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EVALUATION OF TOXICITY AND ANTI-METASTATIC EFFECT OF CERIUM OXIDE NANOPARTICLES IN THE MCF-7 AND T47D BREAST CANCER CELL LINES

Ali Rezvani Dehaghani*[1], Sourena Rezvani Dehaghani[2], Dr. Najmeh Ghanbari[3]

[1]Master Holder of Drug Quality Assurance, Tehran University of Medical Science, Iran, Tehran.
[2]Medicine Student, Qom University of Medical Science, Iran, Qom.
[3]Doctor of Pharmacy, Tehran, University of Medical Science, Iran, Tehran.

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***Corresponding Author**
Ali Rezvani Dehaghani
Master Holder of Drug Quality Assurance, Tehran University of Medical Science, Iran, Tehran.
a-rezvanid@alumnus.tums.ac.ir

ABSTRACT

Aim: in the present study, anti-metastatic and toxicity effects of cerium oxide nanoparticles on the MCF-7 and T47D breast cancer cell lines were determined. **Material and Method:** T47D and MCF-7 breast cancer cell lines and HEK293 normal cell lines were treated with 6.5 mg, 65 and 650 µg and 65 and 650 ng of cerium oxide nanoparticles. MTT analysis was used for the evaluation of nanoparticles toxicity effect. The expression level of MN23 and KAI-I genes were measured by real-time PCR method. **Results:** In the 650 µg and 6.5 mg concentration of CNP, nearly 60% of MCF-7 and T47D were dead, respectively. Also, in the normal cells treated with 6.5 mg of CNP, survival reduced by 25%. According to the gene analysis results, the expression of NM23 and KAI-I anti-tumor genes was not increased in the presence of CNP. **Conclusion:** Based on the present study results, the toxic effect of cerium oxide nanoparticles on breast cancer cells was confirmed. However, the effect was dose and cell line depended. On the other hand, evaluation of anti-metastatic genes expression showed that the cerium oxide nanoparticles could not elevate gene expression and therefore ineffective in controlling cell invasion and probably induced anti-cancer effects via the apoptosis pathway.

KEYWORDS: *Breast cancer, metastasis, cerium oxide nanoparticles, MCF-7, T47D.*

INTRODUCTION

Breast cancer is one of the common types of cancer which causes high mortality among women. Despite many advances in early diagnosis and treatment, cancer remains the leading cause of cancer deaths among women.[1] Different types of breast cancer include ductal carcinoma in situ, lobular carcinoma in situ, inflammatory breast cancer, and recurrent breast cancer. Different treatment strategies were developed for breast cancer, which most common types of treatment include surgery, radiotherapy, and chemotherapy and hormone therapy.[2,3]

Nowadays, nano is one of the main technologies in applied science, especially the pharmaceutical industry. Recently basic and clinical science researchers focused on the using of nanotechnology for cancer treatment.[4] Metal oxide nanoparticles are one of the main compounds and candidates for cancer treatment. A cerium oxide nanoparticle (CNP) contains both Ce3+/Ce4+ in their metal nucleus which surrounded by oxygen atoms. Each of these cations gives a special role to the nanoparticles. Ce3+ led to nanoparticle reduction activity, whereas Ce4+ has oxidative activity. Ce3+/Ce4+ ratio in the CNP surface depends on the microenvironment surrounded the nanoparticles. The CNP nano-drug act via induction of oxidation-reduction reactions. Therefore, the most important application of these nanoparticles is in reactive oxygen species-related mechanisms which play a role in free radicals production. In cancer biology, this mechanism is important in energy production and intracellular activity adjustment. Since cancer is an uncontrolled proliferation of the cell, therefore, this pathway is also impaired in cancer. The antioxidant activity of CNP which acts like superoxide dismutase and catalase were confirmed in previous studies. Absorption of these nanoparticles in normal and tumor cells was confirmed and the cells can harvest these nanoparticles, up to three hours after administration. In one of these studies, clathrin-mediated endocytosis of nanoparticles was introduced. Several studies indicate that these nanoparticles could penetrate to different organelles include nucleus and mitochondria and based on pH differences serve their antioxidant or peroxidative roles.[5]

Cancer metastasis is one of the major causes of cancer-related deaths. Up to 20 different genes known as metastasis process inhibition gens, which among them, NM23 and KAI-1 have an especial role in cancer cells. The NM23 gene plays a role in ATP production form other nucleotides and processing and performance of different enzymes include nucleoside diphosphokinase,

serine/threonine special kinase, pharmexcil phosphate kinase and exonuclease.[6] Systematic reviews indicate that expression elevation of this gene in a patient with breast cancer was related to better prognosis and patient survival.[4,7] Also, in a patient with metastasis, the statistical difference in expression was observed in compression with healthy and patients with benign tumor. Lack of expression in lymph nodes observed in patients with positive lymph node metastasis.[4,5] KAI-1 gene expression was related to metastasis inhibition. The expression of gene reduced in progressive cancer and expansion of metastasis. It has been shown that activation of gene conservation protein, p53, was related to KAI-1 gene.

The aim of the present study is anti-metastatic and toxicity effects of cerium oxide nanoparticles on the MCF-7 and T47D breast cancer cell lines were determined.

MATERIAL AND METHOD

The present experimental practical study was conducted from March to February 2018. MCF-7, T47D, and normal HEK293 cell lines were obtained from the Pasteur Institute of Iran and after that reproduced at 37°C in the presence of 5% CO2. CNP purchased from US Research Nanomaterials Inc. In order to ensure the size and electrical charge of the nanoparticles, size distribution, and zeta analysis were performed. The mean size of nanoparticles was 300 nm with negative charges.

In the present study, MTT assay was used for the determination of CNP cellular toxicity. For this purpose, cell lines treated with 6.5 mg, 65 and 650 µg and 65 and 650 ng of CNP for 48h. The intensity of formazan crystals produced by live cells in the presence of isopropanol was read by ELISA reader (DNM-9602G) at 570 nm wavelength. The percentage of cell viability calculated with the following formula:

$$cell\ viability = \frac{sample\ absorbance}{control\ absorbance} \times 100$$

All concentrations and tests replicated 8 times.

For measurement of gene expression, RNA extraction was performed from MCF-7 and T47D cell lines treated with IC50 concentration of nanoparticles. For RNA extraction, the Trizol Invitrogen kit was used. The spectrophotometric method was used for quantitative and qualitative evaluation of RNA, After RNA extraction, cDNA synthesized by Transgene Biotech AE301-02 kit.

For Real-Time PCR reaction, the final volume of the sample was considered 20 µl which includes 10 pmol of forward and reverse primers of each gene, 35 ng cDNA, 10 µl Master mix and 7 µl RNase free water. In theses experiment, NM23 and KAI-1 were target gene and GAPDH as the reference gene. The used primer sequence was presented in table 1.

Table 1: Primers was used for Real Time PCR reactions.

gene	primers sequences	Duplicate fragment size
NM23	F: ATGGCCAACTGTGAGCGTACC R: CATGTATTTCACCAGGCCGGC	204 bp
KAI-1	F: CTCAGCCTGTATCAAAGTCACCA R: CCCACGCCGATGAAGACATA	183 bp
GAPDH	F; CCCACTCCTCCACCTTTGAC R; CATACCAGGAAATGAGCTTGACAA	74 bp

In the present study, The PCR (ABI 7300, Foster, Applied Biosystems) was as follows: 95°C for 8 min; 95°C for 25 sec and 60°C for 1 min and final section, 72°C for 10 min. Real Time PCR results analysis by ΔΔCt methods and following formula:
ΔCt= Ct target- Ct reference
ΔΔCt= ΔCt T- ΔCt C
ΔΔCt-RQ=2

Where, ΔCt T is the difference between threshold cycle in the target gene and internal control in sample and ΔCt C is the difference between threshold cycle in the target gene and internal control in the control group. This method takes into account the efficiency of the primers so that the amount of product in all cycles is double.

Statistical analysis

The obtained data were analyzed by one way ANOVA and Tukey statistical analysis. $p<0.05$ was considered significant.

RESULTS

Treatment of MCF-7 and T47D breast cancer cell line and HEK293 normal cell whit CNP indicate dose-dependent toxicity of nanoparticles in both cell lines. In the MCF-7 cell line, CNP treatment with 6.5 mg and 65, and 650 µg after 48h cause significant reduction in MCF-7 viability ($p<0.001$) (Figure1).

The result of MTT assay for CNP toxicity on the T47D cell line (Figure 1) showed that treatment with 650 µg and 6.5 mg lead to 40% and 60% reduction in cell viability, respectively which statistically significant ($p<0.001$).

The effect of CNP on HEK-293 as a normal cell line (figure1) indicate a 25% reduction in cell viability in 6.5 mg concentration of nanoparticles ($p<0.001$). In other concentrations, the viability of the HEK293 cell line was constant.

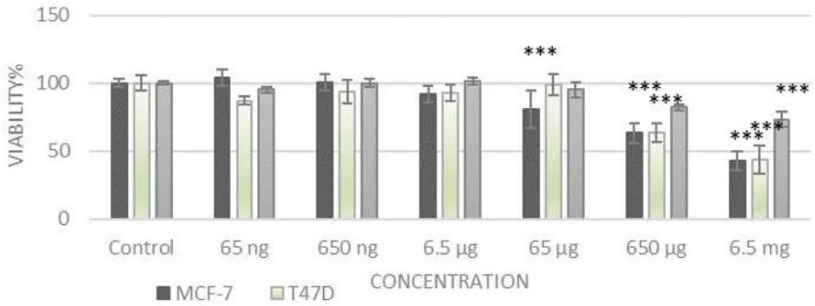

Figure 1: Effect of different concentrations of CNP on MCF-7 and T46D breast cancer cell line and HEK293 normal cell line. Results present as a viability percentage in comparison with control samples and reported as a mean±SD. p<0.05 considered as a significant. (* p<0.001).**

After real-time PCR, the melting curve was used to confirm gene sequences replicated specifically and without contamination and dimer primer. According to figure 2, the presence of a peak for NM23, KAI-1 and GAPDH genes in specific melting temperatures, confirmed their specific replication. Also, the replication diagram of all genes reviewed and calculated Ct was recorded.

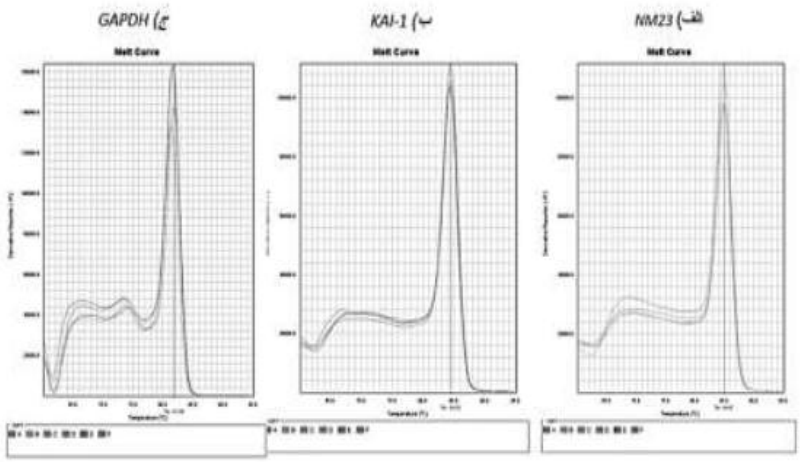

Figure 2: Melting curve of studied genes: a) NM23; b) KAI-1 and c) GAPDH genes. The presence of a peak and same melting temperature indicate specific replication of genes.

After treatment with 600 µg of CNP for 48h, NM23 gene expression in MCF-7 and T47D cell lines was evaluated. Based on our results, NM23 gene expression in comparison with the control gene in the MCF-7 cell line treated with CNP was significantly decreased (p<0.001), whereas, its expression in T47D was not changed (figure 3).

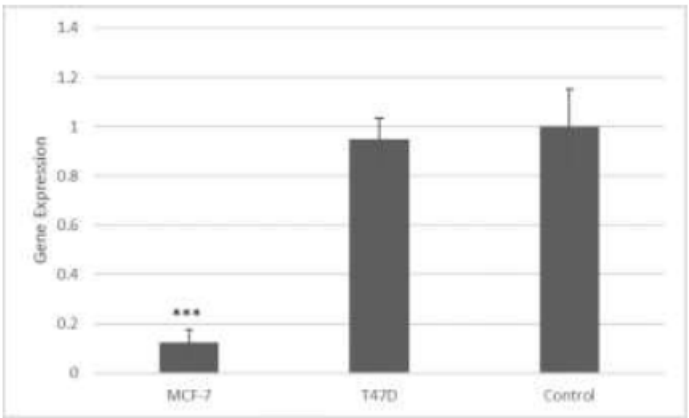

Figure 3. NM23 gene expression in MCF-7 and T47D cell line. a) NM23 gene expression in MCF-7 breast cancer cell line was significantly decreased; b) NM23 gene expression in T47D breast cancer cell line was not changed (* p<0.001).**

The effect of treatment with CNP in KAI-1 gene expression in MCF-7 and T47D cell lines presented in figure 4. The gene expression in both cell lines significantly decreased, whereas this reduction in the MCF-7 cell line was more pronounced.

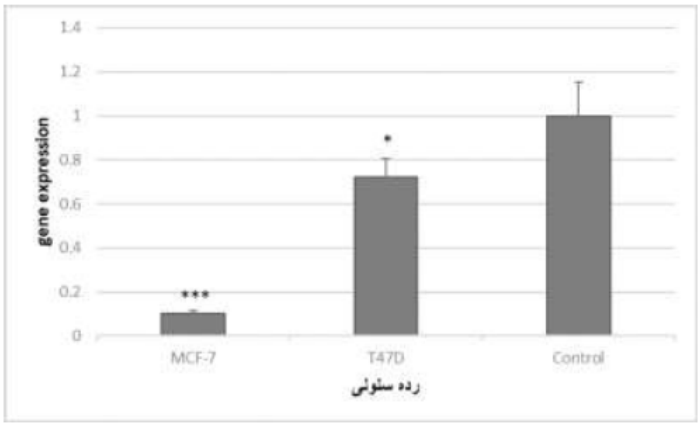

Figure 4: KAI-1 gene expression in MCF-7 and T47D cell line after treatment with CNP. a) KAI-1 gene expression in MCF-7 breast cancer cell line; b) KAI-1 gene expression in T47D breast cancer cell line (*p<0.05; ***p<0.001).

DISCUSSION

Cerium oxide nanoparticles contain a cerium nucleus surrounded by oxygen atoms. Several studies indicate that ambient conditions of cells and internal condition play the main role in their effects and their toxicity effects of theses NPs was dependent on cell line.[8] On the other hand, an anti-cancer and anti-metastatic effect of these NPs was confirmed.[9] Therefore, in the present study, the toxicity effect of CNP on two breast cancer and one normal cell line was evaluated and after that, the possible role of these nanoparticles on metastases inhibition via NM23 and KAI-1 anti-metastatic genes was determined.

The results of the present study, indicate that the viability of the T47D cell line treated with 6.5 mg and 650 µg CNP and MCF-7 cell line treated with 6.5 mg, 65 and 650 µg CNP was reduced. The results suggest that the toxic effect of CNP was dose and cell type-dependent. In the MTT assay on HEK293 cell line as a control group, in the cells treated with 6.5 mg of CNP, a 25% reduction in cell viability was observed. The results of the present study indicate that only high concentrations of CNP have a toxic effect on normal cell lines, whereas low concentrations like 650 µg could cause 50% reduction in MCF-7 and T47D cell line viability. Therefore in the formulation of CNP as an anti-cancer drug, concentration adjustment could cause low side effects on normal cells.

In the study conducted in 2013 on patients with pancreatic cancer, the use of CNP has a protective role of non-cancer cells against oxidative radiotherapy and in addition causes elevation of pH and susceptibility of tumor cells to radiotherapy and elevation of apoptosis. In this study mice with pancreatic cancer were used and drug administrated pre and post-radiotherapy.[10] Colon J et al.[11] in the study on gastrointestinal cancer, indicate that CNP in the pH below 4.3 was activated and has a catalectic effect, and like superoxide dismutase could prevent DNA damage. The pathologic studies indicate that using nanoparticles lead to apoptosis elevation in tumor cells and increases the resistance of healthy cells to ionizing radiation. The results of the present study confirmed the results of previous research about the anti-cancer effects of these nanoparticles. However, the protective and apoptosis effect of CNP on breast cancer was not evaluated in the present study. This must be considered in future studies.

NM23 gene known as a metastatic suppressor gene and their expression in tumor cells were reduced. The expression of NM23 has a direct relationship with metastasis in breast cancer. Therefore, the level of expression could be used as a tool for metastasis evaluation. However, reports in this regard yield conflicting results. Koboko et al,[12,13] could not found a significant relationship between NE23 expression measured by immunohistochemistry method and breast cancer.

KAI-1 genes related to metastasis inhibition and its expression was reduced in end-stage cancer and metastasis progression,[13,14] Based on several reports about the anti-metastatic effect of zinc oxide nanoparticles,[15,16] in the present study, the effects of CNP on the expression of KAI-1 and NM23 were evaluated. The results indicate that NM23 and KAI-1 expression was not increased in cell lines treated with CNP. If cerium oxide nanoparticles would induce metastasis inhibition in breast cancer, these two genes would be expected to increase expression. So it can be argued that CNP was not an anti-metastatic effect on breast cancer or induce the effects via different signaling pathways. In support of this argument can be given the study of Jiri et al,[17] which indicates that CNP could

inhibit the migration and cell invasion in ovarian cancer via inhibition of angiogenesis. Therefore, further studies on breast cancer are needed.

CONCLUSION

In the present study, we show that CNP has dose and cell type-dependent toxicity on breast cancer cell lines. Therefore, the results of using NCP for the treatment of breast cancer are promising, but on the other hand, it has been shown that the effect of this nanoparticle on metastatic suppressor genes is diminishing. It can be concluded that, in relation to the studied genes, CNP could not inhibit metastasis in breast cancer. Further studies are needed to understand these properties of CNPs. For example, evaluation of other important genes in apoptosis and metastasis pathway in cells treated with CNP or increase the efficiency of nanoparticles via conjugation with other anticancer agents leads to a more detailed evaluation of the anticancer mechanisms of this nanoparticle in breast cancer.

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