Cerium Oxide Nanoparticle Effects on Paraoxonase-1 Activity and Oxidative Toxic Stress Induced by Malathion: A Potential Antioxidant Compound, Yes or No?

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Abstract Cerium oxide nanoparticles (CeNPs) are one of the most widely used and important nanoparticles in addition to possessing strong antioxidative properties and inhibiting free radicals. Paraoxonase-1 (PON1) is one of the enzymes that protect the body against damage caused by oxidative stress. The purpose of this study was to investigate the effect of CeNPs on the activity of PON1 as well as biomarkers of oxidative stress in the toxicity of malathion. 48 Albino Wistar male rats with weight range of 180–250 g were randomly divided into 8 groups, Group 1: healthy control, injection of normal saline, Group 2: administration by the malathion 100 mg/kg/day, Group 3: treated with CeNPs 15 mg/kg/day, Group 4: treated with CeNPs 30 mg/kg/day, Group 5: combination of malathion with dose of 100 mg/kg/day and CeNPs 15 mg/kg, Group 6: combination of malathion with dose of 100 mg/kg/day and CeNPs 30 mg/kg for 14 days and 24 h after termination of treatment period, serum and liver tissue samples were collected from all rats. Biochemical test of PON1 activity, oxidative stress biomarkers including total antioxidant capacity (TAC), lipid peroxidation (LPO), total thiol groups (TTG), were carried out. Malathion reduced

plasma TTG levels, TAC and increased LPO in malathion group. However, CeNPs increased TTG, TAC and reduced PON1 activity. Results showed that CeNPs alone had antioxidant properties while with malathion it shows different properties.

Keywords Cerium oxide nanoparticle · Paraoxonase · Malathion · Oxidative stress

Introduction

Organophosphorus (OP) chemicals, which are synthesized to control the pests and insects, are ubiquitously found in the environment [1]. Malathion is an organophosphate compounds used widely as insecticide. It has been increasingly utilized in both control and elimination of unwanted insects and pests in various developing countries around the world. Although irreversible inhibition of acetylcholinesterase (AChE), whether in central or peripheral nervous system, is known as the main mechanism of action of this OP compounds, for the case of malathion, because inhibition of AChE does not explain all of the related symptoms, oxidative toxic stress (OTS) induction is, also, responsible to cause severe cellular damage in different organs including liver and even plasma [2, 3].

Malathion has, additionally, been reported to induce OTS in animals [4, 5] and human [6]. OTS in a living system is the prime reason for the generation of ROS. Furthermore, it also provokes the imbalance between pro-oxidants and antioxidants in the cellular system [7]. Paradoxically, a generation of ROS is both essential and unfavorable to life [8]. There are certain evidences explaining that biochemical changes, due to the excessive

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release of ROS, impose cumulative damage to cellular components such as protein, lipids and DNA which results in causing detrimental effects to mankind. Malathion has, also, been well studied for causing damage by peroxidation to the lipid membrane and, moreover, for disturbing the biochemical balance [5]. Therefore, antioxidants are getting greater attention now-a-days in order to prevent, delay and ameliorate many of health disorders caused by malathion induced OTS in human beings [9]. Liver is important in regulating metabolic functions and diverse physiological processes. In addition, it is involved in detoxification of some drugs and xenobiotics which leads to an increased susceptibility to the toxicity from these agents [10]. Oxidative stress is correlated with cancer, inflammation and multiple organ toxicity [11]. Antioxidants enzymes act as free radical scavenging systems and arrange first-line defense against ROS including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), paraoxonase (PON) and nutritional antioxidants [12]. PON gene is a family that contains three members, PON1, PON2, and PON3. PON1 is primarily synthesized in liver and secreted into plasma. Its natural physiological function revolves around metabolism of toxic oxidized lipids of both low density lipoprotein (LDL) and HDL particles [13]. As such, PON1 may be an indicator of liver damage and has been proposed as a biomarker of hepatotoxicity [13]. Nanoparticles might offer a novel therapeutic alternative for scavenging environmentally elevated ROS [2]. 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 [14].

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 [15]. Cerium oxide nanoparticle (CeNP), meanwhile, have been produced using many different preparation methods such as sol–gel, thermal decomposition, solvothermal oxidation, micro emulsion methods, flame spray pyrolysis and microwave-assisted solvothermal process [16].

One obvious use of the nanoparticles such as cerium oxide nanoparticles (CeNPs) would be for enhancing the performance of antioxidants [17]. Thus, the purpose of this study was to investigation of the CeNPs effect in the antioxidant system, especially PON1 enzyme in the liver and plasma of rats treated with malathion.

Materials and Methods

Chemicals

The CeNPs used in this study (30 nm in diameter, US Research Nanomaterials, Inc Company) were provided by Neutrino Company. Ethylene-diamine-tetra-acetic acid (EDTA), di-thio-nitro-benzoic acid (DTNB), phosphate buffer solution (PBS), tri-chloro-acetic acid (TCA), tetra-ethoxypropane (MDA), tripyridyl-s-tiazine (TPTZ), 2-thiobarbituric acid (TBA), Paraoxon were all purchased from Sigma Co.

Preparation of Nanoparticles

Different doses of CeNPs, for each group, were made based on the average weight of animals (per kg). Nanoparticles were suspended in deionized water. Before injection, a 5 min as sonication time was considered. Then, the suspension of the camps was immediately prepared and vigorously vortexed to avoid precipitation, before each injection.

Ethical Statement

The study design was approved by the Ethics Committee Guidelines of Hamadan University of Medical Sciences.

Experimental In Vivo Treatments and Sample Collection

Male Albino Wistar rats, with weight of 220–250 g, were purchased from animal breeding center of Pasteur Institute, Tehran, Iran. Animals were maintained in polypropylene cages and had freely access to tap water and standard chow. General condition of 12/12 h (light/dark) cycles and room temperature (23–25 °C) were optimized. After 2 weeks of acclimation, rats were randomly divided into 8 groups (5 per each), as noted here: (*Group 1*) Control, receiving normal saline//(*Group 2*) 100 mg/kg/day malathion//(*Group 3*) 15 mg/kg/day CeNPs//(*Group 4*) 30 mg/kg/day CeNPs//(*Group 5*) 60 mg/kg/day CeNPs//(*Group 6*) 100 mg/kg/day malathion plus 15 mg/kg/day CeNPs//(*Group 7*) 100 mg/kg/day malathion plus 30 mg/kg/day CeNPs//(*Group 8*) 100 mg/kg/day malathion plus 60 mg/kg/day CeNPs. Intraperitoneally (IP) injection and handling of all groups were cautiously performed by expert laboratory technicians. At the end of the treatment period, blood samples of rats were collected in tubes containing EDTA. Liver tissues were removed and quickly washed with ice-cold 0.9% NaCl solution. All samples were kept at – 80 °C. For the analysis, liver tissues were homogenized

with PBS (pH = 7.5). Then, homogenates were centrifuged at 3000 g. Supernatants were used to do the tests.

Biochemical Indicators of Liver Function and Malathion Toxicity

Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and cholinesterase activity (ChE) were assayed by the Pars Azmoon kit, Tehran, Iran.

Estimation of Oxidative Stress Biomarkers

Lipid Peroxidation (LPO)

Measurement of LPO was performed, during reaction activated with an acid heating protocol, by thiobarbituric acid reactive substances expressed as the extent of Malondialdehyde (MDA) products. Summarily, samples were first diluted with 1.5 mL TCA (20% w/v) and centrifuged at 3000g. The precipitations were mixed well with 1.5 mL TBA (0.2% w/v) and 1.5 mL H₂SO₄ (0.05 M). Then, an incubation time of 45 min in a boiling water bath was considered. Consequently, by adding 2 mL *n*-butanol to each solution, followed by cooling and centrifuging again, the absorption's were read at 532 nm. Eventually, tetraethoxypropan standard curve was used to determine the concentrations [18].

Total Antioxidant Capacity (TAC)

This test was done by the method of ferric reducing ability of plasma (FRAP), which basically depends on the ability of plasma in reduction of Fe (from 3 positive charges to 2), while TPTZ is available in the environment. A final blue color and good absorbance at 593 nm is gained when the reaction between Fe²⁺ and TPTZ happens [19].

Total Thiol Molecules (TTM)

Plasma total thiol molecules were evaluated using DTNB as a reagent. Briefly, 1 mL of tris buffer (250 mM and EDTA 2 mM, pH = 8) was mixed enough with 50 µL of each sample. Afterwards, by adding DTNB, the reaction between thiol molecules and DTNB resulted in the formation of a yellow complex with a maximum optical absorbance at 412 nm [10].

Paraoxonase-1 (PON1) Activity

The PON1 activity was assessed through quantification of p-nitrophenol formation from paraoxon, as described previously. In brief, freshly prepared paraoxon (1.0 mM) in 290 µL of 50 mM glycine buffer containing 1 mM CaCl₂

(pH 10.5) was incubated with 10 µL of sample, at 37 °C, for 10 min, in 96 well plates. p-nitrophenol formation was later monitored at 412 nm on a microplate reader (Biotrack II plate reader, Amersham Biosciences). The PON1 activity (mU mL⁻¹) was, finally, normalized by intracellular protein level [20].

Protein Extracts from Liver

Livers of control, marathon and CeNPs-fed rats were harvested and washed three times with ice-cold phosphate-buffered saline (PBS). The tissues were, thereafter, homogenized in 10 mM HEPES buffer (pH 7.5) containing 1 mM EDTA, 10 mM NaCl, 20% glycerol and 1 µg/ml each of the following protease inhibitors: aprotinin, phenylmethylsulfonyl fluoride and leupeptin. The nuclei were removed by 5 min centrifugation at 2000g at 4 °C, followed by centrifugation at 100,000g for 30 min at 4 °C. The resulting microsomal pellet was, subsequently, resuspended in a suitable volume of the homogenization buffer for determination of PON1 activity. The total protein concentration of the extract was ascertained by Bradford method (Bio-Rad, Hercules, CA) [21].

Serum and Liver PON1 Activities Measurement

Serum and liver PON1 enzyme activities were determined with paraoxon (Sigma–Aldrich Inc., St. Louis, MO) as the substrate. The activity of PON1 was measured as described by us previously in an aliquot of serum or the microsomal suspension from liver, based on the initial velocity of *p*-nitrophenol production at 37 °C and monitored at 405 nm continuously using SpectroMAX 190 (Molecular Devices, Sunnyvale, CA). 10 µL of serum or microsomal suspension was added into 990 µL of 20 mM Tris–HCl buffer (pH = 7.8, containing 2 mM CaCl₂ and 5 mM of paraoxon). Results were expressed as IU. As a rule, one unit of international enzyme activity was equal to 1 nmol of paraoxon hydrolyzed per minute per ml of serum or per g equivalent of liver microsomes [20].

Data Analysis and Statistics

All of the results were, routinely, expressed as the Mean ± SE for groups. Statistical analysis was carried out by using SPSS 18, and employing one-way analysis of variance (ANOVA) as was followed then by post hoc Tukey test. The level of significant difference, for all measurements, was regarded less than 5% (*p* < 0.05).

Result

Effect of CeNPs and Malathion on the Liver PON1 Activity

In liver homogenate Malathion displayed a significant decrease in PON1 activity when compared to control group; Fig. 1.

Effect of CeNPs and Malathion on the Liver and Serum Oxidative Stress Biomarkers and ChE Activity

In blood Malathion reduced ChE activity, when compared to control group. No significant change in serum ChE activity was presented by CeNPs group in comparison with control and other groups; Fig. 2.

Total Antioxidant Capacity

In blood No significant change in serum TAC level was caused by malathion and CeNPs 15 mg/kg group when compared to control and other groups. Also, TAC concentration increased in CeNPs 30 mg/kg and in groups of co-administration of CeNPs 15 and 30 with malathion, compared to malathion group; Fig. 3.

In liver homogenate Malathion evoked a significant decrease in TAC when compared to control. CeNPs 15 and 30 mg/kg caused a significant increase in TAC as compared to malathion group. Besides, Co-administration of CeNPs 15 and 30 with malathion significantly increased malathion-reduced TAC; Fig. 3.

Lipid Peroxidation

In blood No significant change in serum LPO level was caused by malathion and CeNPs group in comparison with control and other groups; Fig. 4.

Fig. 1 Paraoxonase activity (PON1) in liver homogenate and serum of rats. ^{aa}Significantly different from control group at $p < 0.05$. ^{bb}Significantly different from Mal group at $p < 0.05$. Control; Mal, malathion; CeNPs, Cerium Nanoparticles, CeNPs+Mal, malathion + Cerium Nanoparticles

Fig. 2 Cholinesterase (ChE) activity in serum of rats. ^{aa}Significantly different from control group at $p < 0.05$. ^{bb}Significantly different from Mal group at $p < 0.05$. Control; Mal, malathion; CeNPs, Cerium Nanoparticles, CeNPs+Mal, malathion + Cerium Nanoparticles

Fig. 3 Total antioxidant capacity (TAC) in liver homogenate and serum of rats. ^{aa}Significantly different from control group at $p < 0.05$. ^{bb}Significantly different from Mal group at $p < 0.05$. Control; Mal, malathion; CeNPs, Cerium Nanoparticles, CeNPs+Mal, malathion + Cerium Nanoparticles

Fig. 4 Lipid peroxidation (LPO) in liver homogenate and serum of rats. ^aSignificantly different from control group at $p < 0.05$. ^{aa}Significantly different from control group at $p < 0.05$. ^{bb}Significantly different from Mal group at $p < 0.05$. Control; Mal, malathion; CeNPs, Cerium Nanoparticles, CeNPs+Mal, malathion + Cerium Nanoparticles

In liver homogenate Malathion resulted in a significant increase in LPO, compared to control. Additionally, CeNPs 15 and 30 mg/kg caused a significant decrease in LPO when compared to malathion group. Co-administration of CeNPs 15 and 30 with malathion significantly reduced the malathion-induced LPO; Fig. 4.

Total Thiol Groups

In blood No significant change in serum TTG concentration was caused by malathion and CeNPs group when compared to control and other groups; Fig. 5.

In liver homogenate CeNPs 15 and 30 mg/kg caused a significant increase in TTG compared to malathion group. No significant change was seen in TTG in co-administration of CeNPs 15 and 30 with malathion compared with other groups; Fig. 5.

Discussion

In the last few decades, antioxidant materials have been well accepted as an certified model system to study the OP induced- oxidative toxicity [22]. OTS plays a vital role in malathion toxicity [2]. Thus, indigenous CeNPs were extensively examined for its antioxidative property against malathion. In the current study, the protective role of CeNPs on the malathion-induced OTS and PON1 activity was investigated.

Several reports from the past have performed studies on malathion-induced OTS in liver [21, 23], brain [17] and mitochondria of animals [2]. However, for the first time, we are demonstrating the malathion-induced OTS and toxicity in liver rat. The results of this study showed decrease in ChE activity, induction of LPO and PON activity and, moreover, reduction in TAC and TTG concentrations of the rat liver. Therefore, the ability to generate free radicals by malathion, which may lead to the PON damage, protein oxidation and lipid peroxidation, explicates the overall toxicity and OTS induction by malathion. Furthermore, CeNPs alone changed the levels of ROS in this study. This is in agreement with the recent findings reporting that malathion has induced OTS [24, 25].

Fig. 5 Total thiol groups (TTG) in liver homogenate and serum of rats. ^{aa}Significantly different from control group at $p < 0.05$. ^{bb}Significantly different from Mal group at $p < 0.05$. Control; Mal, malathion; CeNPs, Cerium Nanoparticles, CeNPs+Mal, malathion + Cerium Nanoparticles

Antioxidant enzymes such as PON activity were considered as biomarkers of OTS and liver damage [10]. The results of the experiment showed that CeNPs treatment significantly elevated the reduced OTS levels in liver tissue. Our results supported the pervious study on rat, where in the protective effect of antioxidant compounds was demonstrated [20]. To understand the underlying mechanism behind the role of CeNPs in preventing malathion-induced OTS and to assess the balance of ROS production, levels of enzymatic and non-enzymatic antioxidants were measured. However, in the recent published paper of Pulido-Reyes et al. [26] only the percentage of Ce^{3+}/Ce^{4+} surface content sites was determined to cause the anti- or pro-oxidant behavior, the less Ce^{3+} sites the less pro-oxidant status will be achieved.

Despite the stimulation mechanisms are not totally clears, CeO_2 NPs could stimulate cell proliferation through reduction of intracellular levels of ROS by modulating the expression level of the major antioxidant enzymes [27].

The results of this study confirm and extend previous data which have established malathion-induced impairments in CAT, SOD, GSH, GPx, GR and GST in blood, brain, kidney and liver of rats exposed to malathion [2, 28]. Among the variety of redox systems detected in eukaryotes, GSH is the most important antioxidant outstandingly in the malathion exposure cases in liver; further, the intracellular GSH level appears to be a sensitive marker for the cell overall health and ability to defend toxic challenges [2, 29]. Therefore, it seems that improvement of TTM by CeNPs can be through ROS scavenging effect of these nanoparticles in malathion toxicity. The decreasing level of antioxidants indicated the peroxidation of lipids and thiol groups of protein, which lead to macromolecular damage and liver injury.

Thus, the increase in PON activity may be partially explained by the ability of CeNPs to reduce oxidative stress through scavenging oxidative free radicals.

Hence, the decreased levels of antioxidant enzymes may be the reason for OTS markers accumulation which subsequently causes PON activity. Our results indicated that the activities of PON in the liver of malathion-exposed rats significantly increased.

Similarly, this study elucidates the protective effects of CeNPs treatment in blood and liver against malathion-induced OTS in rats. Thus, these results reveal that the consumption of CeNPs could be a promising biotherapeutic agent against OTS, induced by malathion.

Furthermore, in previous studies, interference of OTS in liver damage was proved and antioxidants usage for protection against these pathways was suggested. In this study, results showed that malathion induced ROS increase, as was declined by using CeNPs nanoparticles due to the antioxidant effects as well as ROS scavenging of these

nanoparticles and usefulness of CeNPs in this respect. The synergistic result of this combination is, likewise, noteworthy and can be the base of further research.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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