Toxicology and Applied Pharmacology 280 (2014) 36–41

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

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

Beta-carotene reduces oxidative stress, improves glutathione metabolism and modifies antioxidant defense systems in lead-exposed workers

Sławomir Kasperczyk [a,*], Michał Dobrakowski [a], Janusz Kasperczyk [b], Alina Ostałowska [a], Jolanta Zalejska-Fiolka [a], Ewa Birkner [a]

[a] Dept. of Biochemistry, School of Medicine with the Division of Dentistry, Medical University of Silesia, ul. Jordana 19, 41-808 Zabrze, Poland
[b] Dept. of Environmental Medicine and Epidemiology, School of Medicine with the Division of Dentistry, Medical University of Silesia, ul. Jordana 19, 41-808 Zabrze, Poland

ARTICLE INFO

Article history:
Received 17 March 2014
Revised 29 June 2014
Accepted 8 July 2014
Available online 16 July 2014

Keywords:
Beta-carotene
Lead poisoning
Oxidative stress
Lipid peroxidation
Antioxidants

ABSTRACT

The aim of this study was to determine whether beta-carotene administration reduces oxidative stress and influences antioxidant, mainly glutathione-related, defense systems in workers chronically exposed to lead.
The population consisted of two randomly divided groups of healthy male volunteers exposed to lead. Workers in the first group (reference group) were not administered any antioxidants, while workers in the second group (CAR group) were treated orally with 10 mg of beta-carotene once a day for 12 weeks. Biochemical analysis included measuring markers of lead-exposure and oxidative stress in addition to the levels and activities of selected antioxidants.
After treatment, levels of malondialdehyde, lipid hydroperoxides and lipofuscin significantly decreased compared with the reference group. However, the level of glutathione significantly increased compared with the baseline. Treatment with beta-carotene also resulted in significantly decreased glutathione peroxidase activity compared with the reference group, while the activities of other glutathione-related enzymes and of superoxide dismutase were not significantly changed. However, the activities of glucose-6-phosphate dehydrogenase and catalase, as well as the level of alpha-tocopherol, were significantly higher after treatment compared with the baseline.
Despite controversy over the antioxidant properties of beta-carotene in vivo, our findings showed reduced oxidative stress after beta-carotene supplementation in chronic lead poisoning.

Introduction

Lead is a ubiquitous environmental toxin that has been detected in almost all phases of biological systems. Even small quantities of lead are harmful to humans and other organisms because they induce a broad range of physiological, biochemical, and behavioral dysfunctions in many parts of the body, including the central and peripheral nervous systems, hematopoietic system, cardiovascular system, kidneys, liver and reproductive systems. As a result, a safe level of lead exposure has not yet been defined (Hsu and Guo, 2002; Massó-González and Antonio-García, 2009).

It has been proposed that the main mechanism involved in lead toxicity is the induction of oxidative stress. It is believed that lead is able to generate reactive oxygen species (ROS), such as singlet oxygen, and alter the function of antioxidant defense system components, including antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) (Casado et al., 2007; Kasperczyk et al., 2012). Moreover, lead inhibits delta-aminolevulinic acid dehydratase (delta-ALAD). This inhibition leads to the accumulation of delta-aminolevulinic acid (delta-ALA), which has pro-oxidant properties. Lead may also induce the generation of ROS by interacting with oxyhemoglobin, which undergoes autoxidation. Enhanced production of ROS leads to cell structure damage, including lipid peroxidation and oxidation of amino acids and nucleic acids (Gurer and Ercal, 2000).

Currently, the only generally approved clinical intervention for lead poisoning is the administration of chelating agents, which bind and remove lead from lead-burdened tissues. However, safety and efficacy are lacking in the use of conventional chelating agents (Gurer and Ercal, 2000). Therefore, new therapeutic approaches must be developed. The potential role of oxidative stress injury in lead poisoning suggests that natural antioxidants may be effective in the treatment; however, this has not been thoroughly investigated.

A few studies on animals suggest that antioxidants can play an important role in abating the toxic effects of lead (Hsu and Guo, 2002; Patrick, 2006). However, insufficient studies have been conducted on humans and further investigation should be performed to clarify the

* Corresponding author. Fax: +48 32 2722318.
E-mail address: kaslav@mp.pl (S. Kasperczyk).

http://dx.doi.org/10.1016/j.taap.2014.07.006
0041-008X/

mechanisms of dietary antioxidant supplementation. In our previous study, we showed that N-acetylcysteine (NAC) reduces oxidative stress and regulates glutathione metabolism in lead-exposed workers (Kasperczyk et al., 2013b). Our satisfactory results prompted us to investigate other antioxidants. In the present study, we focused on beta-carotene.

Beta-carotene is one of the isoprenoid compounds named carotenoids. It is a red-orange pigment found in fruits, vegetables and green leafy plants. It is absorbed in the duodenum and stored in the adipose tissue. In addition to metabolic human functions, carotenes also have antioxidant properties. Beta-carotene is able to quench singlet oxygen without degradation and reacts with free radicals, such as peroxyl, hydroxyl and superoxide radicals. Carotenoids have consistently been shown to prevent or decrease oxidative damage to DNA, lipids and proteins (Agarwal et al., 2012; Chapman, 2012). As a result, beta-carotene is used in the prevention and treatment of many diseases that develop with the participation of oxidative stress (mainly neoplasms and cardiovascular abnormalities) (Rutkowski and Grzegorczyk, 2012) and should be considered as an alternative therapy for chronic lead intoxication which is associated with increased oxidative stress with singlet oxygen overproduction and lipid peroxidation. In light of this, the goal of this study was to determine whether beta-carotene administration reduces oxidative stress and influences antioxidant defense systems in workers chronically exposed to lead.

Materials and methods

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

The exposed population consisted of 85 healthy male volunteers employed in zinc and lead production facilities in Miasteczko Śląskie, Poland. The mean concentration of lead in the air at the facilities was 0.083 ± 0.12 mg/m^3. All participants were recruited by an occupational medicine specialist during prophylactic medical examinations and provided informed consent to the study. Their ages ranged between 22 and 58 years, and they were exposed to lead for 4 to 38 years.

Blood concentrations of lead (PbB) and zinc protoporphyrin (ZPP) served as lead-exposure indices. On average, the levels of PbB and ZPP were measured every three months for two years of observation prior to the start of the study. At the beginning of the study, the mean levels of lead (PbB_{mean}) and zinc protoporphyrin (ZPP_{mean}) in the blood were calculated.

The inclusion criteria were the following: occupational exposure to lead ($PbB_{mean} > 20$ μg/dl and $ZPP_{mean} > 2.5$ μg/g Hb), no significant findings in physical examinations and no history of any chronic disease. Exclusion criteria included contraindications to beta-carotene administration, such as allergy or intolerance to the used beta-carotene preparation.

The examined population was randomly divided into two groups. At the beginning of the study, each participant of the study drew a piece of paper with the group number from a bag. The study participants and investigators were not blinded to the treatment group assignment. Workers in the first group ($n = 50$, reference group) were not administered any antioxidants, drugs, vitamins or dietary supplements. Workers in second group ($n = 35$, CAR group) were treated orally with beta-carotene (Beta Karoten®, Chance) in a dose of 10 mg once a day. The study lasted 12 weeks until the next prophylactic medical examination, when participants were asked to return their empty pill containers to a study investigator to assess compliance with treatment protocol.

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

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 as μg/dl. 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 the 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 amount of protein in the serum was measured by titration. The levels of malondialdehyde (MDA) were determined as per 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. Concentrations of conjugated dienes (CD) were measured according to Corongiu et al. (1989), while the method by Södergren et al. (1998) was used to measure concentrations of lipid hydroperoxides (LHP). The concentrations of CD and LHP were recorded in μmol/l. The method of Reznick and Packer (1994) was used to identify serum protein carbonyl groups (PC). The results were expressed as nmoles 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 levels of GSH were measured as described by Pawelski (1983). The obtained concentrations were expressed as μmoles per gram of hemoglobin (μmol/g Hb).

The method of Oyanagui (1984) was used to measure the activity of superoxide dismutase (SOD) in leukocytes and erythrocytes. The enzymatic activity of SOD was expressed in nitric units. The activity of SOD is equal to 1 nitric unit (NU) when it inhibits nitric ion production by 50%. Activities of SOD were normalized to milligrams of hemoglobin in erythrocytes (NU/mg Hb) and to milligrams of protein in leukocytes (NU/mg protein). The catalase (CAT) in leukocytes and erythrocytes was measured by the Aebi (1984) kinetic method. Catalase activity was expressed as international units per milligram of hemoglobin in erythrocytes (IU/mg Hb) and per milligram of protein in leukocytes (IU/mg protein). Erythrocyte glutathione peroxidase (GPx) activity was measured by the kinetic method of Paglia and Valentine (1967). The activity of GPx was expressed as micromoles of NADPH oxidized per minute normalized to grams of hemoglobin (IU/g Hb). The activities of G6PD and GR in erythrocytes and leukocytes were measured according to Richterich (1971). G6PD activity was expressed as μmoles of NADPH produced per minute normalized to 1 g of hemoglobin in