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Environmental exposure to lead induces oxidative stress and modulates the function of the antioxidant defense system and the immune system in the semen of males with normal semen profile

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ABSTRACT

We investigated the associations between environmental exposure to lead and a repertoire of cytokines in seminal plasma of males with normal semen profile according to the WHO criteria. Based on the median lead concentration in seminal plasma, 65 samples were divided into two groups: low (LE) and high exposure to lead (HE). Differences in semen volume and the pH, count, motility and morphology of sperm cells were not observed between the examined groups. The total oxidant status value and the level of protein sulfhydryl groups as well as the activities of manganese superoxide dismutase and catalase were significantly higher in the HE group, whereas the total antioxidant capacity value and the activities of glutathione reductase and glutathione-S-transferase were depressed. IL-7, IL-10, IL-12, and TNF-α levels were significantly higher in the HE group compared with the LE group. Environmental exposure to lead is sufficient to induce oxidative stress in seminal plasma and to modulate antioxidant defense system.

Introduction

Despite reductions in the environmental emissions of lead, exposure to this xenobiotic is still a public concern, especially in developing countries (Hernández-Ochoa et al., 2005). The annual production of lead is approximately 2.5 million tons globally. Among others, batteries, leaded gasoline, paints, water pipes, insecticides and some cosmetics are the major sources of lead exposure. The general population is exposed to lead primarily through the air, water, soil, food and consumer products (Ayinde et al., 2012). While blood lead content (PbB) comprises 2% of the total body lead, the remaining 95% of absorbed lead is deposited in bones and dentine; the remaining 3% is distributed in other body tissues. Due to redistribution from bones, PbB may remain elevated for years after cessation of exposure. Although the World Health Organization defined a PbB of 40 μg/dl in adults and 10 μg/dl in children as the level of concern, some studies indicate that there is no safe level of exposure (Hosni et al., 2013).

One mechanism by which lead exerts its adverse health effects is by inducing oxidative stress (OS). OS can be defined as an imbalance between the production of reactive oxygen species (ROS) and antioxidant defense mechanisms (Ayinde et al., 2012). Lead is able to both generate ROS and interfere with the human antioxidant defense system. Having a strong affinity for thiol groups, lead impairs the function of many enzymes, (i.e., such as superoxide dismutase, catalase or glutathione peroxidase) and depletes the content of glutathione, which serves as a major thiol antioxidant of the human body (Kasperczyk et al., 2012, 2014). Similarly, lead also affects the immune system; although lead is not cytotoxic to immune cells at low to moderate levels (up to 50 μg/dl), it can still induce the deregulation of their function. Immunomodulatory effects of exposure to lead include an imbalance in T helper (Th) function. Lead induces the Th2 response and, in consequence, may decrease cell-mediated immune responses (Luna et al., 2012). Furthermore, lead may impair humoral immune responses, resulting in a

Abbreviations: LE, low exposure to lead; HE, high exposure to lead; OS, oxidative stress; ROS, reactive oxygen species; MDA, malondialdehyde; TOS, total oxidant status; TAC, total antioxidant capacity; PSH, protein sulfhydryl groups; OSI, oxidative stress index; SOD, superoxide dismutase; Mn-SOD, manganese isoenzyme of SOD; CuZn-SOD, copper zinc isoenzyme of SOD; CAT, catalase; GPx, glutathione peroxidase; GR, glutathione reductase; GST, glutathione S-transferase; G6PD, glucose 6-phosphate dehydrogenase; PbS, concentration of lead in seminal plasma; TBARS, 2-thiobarbituric acid-reactive substance; NU, nitric unit.

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decrease of antibody production. Lead has also been shown to affect the function of granulocytes and monocytes and to modify the cytokine balance (Valentino et al., 2007).

Injury to the male reproductive system is a recognized consequence of lead poisoning (Alexander et al., 1996). Additionally, exposure to lead has been associated with impaired semen quality, including decreased number, motility, and altered morphology of sperm; oxidative DNA damage; perturbed chromatin condensation; altered acrosome reactions and decreased prostate secretory function as well as an increased frequency of spontaneous abortion. The adverse effects of lead on reproductive functions are well established; however, the threshold of their occurrence is still under investigation. Although, most studies indicate no-adverse effects of blood lead levels up to 40–50 μg/dl, some new studies suggest that lead may have adverse impacts on male reproductive health at relatively low levels (Hosni et al., 2013; Awadalla et al., 2011). However, the possible involved mechanisms are poorly understood (Alexander et al., 1998). Therefore, there is a need to evaluate the toxic effect on male reproductive ability following exposure to lead at low-environmental levels (Hernández-Ochoa et al., 2005). Because testicular tissue is susceptible to ROS attack (Ayinde et al., 2012) and because its function is controlled by the neuroendocrine-immune network integrated by cytokines (Seshadri et al., 2009), plausible mechanisms for lead-influenced impaired spermatogenesis are lead-induced OS and the deregulation of the immune system by lead. Therefore, the present study was designed to investigate the associations between environmental exposure to lead and a repertoire of cytokines (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IFN-γ, TNF-α) in the seminal plasma of males with normal semen profile according to the WHO criteria. Additionally, we determined the relationship between lead levels and levels of OS markers (malondialdehyde (MDA), total oxidant status (TOS), total antioxidant capacity (TAC), protein sulfhydryl groups (PSH), oxidative stress index (OSI) as well as several antioxidant enzyme activities-superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), glucose 6-phosphate dehydrogenase (G6PD)) in seminal plasma. In addition, MDA concentration was determined in spermatozoa.

Materials and methods

Study population. Examined group consisted of males who had attended andrology clinic to diagnose infertility. The inclusion criteria were defined as follows: normal semen profile according to the WHO criteria (WHO, 2010) and no drug consumption (including antioxidant medications). The exclusion criteria were defined as follows: smoking habits, alcohol abuse, and a history of any chronic disease, such as diabetes, coronary artery disease, or malignant neoplasm. 114 subjects met inclusion criteria. Among 78 subjects, who additionally agreed to participate in the study, 13 met one or more exclusion criteria. This left 65 study participants. Based on the median of the values of lead concentration in seminal plasma (PbS = 1.00 μg/dl), the subjects were divided into two groups:

1. a group with low environmental exposure to lead (LE)—lead concentration in seminal plasma between 0.40 and 1.00 μg/dl;
2. a group with high environmental exposure to lead (HE)—lead concentration in seminal plasma between 1.01 and 2.70 μg/dl.

Sample collection. Semen was collected on the same day in the morning before the first meal. Semen samples (2–6 ml) were collected by masturbation, at home or at laboratory research facilities, after at least 3 days of sexual abstinence (number of days elapsed since last ejaculation was recorded for each volunteer).

Semen analysis. All of the semen specimens were analyzed according to WHO standards (WHO, 2010), including the assessment of seminal volume, sperm cell density, total sperm cell count, motility, and supravital eosin staining (for percentage of live spermatozoa). Sperm morphology was examined after Papanicolaou staining. The semen samples (1.5 ml) after liquefaction were centrifuged at 6000 *g* for 10 min to separate the spermatozoa from the seminal plasma. The seminal plasma was transferred to fresh tubes and stored at −75 °C until required for the biochemical and lead analyses. Additionally, a 10% spermatozoa lysate in bi-distilled water was made.

Evaluation of lead concentration. Concentration of lead in seminal plasma (PbS) was measured using graphite furnace atomic absorption spectrophotometry Unicam 929 and 939QZ Atomic Absorption Spectrometers with GF90 and GF90Z. Before each assay 1000 μl of modifier (0.1% nitric acid, 0.5% Triton X100 and 0.2% ammonia phosphate) was added to 100 μl of sample and mixed thoroughly before analysis. The calibration curve was prepared according to Norwegian Nycomed company standards. The spectrophotometer was calibrated at the beginning and after each series of assays. Certified Nycomed company standard as an internal control was used—Pb concentration of 4.2 μg/dl. The drying, ashing, and atomizing temperatures for PbS were 600 °C and 1500 °C (Unicam). The concentration of lead in the semen specimens was calculated from a standard curve. Data are shown in μg/dl.

Determination of malondialdehyde (MDA). MDA, a product of lipid peroxidation, was measured fluorometrically as a 2-thiobarbituric acid-reactive substance (TBARS) in seminal plasma according to Ohkawa (Ohkawa et al., 1979) with modifications. Samples were mixed with 8.1% sodium dodecyl sulfate, 20% acetic acid and 0.8% 2-thiobarbituric acid. After vortexing, samples were incubated for 1 h in 95 °C and butanol-pyridine 15:1 (*v*/*v*) was added. The mixture was shaken for 10 min and then centrifuged. The butanol-pyridine layer was measured fluorometrically at 552 nm and 515 nm excitation (Perkin Elmer, USA). TBARS values are expressed as malondialdehyde (MDA) equivalents. Tetraethoxypropane was used as the standard. Concentrations are given in μmol/l plasma and μmol/dl in packed spermatozoa.

Determination of superoxide dismutase (SOD) activity. The method of Oyanagui (Oyanagui, 1984) was used to measure the activity of SOD in seminal plasma. In this method, xanthine oxidase produces superoxide anions which react with hydroxylamine forming nitric ions. This ions react with naphthalene diamine and sulfanilic acid generating a colored product. Concentration of this product is proportional to the amount of produced superoxide anions and negatively proportional to the activity of SOD. Absorbance was measured using an automated analyzer Perkin Elmer at wavelength of 550 nm. The enzymatic activity of SOD was expressed in nitric units. The isoenzymes of SOD, such as Mn-SOD and CuZn-SOD, were also indicated, using KCN as the inhibitor of the CuZn-SOD activity. The activity of SOD is equal to 1 nitric unit (NU) when it inhibits nitric ion production by 50%. Activities of SOD in seminal plasma were expressed in NU/mg protein.

Determination protein. The protein level was measured by means of the A25 biochemical analyzer (BioSystems, Spain) according to the manufacturer's instructions. The results for protein levels are expressed in g/l.

Determination of protein sulfhydryl groups (PSH). PSH concentration was determined as described by Koster (Koster et al., 1986) using DTNB, which undergoes reduction by compounds containing sulfhydryl groups, yielding the yellow anion derivative, 5-thio-2-nitrobenzoate, which absorbs at a wavelength of 412 nm using an automated analyzer Perkin Elmer. The results were shown in μmol/g protein.

Determination of glucose 6-phosphate dehydrogenase (G6PD) activity. The activity of G6PD in seminal plasma was measured according to Richterich (1971) using an automated analyzer Perkin Elmer. G6PD