Turk Neurosurg 30(3):350-353, 2020

Original Investigation

DOI: 10.5137/1019-5149.JTN.21557-17.4

Received: 14.09.2017
Accepted: 24.04.2018
Published Online: 11.05.2018

Effects of Zinc Oxide on mRNA Expression of Genes in Brain Tissue of Rats

Inan GEZGIN[1], Cem OZIC[2]

[1]Dr. Ersin Aslan Education and Research Hospital, Neurosurgery Clinic, Gaziantep, Turkey
[2]Kafkas University, Faculty of Medicine, Department of Medical Biology, Kars, Turkey

Corresponding author: Inan GEZGIN ✉ gezgininan@gmail.com

ABSTRACT

AIM: To investigate the expression of the DR4, DR5, OPG, DcR1, and DcR2 in rat brain tissue.

MATERIAL and METHODS: Thirty rats were used in this study. The rats were divided into three groups as the control group (n=10), tumor group (n=10), and zincoxide (ZnO) nanoparticles (NP) treatment group (n=10). The reverse transcription polymerase chain reaction (RT-PCR) and Western Blotting methods were used to measure the expression of DR4, DR5, OPG, DcR1, DcR2 and β-actin in the brain tissues of all the three groups.

RESULTS: Expression of DR4, DR5, OPG, DcR1, and DcR2 genes were decreased in the tumor group. Overexpression of DR4, DR5, OPG, DcR1, and DcR2 was observed in brain tissues of the ZnO-NP group.

CONCLUSION: Increased expression of the DR4, DR5, OPG, DcR1, and DcR2 genes may play an important role in ZnO-NP treatment of brain tumors.

KEYWORDS: Brain, Nanoparticle, TRAIL, Tumor, Zinc oxide, Rat

INTRODUCTION

Nanoparticles (NP) are commercially used in health and fitness, electronics, food, beverage, cross cutting, appliances, cosmetics, clothing, personal care, sporting goods, sunscreens, and filtration. One of the main nanoparticles added to various materials and products is zinc oxide (ZnO) nanoparticles (11,14). For many years, ZnO nanoparticles have been used in cosmetics. There is very little information on the potential toxicity of ZnO nanoparticles although they are widely used (6,11). Several recent studies have detected the toxicity of ZnO nanoparticles in bacteria, nematodes, algae, cell lines, plants, rat, and mice (7,9,11,22,25) These studies revealed that the cytotoxicity of ZnO nanoparticles is possibly the result of cellular oxidative stress induction through the generation of reactive oxygen species and free radicals (8,11,21). In addition, a few recent studies showed the potential mechanisms of nanotoxicity in brain tumors (1,3,4,11).

It was also shown that the genotoxicity mechanism would correlate with the active oxygen production, oxidative stress, tumor, apoptosis, and anti-oxidant defence mechanisms (11,18).

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is a member of the cytokine TNF superfamily (15,16,24). The TNF family plays an important role in immune regulation. TRAIL causes cell differentiation, cell proliferation, and apoptosis when it binds to TNF receptors (17). Five receptors for TRAIL have been identified including DR4 (TRAIL-R1), DR5 (TRAIL-R2), DcR1 (TRAIL-R3), DcR2 (TRAIL-R4), OPG (5).

To determine the brain tumor etiology, we investigated the over expressions of the DR4, DR5, OPG, DcR1, DcR2 genes in brain tissues. To the best of our knowledge, there is no available study on DR4, DR5, and OPG gene expressions in brain tissues. In this study, we evaluated the effects of ZnO nanoparticles on the gene expression pattern of brain tissue.

Inan GEZGIN : 0000-0003-1284-0464 Cem OZIC : 0000-0001-7676-817X

Total ribonucleic acid (RNA) was prepared from the ZnO nanoparticles, with different surface charges and exposed groups, and the reverse transcription polymerase chain reaction (RT-PCR) and Western Blotting methods were used to detect the complementary deoxyribonucleic acid (cDNA) and protein levels.

MATERIAL and METHODS

Biological Samples

All the procedures adhered to the tenets of the Declaration of Helsinki and were approved by the Institutional Review Board of the Kafkas University (2017/35). Thirty adult Wistar albino white rats were used in this study. Rats were divided into three groups as control (n=10), tumor group (rats were given diethylnitrosamine 150 mg/kg) (n=10), and other group (ZnO-NP 300 mg treatment groups; n=10). ZnO-NP was administered by oral gavage. The total RNA obtained from brain tissues was analyzed to determine the DR4, DR5, OPG, DcR1, and DcR2 gene expression levels.

RT-PCR Analysis

Brain tissues were subjected to RNA isolation employing a previously reported phenol/chloroform extraction method by Chomczynski and Sacchi (2). The TRIzol reagent (Sigma) was used to isolate the total RNA from formalin fixed and paraffin embedded specimens. The total RNA was treated with RQ1 DNAse I (Promega, USA). Reverse transcription (RT) was performed according to the manufacturer's directions (Fermentas, USA) using 1 unit of MMLV reverse transcriptase with 5 µg of total RNA and oligo dT22 primer. PCR was performed with 1 µL of diluted cDNA (1:10) in a total volume of 25 µL using Taq DNA Polymerase enzyme for 27 cycles in the exponential range with DR4, DR5, OPG, DcR1, DcR2, and β-actin primers. The RT-PCR assay was used to detect the differential expressions of DR4, DR5, OPG, DcR1, DcR2, and β-actin between brain tissues. β-actin was used as an internal control to normalize samples.

Western Blot Analysis

Expression of the recombinant protein was induced by the addition of 100 µM isopropyl β–D thiogalactopyranoside (IPTG) in *E. coli* BL21 (DE3) carrying pET16b (+) of DR4, DR5, OPG, DcR1, DcR2, and β-actin, and incubation was continued for a further 3 hours at 37°C. Purification of soluble PON1 through Ni-NTA resin (QIAGEN, Germany) column chromatography was performed according to the manufacturer's instructions. Analysis of the purified proteins on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out with Coomassie brilliant blue staining. Proteins were transferred from the gel to a polyvinylidene fluoride filter (PVDF; Immobilon-P, Millipore). For western blotting, the ProteoQwest colorimetric kit with TMB substrate (Sigma, USA) was used with mouse monoclonal anti-His6 antibody and HRP-conjugated secondary antimouse antibody. Kaleidoscope prestained standards were used as molecular-mass markers (15).

RESULTS

To determine DR4, DR5, OPG, DcR1, DcR2, and β-actin (control) gene expression, thirty rats in the three groups were used. The RT-PCR method was used to investigate the mRNA expression levels of DR4, DR5, OPG, DcR1, DcR2, and β-actin. The mRNA levels of DR4, DR5, OPG, DcR1, DcR2, and β-actin were shown in the control group (Figure 1A), tumor group (Figure 1B) and ZnO-NP group (Figure 1C). The DR4, DR5, OPG, DcR1, DcR2 genes in the tumor group showed a decrease in the gene expression levels when they were analyzed by RT-PCR (Figure 1B); however, the DR4, DR5, OPG, DcR1, DcR2 genes showed an increase in the gene expression levels when they were analyzed by RT-PCR in the ZnO-NP group (Figure 1C). In case of discrepancy, the RT-PCR results were used for classification. Therefore, DR4, DR5, OPG, DcR1 and DcR2 overexpression was confirmed by both RT-PCR.

PCR products were cloned into the pET16b and transformed into *E. coli* BL21 (DE3). SDS-PAGE analysis with Coomassie blue staining showed that the affinity purified recombinant protein was expressed as soluble protein after IPTG induction (Figure 2). The protein can be purified using the nickel agarose. The DR4, DR5, OPG, DcR1, DcR2, and β-actin (control protein) protein showed a decrease in the protein expression levels when they were analyzed by western blotting.

DISCUSSION

It has been demonstrated in recent years that nanomaterials including ZnO-NPs may cause toxic effects on the tissue through genotoxicity, cytotoxicity, the induction of oxidative stress, inflammation (20), and fibrosis (23). These adverse effects are also involved in the onset and progression of neurodegeneration (19). Thus, the relationship between nanomaterial exposure and their capability to induce neurodegeneration has received substantial attention (12).

It has been well demonstrated that the induction of oxidative stress plays a critical role in the common mechanism of nanoparticle toxicity (10). Prolonged and serious oxidative stress may cause an increase in inflammation via the activation of inflammation-related genes (13).

In two studies, when oxidative stress level is increased in the brain, impaired learning and memory abilities, and hippocampal pathological changes were identified especially in old mice following ZnO-NP exposure (11,18). In this study, we used cDNA and protein to study the effects of ZnO nanoparticles on gene expression. The up and down-regulated genes by ZnO nanoparticles exposure belonged to the cytokine TNF superfamily of TRAIL, and 5 genes.

This study has some limitations by the ZnO-NP. The mechanism of the increase expression of the DR4, DR5, OPG, DcR1, and DcR2 genes may play an important role in the ZnO-NP brain tumors.

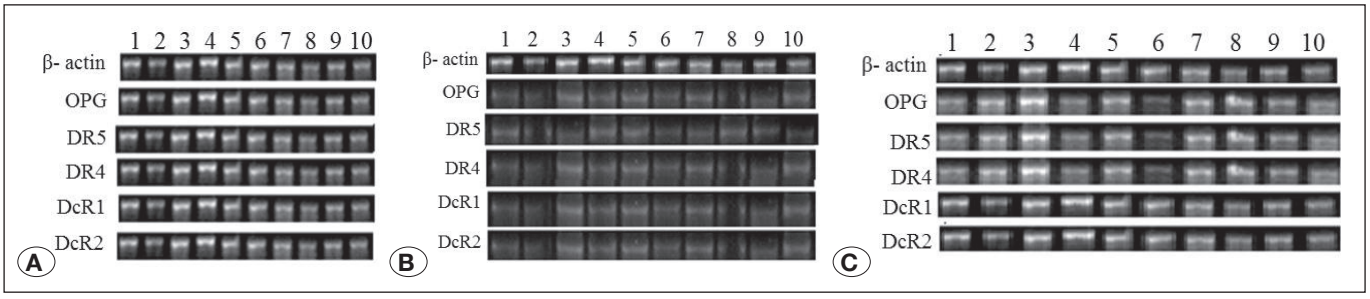

Figure 1: Agarose gel electrophoresis of the RT-PCR amplified fragments DR4, DR5, OPG, DcR1, DcR2, and β-actin (control). Control group **(A)**, tumor group **(B)**, and ZnO-NP group **(C)** brain tissues used DR4, DR5, OPG, DcR1, DcR2, and β-actin (control) expression. After RT-PCR, cDNA products were analyzed with 1% agarose gel including ethidium bromide.

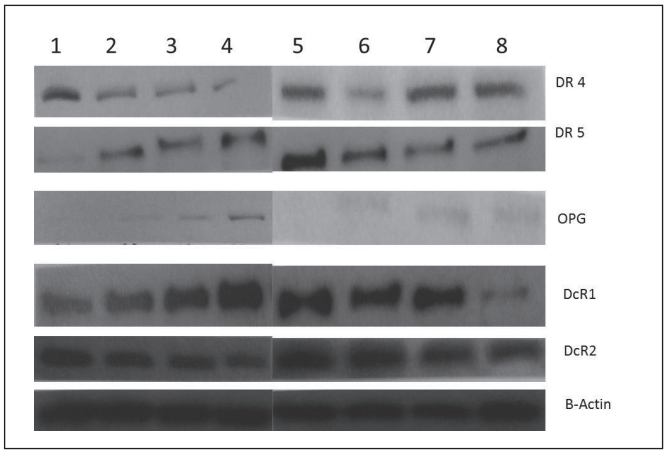

Figure 2: Western blot analysis of affinity purified recombinant DR4, DR5, OPG, DcR1, DcR2, and β-actin proteins for ZnO-NP group. Western blot analysis: DR4, DR5, OPG, DcR1, DcR2, and β-actin proteins were purified with Ni-NTA agarose. Proteins were separated with 12% SDS-PAGE and blotted to PVDF membranes. The membranes were probed with a mouse monoclonal anti-6XHis antibody, 1:1000 (Ni-NTA agarose).

CONCLUSION

The major finding of the study is that DR4, DR5, OPG, DcR1, and DcR2 genes are significantly associated with the risk of brain tumors. We revealed that the DR4, DR5, OPG, DcR1, and DcR2 overexpression was associated with increased risk which may play an important role in ZnO-NP treatment of tumors.

REFERENCES

1. Ahamed M, Akhtar MJ, Raja M, Ahmad I, Siddiqui MK, AlSalhi MS, Alrokayan SA: ZnO nanorod-induced apoptosis in human alveolar adenocarcinoma cells via p53, survivin and bax/bcl-2 pathways: Role of oxidative stress. Nanomedicine 7:904-913, 2011
2. Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Analytical Biochemistry 162:156-159,1987
3. De Berardis B, Civitelli G, Condello M, Lista P, Pozzi R, Arancia G, Meschini S: Exposure to ZnO nanoparticles induces oxidative stress and cytotoxicity in human colon carcinoma cells. Toxicol Appl Pharmacol 246:116-127, 2010
4. Deng X, Luan Q, Chen W, Wang Y, Wu M, Zhang H, Jiao Z: Nanosized zinc oxide particles induce neural stem cell apoptosis. Nanotechnology 20:111-118, 2009
5. Emery J, McDonnell P, Burke M, Deen K, Lyn S, Silverman C, Dul E, Appelbaum E, Fogh J, Fogh JM, Orfeo T: One hundred and twenty-seven cultured human tumor cell lines producing tumors in nude mice. J Natl Cancer Inst 59(1):221–226, 1977
6. Fan Z, Lu JG: Zinc oxide nanostructures: Synthesis and properties. J Nanosci Nanotechnol 5:1561-1573, 2005
7. Franklin NM, Rogers NJ, Apte SC, Batley GE, Gadd GE, Casey PS: Comparative toxicity of nanoparticulate ZnO, bulk ZnO, and ZnCl2 to a freshwater microalga (Pseudokirchneriella subcapitata): The importance of particle solubility. Environ Sci Technol 41: 8484-8490, 2007
8. Huang CC, Aronstam RS, Chen DR, Huang YW: Oxidative stress, calcium homeostasis, and altered gene expression in human lung epithelial cells exposed to ZnO nanoparticles. Toxicol in Vitro 24:45-55, 2010
9. Huang Z, Zheng X, Yan D, Yin G, Liao X, Kang Y, Yao Y, Huang D, Hao B: Toxicological effect of ZnO nanoparticles based on bacteria. Langmuir 24:4140-4144, 2008
10. Karmakar A, Zhang QL, Zhang YB: Neurotoxicity of nanoscale materials. J Food Drug Anal 22: 147–160, 2014
11. Lee SH, Pie JE, Kim YR, Lee HR, Son SW, Kim MK: Effects of zinc oxide nanoparticles on gene expression profile in human keratinocytes. Mol Cell Toxicol 8:113-118, 2012
12. Migliore L, Uboldi C, Di Bucchianico S, Coppedè F: Nanomaterials and neurodegeneration. Environ Mol Mutagen 56: 149–170, 2015
13. Nguyen HX, O'Barr TJ, Anderson AJ: Polymorphonuclear leukocytes promote neurotoxicity through release of matrix metalloproteinases, reactive oxygen species, and TNF-alpha. J Neurochem 102: 900–912, 2007
14. Osmond MJ, McCall MJ: Zinc oxide nanoparticles in modern sunscreens: An analysis of potential exposure and hazard. Nanotoxicology 4:15-41, 2010

15. Ozic C, Arslanyolu M: Characterization of affinity tag features of recombinant Tetrahymena thermophila glutathione-S-transferase zeta for Tetrahymena protein expression vectors. Turk J Biol 36: 513–526, 2012
16. Pitti R, Marsters S, Ruppert S, Donahue C, Moore A, Ashkenzai A: Induction of apoptosis by Apo-2 ligand, a new member of the tumor necrosis factor cytokine family. J Biol Chem 271:12687–12690, 1996
17. Song J, Song D, Pyrzynska B, Petruk K, Van Meir E, Hao C: TRAIL triggers apoptosis in human malignant glioma cells through extrinsic and intrinsic pathways. Brain Pathol 13:539–553, 2003
18. Tian L, Lin B, Wu L, Li K, Liu H, Yan J, Liu X, Xi Z: Neurotoxicity induced by zinc oxide nanoparticles: Age-related differences and interaction. Sci Rep 5: 16117, 2015
19. Urrutia PJ, Mena NP, Núñez MT: The interplay between iron accumulation, mitochondrial dysfunction, and inflammation during the execution step of neurodegenerative disorders. Front Pharmacol 5: 38, 2014
20. Win-Shwe TT, Fujimaki H: Nanoparticles and neurotoxicity. Int J Mol Sci 12: 6267–6280, 2011
21. Xia T, Kovochich M, Liong M, Mädler L, Gilbert B, Shi H, Yeh HI, Zink JI, Nel A: Comparison of the mechanism of toxicity of zinc oxide and cerium oxide nanoparticles based on dissolution and oxidative stress properties. ACS Nano 2: 2121-2134, 2008
22. Yang H, Liu C, Yang D, Zhang H, Xi Z: Comparative study of cytotoxicity, oxidative stress and genotoxicity induced by four typical nanomaterials: The role of particle size, shape and composition. J Appl Toxicol 29:69-78, 2009
23. Yildirim CH, Yucetas SC, Kaya M, Ozic C, Balioglu MB, Ustun H, Tasdemiroglu E, Akbasak A: Alpha-lipoic acid inhibits peridural fibrosis following laminectomy through the inactivation of TGF-β1, PDGF, PAI-1 and IL-6 expressions. Turk Neurosurg 25(1):90-99, 2015
24. Zerafa N, Westwood J, Cretney E, Mitchell S, Waring P, Iezzi M, Smyth M: Cutting edge: TRAIL deficiency accelerates hematological malignancies. J Immunol 175:5586–5590, 2005
25. Zhu X, Zhu L, Duan Z, Qi R, Li Y, Lang Y: Comparative toxicity of several metal oxide nanoparticle aqueous suspensions to Zebrafish (Danio rerio) early developmental stage. J Environ Sci Health A Tox Hazard Subst Environ Eng 43(3): 278-284, 2008