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REVIEW

RELATIONSHIP OF LEAD WITH FREE RADICALS, REACTIVE OXYGEN SPECIES, OXIDATIVE STRESS AND ANTIOXIDANT ENZYMES

Seda VURAL AYDIN *

Kafkas University, Kağızman Vocational School, Department of Pharmacy Services, Kars

ABSTRACT

Heavy metals, which are high in the environment, are substances that have a high toxic effect even at low concentrations. Heavy metals taken into the organism through mouth, respiration and skin cannot be eliminated by the body's excretory pathways. In addition, since they have a durable structure, they participate in the food chain and accumulate in various body tissues. With the increase in industrial activities, heavy metal pollution has also emerged and has increased day by day. Lead is an element that is widely used in industry because it has a low melting temperature. However, it is known that lead, like other heavy metals, has an effect on problems such as environmental pollution and health problems. Occupational diseases such as lead poisoning occur as a result of direct exposure to lead. Direct exposure in this way can also cause death. Lead causes undesirable conditions such as increase of reactive oxygen species (ROS), emergence of oxidative stress and weakening of antioxidant system. Lead disrupts the prooxidant/antioxidant ratio. As a result, effects such as an increase in ROS and thus damage to the basic components of the cell such as lipid, protein and nucleic acid are observed. In particular, lead inhibits enzymes and prevents some enzymes from acting as antioxidants. As a result of exposure to lead, there is a decrease in the defense abilities of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione (GSH) and ascorbic acid in blood and tissues. Lead increases lipid peroxidation and thus causes oxidative damage. Evaluation of the effect of lead at the cellular level is important in terms of developing solutions for the toxic effects of lead. In this study, the effect of lead on the cellular level in the organism and its effects on free radicals, ROS and oxidative stress were evaluated.

Keywords: Antioxidant enzymes, Free radical, Heavy metals, Lead, Oxidative stress

1. INTRODUCTION

Due to industrial development, waste materials are discharged to the environment, primarily soil and water. This situation causes heavy metal accumulation especially in urban areas. Unconsciously releasing heavy metals to the environment creates long-term toxicity in the ecosystem. It also causes health problems in terms of human health. Heavy metals can have a toxic effect on metabolism by changing enzymatic and structural functions when taken by the organism [1]. Most heavy metals can be toxic even at low concentrations. Heavy metals such as arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, silver, zinc is carcinogenic and mutagenic [2]. Toxicity from heavy metals is generally associated with the formation of reactive oxygen species (ROS). ROS increase primarily leads to oxidative stress. Oxidative stress causes changes in the electron transport chain, disruption of macromolecules and finally cellular damage in different tissues and organs [3]. All biological systems are affected because of exposure to lead through air, water and food sources. In this regard, lead is characterized as a dangerous and cumulative environmental pollutant. Regardless of the way it is taken into the body, all chemical forms of lead have a toxic effect [4]. Lead is dispersed into the environment in three forms: metallic lead, lead salts, and organic lead containing carbon [5].

The sources of lead exposure can be listed as leaded gasoline, lead-based paints, lead-containing food cans, ceramic glasses and batteries [4, 6]. Health problems caused by lead have been seen as a public health problem especially in recent years. Therefore, health problems that may arise with lead contact

*Corresponding Author: sedavural76@hotmail.com
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are controlled [7]. The widespread presence of lead in industry and the environment makes lead exposure occupationally important [8]. In addition, exposure to lead through ways such as domestic tap water, food contamination and house dust causes environmental pollution. It can cause behavioral, cognitive, physiological, and biochemical abnormalities on living things [7, 9, 10].

During normal biochemical reactions, compounds called reactive oxygen species (ROS) are formed [11]. Oxidative stress may occur when ROS reaches a high concentration, and the antioxidant mechanism cannot tolerate this situation. In this case, cellular structures may be damaged [12,13]. Macromolecules such as lipid, protein and nucleic acid can be oxidized by ROS. Lipid peroxidation reactions occur as a result of the oxidation of unsaturated fatty acids in the cell membrane structure. Thus, intermediate products are formed with lipid peroxides. The most common of these intermediates is malondialdehyde (MDA). MDA affects the cell membrane and physiological functions of the cell [12, 14].

Reactive oxygen species (ROS) produced in living organisms are detoxified by enzymatic and non-enzymatic antioxidant defense systems [11, 15]. Antioxidant enzymes are generally superoxide dismutases, catalases and glutathione (GSH) peroxidases. Non-enzymatic antioxidants are glutathione (GSH), α-tocopherol, carotenoids, and vitamin C [16, 17].

Lead disrupts the prooxidant/antioxidant balance, causing an increase in ROS. Thus, it damages cellular structures such as lipids, proteins, and nucleic acids [11]. In this review, it is aimed to evaluate the scientific data about the effect of lead at the cellular level, the formation of free radicals, ROS and oxidative stress, and their relationship with lead.

2. FREE RADICALS

Free radicals are formed during physiological and pathological reactions. They have one or more unpaired electrons in their orbitals. They are unstable, low molecular weight and highly reactive molecules that can act independently [12, 17, 18, 19, 20].

Free radicals formed in cells consist of reactive oxygen species (ROS) and reactive nitrogen species (RNS). It is produced against degenerative conditions and various physical-chemical stimuli in the organism [21]. The presence of free radicals in medium or low amounts in the environment provides benefits in signaling and immune mechanisms. The presence of excess free radicals causes lipid peroxidation, inhibition of enzyme activity, and apoptosis. In addition, it causes events such as the destruction of the DNA molecule, which results in undesirable situations such as mutagenesis and carcinogenesis. It causes events such as the destruction of the DNA molecule resulting in undesirable situations such as mutagenesis and carcinogenesis [21, 22, 23].

3. REACTIVE OXYGEN SPECIES (ROS)

Oxygen-derived free radicals are very important for living things [14, 24]. Oxygen radicals are formed when an unstable oxygen uses the electrons of another oxygen together. When oxygen is reduced, short-lived but strong oxidant free oxygen radicals are formed. With the reduction of oxygen, free oxygen radicals with short-lived but strong oxidant properties emerge [24].

ROS is formed as a result of the biochemical reactions of the cell and shows toxic properties. Molecules such as lipid, protein and nucleic acid can be oxidized by ROS. Peroxidation reactions start with the oxidation of unsaturated fatty acids in the membrane structure. As a result of lipid peroxidation, lipid peroxides and intermediate products are formed. Malondialdehyde (MDA), one of these intermediates, affects cell membrane and physiological functions [11, 14, 25].

Major reactive oxygen species; superoxide anion (O^{2-}); hydrogen peroxide (H_2O_2); hydroxyl radical ($\cdot OH$); and singlet oxygen ($^1O_2$), which constitutes a large part of the biologically important free radicals [13]. Reactive oxygen species are detoxified by antioxidants. Thus, the cell keeps the amount of ROS under control. The intracellular ROS concentration is usually around 8-10 molar [26, 27]. ROS, which are formed as a result of oxidative stress, damage the membrane lipids and thus the cell through lipid peroxidation. In addition to lipid peroxidation, lead also causes erythrocyte (RBC) hemolysis by directly causing hemoglobin oxidation. This is due to inhibition of the enzyme Delta-aminolevulinic acid dehydratase (ALAD). Inhibition of ALAD leads to an increase in the concentration of substrate D-aminolevulinic acid (ALA) in blood and urine, thus producing hydrogen peroxide and superoxide radical [25, 28]. The progression of the mechanisms may cause the cell to be vulnerable to oxidative stress and even to death [9].

4. OXIDATIVE STRESS

The deterioration of the balance between the antioxidant defense system and the production of free radicals, that is, the ratio of prooxidant and antioxidant, is defined as oxidative stress [20, 29]. The intracellular ROS concentration may vary in some cases due to the continuous production and consumption of ROS. At levels where the ROS concentration is stable, the amount of ROS produced is equal to the amount of ROS consumed. However, the concentration of ROS may be responsible for the alteration of the oxidative stress state and thus the damage to cells, tissues, and organs. Under normal conditions, the balance between ROS production and consumption is achieved by keeping the ROS ratio constant, while the occurrence of oxidative damage causes the ROS level to increase. In such a case, the increase in ROS level occurs as a temporary, that is acute oxidative, thanks to its antioxidant potential. If the antioxidant potential is not in balance, chronic oxidative stress is seen because of faster production of ROS [27].

Factors such as smoking, heavy metals, radiation, infection cause an increase in oxidative stress. Oxidative stress also damages other macromolecules, especially lipids. As a result, it causes tissue damage, chronic diseases and even death [30]. Oxidative stress plays an important role in the formation of many chronic and degenerative diseases (cancer, autoimmune diseases, cardiovascular diseases, etc.) [12, 22]. Especially since the brain is the organ most affected by oxidative damage, free radicals are the cause of many pathological cases [29].

Oxidative stress forms the basis of especially toxicological studies [20, 29]. For this reason, it is important to investigate the formation mechanisms of free radicals and the responses to these radicals in terms of evaluating oxidative stress [31].

5. RELATIONSHIP OF LEAD WITH ROS AND OXIDATIVE STRESS

Lead increases ROS production and causes oxidative stress. ROS induced by environmental lead damage lipids, proteins, DNA, antioxidant defense systems and cellular structures [32, 33, 34].

When the Pb concentration increases, the balance between reactive oxygen species (ROS) and antioxidants changes. The increase in ROS ($O_2^{\cdot}$, H_2O_2, $NO^{\cdot}$, $ONOO^{\cdot}$, $OH^{\cdot}$) leads to a decrease in antioxidant defense power. Thus, oxidative stress and lead poisoning occur. Lead also affects antioxidant enzyme (Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx)) activity (Figure 1) [35, 36]. Oxidative stress begins with the effect of lead (Pb) due to two different ways. First, ROS such as hydroperoxides, singlet oxygen and hydrogen peroxide (H_2O_2) are produced, and secondly, antioxidant reserves are depleted [9].

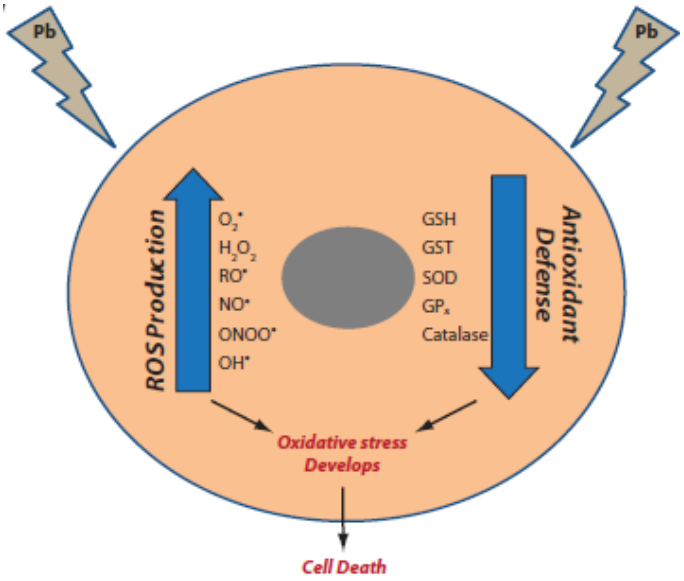

Figure 1. Development of oxidative stress in a lead-exposed cell [9].

Studies on lead exposure show that there are effects such as malondialdehyde (MDA) increase, lipid peroxidation, weakening of antioxidant defense mechanisms [37, 38, 39, 40]. In vivo and in vitro studies of lead exposure reveal increased production of ROS. The resulting oxidative stress causes changes in the structure and composition of fatty acids in the cell membrane [32, 41]. The increase in lipid peroxidation can affect the activities of membrane enzymes, endocytosis and exocytosis, solute transport, and signal transmission [42].

In addition, the enzyme Delta-aminolevulinic acid dehydratase (ALAD) is adversely affected by lead [41, 43]. As a result of the lead effect, D-aminolevulinic acid (ALA) accumulation is observed and this accumulation increases the amount of ROS and thus causes the formation of oxidative stress. It is stated that 4,5-dioxovaleric acid, the oxidation product of ALA, is an effective alkylating agent for DNA [32].

6. RELATIONSHIP OF LEAD WITH ANTIOXIDANT ENZYME ACTIVITY

Increased oxidative stress is one of the main causes of health problems such as cancer, diabetes, asthma, neurodegeneration, inflammation, developmental and reproductive disorders. An imbalance in cellular redox homeostasis is one of the consequences of heavy metal exposure [44]. One of the systems affected by oxidative stress caused by lead exposure is the antioxidant defense system [32].

ROS production is a common phenomenon in normal metabolic activities, but this is tightly regulated by the antioxidant system. Some enzymatic [Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx)] and non-enzymatic (glutathione) antioxidants stand out to control ROS formation. SOD catalyzes the formation of oxygen (O_2) and hydrogen peroxide (H_2O_2) from superoxide radicals by dismutation reaction. CAT and GPx enzymes act to decompose H_2O_2 into water (H_2O) and O_2. The imbalance between the antioxidant system and ROS causes an increase in oxidative stress and thus damage to cellular events [44, 45, 46, 47].

It has been revealed that there are changes in the activities of enzymes such as superoxide dismutase (SOD), glutathione (GSH), catalase (CAT), glutathione peroxidase (GPx) because of lead exposure [48, 49, 50].

Glutathione (GSH) has sulfhydryl groups in its structure and is an important antioxidant. It exists in both reduced (GSH) and oxidized (GSSG) forms. GSH stabilizes reactive oxygen species by donating electrons. After donating electrons, it combines with another glutathione under the action of glutathione peroxidase and forms glutathione disulfide (GSSG). GSH is produced again by glutathione reductase from the formed GSSG. Under oxidative stress conditions, the GSSG concentration is higher than the GSH concentration. Lead neutralizes glutathione by binding to the sulfhydryl groups in the structure of glutathione [9]. In addition, GSH levels decrease when enzymes such as δ-amino levulinic acid dehydratase (ALAD), glutathione reductase (GR), glutathione peroxidase (GPX) and glutathione-S-transferase are inhibited by the effect of lead [5].

As mentioned before, lead has an effect on lipid peroxidation. However, since lead does not participate in oxidation-reduction reactions, this effect occurs indirectly. Lead causes lipid peroxidation by acting on enzymes working against free radicals and GSH levels. As a result of the lead effect, GSH is oxidized to GSSG. The GSH/GSSG ratio is a reliable parameter of oxidative stress. Glutathione, which contains high levels of sulfur, is affected by Pb. Lead binds strongly to -SH groups in the structure of GSH. Thus, the GSH level decreases. In addition, inhibition of glutathione reductase (GR) and glutathione-S-transferase enzymes by lead also negatively affects the maintenance of GSH level [35, 51,52].

SOD and CAT concentrations decrease because of exposure to lead. This situation also disrupts the superoxide radical removal mechanism. Lead can also cause enzyme inhibition by displacing the zinc ion required for the activity of antioxidant enzymes [9].

It has been reported that SOD, CAT and GPx enzymes work irregularly in the renal cortex, renal medulla and thoracic aorta in rats given 100 ppm lead acetate in drinking water. It has been stated that the dysregulation of these enzymes leads to hypertension [53]. It has been observed that blood lead levels of people who are engaged in painting profession are ≤400 μg/L and in this case, SOD and CAT activities decrease. At the same time, an increase in lipid peroxidation was observed [54]. In a study conducted with factory workers, it was stated that lead changed the antioxidant defense by inhibiting the sulfhydryl groups of antioxidant enzymes such as SOD, CAT and GPx [50]. It has been reported that rats exposed to lead acetate have significant decreases in hepatic and erythrocyte GPx, GST, CAT, SOD and GSH contents. At the same time, it was stated that MDA and H_2O_2 concentrations increased significantly. It was observed that antioxidant levels did not improve after removal of lead acetate [55].

7. PREVENTION OF LEAD TOXICITY

Considering the toxic effects of lead, it is almost impossible to remove lead from the body or reverse its harmful effects. Therefore, it is generally preferred to take preventive measures [9]. A three-step preventive approach model is recommended for lead toxicity, which includes an individual intervention, a preventive medicine strategy, and a public health strategy. If lead is detected in the blood, medical intervention is performed to control the poisoning results and prevent lead accumulation. The public health strategy operates at the population level and has a wider impact. The public health strategy aims to reduce lead exposure in living spaces. Public health proposes strategies such as banning the establishment of industrial establishments near the living area and banning the use of lead where another suitable substance can be used [9]. It is reported that nutrition plays an important role in the prevention of toxicity caused by lead. It is stated that nutrients such as vitamins, minerals and flavonoids may be beneficial in protecting the body from lead. These nutrients are effective in eliminating the effects of oxidative stress [32]. It is reported that antioxidant nutrients such as vitamin C, carotenoids, selenium, vitamin E and similar have protective effects [56].

Vitamin C is an important antioxidant that prevents lipid peroxidation and reduces the amount of ROS [56]. It is stated that because of lead exposure, lipid peroxidation with the effect of vitamin C is significantly inhibited in rat liver and brain tissues, and CAT level increases in kidney tissue [57]. It has

been reported that lead-induced ROS production is increased in rat sperm cells. It was observed that the increase in ROS decreased by 40% when water supplementation with vitamin C was given [54]. In animals exposed to lead, vitamin C supplementation exerts a protective effect by significantly affecting lead levels and lead-related biochemical changes in blood, liver, and kidneys [56].

Carotenoids are among the most common fat-soluble phytonutrients. It is stated that carotenoids show antioxidant properties by defending cell membranes and lipoproteins against ROS [59]. By consuming nutrients with rich carotenoid content, tissues and cells are protected from oxidative damage. Carotenoids play a role in scavenging singlet oxygen and peroxyl radicals [60].

β-carotene is found in yellow-orange vegetables, fruits, and green vegetables. β-carotene is found in yellow-orange vegetables, fruits and green vegetables. It shows an antioxidant effect with its activities such as scavenging singlet oxygen, scavenging free radicals and protecting lipids from oxidative degeneration [41].

Lycopene, which is found in red-colored fruits and vegetables such as tomatoes and watermelons, is known to have a strong antioxidant property [59, 61]. In clinical studies, lycopene is characterized as an important micronutrient that plays a role in oxidative stress and cancer-related disorders. Lycopene can reduce oxidative stress by scavenging oxygen-derived radicals and prevent the formation of ROS [62]. Inclusion of lycopene-rich foods in the diet can provide protection against oxidation of lipids, proteins, and DNA [61, 62].

Vitamin E, which is of plant origin, has an important role in the circulatory system, nervous system and reproductive systems [41]. Vitamin E has tocopherol (α, β, ɣ, δ) and tocotrienol (α, β, ɣ, δ) forms. The most bioactive form of vitamin E is α-tocopherol [63]. ROS, which are released because of cell metabolism, affect the polyunsaturated fatty acids of phospholipids in the membranes of cells and organelles. Thus, these fatty acids are converted to hydroperoxides by peroxidation. The number of free radicals increases with the decomposition of hydroperoxides [14]. In this case, vitamin E terminates the lipid peroxidation chain reactions by giving hydrogen to the polyunsaturated fatty acids in the structure of the phospholipids in the membranes [14, 59, 63]. In this respect, vitamin E does not prevent radical formation in an environment with high lipid content. However, it minimizes the formation of secondary radicals [17].

The combined use of vitamin E and other antioxidants is highly effective in preventing or reducing lead toxicity [32]. It is stated that chelation of vitamin E alone or with $CaNa_2EDTA$ (a drug used in lead poisoning) reduces lipid peroxidation in brain and liver tissues of rats [57]. It was determined that aspartate aminotronsferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) activities, cholesterol, triglyceride, LDL levels increased and GSH levels decreased in rats exposed to lead acetate through drinking water. It has been reported that with the concomitant use of vitamins E and C, lipid hydroperoxide levels decrease and GSH levels return to normal. The use of combined doses of vitamins E and C increases the protective effect [64].

In the treatment of oxidative stress caused by lead poisoning, the method of increasing the antioxidant capacity of cells can be used. Antioxidants (pyridoxine, methionine, S-adenosylmethionine, N-acetylcysteine, alpha-lipoic acid, captopril, taurine, homocysteine) which have thiol group (compounds containing -SH group) in their structures bind to compounds that lead tends to bind to, and lead removal is ensured. Thus, oxidative damage is prevented [33].

8. CONCLUSION

As a result of the understanding that lead has industrially important qualities, health problems such as lead poisoning have also emerged. It is known that lead has no biological function and lead exposure

causes serious health problems. The development and effective implementation of individual intervention, preventive medicine strategy and public health strategies are the first measures to be taken against lead toxicity.

Oxidative stress caused by lead is quite severe. ROS production is increased, thus causing damage to DNA, enzymes, proteins and membrane lipids. Increased ROS production causes depletion of antioxidant defense system elements in cells. The use of naturally occurring antioxidants such as some vitamins, carotenoids and herbal antioxidants separately or in combination for the prevention of lead-induced oxidative stress and lead toxicity has been reported in some studies. In addition, the widespread use of sulfur-containing antioxidants as protective and therapeutic is an effective method in preventing oxidative damage. In order to prevent lead toxicity, it is important to carry out and develop experimental studies both in terms of nutrition and the use of sulfur compounds after lead exposure.

CONFLICT OF INTEREST

The author stated that there are no conflicts of interest regarding the publication of this article.

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