Environmental Toxicology and Pharmacology 43 (2016) 175–181

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap

The effects of α-tocopherol administration in chronically lead exposed workers

Sławomir Kasperczyk[a,*], Michał Dobrakowski[a], Aleksandra Kasperczyk[a], Ewa Nogaj[b], Marta Boroń[c], Ewa Birkner[a]

[a] Dept. of Biochemistry, School of Medicine with the Division of Dentistry in Zabrze, Medical University of Silesia in Katowice, ul. Jordana 19, 41-808 Zabrze, Poland
[b] Higher School of Medicine in Sosnowiec, Wojska Polskiego 6, 41-200 Sosnowiec, Poland
[c] Dept. of Department of Physical Hazards Work Physiology and Ergonomics Institute of Occupational Medicine and Environmental Health in Sosnowiec, ul. Kościelna 13, 41-200 Sosnowiec, Poland

ARTICLE INFO

Article history:
Received 2 November 2015
Received in revised form 10 March 2016
Accepted 13 March 2016
Available online 16 March 2016

Keywords:
Alfa-tocopherol
Lead poisoning
Oxidative stress
Lipid peroxidation
Antioxidants

ABSTRACT

The aim of the study was to investigate whether α-tocopherol supplementation for workers who are chronically exposed to lead would normalize/improve the values of parameters that are associated with the lead-induced oxidative stress.

Study population included chronically lead exposed males who were divided into two groups. Workers in the first group (reference group) were not given any antioxidants, while workers in the second group (αT group) received supplementation with α-tocopherol.

After treatment, the blood lead and leukocyte malondialdehyde levels decreased significantly in the αT group compared to the baseline levels and reference group. However, the erythrocyte malondialdehyde, conjugated dienes, and lipofuscin levels significantly increased compared to the baseline levels. The glutathione level significantly increased compared with the baseline.

Effects of supplementation with α-tocopherol on oxidative damage were not satisfactory. Therefore, there is no reason to administer α-tocopherol to workers chronically exposed to lead as a prophylaxis of lead poisoning.

1. Introduction

Although lead emission has decreased considerably, some occupational groups continue to encounter lead exposure at work. It is well established that lead negatively affects the function of the central and peripheral nervous systems, kidneys and hematological, cardiovascular, reproductive, alimentary, osseous and immunological systems. The toxic action of lead, to a large extent, is due to oxidative stress, resulting in oxidative damage to all cell structures, including lipids, DNA, and proteins (Flora et al., 2012; Prokopowicz et al., 2013). Fenton-like reactions play an important role in oxidative stress induced by redox-active metals, such as iron. Lead is a redox-inactive metal. Therefore, there are many proposed mechanisms for lead induced oxidative stress other than valence changes. Firstly, lead is able to disrupt the antioxidant defense system function via glutathione pool depletion, interferences with essential metals needed for antioxidant enzyme activities, and inhibition of sulfhydryl-dependent enzymes. Secondly, lead has been shown to alter lipid composition of cellular membranes resulting in their increased susceptibility to lipid peroxidation. Thirdly, lead is able to generate reactive oxygen species (ROS) indirectly via interaction with oxyhemoglobin. Besides, delta-aminolevulinic acid (ALA), which accumulates as a result of lead-induced inhibition of ALA dehydratase (ALAD), undergoes auto-oxidation and serves as a source of ROS as well (Gurer and Ercal, 2000).

To protect against oxidative damage, organisms have developed a complex antioxidant defense system. The group of enzymatic antioxidants includes superoxide dismutase (SOD), which converts superoxide anion to hydrogen peroxide, as well as catalase (CAT) and glutathione peroxidase (GPx), which utilize hydrogen peroxide. The group of non-enzymatic antioxidants contains a variety of different compounds. These include thiol compounds, such as glutathione, which is responsible for the overall cellular redox regulation in mammalian cells; nutritional trace minerals; and vitamins, including vitamin E (Kasperczyk et al., 2012; Rodríguez-Estival et al., 2011).

* Corresponding author at: Dept. of Biochemistry, Medical University of Silesia, ul. Jordana 19, 41-808 Zabrze, Poland.
E-mail addresses: kaslav@mp.pl, skasperczyk@sum.edu.pl (S. Kasperczyk).

http://dx.doi.org/10.1016/j.etap.2016.03.012
1382-6689/

Vitamin E is a fat-soluble vitamin that contains α-, β-, δ- and γ-tocopherols. Of these, α-tocopherol is the best-known form of vitamin E that protects cell membranes form lipid peroxidation by ROS (superoxide radical anion and lipid peroxyl free radical) scavenging (Flora et al., 2012; Prokopowicz et al., 2013). Several animal studies have shown that vitamin E administration may counteract the deleterious effect of lead poisoning (Flora et al., 2012). In our previous study, we reported that the α-tocopherol level was 17% lower in a group of workers with mean blood lead level under 40 μg/dl and 19% in a group of workers with mean blood lead level above 40 μg/dl (Dobrakowski et al., 2014). In a similar group of workers, we additively reported elevated oxidative stress parameters, including lipid peroxidation markers (Kasperczyk et al., 2013). These results inspired us to investigate whether α-tocopherol supplementation in workers who are chronically exposed to lead would normalize or improve the values of parameters associated with the oxidative stress intensity and antioxidant system function.

2. Materials and methods

2.1. Study population

The Bioethics Committee of the Medical University of Silesia in Katowice approved the experimental protocol (No. NN-6501-36/I/06).

The exposed population included 88 healthy males employed in zinc and lead production facilities in Miasteczko Śląskie, Poland. An occupational medicine specialist recruited all study participants during prophylactic medical examinations, and patients signed informed consent forms before participating in the study. Their mean age was 40.1 ± 8.86 years. They were exposed to lead for an average of 16.1 ± 9.82 years.

The blood concentrations of lead (PbB) and zinc protoporphyrin (ZPP) were evaluated to estimate the level of lead exposure. On average, the PbB and ZPP levels were determined every three months for two years of observation prior to the start of the study. At the beginning of the study, the mean lead (PbB_{mean}) and zinc protoporphyrin (ZPP_{mean}) levels in the blood were calculated.

The inclusion criteria were as follows: occupational exposure to lead (PbB_{mean} > 20 μg/dl and ZPP_{mean} > 2.5 μg/g Hb), no significant findings on physical examination and no history of any chronic disease, such as malignant neoplasm, diabetes, coronary artery disease, or hypertension. Exclusion criteria included contraindications to α-tocopherol administration, such as intolerance to the preparation and vitamin K deficiency.

The examined population was randomly divided into two groups. The study participants and investigators were not blinded to the treatment group assignment. Workers in the first group (n = 50, reference group) were not given any antioxidants, drugs, vitamins or dietary supplements. Workers in second group (n = 38, αT group) were given oral α-tocopherol (Vitaminum E, Medana Pharma Terpol) at a dose of 200 mg once a day. The study lasted until the next prophylactic medical examination, which occurred at 12 weeks. At the end of the study, participants were asked to return their empty pill containers to a study investigator who then assessed compliance with a treatment protocol.

Blood was drawn twice from the study participants at up to three days before the prophylactic medical examination at the start of the study and up to three days before the second prophylactic medical examination at 12 weeks. Biochemical analysis included the aforementioned markers of lead exposure, PbB and ZPP. The following compounds served as oxidative stress biomarkers: the levels of malondialdehyde (MDA) in leukocytes and erythrocytes, plasma levels of conjugated dienes (CD), serum levels of lipid hydroperoxides (LHP), lipofuscin (LPS) levels in erythrocytes, and protein carbonyl groups (PC) in serum. We also determined the level of glutathione (GSH) in erythrocytes and the activities of enzymes involved in the antioxidant defense in blood cells, such as SOD, CAT, GPx, glutathione reductase (GR), glutathione-S-transferase (GST), and glucose-6-phosphate dehydrogenase (G6PD).

2.2. Laboratory procedures

To obtain whole blood, erythrocytes and leukocytes, 14 mL of blood was drawn, by venipuncture, into tubes containing an EDTA solution as an anticoagulant.

PbB was analyzed in the whole blood by graphite furnace atomic absorption spectrophotometry using Unicam 929 and 939OZ Atomic Absorption Spectrometers with GF90 and GF90Z Graphite Furnaces. Data were expressed in μg/dL. The ZPP levels were measured using the Aviv Biomedical Hematofluorometer Model 206. The results were expressed in μg ZPP per gram of hemoglobin (μg/g Hb).

Immediately after blood sampling, 5 mL of whole blood was centrifuged. The plasma supernatant was removed. The sedimented erythrocytes were washed three times through centrifugation with 0.9% sodium chloride solution and then lysed with bidistilled water. Finally, 10% (v/v) hemolysate was prepared.

To isolate the leukocytes, 3 mL of the whole blood was layered over Histopaque-1077 (Sigma-Aldrich), in a 1:1 ratio, and centrifuged for 30 min. Leukocytes (1.5 mL) were collected from the interface and washed three times through centrifugation with 0.9% sodium chloride solution. Finally, the lysate of leukocytes was prepared in 1.5 mL of bidistilled water.

The levels of MDA were determined as described in Ohkawa et al. (1979). The results were recorded as micromoles per gram of protein (μmol/g P) in leukocytes and as nanomoles per gram of hemoglobin (nmol/g Hb) in erythrocytes. The concentrations of CD were measured according to Corongiu et al. (1989). The method by Södergren et al. (1998) was used to measure the concentrations of LHP. The CD and LHP concentrations were recorded in μmol/L. The method described by Reznick and Packer (1994) was used to identify the serum PC. The results were expressed as nanomoles per gram of protein (nmol/g P). The levels of LPS in erythrocytes were measured according to the Jain (1985) method. The results were expressed as relative units (RU) per gram of hemoglobin (the fluorescence of a 0.1 mg/mL solution of quinidine sulfate in sulfuric acid is equal to 100 RU).

The GSH levels were measured as described by Pawelski (1983). The obtained concentrations were expressed as micromoles per gram of hemoglobin (μmol/g Hb). The erythrocyte GPx activity was measured by the kinetic method of Paglia and Valentine (1967). The GPx activity was expressed as micromoles of NADPH oxidized per minute normalized to grams of hemoglobin (IU/g Hb). The activity of GST in leukocytes was measured according to the kinetic method described by Habig and Jakoby (1981). The GST activity was expressed as micromoles of thioether produced per minute and normalized to one gram of protein (IU/g P). The G6PD and GR activities in erythrocytes and leukocytes were measured according to Richterich (1971). G6PD activity was expressed as micromoles of NADPH produced per minute normalized to one gram of hemoglobin in erythrocytes (IU/g Hb) and one gram of protein in leukocytes (IU/g P), whereas the GR activity was expressed as micromoles of NADPH utilized per minute normalized to one gram of hemoglobin in erythrocytes (IU/g Hb) and one gram of protein in leukocytes (IU/g P).

The method of Oyanagui (1984) was used to measure the SOD activity in leukocytes and erythrocytes. The SOD activity was expressed in nitric units. The SOD activity is equal to 1 nitric unit (NU) when it inhibits nitric ion production by 50%. The SOD activities were normalized to milligrams of hemoglobin in erythrocytes