D. Guo, *Cell Prolif* 50 (2017).
- [37] L. Tian, B. Lin, L. Wu, K. Li, H. Liu, J. Yan, X. Liu, and Z. Xi, *Sci Rep* 5 (2015) 16117.
- [38] S. Sruthi and P.V. Mohanan, *Colloids Surf B Biointerfaces*, 133 (2015) 1-11.
- [39] M.G. Amer and R.A. Karam, *Anat Rec (Hoboken)* (2018).
- [40] M. Afifi and A.M. Abdelazim, *Asian Pacific Journal of Tropical Biomedicine*, 5 (2015) 874-877.