



Review Article

CAN ANTIOXIDANTS BE BENEFICIAL IN THE TREATMENT OF
LEAD POISONING?

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Abstract—Recent studies have shown that lead causes oxidative stress by inducing the generation of reactive oxygen species, reducing the antioxidant defense system of cells via depleting glutathione, inhibiting sulfhydryl-dependent enzymes, interfering with some essential metals needed for antioxidant enzyme activities, and/or increasing susceptibility of cells to oxidative attack by altering the membrane integrity and fatty acid composition. Consequently, it is plausible that impaired oxidant/antioxidant balance can be partially responsible for the toxic effects of lead. Where enhanced oxidative stress contributes to lead-induced toxicity, restoration of a cell's antioxidant capacity appears to provide a partial remedy. Several studies are underway to determine the effect of antioxidant supplementation following lead exposure. Data suggest that antioxidants may play an important role in abating some hazards of lead. To explain the importance of using antioxidants in treating lead poisoning the following topics are addressed: (i) Oxidative damage caused by lead poisoning; (ii) conventional treatment of lead poisoning and its side effects; and (iii) possible protective effects of antioxidants in lead toxicity. © 2000 Elsevier Science Inc.

Keywords—Free radicals, Lead poisoning, Antioxidants, Oxidative stress, Treatment

INTRODUCTION

Lead is a ubiquitous environmental toxin that induces a broad range of physiological, biochemical, and behavioral dysfunctions. Its toxicity has been known from ancient times and many studies have explored the mechanisms and symptoms of this toxicity through the years. Because the known mechanisms have not been successful in explaining some of the symptoms of lead poisoning, alternative mechanisms are now being investigated. Recent studies have reported lead's potential for inducing oxidative stress and evidence is accumulating in support of the role for oxidative stress in pathophysiology of lead poisoning.

The currently approved clinical intervention method is to give chelating agents, which bind and remove lead from lead-burdened tissues. Studies indicate, however, that there is a lack of safety and efficacy when conventional chelating agents are used. Despite the knowledge

that lead can induce oxidative stress, the usefulness of antioxidants alone or in conjunction with chelation therapy has not been thoroughly investigated. Considering the fact that some antioxidants can also function as chelators, this dual benefit makes them strong candidates for use in treating lead poisoning.

This review summarizes studies involving the mechanisms of lead-induced oxidative damage, disadvantages of current therapeutic agents, and the beneficial role of antioxidants in treating lead poisoning.

THE MECHANISMS FOR LEAD-INDUCED OXIDATIVE
DAMAGE

A growing amount of evidence indicates that transition metals, especially iron and copper, are able to produce reactive oxygen species (ROS) that result in lipid peroxidation, DNA damage, and depletion of cell antioxidant defense systems. This important role of heavy metals in oxidative damage suggested a new mechanism for an old problem, causing scientists to investigate whether lead is involved in the oxidative deterioration of biological macromolecules. Several theories by which

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transition metals can generate ROS suggest that undergoing redox cycling with reducing agents is an important mechanism. However, lead can not readily undergo valance changes, and therefore, the mechanisms underlying the ability of lead to induce oxidative stress needs to be clarified.

E.D. Willis published the earliest paper regarding lead-induced oxidative stress in 1965 [1]. Some metals with two valence electrons (Co^{2+} , Mn^{2+} , V^{2+} , Cu^{2+} , Fe^{2+} , Fe^{3+}), were shown by Willis to catalyze a rapid rate of oxidation of linoleic and linolenic acid emulsion. Lead, however, was found to have no pro-oxidant catalytic activity with respect to lipid peroxidation. Thirty years later, Yiin and Lin demonstrated marked enhancement in malondialdehyde (MDA) as a result of incubation of linoic, linolenic, and arachidonic acid with lead [2]. This finding was proven by many other studies, which have pointed to either elevated lipid peroxidation or decreased intrinsic antioxidant defense in various tissues of lead-exposed animals [3–7]. Gerber et al. [3] and Shafiq-ur-Rehman et al. [4,5] observed an enhanced rate of lipid peroxidation in brain homogenates of lead-exposed rats. Furthermore, Shafiq-ur-Rehman [4] measured lead concentrations in various brain areas as well as the rate of lipid peroxidation. He concluded that the increase in the rate of lipid peroxidation followed a pattern similar to that of lead concentrations in different regions of the brain. Increased contents of brain thiobarbituric acid-reactive substances accompanied by altered antioxidant defense systems were confirmed by Adanaylo and Oteiza [8] in a recent study. A similar effect in the liver of lead-exposed rats was reported by Sandhir and Gill [6]. Increased peroxidation of hepatic mitochondrial and microsomal membranes in lead-exposed developing chick embryos, observed by Somashekaraiah et al. [7] imply possible involvement of ROS in lead-induced toxicity. Some of these studies underline lead-induced oxidative damage, and activate other groups to further examine the possible mechanisms of identified effect.

Although the mechanisms by which lead induces oxidative stress are not completely understood, evidence indicates that multiple mechanisms may be involved. Any compound or situation that causes oxidative stress does so by accelerating pro-oxidant formation, reducing the antioxidant defense of cells, or by inducing both. The proposed mechanism for lead-induced oxidative stress will be reviewed by addressing its role in the generation of ROS, plus its effect on the antioxidant defense system.

Direct effect of lead on cell membranes

Lead is known to have some toxic effects on membrane structure and functions [9]. The effects on red blood cell (RBC) membranes in particular, are intensely

analyzed because RBCs have a high affinity for lead, contain a majority of the lead found in the blood stream, and are more vulnerable to oxidative damage than many other cells [10–12]. Osmotic and mechanic susceptibilities of RBC were reported to be increase in lead toxicity [13] accompanied by decreased deformability and a shortened life span [14,15]. The biochemical basis for those toxic effects still needs to be answered, however. Activity of some membrane-bound enzymes [16–18] and composition of membrane proteins [19] in RBC were also found to be altered by lead exposure. It is not clear whether oxidative stress is the cause or the consequence of these reported toxic effects of lead, but lead exposure may probably further increase the susceptibility of membranes by altering their integrity via deteriorating their components.

Besides directly inducing the generation of ROS, a molecule can indirectly induce oxidative stress by increasing the vulnerability membranes to the attack of ROS. The major constituents of biological membranes are lipids and proteins. The lipid molecules found in membranes contain hydrophobic, fatty acid side-chains. The “first chain initiation” is the initial step of a peroxidation sequence in a membrane or polyunsaturated fatty acids. This refers to the attack of any species with sufficient reactivity to abstract a hydrogen atom from a methylene group of the fatty acids. The presence of a double bond in the fatty acid weakens the C-H bonds on the carbon atom adjacent to the double bond and therefore makes H removal easier. Therefore, fatty acids with zero, one, or two double bonds are more resistant to oxidative attack than are the polyunsaturated fatty acids that have more than two double bonds [20].

Several studies have focused on the possible toxic effects of lead on membrane components and identified a correlation between these effects and lead-induced oxidative damage. Yiin and Lin demonstrated a marked enhancement in MDA concentrations following incubation of linoic, linolenic, and arachidonic acid with lead [2]. The concentrations of generated MDA were increased with regard to the number of double bonds of fatty acids [2], suggesting possible association of a peroxidation process. Several studies pointed to increased arachidonic acid and the arachidonate/linoleate ratio in liver, serum, and RBC membranes of lead-exposed chicks [21,22]. A mechanism for those changes in the fatty acid composition of membranes was suggested by Knowles and Donaldson [21]. They observed a decrease in the in vitro capacity of the microsomal enzyme system of fatty acid elongation that lengthens linoleic acid (18:2) to the 20 carbon precursor of arachidonic acid (20:4). How such an effect can paradoxically lead to increases in tissue arachidonic acid was suggested to be partially explained by the relative rates of the fatty acid elongation

and desaturation steps [21]. Since fatty acid chain length and unsaturation are important determinants of membrane susceptibility to peroxidation, as mentioned above, the authors suggested that lead-induced arachidonic acid augmentation might be responsible for the enhanced lipid peroxidation in those membranes [22].

On the other hand, Pb^{2+} is shown to bind strongly to phosphatidylcholine membranes in vitro [23]. Shafiq-ur-Rehman and Abdullah reported an alteration of the composition of RBC membrane phospholipids, indicating a decrease in the levels of phosphatidylcholine [24]. Furthermore, in a detailed study which was reported by the same group, lead, phospholipid, and lipid peroxidation levels were determined in various regions of the brains of lead-exposed rats [4]. The percentages of increase in the rate of lipid peroxidation and decrease in the phospholipid level were shown to follow a pattern similar to that of lead concentrations in the brain areas.

Taken together, these data suggest that altered lipid composition of membranes may result in altered membrane integrity, permeability, and function. These would increase the susceptibility to lipid peroxidation.

Lead-hemoglobin interactions

Ribarov and Benov investigated the relation between the hemolytic action of heavy metals and lipid peroxidation [25]. The previous findings that caused them to consider lipid peroxidation as a mechanism in metal-induced hemolysis were reported as: (i) A study done by Levander et al. [14], which suggested that peroxidation of membrane unsaturated fatty acids is directly related to the decreased deformability of RBC from vitamin E-deficient lead-poisoned rats. (ii) The observations from the study of Kumar et al. [26] suggested that the toxicity of copper might be mediated, in part, through the generation of O_2^- in human RBC membranes. According to these studies, Ribarov and Benov found that strong hemolytic agents such as Ag^+ , Hg^{2+} , Cu^{2+} , and Pb^{2+} caused the highest degree of peroxidation [25]. Considering their finding that lead-induced hemolysis is associated with peroxidation of RBC membranes, and the assumption that Pb^{2+} can not initiate peroxidation by direct action on the membrane lipids [1], they investigated a possible indirect mechanism for initiation of lipid peroxidation by lead [27,28]. The interaction of heavy metals with oxyhemoglobin has already been suggested as an important source of O_2^- formation in RBC [29]. Therefore, Ribarov et al. attempted to determine whether lead has a similar effect. They found that Pb^{2+} significantly enhances the autoxidation of hemoglobin in an in vitro liposome model [27]. The inhibition of this effect by superoxide dismutase (SOD) and catalase suggested that O_2^- and H_2O_2 are somehow involved in the process.

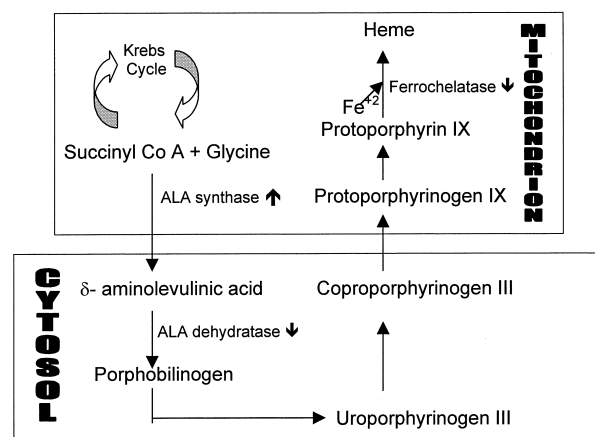


Fig. 1. Effects of lead on heme synthesis.

As a result, they speculated that Pb^{2+} may induce generation of ROS by interacting with oxyhemoglobin, leading to peroxidative damage of RBC membranes [27,28].

δ -Aminolevulinic acid (ALA)-induced generation of reactive oxygen species

One of the targets for lead toxicity is the hematological system. Lead affects this system by: (i) inhibiting the heme and hemoglobin synthesis, and (ii) changing the RBC morphology and survival. A schematic presentation of the effects of lead on heme synthesis is shown in Fig 1. In this pathway, the enzyme that is most sensitive to the toxic effects of lead is probably δ -aminolevulinic acid dehydratase (ALAD). At low blood levels (about 15g/dl), lead inhibits ALAD, a cytosolic sulphhydryl enzyme [30]. Lead also decreases ferrochelatase activity at the last step of the heme synthetic pathway. Failure to condense two molecules of δ -aminolevulinic acid (ALA) to form porphobilinogen by ALAD and to insert iron into protoporphyrin by ferrochelatase results in depressed heme formation. Depressed heme synthesis stimulates ALA synthetase, the first enzyme in the heme biosynthetic pathway, by virtue of negative feedback control. As a consequence, increased production of ALA and decreased condensation of ALA into porphobilinogen result in a considerable amount of ALA both in circulating blood and excreted urine [31,32].

Several studies have been reported showing how accumulated ALA induces ROS generation [33–36]. The evidences for ALA-induced oxidative stress were also reviewed by Bechara [37]. Therefore the present paper summarizes how ALA is involved in ROS generation. ALA undergoes enolization and autoxidation at pH 7.0–8.0. The conversion of the ALA keto form into the ALA enol form is shown to be necessary for autooxidation reactions because levulinic acid, without the -amino

group that is thought to facilitate the enolization, has not been found to be active in oxidation reactions [33,38]. The enolized ALA then autoxidizes and generates superoxide anion, as evidenced by the parallel reduction of ferricytochrome c, and also by electron spin resonance spin trapping experiments [33]. Monteiro et al. reported that ALA/oxyhemoglobin coupled oxidation also results in ROS generation [38]. The steps of the reactions were reported as follows: (i) ALA enol form is generated following tautomerization, (ii) ALA enol acts as an electron donor to molecular oxygen, together with an electron transfer from oxyHb to oxygen resulting in methHb, ALA radical, and H_2O_2 generation [38]. H_2O_2 and O_2^- , which are now present as a result of both ALA and ALA/oxyhemoglobin coupled autoxidation, can interact and generate HO radicals, which have the highest reactivity among ROS. Inhibition of ALA/oxyhemoglobin coupled oxidation by SOD, catalase, and mannitol suggests the involvement of O_2^- , H_2O_2 , and HO, respectively, in the process [38]. Besides oxyhemoglobin, methemoglobin and other ferric and ferrous complexes were also shown to trigger ALA oxidation [33]. This is evidenced by induction of oxygen uptake by ALA in the presence of Fe-ATP and Fe-EDTA complexes as well as oxyhemoglobin and methemoglobin. Finally, as concluded by Monteiro et al. [34], ALA accumulated in saturnism can be suggested as a source of ROS and oxidative damage, which is now accepted as being associated with the pathophysiology of lead poisoning.

Furthermore, Douki et al. demonstrated that the final oxidation product of ALA, 4,5-dioxovaleric acid, is an effective alkylating agent of the guanine moieties within both nucleoside and isolated DNA [39]. The same group reported increased levels of 8-oxo-7,8-dihydro-2'-deoxyguanosine and 5-hydroxy-2'-deoxycytidine in organ DNA of rats chronically treated with ALA, and involvement of HO in ALA-induced DNA damage [40]. Taken together, these findings imply a genotoxic potential of ALA. This possible consequence of ALA accumulation is deserving of consideration in further studies of lead toxicity.

Effect of lead on the antioxidant defense systems of cells

Several studies reported alterations in antioxidant enzyme activities such as SOD, catalase, and glutathione peroxidase (GPx), and changes in the concentrations of some antioxidant molecules, such as glutathione (GSH) in lead-exposed animals [41,42] and workers [43–47]. Although these findings suggest a possible involvement of oxidative stress in the pathophysiology of lead toxicity, it is not clear whether these alterations are the cause of the oxidative damage or a consequence of it. How-

ever, some known biochemical mechanisms of lead toxicity let us hypothesize that some of lead's effects on components of the antioxidant defense system that occur first by lead, might cause an impairment in pro-oxidant/antioxidant balance of cells, resulting in oxidative damage.

Because lead and other metals such as Hg and Cd have a high affinity for sulfhydryl (SH) groups, mercaptides are formed with the SH group of cysteine, and less stable complexes with other amino acid side chains [48]. Lead is shown to inhibit several enzymes having functional SH groups [48]. ALAD is the most known enzyme that is inhibited by lead via direct binding of lead to the SH groups that are essential for the catalytic activity of the enzyme [49,50]. Glucose-6-phosphate dehydrogenase (G6PD), the first enzyme of the pentose phosphate pathway, supplies cells with most of the extra mitochondrial NADPH through the oxidation of glucose-6-phosphate to 6-phosphogluconate. This NADPH keeps GSH at a constant level by providing reducing equivalents for glutathione reductase (GR), which mediates reduction of glutathione disulfide (GSSG) to GSH. G6PD is particularly important in RBC because those cells lack mitochondria. G6PD is known to contain many SH groups, which play a crucial role in maintaining its tertiary structure [51]. In some in vitro studies, G6PD was reported to be inhibited by lead [48,52]. Also, the formation of lead-sulfhydryl complexes was suggested as a plausible mechanism [48,52]. Lachant et al. [52] provided further evidence for lead-SH interactions between lead and G6PD by preventing the loss of G6PD activity when incubating the cells with thiol reagents (GSH and 2-mercaptoethanol) prior to incubation with lead. The same group suggested another mechanism for G6PD inhibition by lead via kinetic studies where lead is indicated as being a noncompetitive inhibitor of both glucose-6-phosphate and NADP for G6PD. The authors concluded that inhibition of the pentose phosphate pathway might then render the lead-treated RBC more susceptible to oxidative damage [52]. However, in vivo studies suggest a more complex effect of lead on G6PD. The pentose phosphate pathway, which has a high reserve capacity, is critical for providing NADPH. The most important regulation of the pathway is the $\text{NADP}^+/\text{NADPH}$ ratio, which is known to change in favor of the oxidized form under oxidative stress conditions. In such a condition, up to 92% of phosphorylated glucose can pass through the pathway, which is normally about 11% [53]. Consistent with this mechanism, increased G6PD activity is reported in RBC of lead-treated rats in one of our latest studies [54] as well as in other studies of lead-exposed animals [55] and workers [56]. However, contradictory results are also reported. Howard [57], Rausa [58], and Calderon-Salinas et al. [59] pointed to decreased G6PD

activity, while Rogers et al. [60] indicated unchanged red cell G6PD following lead exposure. Overall, the accumulated data suggests that exposure to lead can result in an increase or decrease in G6PD activity depending on the concentration of exposed lead, duration of lead exposure, and magnitude of oxidative stress inside the cell [55].

GSH is a tripeptide containing cysteine that has a reactive SH group with reductive potency. Accordingly, GSH plays a vital role in the protection of cells against oxidative stress. It can act as a nonenzymatic antioxidant by direct interaction of the SH group with ROS, or it can be involved in the enzymatic detoxification reactions for ROS, as a cofactor or a coenzyme [61,62]. It possesses carboxylic acid groups, an amino group, a sulfhydryl group, and two peptide linkages as sites for reactions of metals [63]. Pb^{+2} binds exclusively to the SH group [63,64], which decreases the GSH levels [65] and can interfere with the antioxidant activity of GSH.

Another component of the antioxidant defense system, GR, reduces GSSG back to GSH and thereby supports the antioxidant defense system indirectly. GR possesses a disulfide at its active site [66], which was suggested as a target for lead, resulting in the inhibition of the enzyme [6,67]. This inhibition leads to decreased GSH:GSSG ratios that will render cells more susceptible to oxidative damage.

On the other hand, GPx, catalase, and SOD are metalloproteins and accomplish their antioxidant functions by enzymatically detoxifying peroxides, H_2O_2 and O_2^- , respectively. Since these antioxidant enzymes depend on various essential trace elements for proper molecular structure and enzymatic activity, they are potential targets for lead toxicity [55]. Schrauzer [68] indicated antagonistic effects between lead and selenium, resulting in reduced selenium uptake that may affect GPx activity, that requires selenium as a cofactor, and then may increase the susceptibility of the cell to oxidative damage. As shown by Othman and El-Missiry [69], administration of selenium, prior to injection of lead into male rats, produced noticeable prophylactic action against lead by means of increased SOD, GR activity, and GSH content. Although the protective effect was attributed to the formation of inactive selenium-lead complex [70], it was mentioned that such interactions could not be the sole mechanism for the beneficial effects of selenium. Catalase is another major antioxidant enzyme having heme as the prosthetic group. Lead is known to reduce the absorption of iron in the gastrointestinal tract and to inhibit the heme biosynthesis [71]. Decreased catalase activity observed in lead-exposed animals was attributed to the interference of lead by both processes [6,67]. SOD plays an important role in protecting the cells against the toxic effects of O_2^- by catalyzing its dismutation reactions.

The enzyme requires copper and zinc for its activity. Copper ions appear to have a functional role in the reaction by undergoing alternate oxidation and reduction, where zinc ions seem to stabilize the enzyme instead of having a role in the catalytic cycle [20]. Another type, MnSOD, contains manganese at its active site and is not detected in mammalian RBC, but is present in human liver to some extent. Several studies pointed to decreased RBC SOD activity in lead-exposed rats [55,72]. Mylroie et al. observed: (i) high correlation between decreased SOD and decreased copper concentrations in the blood of animals, (ii) no effect on SOD with increased blood lead levels in the presence of normal copper concentrations [72], and (iii) that dietary copper supplementation prevented the Pb-induced decrease in SOD activity [73]. Therefore, they have suggested an indirect inhibitory effect on SOD in vivo due to the lead-induced copper deficiency. Inhibition of SOD activity by lead was also shown in an in vitro study where the authors indicated that this effect of lead can lead to decreased scavenging of ROS and result in oxidative damage [74]. On the other hand, Ariza et al. [75] demonstrated rapid induction of cellular H_2O_2 following treatment of AS52 cells with 1M Pb^{2+} , which they suggested to be increased by the stimulatory effect of lead on the activities of CuZn-SOD and xanthine oxidase, enzymes that produce H_2O_2 .

Overall, these inhibitory effects of lead on various enzymes would probably result in impaired antioxidant defenses by cells and render cells more vulnerable to oxidative attacks.

CONVENTIONAL TREATMENT OF LEAD POISONING AND ITS SIDE EFFECTS

The current therapeutic approach to lead poisoning is to increase the excretion of lead by chelation. Various chelators are available and are prescribed according to the blood lead concentrations of the patient (Table 1). Although chelation has been shown to reduce blood lead levels, the safety and efficacy of the various chelators may be questioned. The efficiency and adverse effects of each of the major chelators prescribed in the United States will be discussed here.

Dimercaprol (BAL in peanut oil)

British antilewisite (BAL; Fig. 2), although originally introduced as an antidote for lewisite, an arsenical chemical warfare gas, is now typically dissolved in peanut oil to form the chelating agent dimercaprol. This lipid soluble, polar compound must be administered intramuscularly, and will combine with lead to form a 2:1 nonpolar complex that is then excreted in bile and urine [76]. Eight

Table 1. Clinical Approach to Chelation Therapy in Children^a

Condition and blood lead concentrations ($\mu\text{g}/\text{dl}$)	Regimen and comments
Encephalopathy (>90 – 100)	BAL + CaNa_2EDTA
Symptomatic without encephalopathy (70 – 90)	BAL + CaNa_2EDTA , CaNa_2EDTA or succimer
Symptoms possible (40 – 69)	CaNa_2EDTA or succimer ($45 \mu\text{g}/\text{dl}$ may be used as lower threshold for chelation)
Biochemical toxicity (25 – 39)	CaNa_2EDTA or succimer (No consensus about chelation)
Excessive exposure (10 – 24)	Chelation therapy not indicated; source identification, education, and nutrition counseling are needed.

^a Modified from [78].

h after treatment, 20% of the BAL is found being excreted in the urine as a dithiol [77]. It is distributed in every tissue because of its high liposolubility and directly chelates the erythrocyte lead [77].

After treatment with BAL, side effects have been reported in up to 50% of patients, with common problems including fever, tachycardia, nausea, vomiting, salivation, watery eyes, sweating, and unpleasant breath

[76–78]. BAL therapy also frequently produces a histamine release, which must be countered with antihistamines [76]. Besides these prevalent adverse effects that accompany BAL treatment, patients with specialized disorders may face more serious risks. Patients with glucose-6-phosphate dehydrogenase deficiency have been shown to experience severe hemolysis [79], while the peanut oil used to prepare BAL may precipitate a reaction in children who are allergic to peanuts [78]. Since BAL forms a toxic chelate with iron, iron supplementation should be avoided during BAL treatment [80].

BAL is often administered in conjunction with calcium disodium ethylenediamine tetraacetic acid (CaNa_2EDTA) to patients with blood lead levels greater than $70 \text{ g}/\text{dl}$ [78]. When lead levels are this high, development of encephalopathy is a risk. BAL has been shown to reduce this risk, while CaNa_2EDTA , another major chelator, has been reported to increase chances of precipitation of encephalopathy and to translocate lead from soft tissues to the central nervous system [76]. Although a combination of BAL and CaNa_2EDTA has been reported to quickly reduce lead levels and decrease the risk of encephalopathy [77], it has also been accompanied by increased vomiting and *elevated* hepatic enzyme activity [76].

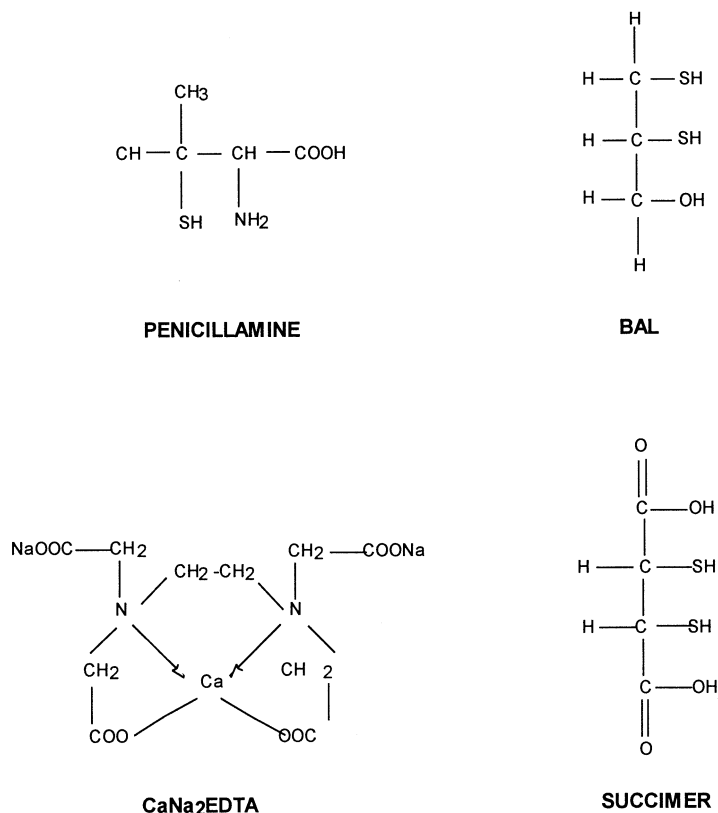


Fig. 2. Chemical structures of chelating agents.

CaNa₂EDTA

CaNa₂EDTA (Fig. 2) was introduced in the 1950s as a lead poisoning treatment. When EDTA was first used therapeutically as its sodium salt it resulted in cardiovascular instability, severe hypocalcemia, and even death in some patients. When it is combined with calcium, sodium, or zinc it forms less toxic compounds. The water-soluble CaNa₂EDTA is recommended for clinical use as a chelator in which the formed lead-EDTA chelate has a higher stability constant [81,82]. Since the absorption of CaNa₂EDTA from the gastrointestinal tract is only around 5%, the oral administration of the compound is not recommended [77,78]. Instead, it can be administered intramuscularly, which is a painful method, or usually by slow intravenous infusion [83]. CaNa₂EDTA is distributed mainly in the extracellular compartments of the body and does not enter the cells because of its ionic form [77]. Therefore it removes lead from the extracellular compartments only [77,84]. In addition, its passage across the blood-brain barrier is very slow [81]. CaNa₂EDTA is not excreted in fecal matter, and 90% of administered CaNa₂EDTA is found not metabolized in the urine 8 h after treatment [85]. When administered, CaNa₂EDTA leads to reduced blood lead levels, a reversal in the hematologic effects of lead toxicity, and an increase in lead levels in urine [76].

However, this chelating agent has many disadvantages. It may cause the redistribution of lead from the bones and kidney to the brain and liver, increase the risk of encephalopathy, and cause renal toxicity [76,81,86]. Since CaNa₂EDTA is not able to cross the blood-brain barrier, it is doubtful whether it is effective in reducing lead levels in the brain or in relieving the effects of lead on neurodevelopmental functioning [87]. Besides increased urinary excretion of lead, because of CaNa₂EDTA's relative lack of specificity, other essential metals such as zinc, copper, iron, cobalt, and manganese are also reported to be excreted and depleted following CaNa₂EDTA therapy [81,88]. Among them zinc diuresis is the most common and severe side effect. Although it may be safe to administer zinc while using CaNa₂EDTA in order to preclude the effects of long-term chelation therapy, this may decrease the effectiveness of therapy [89]. Another disadvantage of the CaNa₂EDTA treatment is its high cost. Since it is administered by slow intravenous infusion, the child needs to be hospitalized. This long treatment protocol may result in a total cost of \$30,000 for a child [81].

The adverse effects of CaNa₂EDTA therapy are also prevalent. Its administration may be responsible for increased renal toxicity [82]. Of 130 children receiving BAL and CaNa₂EDTA treatment, 13% showed signs of nephrotoxicity, while 3% developed acute renal failure.

More than 25% of children who were given intramuscular or intravenous administration of CaNa₂EDTA showed increasing serum urea nitrogen [90].

D-penicillamine

D-penicillamine, also referred to as β,β -dimethyl cysteine, was unintentionally discovered in 1953 as a metabolite of penicillin B in the urine of patients with liver disease. Since then it has been used in the treatment of Wilson's Disease to reduce serum copper levels [91]. It is not an FDA-approved drug for lead poisoning. It is, however, used as treatment for low-level lead toxicity in the blood in the range of 25–40 g/dl, especially in children, since 1956 [92]. It is administered orally, and is typically taken by the patient for 4–12 weeks. It leads to reduced blood lead levels and reversal of hematologic toxicity [78]. D-Penicillamine is a sulfhydryl containing amino acid (Fig. 2). One possible mechanism for its chelating ability is the formation of a simple bond between its sulfhydryl group and lead atom. Other possible mechanisms suggested are as follows: (i) incorporation of lead into a ring structure between the sulfur and adjacent nitrogen atom (Fig. 3), or (ii) a lead atom may be bound between two penicillamine molecules [93].

Like the two previously discussed chelators, D-penicillamine treatment leads to a large number of adverse effects [92]. A study of children being administered D-penicillamine showed that most experienced nausea and vomiting [94]. Eosinophilia was seen in 20% of them [95]. Reversible leukopenia or mild thrombocytopenia was observed in about 10% of children being treated in some other studies [96], while 0.5–1.0% developed angioedema, urticaria, or maculopapular eruptions, requiring a cessation of the therapy [94,97]. Cases of proteinuria, microscopic hematuria, and incontinence have also been reported, although they are less common and may be resolved by reducing the D-penicillamine dosage [96, 98]. D-penicillamine also has the potential for eliminating essential nutrients as pyridoxine, zinc, and iron, as well as lead. Furthermore, its absorption is affected by the diet; it can be reduced 35% when treatment is combined with food or ferrous sulfate, and up to 66% when taken in conjunction with antacids [99,100].

D-penicillamine is a less effective chelator than CaNa₂EDTA, and its overall toxicity profile allows it to be considered as a third choice for lead toxicity treatment after CaNa₂EDTA and succimer [92].

Succimer

Succimer, or 2,3-meso-dimercaptosuccinic acid, is a water soluble analog of dimercaprol. Much less data

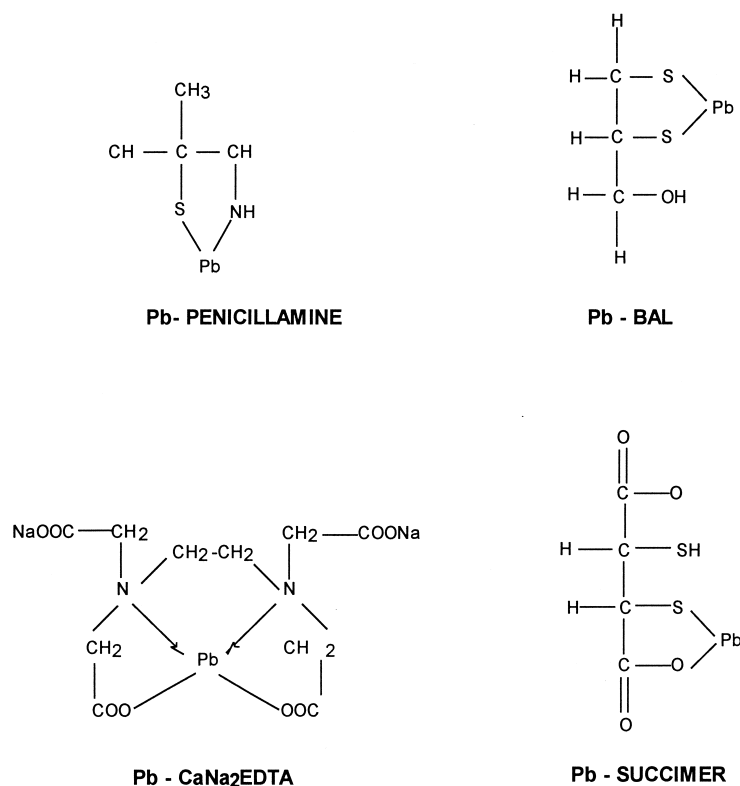


Fig. 3. Chelates formed between lead and d-penicillamine, BAL, CaNa₂EDTA, and succimer.

exists on this agent as compared to the three previously discussed chelators, because administration of succimer has been limited. Succimer is the only chelator in the United States that has been solely approved for pediatric use by FDA; thus no appropriate and large clinical trial has been published with regard to adult treatment with succimer [78,84]. Consequently, relatively little is known about succimer's therapeutic effects on lead poisoning.

Succimer is a water-soluble derivative of BAL and can be administered orally. It has two sulfhydryl groups (Fig. 2), but Pb is reported to be coordinated with the sulfur and oxygen atom. The structure of the chelate formed between meso-succimer and Pb ions in a test tube is shown in Fig. 3 [81]. With regard to the absorption and function of succimer within the body, about 95% of the administered succimer binds to plasma proteins and is primarily distributed in the extracellular compartment [101]. With an elimination half-life of about 48 h, a peak blood concentration of succimer is reached within 2 h [102]. The succimer is metabolized rapidly and extensively excreted through the urine as mixed disulfides [92].

Succimer is most commonly used to treat children who have blood lead levels greater than 45 g/dl and who are not at risk of encephalopathy [78]. Several advan-

tages of succimer make it a good candidate for lead poisoning treatment: (i) Lead doesn't happen to be re-distributed to the brain following succimer treatment, which is a major disadvantage of other chelators. (ii) Its oral availability makes it a preferred agent for the therapy of childhood lead poisoning and allows for administration in an outpatient setting that will decrease the cost of the therapy. (iii) Unlike CaNa₂EDTA, succimer has specific affinity for heavy metals such as lead, arsenic, and mercury, but causes little increase in the excretion of iron, zinc, or calcium [78,81,92]. (iv) Succimer has been shown to decrease the levels of lead in the brain and blood more effectively than CaNa₂EDTA treatment. It mobilizes lead from the soft tissue, brain, liver, kidney, and blood, although it has not consistently reduced lead levels in bone [88,103]. Succimer is more effective than CaNa₂EDTA in producing plumburesis [104], and is not likely to precipitate encephalopathy. (v) Thus far, succimer toxicity has been minimal, with only occasional reports of nausea, vomiting, diarrhea, loose stool, appetite loss, and foul-smelling urine or stools [92,102]. Some children receiving succimer treatment experienced hypersensitivity reactions such as chills, fever, rash, or urticaria [92]. A study of children being treated showed that 12% had mild gastrointestinal symptoms, 5% experienced general malaise, and 4% showed

transient elevation of liver enzymes. Also, some were struck with reversible neutropenia [105]. Because 92–95% of succimer binds to plasma proteins, there is a potential for interactions with other protein-bound drugs [78]. Nevertheless, from what is known, succimer seems to be one of the “best” chelating agents.

Besides its potent chelating effect, succimer appears to have antioxidant activity because of two SH groups in its structure. Our data demonstrated a visible protective effect of succimer on lead-induced oxidative stress in rats. However, since blood lead levels returned to control levels in succimer-treated rats, it remains unclear whether the observed effect of succimer can be attributed to chelation or the potential to act as a thiol antioxidant. Mechanistic studies are missing and needed to determine whether succimer has a potential ROS scavenging activity via its SH groups.

POSSIBLE PROTECTIVE EFFECTS OF ANTIOXIDANTS AGAINST LEAD-INDUCED OXIDATIVE STRESS

As discussed in the first section, induction of ROS by lead and subsequent depletion of antioxidant cell defenses can result in generalized disruption of the pro-oxidant/antioxidant balance in lead-burdened tissues. This could contribute to tissue injury via oxidative damage to critical biomolecules (Fig. 4). In the event that oxidative stress can be partially implicated in lead toxicity, a therapeutic strategy to increase the antioxidant capacity of cells may fortify the long-term effective treatment of lead poisoning. This may be accomplished by either reducing blood and tissue lead levels via chelation, thereby reducing the possibility of lead interacting with critical biomolecules and inducing oxidative damage, or by bolstering the cell's antioxidant defenses through exogenous supplementation of antioxidant molecules (Fig. 4).

Although many investigators have confirmed lead-induced oxidative stress, the usefulness of antioxidants alone or in conjunction with chelation therapy has not been extensively investigated yet. Some groups [4,106–110] investigated the ability of some molecules with antioxidant activity to prevent or treat experimental lead toxicity in animals. Although some of the agents were found to be capable of abating some toxic effects of lead, none of them were discussed as being effective via rebalancing the impaired pro-oxidant/antioxidant ratio following lead exposure.

Vitamin B₆

In 1987, Tandon et al. investigated the effect of Vitamin B₆ in lead intoxication [111]. These authors re-

ported significantly reduced inhibition of ALAD activity and zinc protoporphyrin levels as a result of simultaneous supplementation of vitamin B₆ and lead. Decreased blood, kidney and liver lead levels were also shown in vitamin B₆-supplemented rats, while no effect was observed in their brain lead levels. These beneficial effects of vitamin B₆ on lead toxicity were suggested as being due to the participation of the ring nitrogen atom in the chelation of lead or to a possible interaction between lead and vitamin B₆ at the absorption level [111]. In 1989, McGowan examined the GSH metabolism of lead-exposed rats fed a vitamin B₆-deficient diet [106]. GSH levels in the experimental group were found to be lower than the control values. This involvement of vitamin B₆ in GSH metabolism was explained by the cofactor role of vitamin B₆ for several enzymes in the transsulfuration pathway. Most of the cysteine, the bioprecursor of GSH, is synthesized from dietary methionine in that pathway. Therefore, it was suggested that a lack of vitamin B₆ prevented the involvement of methionine in GSH biosynthesis by limiting the availability of cysteine [106]. The results indicate an indirect antioxidant role for vitamin B₆ in lead-exposed rats via supporting their antioxidant defense systems by inducing GSH biosynthesis. This possible role for vitamin B₆ in lead toxicity was not discussed, however, and was not investigated further.

Zinc

Zinc, on the other hand, was reported to be able to prevent and treat lead intoxication in rats, either alone and/or in combination with methionine or thiamine [107, 108]. In both studies, simultaneous dietary supplementation with “Zn + methionine/thiamine” was found to be most effective in reducing lead-induced inhibition of ALAD activity in the blood and urinary excretion of ALA. Those studies also suggest that supplementation of the combination therapy concurrently with exposure to lead was more effective than treatment after lead exposure. This protective effect for Zn was attributed to a decrease in lead absorption in the gastrointestinal track. In another study [112], Zn was administered to lead-intoxicated rats along with chelating agents CaNa₂EDTA, succimer, and D-penicillamine. Zn was shown to increase the efficacy of chelating agents by potentiating the depletion of blood, hepatic and renal lead, and reversing inhibited blood ALAD activity. Additionally, Zn supplementation was effective in restoring the Zn levels in tissues which were depleted following treatment with chelating agents [112]. Zn has been shown to have an antioxidant effect which was reviewed by Bray and Bettger [113]. Besides some proposed mechanisms for the antioxidant function of Zn, two mechanisms have been elucidated: the protection of sulf-

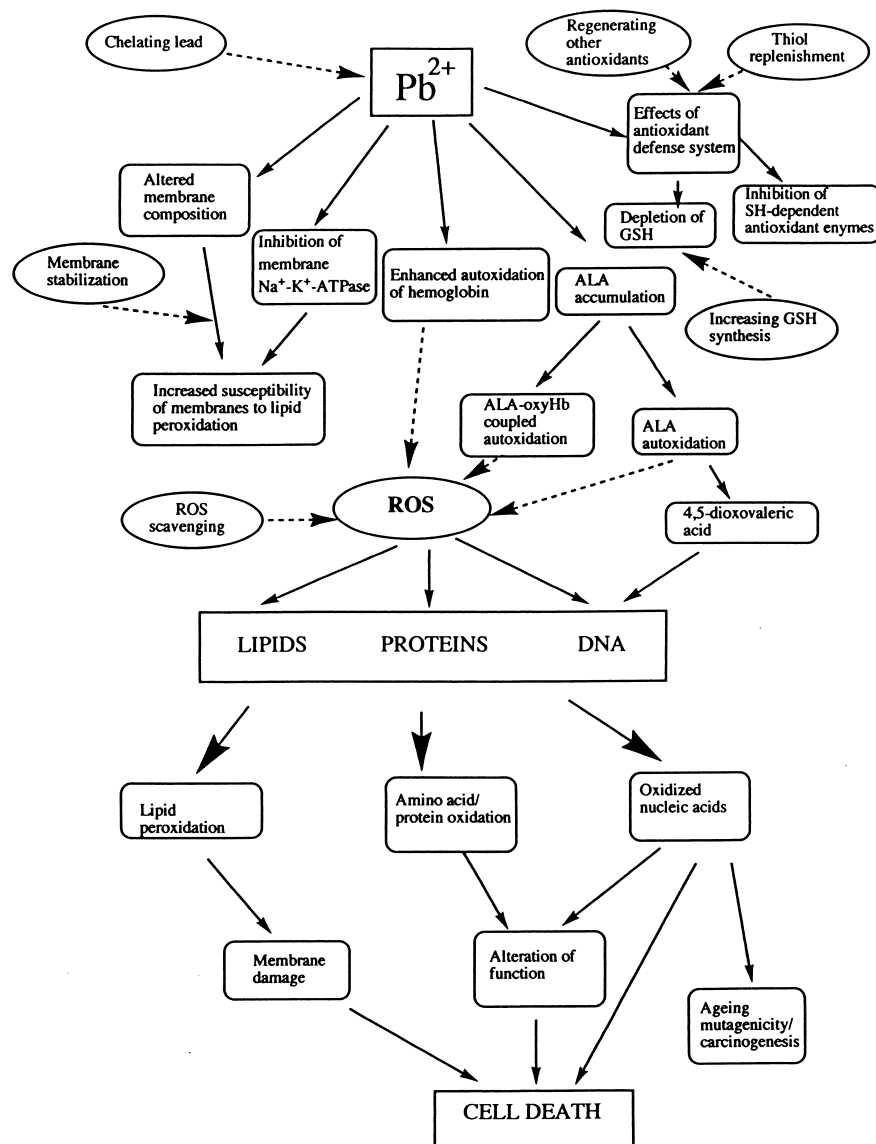


Fig. 4. Possible mechanisms for lead-induced oxidative stress, and proposed targets of antioxidants in lead toxicity.

hydroxyl groups against oxidation and prevention of ROS (HO and O_2^-) production by transition metals. However, none of those studies which indicated the beneficial effect of Zn in lead toxicity [107,108,112] enlightened as to whether this effect is correlated with oxidative stress. Only Flora et al. [108] determined the hepatic GSH of lead-exposed rats following zinc, methionine, and “zinc + methionine” administration. As would be expected, methionine, a precursor of GSH, notably restored lead-induced decreases in hepatic GSH levels, both alone and in combination with Zn. However, the effects of methionine on other oxidative stress parameters in lead toxicity have not been examined.

Vitamin E

Several groups evaluated the remedial effect of Vitamin E, a well-known antioxidant, on lead toxicity. Levander et al. [14] determined the filterability of RBC as well as RBC lipid peroxidation from vitamin E-deficient and vitamin E-supplemented rats. Lead was shown to increase the mechanical fragility of RBC, which makes the RBC less deformable and more susceptible to oxidant stress. Filterability of lead-exposed RBC was estimated by determining the time required for RBC to pass through a polycarbonate filter by visual inspection. The filtration time for RBC from vitamin E-deficient rats

was much greater than that of RBC from vitamin E-supplemented rats. Furthermore, a strong correlation was found between increased lipid peroxidation and decreased filterability of red cells from vitamin E-deficient, lead-poisoned rats. These results indicate that the high vitamin E status of humans may ameliorate lead-induced changes in the deformability of RBC, which makes them more vulnerable to oxidative damage [14]. This preventive role for vitamin E in lead toxicity, implied by Levander et al., is confirmed by another group [110] who found simultaneous supplementation of vitamin E more effective than treatment of lead-exposed animals with vitamin E. This preventive effect for vitamin E was reported to be due to the inhibition of lead absorption.

Ascorbic acid (Vitamin C)

Another antioxidant molecule, ascorbic acid, administered alone or in combination with thiamine to lead-exposed rats and their effect on the efficacy of two thiol metal chelators, succimer and -mercapto—(2-furyl)acrylic acid, were investigated [109]. Both ascorbic acid alone and in combination with thiamine were found to be effective by means of increasing urinary elimination of lead, reducing hepatic and renal lead burden and reversing lead-induced inhibition of the activity of blood ALAD. Thiamine alone, however, did not show any beneficial effects. This beneficial role of ascorbic acid was attributed to its ability to complex with lead [109]. In 1999 Simon and Hudes reported a population-based study that indicates an inverse relation between serum ascorbic acid and blood lead levels among Americans [114]. The authors suggest that higher intake of ascorbic acid may be effective in preventing lead toxicity if a causal relationship is confirmed.

Although improvement by several means of antioxidant administration to lead-exposed animals was reported, there are not many studies in the literature where the effectiveness of an antioxidant in counteracting lead-induced oxidative damage is extensively investigated. It is only recently that the correlation between those beneficial effects of antioxidants and other oxidative stress-related parameters has been investigated.

Ethoxyquin

One of those studies was reported by Donaldson, who determined whether systemic effects of lead, attributed to tissue peroxidation, can be reversed by the dietary antioxidant, ethoxyquin [115]. A peroxidative mechanism for lead toxicity was suggested and ethoxyquin was observed to ameliorate lead toxicity, as assessed by growth inhibition [115].

Selenium

Another study came in 1998 from Egypt, where the role of selenium in lead toxicity was investigated [69]. Selenium is an essential element known to be required for the activity of glutathione peroxidase, thereby having a key role in the antioxidant defense systems of cells. Its efficacy in treatment of free radical-associated diseases has been shown by many studies [116,117]. Selenium administration, prior to lead injection, resulted in pronounced prophylactic action against lead effects in terms of acid and alkaline phosphatases, transaminases (GOT, GPT), total protein, triglycerides, and cholesterol in serum [69]. In addition, oxidative stress-related parameters in two major target organs, the liver and kidney, were analyzed following intramuscular injection of 10 mol/kg sodium selenite, 2 h before administration of 100 mol/kg of lead acetate to male albino rats. Selenium was found to enhance the antioxidant capacity of cells by increasing the activities of SOD and GR, and augmenting the GSH content. Three possible mechanisms were proposed for the protective effect of selenium: (i) formation of an inactive selenium-lead complex; (ii) stimulating radical scavenging by increasing the activity of SOD, thereby increasing the removal of the superoxide radical; and (iii) increasing the antioxidant capacity of cells indirectly by increasing the activity of glutathione reductase, which has a major role in maintaining a sufficient content of GSH in the reduced form [69].

S-adenosyl-L-methionine (SAM)

Since ethanol was reported to potentiate lead-induced inhibition of rat brain antioxidant defense systems [118], benefits of supplementation of SAM, the precursor of GSH, to mice exposed to “lead + ethanol” was investigated by Flora and Seth [119]. SAM was shown to prevent the alterations in some biochemical parameters (blood ALAD, GSH, brain and liver lipid peroxidation, and GSH content) and accumulation of lead in blood, liver, and brain during acute “lead + ethanol” exposure. The results seem promising and the authors suggested that SAM could have a possible therapeutic potential either as a sole (or as an “adjuvant”) agent during chelation therapy by depleting the brain’s lead burden and mitigating the “lead + ethanol”-induced oxidative stress [119].

N-acetylcysteine (NAC)

In 1996 our group began studying the role of antioxidants in lead toxicity in both in vitro and in vivo systems. Our goal was to explore whether lead induces oxidative stress and if so, to investigate the dual benefits of some

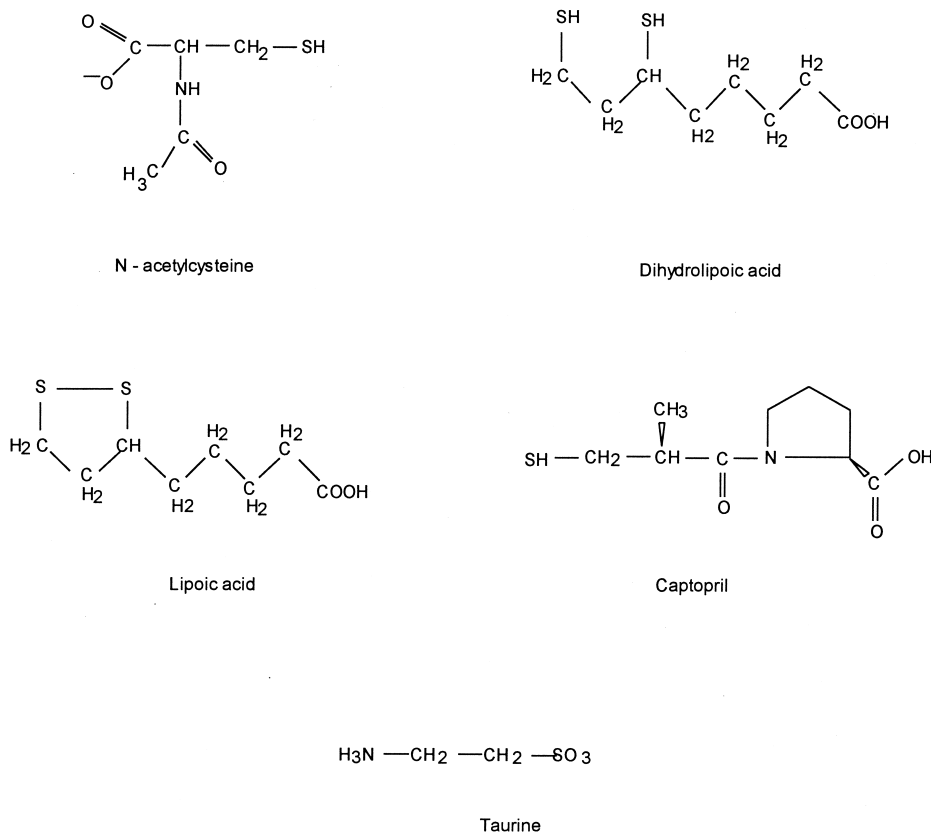


Fig. 5. Chemical structures of NAC, lipoic and dihydrolipoic acid, captopril, and taurine.

thiol compounds in lead toxicity, as antioxidants and thiol chelators. In an *in vitro* model where Chinese hamster ovary (CHO) cells were used, Ercal et al. observed increased oxidative damage in lead-exposed cells by means of a decreased GSH/GSSG ratio, increased MDA levels and catalase activity [120]. A possible remedy for the oxidative imbalance occurring in lead toxicity was suggested by the authors as being replenishment of the GSH supply of the cells. Since direct GSH supplementation has been demonstrated to be ineffective [121], a well-known bioprecursor of GSH—NAC—was used as a supplement for lead-exposed CHO cells and its capacity to minimize lead-induced oxidative stress was investigated [120]. NAC is a thiol-containing antioxidant (Fig. 5) that has been used under several clinical conditions with few adverse side effects [122,123]. Its high toxicity threshold and wide therapeutic window enhances its utility. The antioxidant action is believed to be due to its direct interaction with ROS and/or its ability to stimulate GSH synthesis [124–126]. All of these advantages of NAC imply that a favorable outcome may be obtained by inclusion of NAC in lead poisoning therapy protocol. In our study, NAC supplementation resulted in an increased GSH/GSSG ratio and decreased MDA and catalase activity [120]. Besides this antioxidant function, NAC also

exerted a positive effect on cell survival, which had been dramatically decreased by lead exposure. When taken together, the results support the involvement of ROS in lead toxicity and a possible beneficial role for NAC in therapeutic implications of lead poisoning. These findings were further supported by the same group [120] in an *in vitro* system. Treatment with NAC caused a reduction in indices of oxidative stress in both the brains and livers of lead-exposed C57BL/6 mice. This implies that a thiol-containing antioxidant is capable of mitigating lead-induced oxidative stress.

On the other hand, it has been indicated that NAC has a chelating activity against several heavy metals such as boron, chromium [127], cobalt [128], cadmium, gold, and lead [129]. However, it is suggested that NAC may be ineffective in chelating metals when given orally [130]. Consistent with these results, Ercal et al. found no chelating effect of NAC when orally administered to mice in 5.5 mmol/kg dosages for a week [120]. NAC treatment did not cause any decrease in their blood, liver, or brain lead levels. This finding suggests that NAC administration counteracted *in vivo* oxidative stress without removing lead from target tissues. Therefore, the antioxidant role for NAC appears to be related solely to its free thiol group. In another *in vivo* study, where 5

mmol/kg/d NAC was administered orally to Fisher 344 (F344) rats for a week following exposure to 2000 ppm lead acetate in drinking water for 5 weeks, Gurer et al. observed a slight decrease in blood lead levels (27.3% decrease) [54]. A well-known chelator, succimer, included in the same study to compare and evaluate the chelating efficacy of NAC, dramatically enhanced the clearance of lead from the blood stream (92.8% decrease). Results support the hypothesis that the antioxidant action of NAC could provide some beneficial effects in lead poisoning treatment independent of chelation. Therefore, inclusion of NAC in a chelation-oriented treatment protocol for plumbism seems to be much more effective than using it as a sole agent. Similar effects of NAC were also shown in lenses of lead-exposed F344 rats, where lead induced an oxidative modification of protein sulfhydryl residues and lipids and decreased GSH levels [131].

Neal et al. in 1997 [132] reported other evidence of the beneficial role of NAC in treating lead poisoning. δ -ALA-induced oxidative stress via generation of ROS is suggested to be, in part, responsible for the lead-induced damage [33,35]. Therefore, a study was undertaken to test the hypothesis that ALA accumulation in CHO cells contributes to the cumulative oxidative challenge of lead poisoning, and also to examine whether NAC treatment as an antioxidant can reverse ALA-induced oxidative damage. The results indicate a pro-oxidant effect for ALA by means of a decreased GSH:GSSG ratio, increased MDA levels, and inhibited colony formation [132]. Furthermore, a protective role of NAC in ALA-exposed cells was evidenced by an increase in GSH:GSSG ratios and increased cell survival. This study confirms a therapeutic role for NAC in plumbism that is independent of chelation, and could be solely attributed to NAC's free sulfhydryl group [132].

α -Lipoic acid (LA)

Another antioxidant, LA, was also suggested as being able to abate some of the toxic effects of lead [133]. LA can be synthesized by animals and humans [134], and functions as a cofactor in several multi-enzyme complexes [135]. Its reduced form, dihydrolipoic acid (DHLA), has two free sulfhydryl groups (Fig. 5), and the LA/DHLA redox couple has received great attention in recent studies with regard to its antioxidant potential [134]. Mechanisms of their antioxidant activity are reviewed by Packer et al. [136] and Biewenga et al. [137] in detail. Both LA and DHLA (i) have the ability to scavenge some reactive species, (ii) can regenerate other antioxidants (i.e., vitamins E and C, and GSH) from their radical or inactive forms, and (iii) have metal chelating activity. LA also seems to have a notable advantage over

NAC in opposing GSH loss, since LA is effective in a micromolar range while millimolar NAC is needed for a similar effect [138]. Other criteria, which are important when considering therapeutic applications of an agent, are its absorption and bioavailability, as well as its concentration in target tissues. The capability of LA to cross the blood-brain barrier [139] appears to be an extra advantage because the brain is an important target in lead poisoning.

Incubating lead-treated CHO cells with LA was shown to result in considerably increased cell survival along with attenuated oxidative stress [133]. This is in terms of decreased MDA levels, increased GSH content, and decreased catalase activity. Similar results were found in an in vivo model where F344 rats were exposed to 2000 ppm lead acetate in drinking water for 5 weeks. GSH levels in RBC and brains were found to be elevated following 1 week of 25 mg/kg/d LA administration, whereas RBC, brain, and kidney MDA levels were diminished and catalase, G6PD activities in RBC were returned to the control levels [133]. No chelating action of LA against lead was observed in that study design in terms of no changes in blood, brain, and kidney lead concentrations. Therefore, the beneficial effects of LA on oxidative stress-related parameters do not appear to be related to its ability to remove lead from target cells but are associated with LA's potential for bolstering thiol antioxidant capacity. However, LA was found to have almost no effect on the lenticular redox status following lead exposure [140]. LA administration to lead-exposed rats resulted in significant increases in lenticular cysteine levels with insignificant changes in GSH and MDA [140].

Captopril

Captopril, an angiotensin-converting enzyme inhibitor, was suggested as having antioxidant potential besides its well-known anti-hypertensive action. Its terminal sulfhydryl group (Fig. 5) was suggested as playing a role in scavenging ROS, which was indicated as a mechanism for the antioxidant action of captopril [141–143]. The thiol group in the structure of captopril raised the possibility that it might chelate heavy metals, thereby increasing their excretion [144]. These properties suggested a possible role for captopril in lead toxicity where oxidative stress is thought to be involved. Therefore, Gurer et al. investigated in vivo effects of captopril on lead-induced oxidative stress [145]. The captopril-treated samples showed higher GSH:GSSG ratios in the liver, brain, and kidneys of rats, as well as slightly decreased MDA concentrations. The catalase activity was not significantly affected. Unaltered blood lead concentrations were detected after 1 week of captopril (10mg/d) admin-

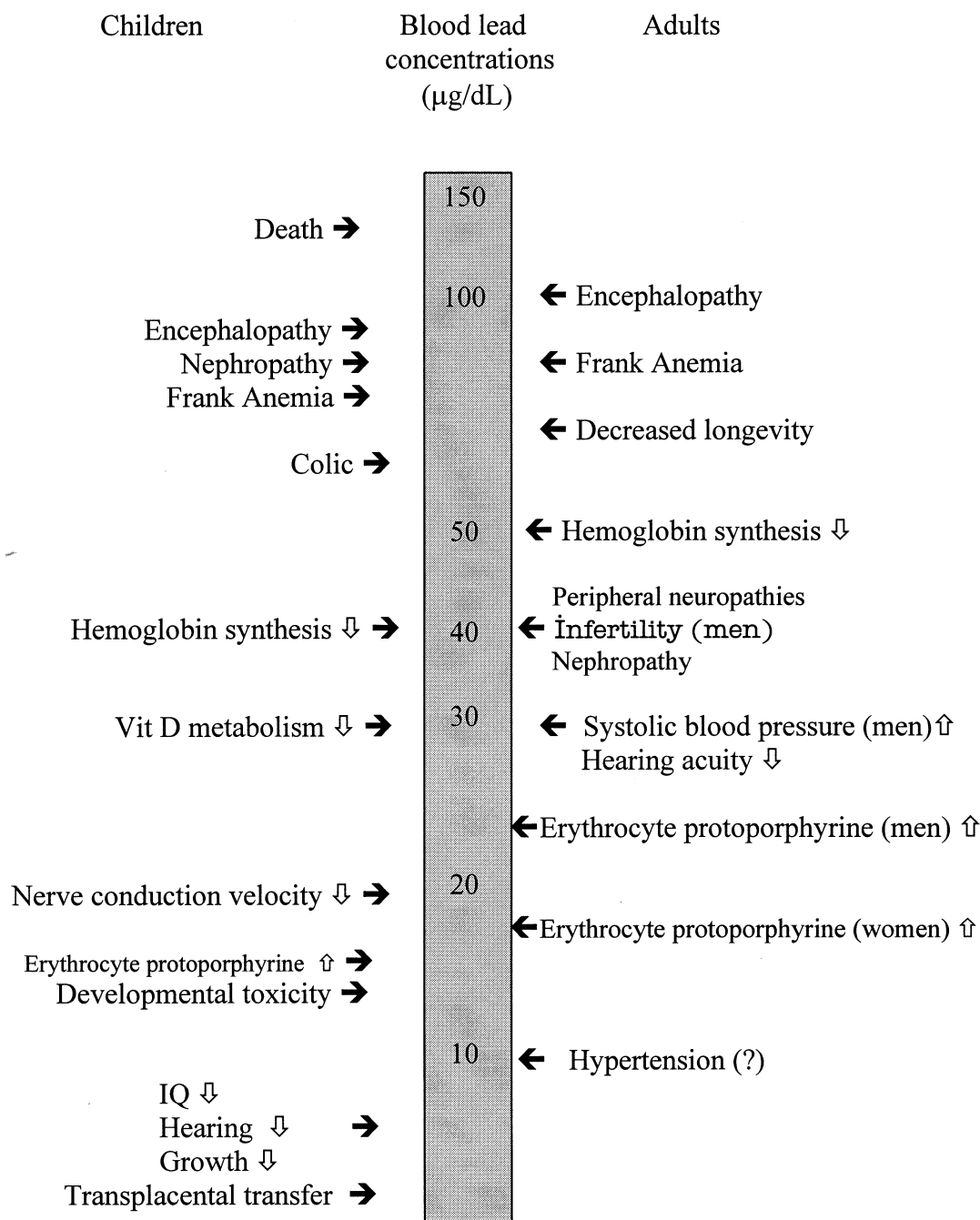


Fig. 6. Effects of inorganic lead on children and adults. (Adapted from [164].)

istration to lead-exposed F344 rats, which indicates that it can only be used along with a chelating agent to treat lead poisoning [145].

Lead-induced hypertension is reported in other studies [146,147], which indicated that increased ROS generated by lead exposure may contribute to increased blood pressure by enhancing inactivation of endothelium-derived nitric oxide [148,149]. A possible role of ROS in lead-induced hypertension was further evidenced by decreased

MDA levels and reduced blood pressure with concomitant administration of vitamin E to lead-exposed animals [150, 151]. Finally, Ding et al. [152] reported that hydroxyl radical may be the radical to blame for the shown endothelial dysfunction in lead toxicity. Therefore captopril's multiple benefits as an antioxidant and antihypertensive agent deserve to be explored to elucidate whether antioxidant effect of captopril can be a possible alternative mechanism for its known antihypertensive effect.

Taurine

Taurine, a semi-essential amino acid has been shown to have a role in maintaining calcium homeostasis, osmoregulation, removal of hypochlorous acid, and stabilizing the membranes [153,154]. Some of the recent data indicate that taurine can act as a direct antioxidant by scavenging ROS [155–158] and/or as an indirect antioxidant by preventing changes in membrane permeability due to oxidant injury [153]. However, some contrary data have also been reported [159]. In our recent studies taurine was shown to have beneficial effects in lead-induced oxidative stress in CHO cells and F344 rats (unpublished data). Dramatically increased cell survival was established in taurine-treated, lead-exposed CHO cells while MDA levels were diminished and GSH levels were increased. Similar effects were found in RBC and the brains and livers of lead-exposed F344 rats. No chelating effect of taurine (1.2 g/kg/d) was indicated by any change in lead concentrations in the blood, brains, livers, and kidneys after taurine treatment. An antioxidant mechanism(s), rather than a chelating activity, seems to underlie this observed effect of taurine against lead-induced oxidative stress. Further studies are needed to understand the antioxidant properties of taurine.

CONCLUSION

Lead poisoning is an old but persistent public health problem throughout the world. Although guidelines for the management of childhood lead poisoning were released by the Centers for Disease Control in 1985 [160] and 1991 [84], a nationwide survey of pediatric lead-poisoning treatment programs indicated that no common approach for the treatment of low-level lead poisoning appears to exist within the lead clinics [161]. Reported adverse effects of conventional chelators and the uncertainty in their efficacy in reversing or preventing the neurotoxic effects of lead (believed to occur in children with 25 µg/dl blood lead concentrations) caused some clinicians to ignore pharmacological intervention in children with low blood lead levels [83] (Table 1). There is an overall consensus on the adverse neurodevelopmental outcomes of low-level (25 µg/dl) lead poisoning during childhood [162,163] (Fig. 6). Investigations pointed to induced oxidative damage even with low blood lead levels.

These facts present a novel approach to strategies for treating lead poisoning by supplementation with antioxidants, either individually or in a combined therapy, with chelating agents. Studies so far suggest that antioxidants can play an extremely important role in abating some toxic effects of lead. Some antioxidants, such as NAC, appear to have a potential for chelating lead and remov-

ing it from the blood stream [54]. Further studies should focus on exploring the dual benefits of these antioxidants as possible chelating and antioxidant agents in treating lead toxicity.

Use of antioxidants brings another option to the therapy: the possibility of therapeutic intervention without removing the patient from the source of lead. Because of the rebound effect of chelators, chelation therapy could not be started when the subject was near lead. Antioxidants, however, are recognized as safe molecules and may be given to subjects with low lead concentrations in their blood even when it is not possible to remove them from exposure to lead. Consequently, experiments are needed to show the effects of antioxidants on the cells or animals that are treated concomitantly for lead exposure. Detailed mechanistic studies are also required to understand the mechanisms underlying the beneficial effects of some antioxidants and to explore the optimum dosage and duration of treatment to obtain better clinical recoveries in lead intoxication cases.

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ABBREVIATIONS

- ALA— δ -aminolevulinic acid
 ALAD—Aminolevulinic acid dehydratase
 BAL—Dimercaprol
 CaNa₂EDTA—Calcium disodiummetilendiamintetraacetic acid
 CHO—Chinese hamster ovary
 CuZnSOD—Copper-zinc superoxide dismutase
 DHLA—Dihydrolipoic acid
 F344—Fisher 344
 6PD—Glucose-6-phosphate dehydrogenase
 GPx—Glutathione peroxidase
 GR—Glutathione reductase
 GSH—Glutathione
 GSSG—Glutathione disulfide
 GST—Glutathione S-Transferase
 H₂O₂—Hydrogen peroxide
 HO—Hydroxyl radical
 LA— α -Lipoic acid
 MDA—Malondialdehyde
 NAC—N-Acetylcysteine
 O₂[−]—Superoxide anion
 RBC—Red blood cell
 ROS—Reactive oxygen species
 SAM—S-adenosyl-L-methionine
 SH—Sulfhydryl (thiol)
 SOD—Superoxide dismutase