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ARSENIC AND LEAD INDUCED FREE RADICAL GENERATION AND THEIR REVERSIBILITY FOLLOWING CHELATION

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Abstract – Health hazards caused by heavy metals have become a great concern to the population. Lead and arsenic are one of the most important current global environmental toxicants. Their toxic manifestations are being considered caused primarily due to the imbalance between pro-oxidant and antioxidant homeostasis and also due to a high affinity of these metals for thiol groups on functional proteins. They also interfere with a number of other body functions and are known to affect central nervous system (CNS), hematopoietic system, liver and kidneys and produce serious disorders. They produce both acute and chronic poisoning, of which chronic poisoning is more dangerous as its very difficult to revert back to normal condition after chronic exposure to these insidious metals present in our life. Despite many years of research, we are still far from an effective treatment of chronic plumbism and arsenicosis. Current approved treatment lies in the administration of chelating agents that forms an insoluble complex with the metal and removes it. They have been used clinically as antidotes for treating acute and chronic poisoning. The most widely used chelating agents are calcium disodium ethylenediamine tetra acetic acid ($CaNa_2EDTA$), D-penicillamine and British anti-lewisite (BAL). Meso 2,3 dimercaptosuccinic acid (DMSA), an analogue of BAL, has been tried successfully in animals as well as in humans. But it is unable to remove the metal from intracellular sites. Effective chelation therapy for intoxication by heavy metals depends on whether the chelating agents are able to reach the intracellular site where the heavy metal is firmly bound. One of the important approaches has been the use of combination therapy. This includes use of structurally different chelators or a combination of an adjuvant/ antioxidant/ herbal extracts and a chelator to provide better clinical/ biochemical recovery. A number of other strategies have been suggested to minimize the numerous problems. This article presents the recent development made in this area with possible directions for future research.

Key words: Arsenic and lead poisoning, free radicals, oxidative stress, chelation therapy, chelating agents, antioxidants, adjuvants, herbal extracts

INTRODUCTION

Heavy metal toxicity represents an uncommon, yet clinically significant, medical condition. The heightened concern for reduction of environmental pollution that has been occurring over the past 20 – 25 years has stimulated active continuing research and literature on the toxicology of heavy metals. If unrecognized or inappropriately treated, heavy metal toxicity can result in significant morbidity and mortality. The periodic table contains 105 elements, of which 80 are considered metals. Toxic effects in humans have been described for less than 30 of these. Many metals are essential to biochemical processes, and others have found therapeutic uses in medicine. While the toxic effects of these substances are a widespread concern in the modern industrial context. However, occupational exposure to heavy metals has accounted for the vast majority of poisonings throughout human history. Hippocrates described abdominal colic in a man who extracted metals, and the pernicious effects of arsenic and mercury among smelters were known even to Theophrastus of Erebus (370-287 BC). Virtually all metals can produce toxicity when ingested in sufficient quantities, but there are several which are especially important because either they are so pervasive, or produce toxicity at such low concentrations among which lead and arsenic are known to be most common. Intentional or unintentional ingestion of arsenic has been notorious as a means of suicide and homicide.

Abbreviations: **$CaNa_2EDTA$**: calcium disodium ethylenediamine tetra acetic acid; **BAL**: D-penicillamine and British anti-lewisite; **DMSA**: Meso 2,3 dimercaptosuccinic acid.

Oxidative Stress/ Free radicals mediated toxicity

It has been observed that oxygen is both life-sustaining and life-threatening inhalant. During the past two decades, the evidence supporting the deleterious effects of oxygen free radicals in

many pathological processes has grown considerably (1). Free radicals may play an important role in several pathological conditions of the CNS where they directly injure tissue and their formation may also be a consequence of tissue injury (1). Recently, attention has also been focused on the contribution of oxygen free radicals to brain dysfunction and brain cell death after brain injury such as cerebral ischemia and head trauma. Free radicals produce tissue damage through multiple mechanisms, including excito-toxicity, metabolic dysfunction, and disturbance of intracellular calcium homeostasis (2). Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons. The presence of unpaired electrons usually confers a considerable degree of reactivity upon a free radical. Those radicals derived from oxygen represent the most important class of such species generated in living systems (3). Oxidative stress, a condition describing the production of oxygen radicals beyond a threshold for proper antioxidant neutralization has been implicated as an important mechanism for arsenic and lead induced toxicity.

Many studies have focused on metal-induced toxicity and carcinogenicity, emphasising their role in the generation of reactive oxygen and nitrogen species in biological systems, and the significance of this therein (3-9). Metal-mediated formation of free radicals may cause various modifications to DNA bases, enhanced lipid per-oxidation, and changes in calcium and sulfhydryl homeostasis.

ROS can be produced from both endogenous and exogenous substances. Potential endogenous sources include mitochondria, cytochrome P-450 metabolism, peroxisomes, and inflammatory cell activation (10). Mitochondria have long been known to generate significant quantities of hydrogen peroxide. The hydrogen peroxide molecule does not contain an unpaired electron and thus is not a radical species. Under physiological conditions, the production of hydrogen peroxide is estimated to account for about ~2% of the total oxygen uptake by the organism. However, it is difficult to detect the occurrence of the superoxide radical in intact mitochondria, most probably in consequence of the presence of high SOD activity therein. Generation of the superoxide radical by mitochondria was first reported more than three decades ago by Loschen and Flohe (11). After the determination of the ratios of the mitochondrial generation of superoxide to that of hydrogen peroxide, the former was considered as the stoichiometric precursor for the latter.

Mitochondria have been described as the “power house” of the cell because they link the energy-releasing activities of electron transport and proton pumping with the energy conserving process of oxidative phosphorylation to harness the value of foods in the form of ATP. Mitochondria generate approximately 2–3 nmol of superoxide/min per mg of protein, the ubiquitous presence of which indicates it to be the most important physiological source of this radical in living organisms (10). Since mitochondria are the major site of free radical generation, they are highly enriched with antioxidants including GSH and enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), which are present on both sides of their membranes in order to minimise oxidative stress in the organelle (12). Superoxide radicals formed on both sides of mitochondrial inner membranes are efficiently detoxified initially to hydrogen peroxide and then to water by Cu, Zn-SOD (SOD1, localised in the intermembrane space) and Mn- SOD (SOD2, localised in the matrix).

The generation of various free radicals is closely linked with the participation of redox-active metals (7). The redox state of the cell is largely linked to an iron (and sometimes copper) redox couple and is maintained within strict physiological limits. It has been suggested that iron regulation ensures that there is no free intracellular iron; however, in vivo, under stress conditions, an excess of superoxide releases “free iron” from iron-containing molecules. The release of iron by superoxide has been demonstrated for [4Fe–4S] cluster-containing enzymes of the dehydratase-lyase family (13). The released Fe(II) can participate in the Fenton reaction, generating highly reactive hydroxyl radical ($Fe(II) + H_2O_2 \rightarrow Fe(III) + {\bullet}OH + OH^-$). Thus under stress conditions $O_2^{\bullet-}$ acts as an oxidant of [4Fe–4S] cluster-containing enzymes and facilitates •OH production from H_2O_2 by making Fe(II) available for the Fenton reaction (4-7). The superoxide radical participates in the Haber-Weiss reaction ($O_2^{\bullet-} + H_2O_2 \rightarrow O_2 + OH^{\bullet} + OH^-$) which combines a Fenton reaction and the reduction of Fe(III) by superoxide, yielding Fe(II) and oxygen

($Fe(III) + O_2{\bullet}- \rightarrow Fe(II) + O_2$) (Liochev and Fridovich , 2002)

The following article is a compilation of the toxic effects of two important toxic heavy metals namely arsenic and lead and the pharmacologic agents and rationales used to treat them.

ARSENIC POISONING

Arsenic (usually as arsenic trioxide, As_2O_3) is well known as a poison and has been discovered to be a carcinogen in humans. Arsenic occurs naturally in the environment as an element of the earth's crust. Arsenic is combined with other elements such as oxygen, chlorine, and sulfur to form inorganic arsenic compounds. Exposure to higher-than-average levels of arsenic occurs mainly in workplaces, near or in hazardous waste sites, and areas with high levels naturally occurring in soil, rocks, and water. Exposure to arsenic at low levels for extended periods of time can cause a discoloration of the skin and the appearance of small corns or warts. Exposure to high levels of arsenic can cause death. The natural occurrence of arsenic in groundwater constitutes a setback in the provision of safe drinking water to millions of citizens in Asia. There are millions of people at risk in the world because they drink water containing carcinogenic amounts of arsenic (14,15). Chronic exposure to inorganic arsenic can lead to cancer of the skin, lungs, bladder and liver if the exposure is via ingestion (16). Lung cancer can occur if exposure is by inhalation (17). The first case of arsenicosis which was revealed in West Bengal in early 1980s was the outcome of ground water arsenic poisoning in Bangladesh, and since its detection in 1993, cases of arsenic poisoning have been increasing in an alarming way. Arsenic is reported to occur at high concentrations in the water supply of communities in diverse countries such as India, Nepal, Vietnam, China, Argentina, Mexico, Chile, Taiwan, Mongolia and United States of America. Recent reports proved that number of countries and many states in India (Uttar Pradesh, Bihar, Jharkhand, West Bengal, Assam, Manipur and Bangladesh) in the Ganga-Meghna-Brahmaputra (GMB) plain an area of 569,749 Km^2, with a population of over 500 million are at risk from ground water arsenic concentration and its health effects. The British Geological Survey (BGS) in 2001 estimated that 46% of all (10 million) shallow tube wells in Bangladesh are contaminated with arsenic at concentrations exceeding the World Health Organization's (WHO) guideline concentration of 0.01 mg/L.

The toxicity of this environmental toxicant is complex and depends, in part, on its chemical form, dose, route and duration of exposure, degree of accumulation, rate of clearance and animal species. Arsenic can exist in three possible oxidation states: element (0), trivalent (+3 e.g. Arsenite or -3 eg. Arsine) and pentavalent (+5, eg. arsenate). Because it has multiple and inter-convertible oxidation states, arsenic can participate in a number of chemical and biological reactions, including oxidation–reduction reactions, acid–base reactions, covalent interactions with most non-metals and metals and methylation–demethylation reactions. In general, inorganic forms of arsenic (eg. arsenite and arsenate) are more toxic than organic forms (e.g. methyl arsonate, dimethyl arsenite or arsenobetaine). The toxicity of different arsenic species varies in the order: arsenite > arsenate > mono-methyl arsonate (MMA) > dimethyl arsenite (DMA).

Both inorganic and organic arsenic are absorbed from the gastrointestinal tract; however, arsenic toxicity results from absorption of trivalent and pentavalent inorganic arsenic. After absorption, arsenic is cleared rapidly from the blood and during its "first pass" phase it reaches the liver where it is detoxified by conversion into MMA and DMA. Arsenic metabolism is characterized by two sequential reactions (18,19) (Figure 1):

(a) The reaction of pentavalent arsenic to trivalent arsenic in the presence of glutathione(20);
(b) Oxidative methylation reaction, in which the trivalent forms of arsenic are sequentially methylated to form mono, di and trimethylated products using S-adenosyl methionine (SAM) as methyl donor and GSH as an essential co-factor. Arsenic methylation occurs primarily in liver.

Many studies confirmed the generation of free radicals during arsenic metabolism in cells (21). Interestingly, some recent reports have provided experimental evidence that arsenic-induced generation of free radicals can cause cell damage and death through activation of oxidative sensitive signaling pathways (22). Arsenic-mediated generation of reactive oxygen species is a complex process which involves the generation of a variety of ROS including superoxide ($O_2^{\bullet -}$), singlet oxygen ($^1O_2$), the peroxyl radical (ROO•), nitric oxide (NO•), hydrogen peroxide (H_2O_2), dimethylarsinic peroxyl radicals

([$(CH_3)_2AsOO•$]) and also the dimethylarsinic radical [$(CH3)_2As•$].

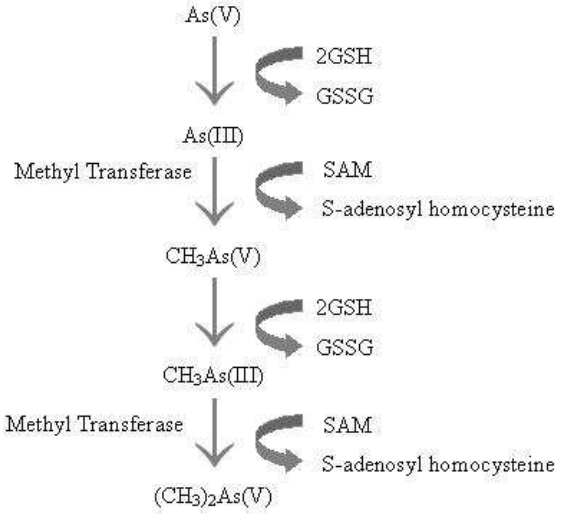

Figure 1. Figure showing the sequential reactions of arsenic metabolism

The exact mechanism responsible for the generation of all these reactive species is not yet clear, but some workers have proposed the formation of intermediary arsine species (21). Another route to the production of H_2O_2 was suggested, involving the oxidation of As (III) to As (V) which, under physiological conditions, results in the formation of H_2O_2:

$$H_3AsO_3 + H_2O + O_2 \rightarrow H_3AsO_4 + H_2O_2$$

In recent studies concerning the mechanism of arsenite toxicity in the brain it was reported that some of its effects have been traced to the generation of the hydroxyl radicals (23). The time-evolution of the formation of the hydroxyl radical in the striatum of both female and male rats who underwent a direct infusion of different concentrations of arsenite was investigated. The treatment with arsenite induced significant increases of hydroxyl radical formation. These results support the participation of hydroxyl radicals in arsenic-induced disturbances in the central nervous system. Arsenic is a well-established human carcinogen (24). Arsenic compounds bind to SH groups and can inhibit various enzymes, including glutathione reductase. Studies support the hypothesis that arsenic may act as a co-carcinogen-not causing cancer directly, but allowing other substances, such as cigarette smoke and UV radiation, to cause DNA mutations more effectively (25) (Figure 2). Arsenic is one of the few species besides vinyl chloride that causes angiosarcoma, which provides a good indication of the potency of arsenic as a cancer-causing agent.

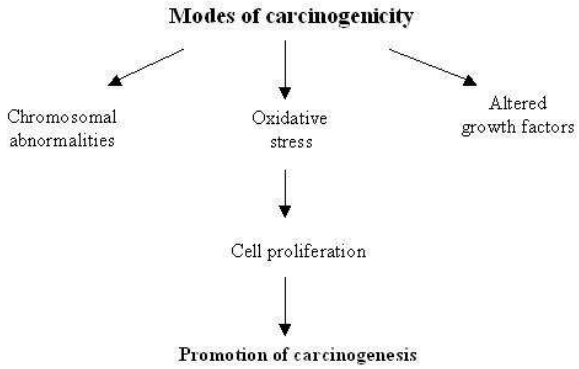

Figure 2. Figure showing the modes of carcinogenicity of arsenic

LEAD POISONING

Occupational lead poisoning has been a recognized health hazard for more than 2,000 years. Characteristic features of lead toxicity, includes anemia, colic, neuropathy, nephropathy, sterility and coma. Lead serves no useful biologic function in the human body. Over the past several years, concern has increased over the health effects of low-level lead exposure and the "normal" body burden of lead. In the occupational setting, the present "no-effect" level for lead exposure is currently being re-evaluated as more sensitive measures of the physiologic effects of lead are made available through clinical investigations. The biochemical basis for lead toxicity is its ability to bind the biologically-important molecules, thereby interfering with their function by a number of mechanisms (Figure 3). Lead has been reported to impair normal metabolic pathways in children at very low blood levels (26,27). At least three enzymes of the heme biosynthetic pathway are affected by lead and at high blood lead levels the decreased heme synthesis which leads to decreased synthesis of hemoglobin. Blood lead levels as low as 10 µg/dL have been shown to interfere with one of the enzymes of the heme pathway, δ-aminolevulinic acid dehydratase.

Accumulating evidences have shown that lead causes oxidative stress by inducing the generation of reactive oxygen species (ROS) and weakening the antioxidant defence system of cells (28-30). Depletion of cells' major sulfhydryl reserves seems to be an important indirect mechanism for oxidative stress that is induced by redox-inactive metals (5,31). When GSH is reduced by lead, GSH synthesizing systems start making more GSH from cysteine via the γ-glutamyl cycle. GSH is usually not

effectively supplied, however, if GSH depletion continues because of chronic exposure (32). Several enzymes in antioxidant defense system may protect this imbalance but they also get inactive due to direct binding of lead to the enzymes' active sites, if the sites contain sulfhydryl group e.g. ALAD. Further, zinc which usually serves as a cofactor of many enzymes could be replaced by lead, thereby making the enzyme inactive.

The increased lipid peroxidation and inhibition of enzymes responsible to prevent such oxidative damage have demonstrated lead induced oxidative injury (33). Lead induced disruption of the prooxidant/antioxidant balance could induce injury via oxidative damage to critical biomolecules. The possible mechanisms resulting in the formation of free radicals include generation of superoxide ion (34). A significant decrease in the activity of tissue superoxide dismutase (SOD), a free radical scavenger and metalloenzyme (zinc/copper) on lead exposure have been reported (35,36). This could be due to an increase in lead concentration in these tissues and their possible reaction with this enzyme (37) thereby, reducing the disposal of superoxide radicals. Catalase activity too has been shown to increase in kidney.

Catalase is an efficient decomposer of H_2O_2 and known to be susceptible to lead toxicity (38). Lead induced decrease in brain GPx activity may arise as a consequence of impaired functional groups such as GSH and NADPH or selenium mediated detoxification of toxic metals (39). While, antioxidant enzyme glutathione S-transferase (GST) is known to provide protection against oxidative stress and the inhibition of this enzyme on lead exposure might be due to the depletion in the status of tissue thiol moiety. These enzymes are important for maintaining critical balance in the glutathione redox state. Production of GSH is considered to be the first line of defense against oxidative injury and free radical generation where GSH functions as a scavenger and a co-factor in metabolic detoxification (40). GSH has carboxylic groups, an amino group, a sulfhydryl group and two peptide linkages as sites for the reaction of lead. Its functional group, -SH plays an important role in lead binding. Several reports have demonstrated that GSH is decreased in the brain, liver and eye lens of rats exposed to lead (31).

SYMPTOMS OF LEAD TOXICITY

Lead is known to cause acute, sub-chronic and chronic toxicity. The most commonly used biological marker is the concentration of lead in blood. The concentration of lead in plasma is very low and thus is not recommended.

Acute Toxicity

Acute lead toxicity occurs at blood levels of 100-120 µg/dL in adults and 80-100 µg/dL in children. It results from inhalation of large quantities of lead due to occupational exposure among industrial workers and in children through ingestion of large oral dose from lead based paint on toys. The clinical symptoms of acute poisoning are characterised by metallic taste, abdominal pain, vomiting, diarrhoea, anaemia, oliguria, collapse and coma.

Chronic toxicity

Symptoms of chronic toxicity may appear in adults at blood lead levels of 40-60 µg/dL. This is more common and can be described in three stages of progression: The early stage is characterised by loss of appetite, weight loss, constipation, irritability, occasional vomiting, fatigue, weakness, gingival lining on gums and anaemia; The second stage is marked by intermittent vomiting, irritability, nervousness, tremors and sensory disturbances in the extremities, most often accompanied by stippling of red blood cells; and the third severe stage of toxicity is characterised by persistent vomiting, encephalopathy, lethargy, delirium, convulsions and coma.

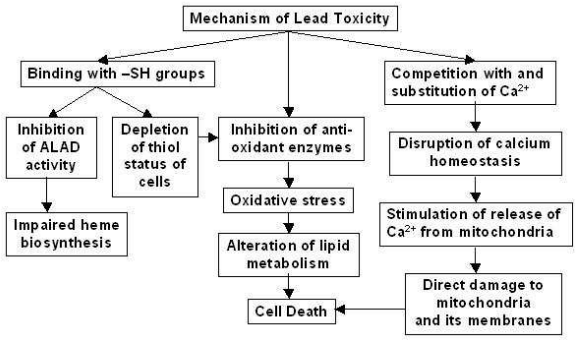

Figure **3** Figure depicting the mechanisms of lead toxicity

It can thus be concluded that inhibitory effect of lead on antioxidant enzymes and glutathione appear to impair the cells' antioxidant defenses and render them more susceptible to oxidative attacks.

TREATMENT OF METAL POISONING

Chelation therapy

Chelating agents are organic compounds capable of linking together metal ions to form complex ring-like structure called chelates. 'Chelate' is a Greek word meaning the claws of a lobster. Chelators act according to a general principle: the chelator forms a complex with the toxic ion, and these complexes reveal a lower toxicity and are more easily eliminated from the body through the excretory system.

It is of great importance that the chemical affinity of the complexing agent for the toxic metal ion should be higher than the affinity of the metal for the sensitive biological molecules. Thus, chemical measurement of the stability constants of the metal-complexes formed may give a first indication of the effectiveness of a particular chelating agent. An ideal chelating agent should possess the characteristic like, greater affinity for the toxic metal that has to be chelated, low toxicity, rapid elimination of metal, high water solubility, ability to penetrate cell membrane, administered orally, ability to chelate with natural chelating groups found in biological system, minimal metabolism etc.

The metal chelate complexes have a reduced tendency to undergo exchange reactions once they are formed. However, it is frequently advantageous to use a preferred donor atom in a chelating agent of lower density. It is also necessary to keep in mind that the introduction of the chelating agent into any intracellular space requires its passage through the cell membrane. This passage can be accomplished either (a) by passing through the lipid part of the membrane as an uncharged molecule or (b) via utilizing one of the anion/cation transport systems present in the membrane. There is a hypothesis that large ion complex with a positive charge will pass out of a cell very slowly because of their inability to pass through either the lipid portion of the cellular membrane or the cation transport system designed to move ions with +1 or a +2 charge across the membrane. Another important property of metal complexes is the stereochemistry of the toxic metal ion. Chelating agents tie up all the coordination position of a metal ion (41-46). It should be noted that metal chelating agents usually contain more than one functional group, in order to provide a chemical 'claw' to chelate the toxic metal.

Conventional chelating agents

The most commonly used chelating agents that have been the forerunners in chelation therapy belong to the polyaminocarboxylic groups. As the name indicates, these chelators utilize the amino and the carboxylic groups to scavenge the toxic metal from the system. In this category, calcium disodium ethylene diamine tetra acetic acid ($CaNa_2EDTA$) is a derivative of ethylene diamine tetra acetic acid (EDTA), a synthetic polyamino-polycarboxylic acid was used for the treatment of metal poisoning and had been the mainstay of chelation therapy for many years. Another member belonging to this family is diethylene triamine pentaacetic acid DTPA is a synthetic polyaminocarboxylic acid with properties similar to EDTA (47). It can be affirmed that EDTA does not penetrate cell membranes and has a biological half-life of 50-60 minutes; 90% is excreted within 6-8 hours after administration. Renal clearance is mainly through active tubular secretion without any significant re-absorption. Variations in the pH and diuresis do not affect the excretion rate (48). $CaNa_2EDTA$ has the LD_{50} value of 16.4 mmol/kg in mouse (49). Intravenous administration of this drug results in good absorption but very painful at the injection site. Hence intravenous injection could be given either by diluting in 5% dextrose or saline (49). Hypocalcaemia is reported with the administration of Na_2EDTA. CaEDTA has the major toxic effects on the renal system causing the necrosis of tubular cells. Severe, hydropic degeneration of proximal tubule cells has also been reported. These lesions along-with some alterations in the urine like hematuria, proteinuria and elevated BUN are generally reversible when the treatment ceases. Another side effect of EDTA is its ability to chelate various essential metals endogenous to the body, zinc in particular (50,51). Zinc administration during EDTA administration is generally recommended to reduce toxicity (50).

It has been well established that administration of EDTA during pregnancy can result in teratogenic effects especially when administered between days 11 to 14 at doses comparable to humans (52). Tuchmann-Duplessis and Mercier-Parort (53) were the first to report teratogenic effect of EDTA. Absorption

into the circulation, potential interaction with essential trace elements, and the stress associated with the administration of the compound were suggested to be the possible factors involved in the differences in EDTA-induced maternal and developmental toxicity (54). Brownie et al. (52) also reported teratogenic effects. Another reported disadvantage of $CaNa_2EDTA$ is that it redistributes lead to the brain. Cory Slechta et al. (55) and Flora et al. (56) in separate studies provided evidence that rat given lead as lead acetate in their drinking water and then treated with $CaNa_2EDTA$ mobilized lead from their tissues and redistributed to brain and liver on the first day of treatment. The large number of side effects due to the administration of these chelating agents prompted in the commercialization of chelators containing thiol or sulfhydryl groups.

D-Penicillamine (DPA) is 3,3 dimethylcysteine, a sulfhydryl containing amino acid, first introduced in clinical practice by Walshe (57) but was tried by Ohlsson (58) as an antidote for low or mild lead poisoning. It can penetrate cell membranes and then get metabolized. It can be absorbed through the gastro intestinal tract and thus can be administered orally. Its absorption from the gastrointestinal tract is between 40 to 70% (59). It is fairly stable as its SH group is very resistant to oxidation *in vivo*, attack from enzymes such as cysteine desulfhydrase and L-amino acid oxidase, compared to other monothiols. Excretion of DPA through urine is very fast. Small amount is also reported to cross hepatocyte membrane and excreted through bile. However, the major toxic effect of DPA is antagonizing pyridoxine and inhibiting pyridoxine dependent enzyme such as transaminases. Other toxic effects include hypersensitive allergic reactions like fever, skin rashes, leucopoenia and thrombocytopenia (60). In few reports nephrotoxic effects too have been observed along with penicillin allergic reaction in sensitive individual due to cross reactivity. Prolonged treatment may also lead to anorexia, nausea, vomiting in human. Apart from this, DPA is also a well recognized teratogen and lathyrogen that causes skeletal, palatal, cutaneous and pulmonary abnormalities (61-63). As compared to other chelators, the developmental toxicity of DPA is abundant in both human and experimental animals. First report on human embryopathy associated with DPA was published by Mjolnerod et al. (64). Author described the effect of DPA on the infant with generalized connective tissue defects including lax-skin, hyperflexibility of joints, vein fragility, varicosities and impaired wound healing, the child died at a age of 7 weeks. Since DPA chelated copper, it was hypothesis that the drug might be teratogenic (65). Various investigations were performed in the early eighties to test the hypothesis (65-68) and it was observed that when pregnant rats were given DPA along with their diet, there was a high incidence of malformations. The frequency of reabsorption and the frequency and severity of malformations increased in the rats in a dose dependent manner (67). However, literature also suggests that the administration of DPA during pregnancy protects the mother from the relapse of Wilson's disease, while it would carry few risks to the fetus (69). DPA have been tried safely throughout pregnancy in women with Wilson's disease, suggesting that the excessive copper stores improve tolerance (70). The American Academy of Pediatrics (71) recommends pencillamine use only when unacceptable adverse reactions to both DMSA and EDTA have occurred. However, Kreppel et al. (72) reported that pencillamine was ineffective in reducing arsenic burden in rats.

It is clear from above that most of the conventional chelators are compromised with many side effects and drawbacks and there is no safe and effective treatment available for arsenic and lead poisoning. In the recent past some newer strategies were adopted to find a solution to this problem. In the following paragraphs some of these strategies have been discussed in brief.

SYNTHESIS OF NEW CHELATORS

Thiol chelators:

In the early eighties it was shown that some newer complexing agents like DMPS and DMSA were effective against mercury, arsenic and lead poisoning. When compared to BAL these newer chelating agents were of significant lower toxicity and moreover they could be administered orally or intravenously (73). In addition to their heavy metal chelating properties, these agents have a dithiol group that may act as an oxygen radical scavenger and thus inhibit lipid peroxidation (74-76). Chemical structures of some of the newer thiol chelators are summarized in Figure 4.

Sodium 2,3 dimercaptopropane 1-sulphonate (DMPS)

DMPS was first introduced in Soviet Union in the 1950s as `Unithiol'. DMPS is mainly distributed in the extra cellular space; it may enter cells by specific transport mechanism. After i.p. injection of lethal doses the animals were highly irritable for some minutes before they became apathetic and breathing ceased (49,77). DMPS is rapidly eliminated from the body through the kidneys. The serum half-life is about 20 to 60 minutes. Following oral administration, about 60 to 30% of the administered dose is absorbed in dogs (78) and 30 % in rats (49,79), and plasma peak levels are reached after 30 to 45 minute (78). Rapid oxidation of DMPS after intravenous administration to disulfide forms is well reported in blood (80,81). Fifteen minutes after iv administration of DMPS (3 mg/kg) to humans only 12% of the total DMPS was oxidized to disulfides (82). DMPS is not involved in important metabolic pathway and parts of administered substance are excreted in an unchanged form. By the parenteral route the LD_{50} for various species is about 1 g/kg to 2 g/kg. No major adverse effects following DMPS administration in humans or animals have been reported (83). However, a dose dependent decrease in the copper contents was found in the serum, liver, kidneys and spleen. Information regarding the developmental toxicity of DMPS is rather scarce. No abnormalities in the offspring with chronic oral DMPS treatment are reported. Oral administration of DMPS did not adversely affect late gestation, parturition, or lactation in mature mice and fetal and neonatal development does not appear to be adversely affected(84).

DMPS although known for its antidotal efficacy against mercury, it has been reported to be an effective drug for treating arsenic poisoning. This drug too can be administered both orally and intravenously. An oral dose of 100 mg/kg thrice a day for 10-12 days is effective against mild arsenic poisoning while no recommendation for treating chronic arsenic poisoning is available (85). In experimental animals, i.p. administration of DMPS increased the lethal dose of sodium arsenite in mice by four folds. A quantitative evaluation of three drugs reveals that DMPS is 28 times more effective than BAL in arsenic therapy in mice (86), while DMSA and DMPS are equally effective. DMPS also appeared to be effective at least in reducing the body lead and gold burden (79,87). In children, 5 mg/kg per single dose should be given (88). Oral DMPS treatment in adults may be given with an initial dose of 100-300 mg and continued with 100 mg every 6 or 8 hours. In children, the oral dosage is 5 mg/kg/day.

Succimer or meso 2,3-dimercaptosuccinic acid (DMSA)

The one chemical derivative of dimercaprol, which has gained more and more attention these days, is DMSA also known as Succimer. Succimer is an orally active chelating agent, much less toxic than BAL and its therapeutic index is about 30 times higher (89). US FDA has approved this compound in 1991 for the treatment of children whose blood lead concentration was above 45 μg/dL (90). The empirical formula of DMSA is $C_4H_6O_4S_2$ and its molecular weight is 182.21. It's a weak acid soluble in water (49).

DMSA distribution is predominantly extra cellular since it is unable to cross hepatic cell membrane and excreted by the kidney with a half-life of about two days (73). Over 95% of blood DMSA is bound mainly to albumin (73,91). DMSA appears to be transported by plasma albumin. It has been reported that 2-4 hours after DMSA administration only 12% of meso DMSA excreted in urine was unaltered whereas about 88% oxidized to form disulfates (DMSA attached to one or 2 cysteine molecules). No mixed disulfates are found in the blood (91-94). The absorption of DMSA after oral administration is about 60%. Studies addressing the possibility that DMSA may chelate metal stored in the gut, because a significant percentage of an oral dose is not absorbed, have yet to be elucidated (92).

The LD_{50} values of sodium salts of DMSA in mice are: iv 2.4, im 3.8, ip 4.4 and po 8.5 g/kg, respectively. Using a percutaneous route, the acute LD_{50} for rats and mice is about 2 g/kg. Graziano et al. (95) reported that i.p. administration of 200 mg/kg DMSA could produce only a marginal change in growth but did not elicit any appreciable change in histopathological alterations in tissue or cause hematological or biochemical change in blood. No significant loss of essential metals like zinc, iron, calcium or magnesium was observed. A slight increase in transaminase activities in serum of human and animals has been reported after DMSA treatment (95,96). Adverse reaction to DMSA includes gastrointestinal discomfort, skin reaction, mild neutropenia and elevated liver

enzymes. No redistribution of lead also occurred on DMSA administration in rats (55).

Orally administered DMSA caused no marked adverse reactions but some sulphurous odor in the mouth, weakness, abdominal distension and anorexia. These reactions were mild and disappeared quickly after withdrawal of DMSA. No pathological findings were observed in the blood, urine, ECG or ultrasonography of liver and spleen.

It has also been found that DMSA resulted in low maternal liver copper and calcium concentration whereas high iron levels, the fetal copper, calcium and zinc levels decreased (97). Although the results suggest that DMSA induced developmental toxicity was due to an induced zinc deficiency, additional investigations showed that the embryo/fetal toxicity of DMSA might be mediated, at least in part, through altered fetal copper metabolism (98). In contrast, the oral route did not cause any adverse affects on the offspring survival and development (99).

DMSA has been tried successfully in animal as well as in few cases of human arsenic poisoning (100). DMSA has been shown to protect mice due to lethal effects of arsenic. A subcutaneous injection of DMSA provided 80-100% survival of mice injected with sc sodium arsenite (101). Flora and Tripathi (100) also reported a significant depletion of arsenic and a significant recovery in the altered biochemical variables of chronically arsenic exposed rats. This drug can be effective if given by either oral or i.p. route. Patients treated with 30 mg/kg DMSA per day for 5 days showed significant increase in arsenic excretion and a marked clinical improvement. In a double blind, randomized controlled trial study conducted on few selected patients from arsenic affected West Bengal (India) regions with oral administration of DMSA suggested that DMSA was not effective in producing any clinical or biochemical benefits or any histopathological improvements of skin lesions (102). In an experimental study recently conducted, provided an *in vivo* evidence of arsenic induced oxidative stress in number of major organs of arsenic exposed rats and that these effects can be mitigated by pharmacological intervention that encompasses combined treatment with N-acetylcysteine and DMSA (103).

US FDA has recently licensed the drug DMSA for reduction of blood lead levels. It was reported that EDTA increases the lead content in the brain due to redistribution (55). DMSA when administered either alone or in combination with EDTA decreases the lead concentration in the brain (55,56). Besunder et al. (104) too recently confirmed these findings in rats and recommended the administration of DMSA and EDTA to children hospitalized for combined chelation therapy.

Esters of Succimer (DMSA)

A large number of esters of DMSA have been synthesized for achieving optimal chelation as compared to DMSA. These esters are mainly the mono and dimethyl esters of DMSA that have been studied experimentally with the aim of enhancing tissue uptake of chelating agents (93). In order to make the compounds more lipophillic the carbon chain length of the parent DMSA was increased by controlled esterification with the corresponding alcohol (methyl, ethyl, propyl, isopropyl, butyl, isobutyl, pentyl, isopentyl and hexyl). A large number of esters have been synthesized and are being tried for the treatment of metal poisoning. It has also been reported that these mono and diesters have a better potential in mobilizing cadmium and lead from the tissues in mice (105,106). Rivera et al. (107) reported that that the dimethyl ester of DMSA (meso-DiMeDMSA) increased the excretion of cadmium. They also reported that when rabbit liver metallothionein was incubated with the diester, 32% of the cadmium and 87% of zinc bound metallothionein was removed from the system (108). Although, the diester entered the cell but it caused severe zinc depletion (108). Singh et al. (109) examined the efficacies of three diesters of DMSA and found that these diesters were effective in reducing the soft organ lead concentrations when compared to BAL. Kreppel et al. (110) reported the therapeutic efficacy of six analogues of DMSA in mice. They administered mice with a single LD_{80} dose of arsenic trioxide followed by a single dose of these six analogues of DMSA. They found that meso 2,3-di(acetylthio) succinic acid (DATSA) and 2,3-di(benzoylthio) succinic acid (DBTSA) increased the survival rates by 29% and 43% respectively when administered via gastric tube (i.g) and 89% when administered intraperitoneally (i.p). Administration of dimethyl DMSA (DMDMSA) through i.g and i.p and diethyl DMSA (DEDMSA), di-n-propyl DMSA (DnPDMSA) and diisopropyl DMSA (DiPDMSA) through i.g route did not reduce the lethality. While the i.p. administration of DnPDMSA increased the survival rate by 72%

whereas DEDMSA and DiPDMSA increased it by 86% (110). Kreppel et al. (111) also reported the effects of 4 monoesters of DMSA in increasing the survival and arsenic elimination in various organs in mice. It was observed that all the monoesters, MiADMSA (mono- isoamyl), MnDMSA (mono n-amyl), MnBDMSA (mono n-butyl) and MiBDMSA (mono i-butyl) markedly decreased the arsenic content in most of the organs as soon as 1.5 hrs after administration. They found that MiADMSA and MnADMSA were the most effective in increasing the survival of mice (111). Similar studies were also performed by Flora et al. (112) where they investigated the effect of DMDMSA, DEDMSA DiPDMSA and diidoamyl DMSA (DiADMSA) on sub chronically arsenic treated rats. The results suggested that the diesters reduced the arsenic burden in blood and soft tissue but were only moderately effective in reversing the biochemical recoveries when compared to DMSA (112).

Walker et al. (106) studied the effects of seven different monoalkyl esters of DMSA on the mobilization of lead in mice and observed that after a single parenteral dose of the chelator DMSA there was a 52% reduction in the lead concentrations while with the monoesters the reduction varied from 54% to 75%. Jones et al. (105) reported the efficacy of ten different monoesters through oral and i.p. route on cadmium mobilization in mouse. Out of the ten monoesters studied they found MiADMSA to be the most effective in reducing the cadmium concentrations from the liver and kidneys.

In all of the reported literature, it was observed that the analogues of DMSA were capable of crossing the membranes and were more effective in reducing the metal burden in acute and sub-chronic metal intoxication. Most of the studies have also suggested that the monoesters are more effective in treatment of experimentally induced metal intoxication.

Monoisoamyl DMSA (MiADMSA)

Among these new chelators, monoisoamyl ester of DMSA (MiADMSA; a C_5 branched chain alkyl monoester of DMSA) has been found to be the more effective than DMSA in reducing cadmium and mercury burden (113,114). It is reported that the toxicity of DMSA with LD_{50} of 16 mmol/kg is much lower than the toxicity of MiADMSA with LD_{50} of 3 mmol/kg but lesser than BAL (1.1 mmole/kg). The interaction of MiADMSA and DMSA with essential metals is same. Mehta and Flora (115) reported for the first time the comparison of different chelating agents (3 amino and 4 thiol chelators) on their role on metal redistribution, hepatotoxicity and oxidative stress in chelating agents induced metallothionein in rats. We suggested that out of all the 7 chelators, MiADMSA and DMSA produced the least oxidative stress and toxicity as compared to all other 5 chelators (115). However, no reports are available about the toxicity of this metal complexing agent except for its developmental toxicity. No observed adverse effect levels (NOAELs) for maternal and developmental toxicity of MiADMSA were 47.5 mg/kg and 95 mg/kg/day respectively indicating that MiADMSA would not produce developmental toxicity in mice in the absence of maternal toxicity (116). Bosque et al., (117) reported that administration of MiADMSA through the parenteral route to pregnant mice during organogenesis produced maternal toxicity at a dose of 95 and 195 mg/kg with a significant decrease in the body weight and an increase in the liver weights. They also reported that MiADMSA caused embryo/fetotoxicity at a dose of 190 mg/kg by significantly increasing the embryo lethality and non-significant increase in the skeletal defects. Taubeneck et al. (98) showed that the developmental toxicity of DMSA is mediated mainly through disturbed copper metabolism and this may also be true for MiADMSA. Recently, our group was the first to report the toxicological data of MiADMSA when administered in male and female rats (118-120) through the oral as well as the intraperitoneal route (25, 50 and 100 mg/kg /3 weeks). We observed that there was no major alteration in the heme biosynthesis pathway except for a slight rise in the zinc protoporphyrin levels suggesting mild anemia at the highest dose. The oral route of administration was also seen to be better when compared to the ip route based on the histopathological studies of the liver and kidney tissues. MiADMSA was seen to be slightly more toxic in terms of copper loss and some biochemical variable in the hepatic tissue in females as compared to male rats. The studies concluded that the administration of MiADMSA in female rats is confounded with side effects and may require caution during its use (118-120). Since administration of a chelating agent during pregnancy is always with caution, we studied the effects of MiADMSA administration from day

14 of gestation to day 21 of lactation at different doses through oral and ip routes to examine the maternal and developmental toxicity in the pups (121). Results suggested that MiADMSA had no effect on length of gestation, litter-size, sex ratio, viability and lactation. No skeletal defects too were observed following the administration of the chelator. However, MiADMSA administration produced some marginal maternal oxidative stress at the higher doses (100mg/kg and 200 mg/kg) based on thiobarbituric acid reactive substances (TBARS) in RBCs and decrease in the δ-aminolevulinic acid dehydratase (ALAD) activity. MiADMSA administration too caused some changes in the essential metal concentration in the soft tissues especially the copper loss in lactating mothers and pups, which would be of some concern. Apart from copper, changes too were observed in the zinc concentrations in mothers and pups following administration of MiADMSA. The study further suggested that the chelator could be administered during pregnancy as it does not cause any major alteration in the mothers and the developing pups (118). Since chelating agents are administrable to individuals of all ages, we investigated the effect of MiADMSA administration in different age groups of male rats (young, adult and old rats) based on the fact that whether MiADMSA, a dithiol agent was a pro-oxidant or an antioxidant (118). Results suggested that MiADMSA administration increased in activity of ALAD in all the age groups and increased blood GSH levels in young rats. MiADMSA also potentiated the synthesis of MT in liver and kidneys and GSH levels in liver and brain. Apart from this it also significantly reduced the GSSG levels in tissues. MiADMSA was found to be safe in adult rats followed by young and old rats (118,119).

A large number of reports are available on the therapeutic efficacy of the MiADMSA (122, 123). Pande et al. (122) found that MiADMSA was effective in prevention and treatment of acute lead intoxication. Walker et al. (106) reported that MiADMSA administration reduced the brain lead concentrations by 75% when compared to 35% with DMSA whereas the ip administration reduced kidney lead levels by 93% while oral administration reduced the kidney lead by 94% (106). MiADMSA completely prevented the testicular damage after intraperitoneal administration of cadmium chloride at a dose of 0.03 mmol/kg (113). Jones et al. (105) reported that MiADMSA enhanced the cadmium elimination through urine by 3.6% compared to 0.02% of the controls and 24% in faeces compared to 0.11% in controls.

Therapeutic effects of MiADMSA against mercury burden have shown that MiADMSA is capable of decreasing mercury concentration by 59% and 80% after two doses when compared to DMSA (25% and 54% respectively). The total corporal mercury burden of 29.25μg was reduced to 21.06μg with DMSA after a single injection of 0.5 mmol/kg. The same dose of MiADMSA effected a reduction to 12.09 μg (114). Belles et al. (124) assessed the protective activity of MiADMSA against methyl mercury-induced maternal and embryo/fetal toxicity in mice. Oral methyl mercury administration increased the number of resorptions, decreased fetal weights and increased skeletal abnormalities. MiADMSA administration could not reverse the embryo lethality but fetotoxicty was significantly reduced by the administration of these agents at different doses.

Recently, Flora et al. (125) reported the effect of MiADMSA on the reversal of gallium arsenide (GaAs) induced changes in the hepatic tissue. Rats were exposed for 24 weeks with 10 mg/kg GaAs, orally, once daily and treated with 0.3 mmol/kg of MiADMSA or DMSA for two courses. They observed that MiADMSA was better than DMSA in mobilizing arsenic and in the turnover of the GaAs sensitive biochemical variables. Histopathological lesions, also responded more favorably to chelation therapy with MiADMSA. In another study, dose dependent therapeutic potential of MiADMSA was compared with monomethyl ester and DMSA in sub-chronically GaAs treated rats and it was found that MiADMSA was highly effective in the reversal of altered biochemical variables and in the mobilization of arsenic (126).

Dose and route dependent efficacy of MiADMSA against chronic arsenic poisoning has also suggested that the chelator is highly effective through oral route in reversing the arsenic induced changes in the variables indicative of oxidative stress in major organs as well as in mobilization of arsenic (127). Kreppel et al. (111) reported that MiADMSA was effective in increasing the survival of arsenic exposed mice when compared to its parent DMSA.

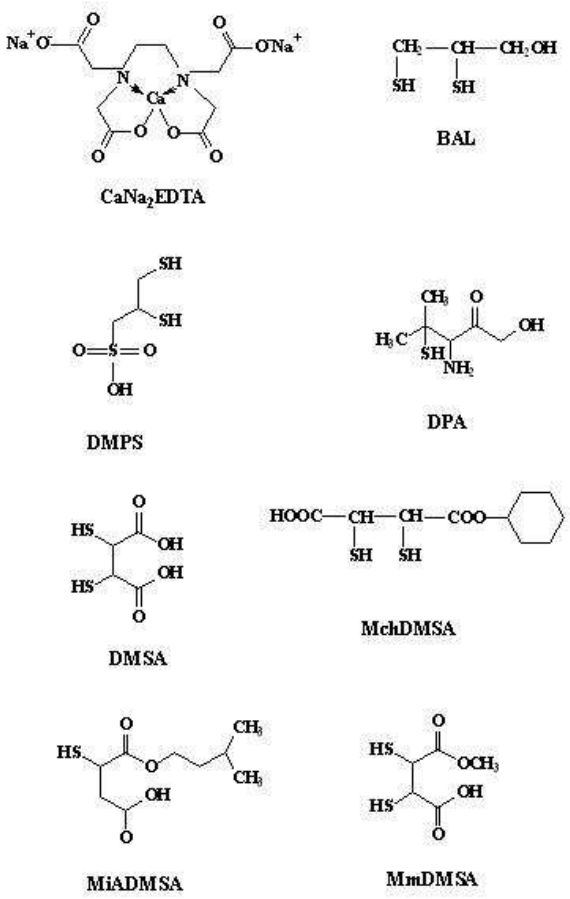

Figure 4. Figure showing the structures of common chelating agents

Despite a few drawbacks/side effects associated with MiADMSA, the above results suggest that MiADMSA may be a future drug of choice owing to its lipophilic character and the absence of any metal redistribution. However, significant copper loss requires further studies. Moderate toxicity after repeated administration of MiADMSA may be reversible after the withdrawal of the chelating agent.

Role of Micronutrients or Adjuvants

One of the best measures to minimize heavy metal exposure is by maintaining nutritional health. Absorption of lead for an instance is increased in subjects with deficiencies in iron, zinc, vitamins (like thiamine); thus maintaining good nutrition minimizes dietary absorption of lead. A new trend in chelation therapy has also emerged recently, which is to use combination therapy instead of monotherapy with chelating agents. Vitamins, essential metals or amino acid supplementation during chelation therapy has been found to be beneficial in increasing metal mobilization and providing recoveries in number of altered biochemical variables. Since the defense of biological system against damage caused by activated oxygen involves a battery of interrelated protective agencies, the micronutrients which have come to be regarded as antioxidant nutrients lie functionally at the heart of this protective mechanism and includes vitamins such as α-tocopherol, ascorbic acid etc. These antioxidants when given either alone or in combination with a chelating agent proved to be effective in mobilizing metal from soft as well as hard tissue. It is now well known that most of the heavy metals with special reference of lead and arsenic cause their toxicity by the involvement of reactive oxygen species (ROS). These metals bind to biological molecules and produce different free radicals that in turn attack the building blocks of the biological systems. 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, 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. The important role of heavy metals in oxidative damage suggested a new mechanism for an old problem, whether lead is involved in the oxidative deterioration of biological macromolecules. Although several mechanisms have been proposed to explain the lead-induced toxicity (128), none of the mechanisms have been yet defined explicitly. Recent studies suggest oxidative stress as one of the important mechanisms of toxic effects of lead (129,130). The oxidative stress has also been implicated to contribute to lead associated tissue injury in the liver, kidneys and brain (131,132). Indirect *in vivo* evidence of oxidative involvement in lead induced pathotoxicity was demonstrated by alleviation of oxidative stress in the erythrocytes after treatment with thiol containing proven antioxidants, N-acetyl cysteine and a succimer in lead exposed rats (130). Deficiency of several essential nutrients namely vitamins and essential elements, has been shown to exacerbate the toxic effects of metals, and supplementation of such nutrients ameliorates the toxicity. In addition to the role of micronutrients in modifying metal toxicity, these nutritional components can also act as complimentary chelating agents

(adjuvants) increasing the efficacy of a known chelator, or by acting independently.

Calcium

Interaction between lead and calcium occurs at several sites in the body, including cellular mechanisms that regulate ion transport across membrane (133). Calcium deficiency decreases lead clearance and increases lead absorption whereas, calcium excess only decreases lead clearance slightly and has little effect on lead absorption. Six and Goyer (134) reported that by lowering dietary calcium deficiency from 0.7 to 0.1% significantly enhanced the body lead burden of adult rat exposed to 200 ppm lead in drinking water for 10 weeks. A significant increase in tissue lead, urinary delta-aminolevulinic acid (ALA) and renal intranuclear lead inclusion bodies was also observed in lead exposed rats consuming low calcium. Kostial et al. (135) recommended adequate calcium (940 mg/day) especially for pregnant and lactating women (to prevent bone resorption) and for children (to enhance bone mass formation). Further work in this area will be useful particularly in view of few recent reports where, it has been reported that coprophagy may be a serious complication in the rat model system as both calcium and lead may be recycled.

The mechanism by which calcium interferes with lead absorption is not clear however; few interesting studies using ligated isolated loop technique suggest that calcium intake rather than calcium status of the animals modulate lead absorption. These studies also demonstrated that at-least in part calcium appears to inhibit lead absorption via competition for common binding sites on intestinal binding proteins.

Iron

Iron functions mainly in the regulation of oxidative processes. It is a component of heme compounds that transport oxygen, cytochrome that function in the electron transport chain and metalloprotein (136,137). Subjects consuming low iron diet had tissue lead concentration significantly higher than subjects consuming adequate iron. Further, excess iron uptake decreased blood; femur and kidney lead concentration while the low iron increased the tissue lead concentration (138-141). Very limited information of whether or not neurobehavioral changes and cognitive impairment are more extreme in iron deficient, lead toxic children than in either condition, are available. The role of iron and lead in haem synthesis is well-understood. The cellular basis for greater susceptibility of non-iron deficient animals to lead is that limited iron in the mitochondria apparently enhances the impairment by lead of iron utilisation for heme synthesis. Additional studies have demonstrated the capacity of MT to attenuate the lead-induced inhibition of blood aminolevulinic acid dehydratase (142). Existence of MT-like protein in erythrocyte that binds lead and possibly protects against lead toxicity by rendering lead unavailability for retention in the target organs.

Zinc

When dietary zinc is increased over requirement level, it reduces trace metal absorption. Lead and zinc are competitive at tissue sites, which would account for at-least part of the protective effect of zinc on lead toxicity. Victery et al. (143) examined the excretion of lead, zinc and calcium in rats exposed to different levels of lead. Adult male rats were fed Teklad AIN-76 diet containing 5.2 g Ca and 0.0314 g Zn /Kg and received 0, 200, 500 or 1000 ppm lead as acetate. Brain zinc concentration decreased significantly in animals while, plasma, erythrocyte and kidney zinc levels remained unchanged by lead exposure. We reported the influence of orally supplemented zinc in preventing lead intoxication in experimental animals (144). Thus, the protective effect of zinc against lead toxicity could be attributed to a decrease in metal absorption in the gastrointestinal tract. Zinc could also be competing for and effectively reducing the availability of binding sites for trace metal uptake. Enhanced zinc also increases the renal and hepatic contents of metallothionein and causes detoxification through metal binding in this form.

Arsenic is capable of inducing an increase in MT levels suggesting the possible role of this cysteine rich low molecular weight protein. Kreppel et al. (145) however, reported that zinc induced increase in MT do not seems to be responsible for the protective role of pre-administered zinc against arsenic induced lethality. Zinc pre-treatment however afforded an increase in arsenic elimination. Studies on the effect of zinc on mercury exposure have focussed mainly on inorganic mercury rather than organic mercury. It is believed that zinc may reduce lipid peroxidation by increasing the activities of enzymes like glutathione peroxidase (GPx) to

ameliorate the sign of mercury induced neurotoxicity.

Selenium

Selenium is a required dietary element for health but it is also a toxic material. It is an integral component of ubiquitous enzyme glutathione peroxidase, an antioxidase enzyme. This enzyme together with superoxide dismutase, catalase and vitamin E neutralises reactive oxygen species (ROS). Selenium is known to affect the distribution of many toxic metals. Role of selenium in lead intoxication has rather been controversial. Cerklewski and Forbes (146) investigated the effect of low and high dietary selenium on toxicity of dietary lead male rats and suggested that low dietary levels mildly protects against toxic effects of lead while, at high levels it exaggerates the lead toxicity. Rastogi et al. (147) observed that selenium and lead protect against toxicity of each other. Enzymatic activity of ALAD and Cytochrome P-450 in liver was normal in rats exposed concomitantly to selenium and lead. We also suggested that oral administration of selenium could partly prevent lead toxicity during the course of simultaneous administration (148). Othman and El-Missiry (149) reported that intramuscular injection of selenium prior to lead exposure provided prophylactic action against lead effects and observed that selenium enhances the autooxidant capacity of the cells by increasing the activity of the superoxide dismutase, glutathione reductase and glutathione content.

Interaction of arsenic and selenium promotes the biliary excretion of exogenous selenium and selenite also augments the excretion of arsenic into bile. Few authors suggested that arsenic augmented the hepatobiliary transport of selenium and facilitated accumulation of selenium in red blood cells. Selenium in turn facilitated the biliary excretion of arsenic (150-152). Glattre et al. (153) studied the distribution and interaction of arsenic and selenium in rat thyroid. Combined arsenic plus selenium administered group exhibited same selenium concentration as the sum of the mean selenium concentration in the groups pre-treated with arsenic or selenium alone suggesting both arsenic and selenium accumulate in thyroid tissue. Post-mortem examination of thyroid following arsenic exposure indicated toxic changes whereas, only minor changes were observed in selenium or arsenic plus selenium treated group (153).

Copper

Copper is a component of the mitochondrial electron transport chain, functions in iron absorption and mobilization and maintenance of neurotransmitter levels in brain (154,155). Adequate intake of copper has been reported to prevent lead-induced anemia, which was developed when dietary copper was low (156). However, in an conflicting report animals fed low, adequate and high copper to the lead exposed rats, exhibited a pronounced decrease in lead contents in animals fed low calcium diet while, high copper diet produced an increase tissue lead accumulation. There are few reports concerning cadmium-copper interaction. Dietary copper supplementation reduces mortality rate and severity of anemia, in experimental animals. Cadmium exposure has been reported to produce disturbances in the copper metabolism particularly depletion of plasma copper and copper sensitive ceruloplasmin. Beneficial effect of copper has been attributed to the competition between cadmium and copper for binding to the metallothionein (MT). Copper may displace cadmium for MT because of its higher affinity for the protein (157,158).

Role of Antioxidants

Induction of reactive oxygen species by metal and subsequent depletion of antioxidant cell defenses can result in disruption of the pro-oxidant / antioxidant balance in mammalian tissues. In the event that oxidative stress can be partially implicated in metal toxicity, a therapeutic strategy to increase the antioxidant capacity of cells may fortify the long term effective treatment of metal poisoning. This may be accomplished by either reducing the possibility of metal interacting with critical biomolecules and inducing oxidative damage, or by bolstering the cells antioxidant defenses through endogenous supplementation of antioxidant molecules. Although many investigators have confirmed lead induced oxidative stress, the usefulness of antioxidants along or in conjunction with chelation therapy has not been extensively investigated yet. Recently we (122) explored the therapeutic efficacy of antioxidant along with a chelating agent during the removal of lead in rats. Some groups (50,159-163) investigated the ability of some molecules with antioxidant activity to prevent or treat experimental lead toxicity in animals.

The following part apprises with some antioxidants that have been tried in treatment of metal poisoning with special reference to lead and arsenic (Figure 5).

N-Acetyl cysteine (NAC)

NAC is a thiol-containing antioxidant that has been used to mitigate various conditions of oxidative stress. Its antioxidant action is believed to originate from its ability to stimulate GSH synthesis, therefore maintaining intracellular GSH levels and scavenging reactive oxygen species (ROS) (164,165). Besides the antioxidant potential, NAC also has some chelating properties against lead (166). One of the first reports by Pande et al., (122) suggested that NAC could be used both as preventive as well as a therapeutic agent along with MiADMSA/DMSA in the prevention and treatment of lead intoxication in rats. Pande et al (122) reported that simultaneous administration of NAC with succimer reversed the altered ALAD and TBARS levels, increased the reduced glutathione levels and decreased the lead levels, apart from this the study too highlighted the favorable response of NAC in post-exposure treatment along with succimer (122). Combined administration of NAC and succimer post arsenic exposure led to a significant turnover in variables indicative of oxidative stress and removal of arsenic from soft organs (103). A recent report suggested that co-administration of NAC along with succimer in sub-chronically lead exposed rats, reduced oxidative stress significantly by lowering the TBARS levels, oxidized glutathione levels along with the decrease in the lead burden on the soft tissues especially the brain (167).

Melatonin

Melatonin, N-acetyl-5-methoxy triptamine, is a hormonal product of the pineal gland that plays many roles within the body including control of reproductive functions, modulation of immune system activity, limitation of tumorigenesis and effective inhibition of oxidative stress (168). One major function of melatonin is to scavenge radicals formed in oxygen metabolism (168,169), thereby potentially protecting against free radical induced damage to DNA, proteins and membranes (168,170). It has been shown that melatonin stimulates the antioxidative enzyme GPx in the brain, thus providing indirect protection against free radical attack (171). In animal experiments, metatonin prevented the induction of free radical damage by a variety of conditions including ingestion of toxins, ionizing radiation, ischaemia, reperfusion and excessive exercise (172-176). Melatonin has a molecular weight of 232 and is both lipid (177,178) and water soluble (179), although its solubility in lipid is clearly greater.

α-Lipoic acid (LA)

α-lipoic acid is a naturally occurring antioxidant and is able to abate some of the toxic effects of lead (180). It functions as a cofactor in several multienzyme complexes (181). Its reduced form, dihydrolipoic acid (DHLA), has two free sulfhydryl groups and the two forms LA/DHLA possess a great antioxidant potential (182). 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. Lipoic acid also have an advantage over NAC in opposing GSH loss, since LA is effective in a micromolar range while millimolar NAC is needed for a similar effect (183). The capability of LA to cross the blood brain barrier (184) is an extra advantage because the brain is an important target in lead poisoning.

Vitamin E (α-tocopherol)

Various vitamins have been found to reduce the toxic manifestation of lead (185,186). Dietary oral supplementation with these vitamins often lessens the severity of lead poisoning by inhibiting the lead absorption or interaction at the macromolecular site of physiological action (186-188).

The antioxidant function of vitamin E has also been proposed in cadmium induced brain damage (189). It also appears that the protective effect of vitamin E in lead toxicity is attributed mainly to its antioxidant property. Anemia, splenomegaly and increased fragility of red blood cells in lead toxicity of vitamin E deficient rats have been reported (190-193). Vitamin E which is a low molecular mass antioxidant interact directly with the oxidizing radicals (194,195) and protect the cells from reactive oxygen species (196). The lipid soluble, non-enzymatic antioxidant, α-tocopherol checks the lipid peroxidation through limiting the propagation of chain reaction of lipid peroxidation (197). Lead poisoning has been shown to cause a marked anaemia in vitamin E deficient rats indicating a possible involvement of this vitamin in the synthesis of heme protein. It is believed that

vitamin E, as a scavenger of free radicals, might be reacting with methyl radicals that might be formed in the breakdown to provide protection. Addition of vitamin E may also alleviate arsenic toxicity. The protective mechanism of vitamin E could be attributed to its antioxidant property or its location in the cell membrane and its ability to stabilize membrane by interacting with unsaturated fatty acid chain. Flora et al (198) reported that administration of Vitamin C or vitamin E when given in combination with succimer or its monoisoamyl derivative (MiADMSA) produced profound recoveries in sub-chronically lead exposed rats. Although the group suggest that vitamin C was better in providing clinical recoveries and Vitamin E was equally efficient in decreasing the lead burden from the tissues.

Vitamin C (Ascorbic acid)

Vitamin C is a low molecular mass antioxidant that interacts directly with the oxidizing radicals (195) and protect the cells from reactive oxygen species (196). It is well known that ascorbic acid enhances the absorption of dietary iron by increasing the solubility of iron at the alkaline pH of the intestine and by maintaining the ferrous iron in its reduced oxidation state (199). Vitamin C scavenges the aqueous reactive oxygen species (ROS) by very rapid electron transfer that thus inhibits lipid peroxidation (199). It acts mainly as an antioxidant molecule and its beneficial effects could be attributed to its ability to complex with lead (188). Vitamin C. Animal studies have suggested an antagonistic effect of ascorbic acid on lead absorption and toxicity and ascorbic acid may even chelate lead as effectively as EDTA. However, studies in humans have shown some mixed results. In a study with 78 male workers, 38 received vitamin C and 38 were given placebo (200). They found no effect of ascorbic acid on absorption or excretion of lead. However, 47 psychiatric patients receiving ascorbic acid and zinc showed reduced blood lead concentration (200). Simon and Hudes investigated the association between ascorbic acid concentration and the prevalence of elevated blood lead concentration in 19, 578 participants ages 6 years and older in National Health and Nutrition Examination Survey 1988-94 (NHANES III) (201). Serum ascorbic acid concentration was inversely associated with prevalence of elevated blood lead concentration in children. However, no relationship between dietary ascorbic acid intake and blood lead concentration has been reported. Vitamin C has been shown to be effective against methyl mercury intoxication however; no definite conclusion regarding its beneficial effects could be drawn.

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 (202,203). The highest concentrations of taurine occur in developing brain, at which time the concentrations of other free amino acids tend to be low (203).

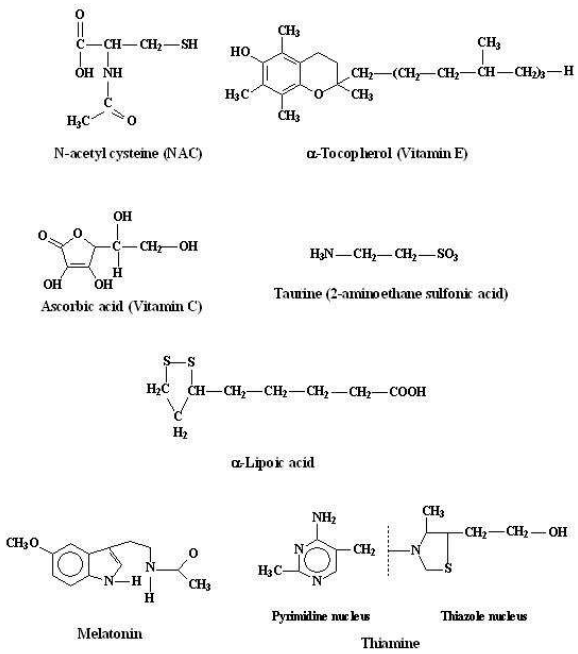

Figure 5. Chemical structures of antioxidants known for their protective efficacy against arsenic and lead

Some of the recent data indicate that taurine can act as the direct antioxidant by scavenging ROS and/or as an indirect antioxidant by preventing changes in membrane permeability due to oxidant injury (202). The zwitterionic nature of taurine gives it high water solubility and low lipophilicity. Consequently compared with carboxylic amino acids, diffusion through lipophillic membranes is slow for taurine (203). In the studies conducted by Gurer and Ercal (165), taurine was shown to have beneficial effects in lead induced oxidative stress in Chinese Hamster Ovary (CHO) cells and F344 rats (165). There was an increased cell survival 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 F-344 rats. In the above study, 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 mechanisms rather than a chelating activity, seems to underlie this observed effects of taurine against lead-induced oxidative stress.

Although a lot of work has been done for the treatment of lead and arsenic poisoning, still we are far away from having a safe, specific and effective chelating agent for the treatment against these deadly toxic metals. Besides, further knowledge is needed in several basic research areas within the field of *in vivo* chelation of metals and call for studies on (a) Molecular mechanism of action of clinically important chelators, (b) Intracellular and extra cellular chelation in relation to mobilization of aged metal deposits and the possible redistribution of toxic metal to sensitive organs as the brain, (c) Effect of metal chelators on biokinetics during continued exposure to metal, especially possible enhancement or reduction of intestinal metal uptake, (d) Combined chelation with lipophilic and hydrophilic chelators, which presently has a minimal clinical role, (e) Use of antioxidants, micronutrients or vitamins as complimentary agents or antagonists (f) Minimization of the mobilization of essential trace elements during long-term chelation, and (f) Fetotoxic and teratogenic effects of chelators.

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