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Comparison the Effects of Cerium Nanoparticles (CeNP) and Cerium Oxide (CeO2) on Oxidative Toxic Stress in Human Lymphocytes

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Abstract

Background: Cerium nanoparticles (CeNP) have an interesting potential in drug delivery, gene therapy, molecular imaging and medicine. The present study focuses on the effect of exposure of cerium nanoparticles and Cerium oxide (CeO2) on oxidative stress biomarkers in human lymphocytes.

Materials and Methods: Lymphocytes were incubated for 24 , 48 and 72 h at 37 °C with different concentrations (15, 30, 60 and 120 mM) of CeNP and CeO2. In lymphocytes samples evaluated oxidative stress biomarkers such as total antioxidant capacity (TAC), lipid peroxidation (LPO) and catalase activity (CAT). All data were analyzed by one way ANOVA with Tukey post hoc test. The results showed that CeNP after 24 ,48 and 72 hours caused a increase in the TAC compared to CeO2 120mg/kg group. CeNP decreased LPO level and CAT activity compared with CeO2 120mg/kg group.

Results: The present study showed that after treatment for 24, 48 and 72 hours, CeO2 is able to induce oxidative toxicity in human lymphocytes.

Conclusion: The results suggest that ROS intermediates are responsible for Ce-induced oxidative damage in experimental lymphocytes. Therefore, CeNP plays an important role in the alteration of oxidative injuries of human lymphocytes

Keywords: Cerium nanoparticle; Lymphocyte; Oxidative stress

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Introduction

Cerium is a member of the lanthanide series of metals and is the most abundant in the earth's crust of the rare-earth elements. Elemental cerium is an iron-gray, ductile, malleable metal. Cerium metal is very reactive and is a strong oxidizing agent that is stabilized when associated with an oxygen ligand (1). Cerium is found in nature along with other lanthanide elements in the minerals alanite, bastanite, monazite, cerite, and samarskite; however, only bastanite and monazite are important sources commercially (2). Cerium oxide nanoparticle (CeNP), mean while, have been produced using many different preparation methods such as sol-gel, thermal decomposition, solvothermal oxidation, microemulsion methods, flame spray pyrolysis and microwave-assisted solvo-thermal process (3). A synthetic method has recently been reported of being capable of producing homogeneous cerium oxide nanoparticles of 2 nm in average size by merely mixing cerium sulphate and ammonia solution at room temperature (4). CeNPs have distinctive physico-chemical properties, including a high electrical and thermal conductivity, surface-enhanced Raman scattering, chemical stability, catalytic activity and non-linear optical behavior (4, 5).

Moreover, nanoparticles (NPs) like CeNP are known to induce reactive oxygen species (ROS) in various cell types (6). In spite of this, the link between CeNPs and oxidative stress is not well established. Most often, the harmful effects of ROS may be manifested

through damage of DNA, oxidations of polyunsaturated fatty acids in lipids and oxidations of amino acids in proteins (7, 8). Also, NPs can undergo a series of processes like binding and reacting with proteins, phagocytosis, deposition, clearance and translocation. On the other hand NPs can elicit a spectrum of tissue responses such as cell activation, generation of ROS, inflammation and cell death (9). Some of these studies provided sample evidence that the cytotoxicity of CeNPs may be partially due to their induction of cellular oxidative stress through the generation of free radicals and ROS (10). This is of clinical significance because certain pathological conditions such as inflammation is associated with elevated oxidative stress and this may in turn alter the sensitivity of cells and tissues to potentially cytotoxic CeNPs increasing their market value (11, 12). Therefore, this study aimed to compare antioxidant effects of CeNPs and CeO_2 in different dose and various time in lymphocyte of human by in vitro study.

Materials and Methods

Reagents and Chemicals

Tetraethoxypropane (MDA), 2-thiobarbituric acid (TBA), trichloroace tic acid (TCA), n-butanol, 2, 4, 6 tri pyridyl-s-tiazine (TPTZ), H2O2 and Cerium oxide (CeO2) were used in this study. All other chemicals were obtained from the Sigma. The Ce NP (30 nm, US Research Nanomaterials, Inc company) used in this study were supplied by Notrino compony. The nanoparticle was suspending in deionized water.

Table 1. Oxidative toxic stress biomarkers in human lymphocytes incubated after 24 hour.

Time (24 hour)	FRAP (µmol/ml) Mean ± SE	LPO (µmol/ml) Mean ± SE	CAT(µ/ml) Mean ± SE
CeO 15 µmol/mL	0.12 ± 0.01*	3.8 ± 0.04*	2.3 ± 0.01*
CeO 30 µmol/mL	0.33 ± 0.01*	3.9 ± 0.02*	1.3 ± 0.01
CeO 60 µmol/mL	0.87 ± 0.01*	4.8 ± 0.08*	1.15 ± 0.02
CeO 120 µmol/mL	0.92 ± 0.01	1.09 ± 0.05	1.28 ± 0.01
CeNPs 15 µmol/m	0.47 ± 0.01*	1.19 ± 0.02	0.7 ± 0.03*
CeNPs 30 µmol/mL	1.7 ± 0.04*	1.2 ± 0.03	0.35 ± 0.01*
CeNPs 60 µmol/mL	2.7 ± 0.01*	1.44 ± 0.07	0.51± 0.01*
CeNPs 120µmol/mL	0.63 ± 0.01	2.25 ± 0.3*	1.19 ± 0.01

*Significantly different from CeNP15 mM group at $p < 0.05$.

Isolation of Lymphocytes

Blood sample (2 ml) from a healthy human donor was collected in conical centrifuge tube and centrifuged at 2,000 rpm for 15 min. The whitish portion of blood, formed just below the upper plasma layer, was separated and collected into fresh microcentrifuge tube. Collected cells were washed twice with 0.5 ml NH4Cl (0.85%) to remove contaminant RBC. Lymphocytes that appeared in the form of white pellet were subsequently resuspended into PBS (phosphate buffer saline) and stored at 4 °C (13).

Incubation of human lymphocytes with CeNPs and CeO_2

Human lymphocytes were incubated with CeNPs and CeO_2 at the following concentrations: 15, 30, 60 and 120 µmol/mL, for 24, 48, and 72 h. Following treatment, the lymphocytes were processed for oxidative stress biomarkers.

Oxidative stress biomarkers

Measurement of lipid peroxidation (LPO)

The LPO product in tissues was determined by thiobarbituric acid (TBA) reagent expressed as the extent of malondialdehyde (MDA) productions during an acid heating reaction. The calibration curve of tetraethoxypropane standard solutions was used to determine the concentrations of TBA+MDA adducts in samples (14).

Measurement of total antioxidant capacity (TAC)

It was measured by the ferric reducing ability of plasma (FRAP) method. This method is based on the ability of plasma to reduce Fe^{3+} to Fe^{+2} in the presence of TPTZ. The reaction of Fe^{2+} and TPTZ gives a complex with blue color and maximum absorbance in 593 nm (15).

Measurement of CAT activity

Catalase activity was assayed in the samples by measuring the absorbance decrease at 240 nm in a

reaction medium containing H2O2 (10 mM), sodium phosphate buffer (50 mM, pH = 7.0). One unit of the enzyme is defined as 1 mol H2O2 as substrate consumed/min, and the specific activity is reported as units/ml sample (16)**.**

Statistical analysis

Mean and standard error values were determined for all the parameters and the results were expressed as Mean±SE. All data were analyzed with SPSS Version: 11 employing one-way ANOVA followed by Tukey post hoc test. Differences between groups was considered significant when $P < 0.05$.

Ethics Statement

The study protocol has been approved by the Institutional Review Board of Hamadan University of Medical Sciences, Hamadan, Iran.

Results

LPO

Ce NP caused a significant decrease in LPO level in 15,30 and 60 µmol/mL when compared to control group and CeO2 15, 30, 60 and 120 µmol/mL after 24 hours($p < 0.05$). Ce NP caused a significant decrease in LPO level when compared to control and CeO2 groups after 72 hours ($p < 0.05$). No significant difference was observed LPO between groups after 48 hours, tables 1, 2 and 3.

Table 2. Oxidative toxic stress biomarkers in human lymphocytes incubated after 48 hour.

Time (48 hour)	FRAP (µmol/ml) Mean ±SE	LPO (µmol/ml) Mean ±SE	CAT (µ/ml) Mean ±SE
CeO 15 µmol/mL	0.16 ± 0.01*	1.81 ± 0.04	1.01 ± 0.03
CeO 30 µmol/mL	0.88 ± 0.01*	1.5 ± 0.04	1.28 ± 0.01
CeO 60 µmol/mL	0.41 ± 0.01*	1.2 ± 0.06	2.13 ± 0.01
CeO 120 µmol/mL	0.87 ± 0.01	1.8 ± 0.06	2.08 ± 0.01
CeNPs 1 µmol/mL	0.15 ± 0.01*	1.35 ± 0.02	0.09 ± 0.02*
CeNPs 30 µmol/mL	*2.7 ± 0.04	1.48 ± 0.05	0.05 ± 0.01*
CeNPs 60 µmol/mL	1.8 ± 0.04*	1.5 ± 0.06	0.70 ± 0.01*
CeNPs 120 µmol/mL	0.36 ± 0.01*	1.6 ± 0.03	2.27 ± 0.01

*Significantly different from CeNP15 mM group at $p < 0.05$.

TAC

CeNP caused a significant increased in TAC in 15,30 and 60 µmol/mL when compared to control group and CeO2 15,30,60 and 120 µmol/mL after 24, 48 and 72 hours ($p < 0.05$). CeO2 caused a significant decrease in TAC level when compared to control group after 24, 48 and 72 hours ($p < 0.05$), Table1, 2, 3.

CAT

Ce NP caused a significant decrease in CAT activity in 15, 30 and µmol/mL when compared to control group after 24, 48 and 72 hours ($p < 0.05$). No significant difference was observed CAT activity between groups in CeO2 after 24, 48 and 72 hours, tables 1, 2, and 3.

Discussion

The aim of this study was to comparison oxidative stress biomarkers between CeNPs and CeO_2 in isolated human lymphocyte: in vitro study. Our results demonstrate that CeNP decreased the oxidative toxic stress in low doses, as shown by a decreased in CAT activity, LPO level and increase TAC level in 15, 30 and 60 µmol/mL, but in 120mg/kg decrease TAC level and increase CAT activities in this group compared the other groups.

The present study provides the first evidence that CeNPs act as antioxidants that prevent oxidative stress and suppress inflammatory response. Also, this study has demonstrated that CeO causes an increase in intracellular ROS, which may have the ability to cause oxidative damage in isolated human lymphocytes. Cellular response to ROS may depend on the reduction oxidation state in the cell. If the amounts of oxidants do not exceed the reducing ability of the cell, ROS are involved in physiological functions such as signal transduction (17, 18). However, when ROS levels exceed the antioxidative ability of the cell, the cell undergoes oxidative stress, finally leading to cell death (9). Our findings provide the evidence that CeNPs can decrease ROS production, inhibit inflammation, and preserve antioxidant enzymes and LPO content in a biological system. These effect or molecules activated under

prolonged oxidative stress relate chronic inflammation to malignant transformation, in particular to the invasive potential of cells, at least at a molecular level (19, 20).

Table 3. Oxidative toxic stress biomarkers in human lymphocytes incubated after 72 hour.

Time (72 hour)	FRAP (umol/ml) Mean ±SE	LPO (nmol/ml) Mean ± SE	CAT (U/ml) Mean ± SE
CeO 15 µmol/mL	0.5 ± 0.01*	2.11 ± 0.06*	0.64 ± 0.01*
CeO 30µ mol/mL	0.34 ± 0.01*	3.4 ± 0.3	1.4 ± 0.01
CeO 60 µmol/mL	0.5 ± 0.01*	4.2 ± 0.06	1.15 ± 0.01
CeO 120 µmol/mL	0.19 ± 0.01	4.8 ± 0.2	2.9 ± 0.02
CeNPs 15 µmol/mL	1.07 ± 0.01*	*1.8 ± 0.06	0.22 ± 0.01*
CeNPs 30 µmol/mL	1.4 ± 0.02*	1.8 ± 0.06*	0.25 ± 0.01*
CeNPs 60 µmol/mL	1.9 ± 0.01*	2.1 ± 0.09*	0.67 ± 0.01*
CeNPs 120 µmol/mL	2.3 ± 0.01*	2.5 ± 0.07	0.67 ± 0.01*

*Significantly different from CeNP15 mM group at $p < 0.05$.

Our results showed that treatment with CeNP lowered the content of intracellular ROS and decreased the level of LPO after 24, 48 and 72 hours treatment. But these results indicated that suitable time is 24 h after incubation for all oxidative stress biomarkers. On the other hand, inflammatory cells also produce soluble mediators, such as metabolites of arachidonic acid, cytokines, and chemokines, which act by further recruiting inflammatory cells to the site of damage and producing more reactive species. As an example, mutations in the rat sarcoma viral oncogene (Ras) induce an inflammatory response. The results of this study have been shown that CeO2 are more toxic than CeNPs. Other studies reported that CeNPs have antioxidative properties. In the present study we investigated antioxidative properties in different dose comparison with CeO2. Since CeNP are used in an increasing number of applications, further studies are required to elucidate the mechanism of action of these compounds, to distinguish the implication of their widespread use (21). In conclusion, the present studies show that after treatment for 24, 48 and 72 hours, CeO2 is able to induce oxidative toxicity in human lymphocytes. The results suggest that ROS intermediates are responsible for CeO_2induced oxidative damage in experimental lymphocytes and their effects of CeNPs are dose dependent. Therefore, CeNP plays an important role in the alteration of oxidative injuries of human lymphocytes.

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Authors' contributions
KA Design of the study and doing labratory methods. YH Sampling and Help to labratory methods. HA Help to labratory methods. MF Doing statistical analysis. RA Help in design of the study and English editing of the paper.

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Conflict of interest
There is no conflict of interests

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