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Cerium Lanthanide Effect on Growth of AGS Cell Line with the Presence of Transferrin in Vitro

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Abstract

***Background*:** Cerium is a trace element and a member of the lanthanide group. Cerium cation is similar to ferric ions with regard to transferring binding, suggesting transferrin-receptor mediated transport could be possible to uptake the element. Therefore the aim of the present study was to survey of cytotoxic activity of cerium in the presence of transferring on growth of adenocarcinoma gastric stomach (AGS) cell line in vitro.

***Materials and Methods*:** Adenocarcinoma gastric stomach cells obtained from Pasteur institute were cultured on RPMI-1640 medium and the effect of cerium lanthanide with 0.1, 1, 10, 100 (µM) concentration with and without transferrin in 24 h and 48 h incubation periods was investigated by 3-(4,5-dimethylthiazol-2-Yl)-2,5-diphenyl tetrazolium bromide (MTT) and lactate dehydrogenase (LDH) assay.

***Results*:** The results obtained from LDH assay showed that cerium without transferrin and cerium with transferrin decreased survival AGS cells significantly. Also results obtained from MTT assay showed that cerium without transferrin and cerium with transferrin decreased survival AGS cells significantly.

***Conclusion*:** In our results cerium could induce the inhibition of cell growth but the percent of growth inhibition could be higher with presence of transferrin. Our results indicate that at a certain concentration, the cerium compounds could inhibit the growth of cancer cells.

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Introduction

Gastric cancer is the fourth most common cancer in the world and adenocarcinoma, or glandular cancer of the stomach is the most common form. Adenocarcinoma can be considered as uncontrolled grow of malignant cells in the stomach. The disease is caused deaths worldwide; gastric cancer annually kills about 1 million people worldwide [1]. The prevalence in men's is doubles in comparison to women [2]. Over the past three decades, the study of materials with biological effects on cancer cell lines is under consideration of the scientific community. Currently, increasing attention has been directed to use lanthanides in the treatment of various cancers including gastric cancer, the inhibitory effects and cytotoxic properties of some lanthanides and the action mechanism of these elements has been researched on various cancer cell lines [3, 4]. The accumulation mechanism of these elements within the cell and interferences with the normal physiological functions of the cells has not been clearly identified [5]. On the other hand it has been found that the different cancer cell lines indicates the higher iron needs than in normal cells and non-cancerous cells [6]. Research on the transfer mechanism of some elements and compounds into the cell has been identified the expression of transferrin-mediated transport mechanism like iron transport into the [7]. Given that transferrin may tend the lanthanides similar to iron, the question is that whether the presence of small amounts of transferrin may be cause anticancer effects of these trace elements in several hundred times. So to destroy cancer cells which have so much of transferrin receptors comparison to normal cells can be used from transferrin [8]. The presence of lanthanide trace elements and cerium in mineral drinking water because of washing the rocks in the path of rain water has made doubled the need for such studies. So that cerium out of the soil is equal to 50 parts per million. Cerium of the sea 1.5 part per trillion, and there isn't any cerium in atmosphere [8]. Today, the pharmacological properties of lanthanide elements and cerium are considered if they have a therapeutic effect can be used from [9]. It hasn't been carried out any study on anticancer properties of cerium (lanthanides) on the growth and survival of gastric cancer cell line (AGS) in vitro in the presence of transferrin. The aim of this study was to evaluate the anticancer effects of cerium on growth and survival of gastric cancer cell line (AGS) in vitro in the presence of transferrin is.

Materials and Methods

In this experimental study, the concentrations of 1, 10, 100 and 1000 µM from cerium sulfate were obtained with 0.22 µ microbiological filters was filtered and the concentration of 20 µl of 100 µM sodium sulfate was used as a control. One hundred eighty µM of cell suspension cell lines were used. The cells were plated in RPMI 1640 culture media and 96 well microplates at a concentration of 5×10^4 cell/ml. In wells that the cancer cells were cultured with 4 different concentrations of sulfate cerium, was added 120 micrograms of transferrin in labeled rows. First, a cell suspension with a concentration 5×10^4 cell/ml was prepared in Falcon, and then was exploited in a 96-well plate as follows:

1. In the 3 first wells 200 µl was poured RPMI 1640 culture media without cells.
2. In the subsequent 3 wells of first row was poured 200 µl of cell suspension with RPMI 1640 medium.
3. In the third 3 wells of first row was poured 180 µl of cell suspension with RPMI 1640 medium plus 20 µl of 100 µM sodium sulfate as a negative control.
4. In the fourth 3 wells of first row was poured 180 µl of cell suspension plus 20 µl of 120 µg of transferrin.
5. In the first 3 wells of second row was poured 180 µl of cell suspension plus 20 µl of 1 µM cerium sulfate.
6. In the second 3 wells of second row was poured 180 µl of cell suspension plus 20 µl of 1 µM cerium sulfate plus 120 µg of transferrin.
7. In the third 3 wells of second row was poured 180 µl of cell suspension plus 20 µl of 10 µM cerium sulfate.
8. In the fourth 3 wells of second row was poured 180 µl of cell suspension plus 20 µl of 10 µM cerium sulfate plus 120 µg of transferrin.
9. In the first 3 wells of third row was poured 180 µl of cell suspension plus 20 µl of 100 µM cerium sulfate.
10. In the second 3 wells of third row was poured 180 µl of cell suspension plus 20 µl of 100 µM cerium sulfate plus 120 µg of transferrin.
11. In the third 3 wells of third row was poured 180 µl of cell suspension plus 20 µl of 1000 µM cerium sulfate.
12. In the fourth 3 wells of third row was poured 180 µl of cell suspension plus 20 µl of 1000 µM cerium sulfate plus 120 µg of transferrin.
13. Samples were incubated at 37°C for 24 and 48 hours in an incubator containing 5% CO_2 and 95% humidity. Using MTT assay, the percent of cell viability was measured by ELISA reader in 540 nm.

To investigate the anticancer effects of cerium on (AGS) cell line after sample preparation and adding different serial cerium concentrations in the presence and absence of transferrin and passing the time of incubation, the cerium induced cytotoxicity was determined by the MTT method and measuring LDH activity released from damaged cells. Cytotoxic effects of different concentrations cerium sulfate in the presence of 120 mg of transferrin on the cell line colorimetric method [10]

In this technique the tetrazolium salt 3 (4, 5-Thiazole dimethyl-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT), which is a solution reduced by mitochondrial succinate dehydrogenase of living cells and form indloublr purple formazan which soluble in DMSO and measured by ELISA Reader in 540 nm to calculate the cell viability. A simple and accurate colorimetric method for determining cytolysis is measuring LDH activity released from damaged cells, for measurement of cytotoxicity was used Roche company lactate dehydrogenase assay kit. Percentage of cell survival calculated by below formula:

Percentage of cell survival=(absorbance of test /absorbance of control)×100

The results are reported as mean±SD. Results showed significant mean value of (p<0.05) with statistical difference between the groups using SPSS-13 software and Mann-Whitney U test.

Results

MTT results showed that cerium alone and in the presence of transferrin was significantly decreased in cell survival of AGS cell line (p=0.043). Cerium effects alone and in the presence of transferrin carrying cell death in cancer cell lines with increasing concentrations, this increase is expected in terms of a linear relationship. The results showed that in different concentration of cerium in the presence of transferrin had a greater effect on the rate of cell death in cancer cell lines comparison to absence of transferrin (Fig. 1). Also the results showed that at 48 hours of incubation had a greater significance on the AGS cell line (p=0.003) comparison to 24 hours of cell line incubation. The results of LDH assay showed that cerium alone and in the presence of transferrin was significantly decreased in cell survival of AGS cell line (p=0.001). cerium effects alone and in the presence of transferrin carrying cell death in cancer cell lines with increasing concentrations, this increase is expected in terms of a linear relationship. The results showed that in different concentration of cerium in the presence of transferrin had a greater effect on the rate of cell death in cancer cell lines comparison to absence of transferring (p=0.02) (Fig. 2). Also the results showed that at 48 hours of incubation had a greater significance on the AGS cell line (p=0.003) comparison to 24 hours of cell line incubation (Fig. 2).

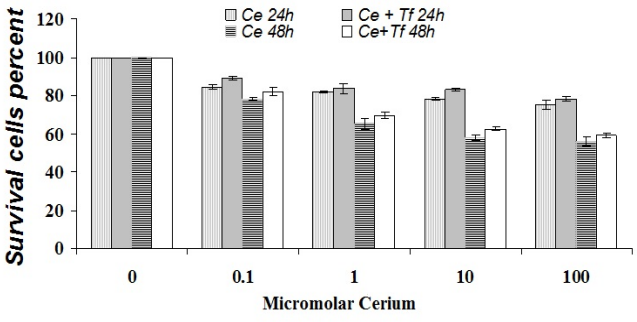

Figure 1. Effects of serial four concentrations of cerium alone or in the presence of transferrin on the AGS cell line with MTT assay technique at 24 and 48 hours

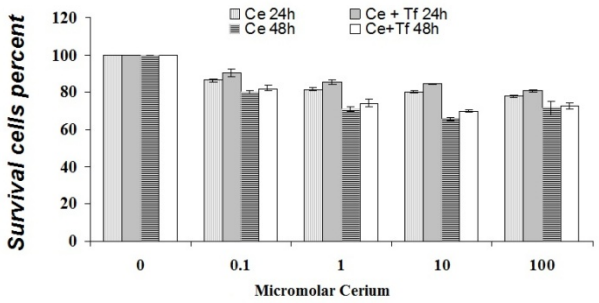

Figure 2. Effects of serial four concentrations of cerium alone or in the presence of transferrin on AGS cell line with LDH assay technique at 24 and 48 hours

Discussion

The results showed that different concentrations of cerium with out transferrin reduce growth and survival of AGS cancer cells. Reduction in cell survival is more observed in the presence of transferrin at different concentrations of cerium. In AGS cells, at concentration of 100 µM of cerium, cancer cell survival decreased significantly after 24 h and 48 h in presence of cerium alone and also in presence of cerium and transferrin. Previous studies have shown that cerium concentration of 10 µM can be reduced tumor growth of HeLa cells after 48 and 72 h of incubation. In the same study it was shown that concurrent use of cerium and transferrin significantly decreased HeLa cell proliferation comparison to use cerium alone. This study showed that cerium alone reduced tumor growth of HeLa cells and with transferrin further reduce of HeLa cancer cell growth can be observed. In another study it was shown that cerium alone and cerium with transferrin reduced tumor growth of MCF-7 cells. The results of these studies are in agreement with the result of present study [11]. Transferrin is necessary to transport iron in all living organisms, particularly for cancer cell proliferation, increased expression of transferrin receptors on the surface of cancer cells is the reason of more iron metabolic demand [1, 12 ,13], different levels of transferrin receptor expression has been observed in cell lines such as epithelial cells, cervical cancer cells, gastric cancer and breast cancer which the amount of available iron is essential for cancer cell proliferation and promotion of metastasis [14]. Thus, the main role of transferrin has been shown as an iron-dependent growth factor for cell growth including cancer cells [11, 15]. In present study was well observed this phenomenon for AGS cells in the presence of transferrin and iron competitive element-cerium. The results are consistent with previous studies that transferrin increases cell proliferation in cancer cells, however, if the element is a competitive alternative to iron onto transferrin inhibits the growth of cancer cells. Therefore, this phenomenon can be introduce cerium as candidates to inhibit cancer cell activity [16] and more effectiveness of cerium in the presence of transferrin show that this element may have a similar mechanism of endocytosis mediated by transferrin receptor [17]. Due to the similarity of the chemical properties of cerium with iron ions and given that cancer cells generally express much higher transferrin receptors than the normal cells. Therefore may be inhibiting malignant cell type's dependent to metal ion with such substances and mechanisms [18-20]. Studies have also shown that cerium has antioxidant role. Cerium antioxidant role could also consider as one of the mechanisms to inhibit of malignant cell growth is [21, 22]. Entries so far little is known about the effects of cerium in controlling the growth of cancer cells, the biochemical similarities between cerium and iron ions, especially in the case of binding to transferrin, possibly can help to predict the physiological role cerium in treatment of cancer. In conclusion, the present study is part of the picture can describe how cerium ion and transferrin with cerium ion affect on cell growth inhibition in gastric cancer, but the actual mechanism of action remains unclear who will be conducting further studies to understand.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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