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Nanoparticles Induce Oxidative Stress in HT-29 Colon Adenocarcinoma Cell Line After 24 and 48 Hour Exposure

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Abstract: Nanoparticle research is currently an area of intense scientific research, due to a wide variety of potential applications in biomedical, optical, and electronic fields. Nanoparticles are of great scientific interest as they are effectively a bridge between bulk materials and atomic or molecular structures. Superoxide dismutase (SOD), Glutamine synthetase (GS), Catalase (CAT) are some of the defense mechanisms against cellular oxidative stress, especially against free oxygen radicals. In this study, we aimed to investigate that Ag, SiO_2 and ZnO nanoparticles affect cancer cell lines (HT-29) and relationship between SOD, GS and CAT. We investigated that alterations in gene expressions of SOD, GS and CAT caused by exposure to nanoparticles in HT-29 cells. The difference between the Ct values (ΔCt) of the gene of interest was calculated for each experimental sample. As a result of Ag, SiO_2 and ZnO nanoparticle application, there was a 2-fold increase in SOD and CAT expression in the first 24 hours compared to control. As a result of 48 hours of application, it was observed that Ag nanoparticles caused 4-fold increase in SOD and 6-fold statistically significant increase in CAT and GS expression of SiO_2 nanoparticles. Consequently, after 48 hours of nanoparticle application, SiO_2, CAT and GS expression were more effective than 24-hour application. Our results suggest that nanoparticles may cause increased oxidative stress in colon cells and may have therapeutic properties by affecting cancer cells in these aspects.

Keywords: Colon Adenocarcinoma, Nanoparticles, Oxidative Stress, SOD, GS, CAT

1. Introduction

Materials with dimensions between 1 and 1000 nm are counted as nanomaterials. Since the ratio of nanomaterials to volume of surface areas are much greater than their macro-size states, this free surface energy increases the reactivity of nanoparticles. Since they are generally in the form of needles, they provide an advantage in passing the drugs which they carry with them easily to pass through the cell membrane. While many studies are currently being conducted on the clinical suitability of nanomaterials, there are some studies that are the promising treatments and diagnosis of diseases, especially cancer, due to the physical and chemical properties of these materials [1].

Oxidative stress includes the formation of free radicals, in particular reactive oxygen species such as superoxide molecule, singlet oxygen, hydrogen peroxide and hydroxyl radicals (OH) and other reactive oxygen species. Hydrogen peroxide is a stable ROS and increases under various stress conditions. Most of the hydrogen peroxide (H_2O_2) formed in the cells is formed by catalyzing superoxide radicals with superoxide dismutase enzyme [2, 3]. Superoxide anion, H_2O_2 and hydroxyl radical are biological products produced by reduction of oxygen molecule. Therefore, free radicals play an important role in many disease processes, such as myocardial infarction, diabetes, cancer, cataract, rheumatoid arthritis, infertility, respiratory, nervous and urinary system diseases, have been reported by many researchers [4]. Superoxide dismutase is an enzyme that alternately catalyzes the decomposition of superoxide radical into ordinary

molecular oxygen or hydrogen peroxide [5, 6]. Glutamine synthetase is an enzyme that plays an important role in nitrogen metabolism by catalyzing the condensation of glutamate and ammonia to form glutamine [7-10]. Catalase is a common enzyme found in almost all living things and its separates hydrogen peroxide into water and oxygen [11]. Antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) Glutamine synthetase (GS) protect the organism against these radicals [4, 5, 12].

2. Material and Methods

An cellular oxidative stress pathway indicators for expression of superoxide dismutase, glutamine synthetase and catalase was used to investigate alterations in gene expression caused by exposure to nanoparticles in HT-29 cells. After treatment with IC_{50} concentrations of nanoparticles approximately $5x10^6$ cells were collected for RNA extraction using with Rneasy Mini Kit (Qiagen, Valencia, CA, USA) [13].

2.1. Cell Lines

The cells were incubated with Dubelco's Modified Eagle Medium (EMEM) (Thermo Fisher 11855-092) containing 10% fetal bovine serum (FBS) (Thermo Fisher 1050064) for vital activities in an incubator at 37°C with 5% CO_2. All applications to cells were made in a horizontal air flow safety cabinet and aseptic rules were followed in order to prevent infection of cells. In addition, 1% antibiotic antimycotic (Thermo Fisher 15240062) was added to medium of cells to prevent growth of microorganisms. The experiments were performed between 10 and 20 cell passages. Cells were viable in $75cm^2$ flasks during experiments. Cells were exchanged twice a week and passaged 1:4 when 90% cell concentration was reached in flask [14]. These cell lines were applied with control group that introduced same amount of solvent as nanomaterials. After this application, MTT test was performed and lethal dose (LD_{50}) value was calculated by probit analysis. As a result of LD of this test, gene expression was investigated in nanoparticle with sufficient LD_{50} value. Cells were used in the research by determining live/dead ratios in hemocytometer under inverted microscope. Cell and reagent-free medium were used as negative control. In study, each parameter was studied in triplicate and cells were incubated up to 24 and 48 hours.

2.2. Nanoparticles Doses

In the study, it was mainly found that HT-29 cells were found to be 50% viable in the MTT test, 1.58 ppm for zinc nanoparticles in 24 hours application and 20.4 ppm in 48 hours, for silver 2.04 ppm in 24 hours and 23 ppm, for silicon nanoparticles 5 ppm in 24 hours and 42, 37 ppm dosages for 48 hours application.

2.3. RNA Extraction and RT-PCR Analysis

Total RNA from cells was extracted with Trizol PureLink™ RNA Mini Kit (Cat no: 12183018A, Invitrogen, USA) and the quantity and quality of RNA was determined by Nanodrop Spectrometer the Nano-200 Aosheng, and confirmed by gel electrophoresis, which contains 1.5% agarose and formaldehyde. Reversely transcribed to Superscript II (Invitrogen, USA). SYBR green dye (Takara Bio Inc., Japan) was used for amplification of cDNA. Real-time PCR reactions were carried out by using SYBR green dye Gene Expression Assays (Applied Biosystems), which were designed for SOD, GS and CAT. Results of RT-PCR reactions were analyzed by using ΔCt method. mRNA levels and the standard actin (ACTB) were measured by Real-time PCR was performed using Applied Biosystems™ StepOnePlus™ Real-Time PCR System. The sequences of primers of the target gene superoxide dismutase (SOD, NCBI, Entrez Gene ID: 6647), glutamine synthetase (GLUL, NCBI, Entrez Gene ID: 2752), Catalase (CAT, NCBI Entrez Gene ID: 847) and actin (ACTB, NCBI Entrez Gene ID: 60) [15]

2.4. Statistic Analysis

Statistical significance between all groups was calculated with SPSS v.19 one-way ANOVA and $p<0.01$ and $p<0.05$ were taken.

3. Results

According to our findings, in the treatment of cells with nanoparticles, HT-29 cells survived in 24-hour nanoparticle application 50% of the doses of Ag, Zn, SiO_2 respectively 1.5, 2, 5 ppm, while 48 hours MTT viability observed doses respectively 20, 23, 42 ppm. According to the results of the dose increase, 24-hour lethal doses of the nanoparticles were at very low doses, while the lethal dose amount increased approximately ten times after the 48-hour administration compared to 24 hour application ($p<0.01$).

3.1. Gene Expression After 24 Hours

As a result of this study, we used SOD, GS and CAT expression as an indicator of oxidative stress as a result of application of related nanoparticles. SOD expression was increased 3 times more in Ag and ZnO group than nanoparticle application and 2 times statistically significant increase in SiO_2 group (Figure 1A). Expression of CAT in Ag and ZnO nanoparticles groups were increased by 4 times compared to control and 3 times with SiO_2 (Figure 1B). Interestingly, however, the increase in GS was less significantly than SOD and CAT. ZnO and Ag treated cells increase were approximately 0.5 times and SiO_2 increase was twice (Figure 1C).

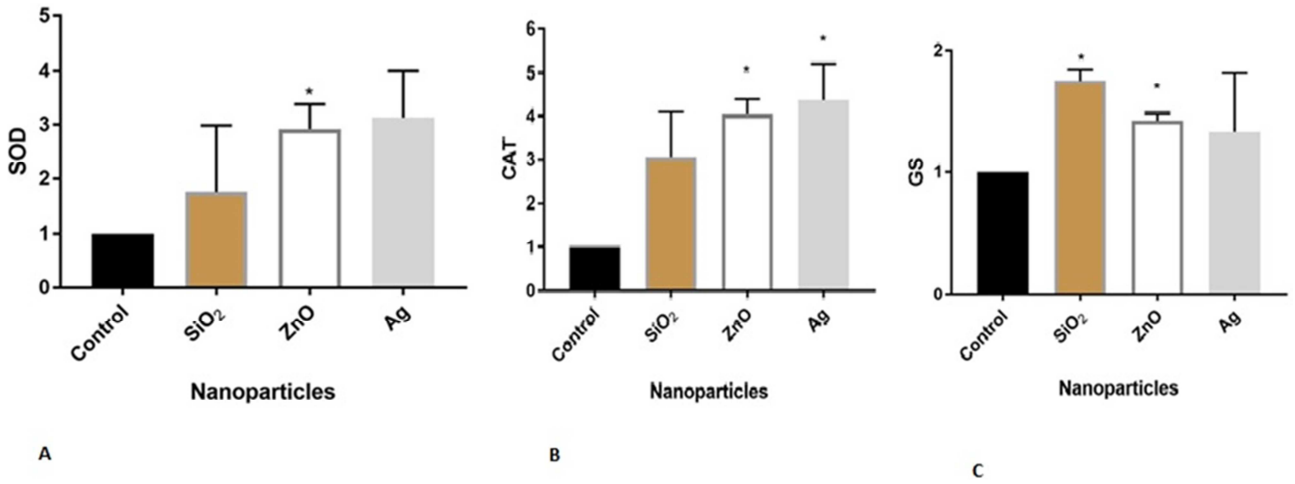

Figure 1. *After 24 hours nanoparticle application A: SOD expression. B: CAT expression C: GS expression.*

3.2. Gene Expressions After 48 Hours

As a result of the 48-hour application of these nanoparticles, increase in expression in SOD was approximately 4-fold in the Ag and increase approximately 2 and 1-fold in ZnO and SiO_2 respectively (Figure 2A). The increase in GS was 4-fold in SiO_2 treated group while the increases in ZnO and Ag were relatively low and 2-fold and 3-fold GS expression respectively (Figure 2B). However, CAT expression was examined, the increase in CAT expression in the SiO_2 group increased 6-fold compared to the control and became the highest expression level in the study. The increase in Ag group was 5-fold, whereas the increase in ZnO group was 3rd times (Figure 2C).

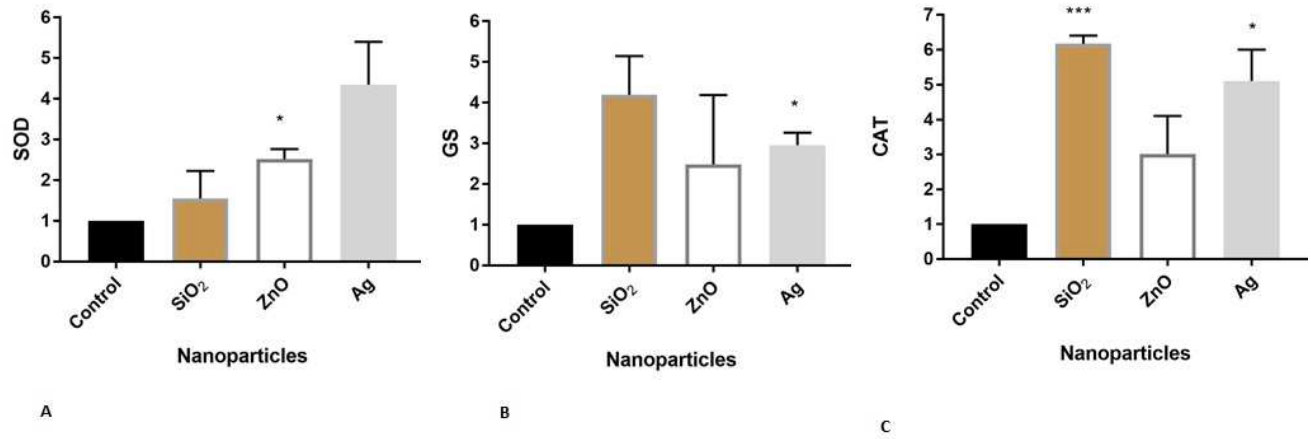

Figure 2. *After 48 hours nanoparticle application A: SOD expression. B: CAT expression. C: GS expression.*

As a result of the 48-hour application of these nanoparticles, increase in expression that SOD was approximately 4-fold in the Ag and increase approximately 2 and 1-fold in ZnO and SiO_2 respectively (Figure 2A). The increase in GS was 4-fold in SiO_2 treated group while the increases in ZnO and Ag were relatively low and 2-fold and 3-fold GS expression respectively (Figure 2B). However, CAT expression was examined, the increase in CAT expression in the SiO_2 group increased 6-fold compared to the control and became the highest expression level in the study. The increase in Ag group was 5-fold, whereas the increase in ZnO group was 3rd times (Figure 2C).

4. Discussion

In nanoscale, the surface area of the particles increases so that they can be more penetrated into the cell. For example; gold particles are almost universally very stable molecules in micro-dimensions, whereas nanoscale can be highly reactive with other molecules in using of nanomedicine [16]. Furthermore, it was also reported that nanoparticles were agglomerated due to their inhomogeneous solvent structures during application. Therefore, they could not interact well with biological systems and different effects could be observed [17].

When the literature on this subject is searched, it is seen that especially gold nanoparticles are more researched, gold conjugated with protein peptides can interact with cancer cell DNA and break DNA bonds and increase apoptosis through mechanisms [18]. In another study, it was found that gold nanoparticles decreased bone mineralization by changing the surface electrical properties [19]. It has been shown that

silver nanoparticles can have toxic effects due to the increase of SOD and GSH in HepG2 human liver cells and that the toxicity of silver nanomolecules does not change regardless of their size [20]. In another study with gold nanoparticles, triangular shapes with pointed ends from star, rods or triangular shaped nanoparticles were shown to be more effective especially entering inside cells and it was stated that it could change its toxicity [21]. In addition, silver nanoparticles have been shown to have strong apoptotic effect due to ROS and thus have intracellular toxic properties [22]. In addition, silver nanoparticles have been shown to be cytotoxic in lung cancer cells via ROS mechanism and mitochondrial damage, and antioxidant applications have been shown to decrease this cytotoxic effect before application [23]. In another study, it was shown that poly vinyl pyrrolidone coated silver nanoparticles induce apoptosis in human monocytic leukemia cell lines by ROS mechanism, but not oxidative stress in HepG2 and Caco cells [24].

Our results were quite interesting. As a result of 24 and 48 hours application of HT-29 cells and IC_{50} ratio was increased approximately 10 times in the 24 hour interval. In 24 hours, the average doses from 1.5 to 5 ppm of these cells were quite lethal, whereas in the same cell line the lethal doses increased approximately 10 times after 48 hours. We could not reach a clear information in the literature to explain this, but probably reason is that the oxidative defense mechanisms of cancer cells are quite effective in the second 24-hour period. In fact, considering 48-hour data, it has been suggested that increase in CAT expression may be effective. We found that in 24-hour period, Ag particles increased CAT and GS expression, while SiO_2 particles increased GS expression specifically increases in SOD, GS, and CAT expressions over the 48-hour period compared to the 24-hour period indicate that the nanoparticles were applied to induce hydrogen peroxide formation, especially by water-based ionization, and may increase CAT and SOD activation in response to this by the cells. We think that ammonia in the environment may be increased due to protein and amino acid degradation of SiO_2 nanoparticle and it may have induced GS increase.

5. Conclusions

Since there are no other studies showing dose and oxidative stress in 24-48 hour like our study, we think that it may be more informative to investigate the stress caused by nanoparticles on cancer cells by shorter and wider time intervals with dose adjustment. Our results in our present study support other studies in which nanoparticles have been shown to be oxidative stress enhancing factors. In addition, it was observed that lethal doses of the same nanoparticles could change in different time periods. Therefore, we showed that the lethal doses of nanoparticles in colon cells are effective during the first 24 hours, during which time the therapeutic effect can be increased by additional apoptosis enhancing or lethal applications such as chemotherapy. In the future, we anticipate that nanoparticles can be used as therapeutic agents with other mechanisms such as chemo-radiotherapy. Limitations of our study, the effects of Ag, SiO_2 and ZnO nanoparticles SOD, CAT, GS expressions were investigated at the RNA level but the activity of these enzymes was not measured that it was limited our results. In addition, the fact that morphological changes in the structure of the cells could not be observed by microscope and the effects of these nanoparticles on DNA and RNA were not shown that it is another limitation of our study.

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References

[1] R. G. Haverkamp, A. T. Marshall, D. van Agterveld, (2007). Pick your carats: nanoparticles of gold–silver–copper alloy produced in vivo. Journal of Nanoparticle Research.; 9 (4): 697-700.

[2] J. S. Han, J. H. Lee, J. Y. Kong, J. Kim, S. S. Choe, et al. (2019). Hypoxia Restrains Lipid Utilization via Protein Kinase A and Adipose Triglyceride Lipase Downregulation through Hypoxia-Inducible Factor. Molecular and cellular biology: 39 (2): e00390-18.

[3] A. Holley, J. Pitman, J. Miller, S. Harding, P. Larsen, (2017). Glutathione peroxidase activity and expression levels are significantly increased in acute coronary syndromes. Journal of Investigative Medicine. 65 (5): 919-25.

[4] L. Banjac, G. Banjac, J. Kotur-Stevuljević, V. Spasojević-Kalimanovska, T. Gojković, N. Bogavac-Stanojević, et al. (2018). Pro-Oxidants and Antioxidants in Retinopathy of Prematurity. Acta clinica Croatica: 57 (3): 458-63.

[5] J. Momen-Beitollahi, A. Mansourian, F. Momen-Heravi, M. Amanlou, S. Obradov, M. Sahebjamee, (2010). Assessment of salivary and serum antioxidant status in patients with recurrent aphthous stomatitis. Med Oral patol Oral cir bucal. 15 (4): e557-61.

[6] A. J. Bott, S. Maimouni, W-X. Zong, (2019). The Pleiotropic Effects of Glutamine Metabolism in Cancer. Cancers. 11 (6): 770.

[7] J. Yang. (2018). The role of reactive oxygen species in angiogenesis and preventing tissue injury after brain ischemia. Microvascular research: 123; 62–67.

[8] R. Alili, V. Nivet-Antoine, A. Saldmann, J-L. Golmard, C-H. Cottart, C. Laguillier, et al. (2018). Human catalase gene promoter haplotype and cardiometabolic improvement after bariatric surgery. Gene: 656: 17-21.

[9] T. Nauser, J. M. Gebicki. (2019). Fast reaction of carbon free radicals with flavonoids and other aromatic compounds. Archives of biochemistry and biophysics: 108-107.

[10] Y. Chen, S. Zheng, Z. Ju, C. Zhang, G. Tang, J. Wang, et al. (2018). Contribution of peroxisomal docking machinery to mycotoxin biosynthesis, pathogenicity and pexophagy in the plant pathogenic fungus Fusarium graminearum. Environmental microbiology. 20 (9): 3224-45.

[11] A. H. Huang, (2012). Plant peroxisomes: Elsevier; pp 112-115.

[12] A-K. Mix, U. Cenci, T. Heimerl, P. Marter, M-L. Wirkner, D. Moog. (2018). Identification and Localization of Peroxisomal Biogenesis Proteins Indicates the Presence of Peroxisomes in the Cryptophyte Guillardia theta and Other "Chromalveolates". Genome biology and evolution. 10 (10): 2834-52.

[13] C-C. Huang, R. S. Aronstam, D-R. Chen, Y-W. Huang. (2010). Oxidative stress, calcium homeostasis, and altered gene expression in human lung epithelial cells exposed to ZnO nanoparticles. Toxicology in vitro. 24 (1): 45-55.

[14] T. J. Goodwin, J. M. Jessup, D. A. Wolf. (1992). Morphologic differentiation of colon carcinoma cell lines HT-29 and HT-29KM in rotating-wall vessels. In Vitro Cellular & Developmental Biology-Animal. 28 (1): 47-60.

[15] K. Ferro, D. Ferro, F. Corrà, R. Bakiu, G. Santovito, J. Kurtz J, (2017). Cu, Zn superoxide dismutase genes in Tribolium castaneum: evolution, molecular characterisation, and gene expression during immune priming. Frontiers in immunology. 8: 1811.

[16] B. Yadav, R. Srivastava, C. Dwivedi, P. Pramanik. (2008). Moisture sensor based on ZnO nanomaterial synthesized through oxalate route. Sensors and Actuators B: Chemical. 131 (1): 216-22.

[17] A. Albanese, C. W. Chan. (2011). Effect of gold nanoparticle aggregation on cell uptake and toxicity. ACS nano. 5 (7): 5478-89.

[18] K. Banerjee, V. R. Rai, M. Umashankar. (2019). Effect of peptide-conjugated nanoparticles on cell lines. Progress in biomaterials. 8 (1): 11-21.

[19] X-Q. Liu, R-Z. Tang. (2017). Biological responses to nanomaterials: understanding nano-bio effects on cell behaviors. Drug delivery. 24 (2): 1-15.

[20] W. Liu, Y. Wu, C. Wang, H. C. Li, T. Wang, C. Y. Liao, et al. (2010). Impact of silver nanoparticles on human cells: effect of particle size. Nanotoxicology. 4 (3): 319-30.

[21] Js. Mosquera, M. Henriksen-Lacey, I. García, M. Martínez-Calvo, Js. Rodríguez, J. L. Mascareñas, et al. (2018). Cellular uptake of gold nanoparticles triggered by host–guest interactions. Journal of the American Chemical Society. 140 (13): 4469-72.

[22] K. Kang, H. Jung, J-S. Lim. (2012). Cell death by polyvinylpyrrolidine-coated silver nanoparticles is mediated by ROS-dependent signaling. Biomolecules & therapeutics. 20 (4): 399.

[23] R. Foldbjerg, D. A. Dang, H. Autrup. (2011). Cytotoxicity and genotoxicity of silver nanoparticles in the human lung cancer cell line, A549. Archives of toxicology. 85 (7): 743-50.

[24] R. Foldbjerg, P. Olesen, M. Hougaard, D. A. Dang, H. J. Hoffmann, H. Autrup. (2009). PVP-coated silver nanoparticles and silver ions induce reactive oxygen species, apoptosis and necrosis in THP-1 monocytes. Toxicology letters. 190 (2): 156-62.