Strategies for Safe and Effective Therapeutic Measures for Chronic Arsenic and Lead Poisoning

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Abstract: Strategies for Safe and Effective Therapeutic Measures for Arsenic and Lead Poisoning: Kiran Kalia, et al. Department of Biosciences, Sardar Patel University, India—Exposure to toxic metals remains a widespread occupational and environmental problem in world. There have been a number of reports in the recent past suggesting an incidence of childhood lead poisoning and chronic arsenic poisoning due to contaminated drinking water in many areas of West Bengal in India and Bangladesh has become a national calamity. Low level metal exposure in humans is caused by air, food and water intake. Lead and arsenic generally interferes with a number of body functions such as the central nervous system (CNS), the haematopoietic system, liver and kidneys. Over the past few decades there has been growing awareness and concern that the toxic biochemical and functional effects are occurring at a lower level of metal exposure than those that produce overt clinical and pathological signs and symptoms. Despite many years of research, we are still far from an effective treatment of chronic plumbism and arsenicosis. Medical treatment of acute and chronic lead and arsenic toxicity is furnished by chelating agents. Chelating agents are organic compounds capable of linking together metal ions to form complex ring-like structures called chelates. They have been used clinically as antidotes for acute and chronic poisoning. 2, 3-dimercaprol (BAL) has long been the mainstay of chelation therapy for lead or arsenic poisoning. Meso 2, 3-dimercaptosuccinic acid (DMSA) has been tried successfully in animals as well as in a few cases of human lead and arsenic poisoning. DMSA could be a safe and effective method for treating lead or arsenic poisoning, but one of the major disadvantages of chelation with DMSA has been its inability to remove lead from the intracellular sites because of its lipophobic nature. Further, it does not provide protection in terms of clinical/biochemical recovery. A new trend in chelation therapy is to use combined treatment. This includes the use of structurally different chelators or a combination of an adjuvant and a chelator to provide better clinical/biochemical recovery in addition to lead mobilization. The present review article attempts to provide update information about the current strategies being adopted for a safe, effective and specific treatment for two major toxic metals or metalloid.

Key words: Chronic lead and arsenic poisoning, Oxidative stress, Chelation therapy, Drawbacks of chelation, New Chelating agents, DMSA monoesters, Combination Therapy, Biochemical changes, Herbal medicine

Received July 16, 2004; Accepted Oct 12, 2004

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British Anti-Lewisite (BAL) was one of the first chelating agents to be developed as an antidote for war gas, dichlorovinyl arsine (Lewisite) during the Second World War. The breakthrough in the development of chelation therapy came after the introduction of ethylene diamine tetraacetic acid (EDTA), initially to combat lead intoxication. The value of EDTA as a clinical chelating agent was reduced by the need for slow intravenous administration, low intestinal uptake, exclusive extra-cellular action and high stability constants with essential metals.

### Chelating agents and Chelation

Chelating agents are organic compounds capable of linking together metal ions to form complex ring-like structures called ‘chelates’. Chelate is a Greek word meaning the claws of a lobster. Chelators form a complex with the toxic metal ion and these complexes are easily eliminated from the body through the excretory system and also show low toxicity. 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 so that 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 such characteristic as greater affinity for the toxic metal that has to be chelated, low toxicity, rapid elimination of the metal, high water solubility, ability to penetrate cell membrane, oral administration, ability to chelate with natural chelating groups found in the biological system, and minimal metabolism, etc.

The metal chelate complexes have a reduced tendency to undergo exchange reactions once they are formed, but 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) by utilizing one of the anion/cation transport systems present in the membrane. There is a hypothesis that a large ion complex with a positive charge will pass out of a cell very slowly because of its inability to pass through either the lipid portion of the cellular membrane or the cation transport system designed to move ions with a +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 positions of a metal ion. Metal chelating agents generally contain more than one functional group, in order to provide a chemical claw to chelate the toxic metal.

### Conventional chelating agents and their drawbacks

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 (CaNa₂EDTA) is a derivative of ethylene diamine tetra acetic acid (EDTA); a synthetic polyamino-polycarboxylic acid which was used for the treatment of metal poisoning and had been the mainstay of chelation therapy for many years. Another member of this family is diethylene triamine pentaacetic acid (DTPA), a synthetic polyaminocarboxylic acid with properties similar to EDTA. CaNa₂EDTA has the LD₅₀ value of 16.4 mmol/kg in mouse. 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. Hypocalcaemia is reported with the administration of CaNa₂EDTA. CaNa₂EDTA 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 such as hematuria, proteinuria and high 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. Zinc administration during EDTA administration is generally recommended to reduce toxicity.

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 those for humans. 2–3% disodium EDTA when given in feed along with 100 ppm of zinc to pregnant rats from gestation day 6 through 21 resulted gross congenital malformations which included clubbed legs, micro or anophthalimia, micro or agnathia, cleft palate, fused or missing digits, brain malformation and curly, short or missing tail in the young. The use of EDTA to remove endogenous zinc appeared to offer a mechanism for studying the effects of short-term zinc supplementation at critical periods in pregnant zinc deficient rats. Kimmel showed that effects on teratogenicity varied with the route of administration of EDTA. Subcutaneous administration of the disodium salt at a dose of 375 mg/kg was only maternally toxic, but did not cause any malformation, whereas gavage administration resulted in more signs of toxicity. Absorption into the circulation, potential interaction with essential trace elements, and the stress associated with the administration of the compound were suggested to be possible factors involved in the differences in EDTA-
induced maternal and developmental toxicity\textsuperscript{20}. Brownie \textit{et al.} also reported teratogenic effects\textsuperscript{37}. Another reported disadvantage of CaNa\textsubscript{2}EDTA is that it redistributes lead to the brain. Cory Slechta \textit{et al.} \textsuperscript{21} and Flora \textit{et al.} \textsuperscript{22} in separate studies provided evidence that rats given lead as lead acetate in their drinking water and then treated with CaNa\textsubscript{2}EDTA mobilized lead from their tissues and redistributed it 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 the commercialization of chelators containing thiol or sulfhydryl groups.

D-Penicillamine (DPA) is 3, 3 dimethylcysteine, a sulphydryl containing amino acid, is as an antidote to low or mild lead poisoning\textsuperscript{23, 24}. It can penetrate cell membranes and then get metabolized. It can be absorbed through the gastrointestinal tract and therefore can be administered orally, but the major toxic effect of DPA is antagonizing pyridoxine and inhibiting pyridoxine dependent enzymes such as transaminases. Other toxic effects include hypersensitive allergic reactions such as fever, skin rashes, leucopoenia and thrombocytopenia\textsuperscript{26}. In few reports nephrotic effects have also been observed along with penicillin allergic reaction in sensitive individuals due to cross reactivity. Prolonged treatment may also lead to anorexia, nausea and vomiting in humans. Apart from this, DPA is also a well recognized teragen and lathyrogen that causes skeletal, palatal, cutaneous and pulmonary abnormalities\textsuperscript{27–29}. The developmental toxicity of DPA is abundant in both humans and laboratory animals. The first report on human embroyopathy associated with DPA was published by Mjolnerod \textit{et al.}\textsuperscript{30} Since DPA chelated copper, it was hypothesized that the drug might be teratogenic\textsuperscript{31}. Various investigations were performed in the early eighties to test the hypothesis\textsuperscript{31–34} and a high incidence of malformations were reported. The frequency of reabsorption and the frequency and severity of malformations increased in the rats in a dose dependent manner\textsuperscript{35}. Nevertheless, the literature also suggests that the administration of DPA during pregnancy protects the mother from the relapse of Wilson’s disease, whereas it would carry few risks to the fetus\textsuperscript{35}. DPA has been tried safely throughout pregnancy in women with Wilson’s disease, suggesting that the excessive copper stores improve tolerance\textsuperscript{36}. The American Academy of Pediatrics recommends Penicillamine use only when unacceptable adverse reactions to both DMSA and EDTA have occurred\textsuperscript{37}, but Kreppel \textit{et al.} reported that Penicillamine was ineffective in reducing arsenic burden in rats\textsuperscript{38}.

Meso 2, 3- dimercaptosuccinic acid (DMSA), is a water-soluble, sulphydryl containing a compound which is an effective oral chelator for lead and arsenic. DMSA contains two sulphydryl (-SH) groups and an analogue of dimercaprol. DMSA has been shown to be an effective chelator of lead, reducing blood lead levels significantly. Clinical symptoms and biochemical indices of lead toxicity also improved\textsuperscript{39, 40}. Animal studies suggest that DMSA is an effective chelator of soft tissue but it is unable to chelate lead from bones\textsuperscript{42, 43}. In an interesting study by Ercal \textit{et al.}, lead induced biochemical variables suggestive of oxidative stress responded moderately to treatment with DMSA, and there was a marked reduction in the lead concentration in blood, liver and brain\textsuperscript{42}. Miller suggested that the protocol for lead toxicity is to identify and remove the environmental exposure and use DMSA \textit{10 mg/kg} three times a day for the first five days followed by 14 days at \textit{10 mg/kg} twice a day\textsuperscript{43}. DMSA has also been tried successfully in animals as well as in a few cases of human arsenic poisoning. DMSA has been shown to protect mice from the lethal effects of arsenic. We also reported a significant depletion of arsenic and a significant recovery in the altered biochemical variables of sub-chronically arsenic exposed rats\textsuperscript{44}. A number of other studies which appeared in the recent past have recommended that DMSA could be safe and effective for treating arsenic poisoning. But in an interesting perspective, a double blind randomized controlled clinical trial study conducted on a 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 in skin lesions\textsuperscript{45}. Fournier \textit{et al.} reported cases of heavy metal poisoning in humans who were treated with DMSA\textsuperscript{46}. In 9 subjects with lead poisoning DMSA decreased blood lead concentration by 35 to 81% and induced a 4.5 to 16.9 fold increase in mean daily urinary excretion of the metal. An experimental study by us recently provided \textit{in vivo} evidence of arsenic induced oxidative stress in a 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\textsuperscript{47}. In an interesting study reported by Tallis, three cases of acute lead arsenate poisoning in South Australia during a 12 month period were described\textsuperscript{48}. The case reports provided a number of features of a characteristic clinical syndrome which may follow ingestion of lead arsenate. The patients were treated with EDTA and/ or BAL. The study suggests that lead poisoning could be effectively treated by EDTA but it is controversial as to whether chelation with dimercaprol prevents arsenical neuropathy. The above studies therefore in general recommend that DMSA has serious limitations in chelating arsenic from intracellular sites and can only be useful for acute cases of arsenic poisoning.

Sodium 2, 3-dimercaptopropane sulfonate (DMPS) is another analogue of BAL and is mainly distributed in the
extracellular space and it may enter cells by means of a specific transport mechanism. DMPS is rapidly eliminated from the body through the kidneys. No major adverse effects after DMPS administration to humans or animals have been reported, but a dose dependent increase in copper content was found in the serum, liver, kidneys and spleen. Oral administration of DMPS also did not adversely affect late gestation, parturition or lactation in mature mice, and fetal and neonatal development do not appear to be adversely affected. DMPS, although known for its antidotal efficacy against mercury, has also been reported to be an effective drug for treating lead and arsenic poisoning. This drug can be administered both orally and intravenously. An oral dose of 100 mg/kg thrice daily for 10–12 d is effective against mild arsenic poisoning but no recommendation for treating chronic arsenic poisoning is available. A quantitative evaluation of three drugs reveals that DMPS is 28 times more effective than BAL in arsenic therapy in mice, and DMSA and DMPS are equally effective. Guha Majumder et al. evaluated the efficacy of DMPS in a single blind placebo control trial of patients suffering from chronic arsenic poisoning in West Bengal, India. DMPS was given in a 100 mg capsule dose 4 times a day for 7 d for four courses with a one-week drug free period between courses. There was a significant decrease in clinical scores from pre-treatment to post treatment values amongst both DMPS and placebo groups. There was however, no change in skin histology, haematology and liver function test parameters in the patients before and after the therapy with DMPS or a placebo. And no side effects were noticed in patients treated with DMPS. Detailed toxic effects of the above-mentioned chelating agents have already been discussed by us in an earlier article.

It is therefore clear from the 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.

1. Synthesis of New Chelators

In the early eighties it was shown that some newer complexing agents such as DMPS and DMSA were effective against arsenic and lead poisoning. When compared to BAL these newer chelating agents were of significantly lower toxicity and they could be administered orally or intravenously. 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.

Esters of Succimer (DMSA)

A large number of esters of DMSA have been synthesized for achieving optimal effects of chelation 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. In order to make the compounds more lipophilic, 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 of mice. Rivera et al. reported 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. The diester entered the cells but it caused severe zinc depletion. Singh et al. examined the effects of three diesters of DMSA and found that these diesters were more effective than BAL in reducing soft organ lead concentrations. Kreppel et al. reported the therapeutic efficacy of six analogues of DMSA in mice. They gave mice a single LD50 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 89% when administered intraperitoneally (i.p). Administration of dimethyl DMSA (DMDMSA) through i.g and i.p and diethyl DMSA (DEDSMA), di-n-propyl DMSA (DiPDMSA) and diisopropyl DMSA (DiPDMPS) through the i.g route did not reduce the lethality, whereas the i.p administration of DnPDMSA increased the survival rate by 72% whereas DEDMSA and DiPDMSA increased it by 86%. Kreppel et al. 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-amy), MnBDMSA (mono n-butyl) and MiBDMSA (mono i-buty) noticeable decreased the arsenic content in most of the organs as soon as 1.5 h after administration. They found that MiADMSA and MnADMSA were the most effective in increasing the survival of mice. We also performed similar studies where the effects of DMDMSA, DEDMSA DiPDMPSA and diisoamyl DMSA (DiADMSA) on sub chronically arsenic treated rats were investigated. The results suggested that the diesters
reduced the arsenic burden in blood and soft tissue but were only moderately effective in reversing the biochemical recovery when compared to DMSA\textsuperscript{40}.

Walker et al. 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 whereas in the monoesters the reduction varied from 54\% to 75\%\textsuperscript{61}). Jones et al. reported the efficacy of ten different monoesters through the oral and i.p. routes on cadmium mobilization in mouse\textsuperscript{60}). Out of the ten monoesters studied they found MiADMSA to be the most effective in reducing the cadmium concentrations in the liver and kidneys.

In most of these published reports, it has been observed that the analogues of DMSA were capable of crossing the membranes and were more effective than other analogues in reducing the metal burden in acute and sub-chronic metal intoxication. These studies have also suggested that the monoesters are more effective in the treatment of experimentally induced metal intoxication.

**Monoisoamyl DMSA (MiADMSA)**

Among these new chelators, monoisoamyl ester of DMSA (MiADMSA; a C\textsubscript{5} branched chain alkyl monoester of DMSA) has been found to be the more effective than DMSA in reducing the cadmium and mercury burden\textsuperscript{67, 68}). It is reported that the toxicity of DMSA with an LD\textsubscript{50} of 16 mmol/kg is much lower than the toxicity of MiADMSA with an LD\textsubscript{50} of 3 mmol/kg but less than BAL (1.1 mmole/kg). The interaction of MiADMSA and DMSA with essential metals is the same. Mehta and Flora reported for the first time the comparison of different chelating agents (3 amino and 4 thiol chelators) and their role in metal redistribution, hepatotoxicity and oxidative stress in chelating agents induced metallothionein in rats\textsuperscript{69}). We suggested that out of all the 7 chelators, MiADMSA and DMSA produced less oxidative stress and toxicity than 5 other chelators\textsuperscript{54}), but no major reports are available about the toxicity of this metal complexing agent except for its developmental toxicity.

The no observed adverse effect levels (NOAELs) for maternal and developmental toxicity of MiADMSA were 47.5 mg/kg and 95 mg/kg/d, respectively, indicating that MiADMSA would not produce developmental toxicity in mice in the absence of maternal toxicity\textsuperscript{70}). Bosque et al. 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 body weight and an increase in liver weight\textsuperscript{71}). They also reported that MiADMSA caused embryofetotoxicity at a dose of 190 mg/kg by significantly increasing embryo lethality and a non-significant increase in skeletal defects. Taubeneck et al. showed that the developmental toxicity of DMSA is mediated mainly through disturbed copper metabolism and this may also be true for MiADMSA\textsuperscript{72}).

Recently, our group was the first to report the toxicological data on MiADMSA when administered in male and female rats\textsuperscript{73, 74}) through the oral as well as the intraperitoneal route (25, 50 and 100 mg/kg/3 wk). 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 than the ip route based on histopathological studies of liver and kidney tissues. MiADMSA was seen to be slightly more toxic in terms of copper loss and some biochemical variables in the hepatic tissue of females as compared to male rats. The studies concluded that the administration of MiADMSA to female rats is confounded with side effects and may require caution during its use\textsuperscript{73, 74}). Since administration of chelating agents during pregnancy always requires 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\textsuperscript{75}). Results suggested that MiADMSA had no effect on the length of gestation, litter-size, sex ratio, viability and lactation. And no skeletal defects were observed after the administration of the chelator. But, MiADMSA administration produced some marginal maternal oxidative stress at higher doses (100 mg/kg and 200 mg/ kg) based on thiobarbituric acid reactive substances (TBARS) in RBCs and a decrease in the δ-aminolevulinic acid dehydratase (ALAD) activity. MiADMSA administration also 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 were also observed in the zinc concentrations in mothers and pups after the 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\textsuperscript{76}). 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 di-thiol agent, was a prooxidant or an antioxidant\textsuperscript{76}). Results suggested that MiADMSA administration increased the 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 safest in adult rats, followed by young and old rats\textsuperscript{77, 138, 159}).

A large number of reports are now available on the...
therapeutic efficacy of MiADMSA. Pande et al. found that MiADMSA was effective in the prevention and treatment of acute lead intoxication. Walker et al. reported that MiADMSA administration reduced brain lead concentrations by 75% when compared to 35% with DMSA, whereas ip administration reduced kidney lead levels by 93% whereas oral administration reduced kidney lead by 94%. MiADMSA completely prevented testicular damage after intraperitoneal administration of cadmium chloride at a dose of 0.03 mmol/kg.

Recently, Flora et al. reported the effect of MiADMSA on the reversal of gallium arsenide (GaAs) induced changes in hepatic tissue. Rats were exposed for 24 wk to 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.

We also reported the dose dependent effects of monoisoamyl and monomethyl esters of meso 2, 3-dimercaptosuccinic acid (DMSA) (0.1, 0.3 and 0.5 mmol/kg, i.p. once daily for 5 days) to offset the characteristic biochemical, immunological, oxidative stress consequences and DNA damage (based on DNA fragmentation and comet assay) after sub-chronic administration of gallium arsenide, and the mobilization of gallium and arsenic were examined. The study concluded that administration of DMSA monoesters (particularly MiADMSA) is of benefit in the removal of arsenic from gallium arsenide exposed rats, and recovery in some of the GaAs sensitive biochemical, immunotoxic variables and DNA damage, after 5 days of chelation therapy. We also recommended the use of MiADMSA for treating cases of GaAs intoxication for these reasons: (i) there was better recovery in the altered heme biosynthesis pathway (ii) better recovery in the tissue damage/oxidative stress, immunological variables and to some extend in DNA repair. Although, a significant recovery in the altered parameters indicative of oxidative stress, due to GaAs exposure could not be achieved, it may perhaps require two or three courses of 5 day treatment to achieve the optimum effects.

Dose and route dependent efficacy of MiADMSA against chronic arsenic poisoning has also suggested that the chelator is highly effective through the 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. Kreppel et al. reported that MiADMSA was more effective in increasing the survival of arsenic exposed mice than its parent DMSA.

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, but significant copper loss requires further study. Moderate toxicity after repeated administration of MiADMSA may be reversible after the withdrawal of the chelating agent.

2. Natural Antagonists: Scavengers of Toxicity I: Role of Micronutrients

Defense of the 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 include vitamins such as α-tocopherol and ascorbic acid, etc. Antioxidants such as vitamins, 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 to lead and arsenic cause their toxicity due to 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. 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. Several mechanisms have been proposed to explain the lead-induced toxicity. Recent studies suggest oxidative stress as one of the important mechanisms of the toxic effects of lead. Oxidative stress has also been seen to contribute to lead associated tissue injury in the liver, kidneys and brain. 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. 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 membranes. A significant increase in tissue lead, urinary delta-aminolevulinic acid (ALA) and renal intranuclear lead inclusion bodies was observed in lead exposed rats consuming low calcium. Silbergeld et al. observed that calcium intake was negatively correlated with the blood lead level. Not many epidemiological data on the relationship between dietary calcium intake and the blood lead level in normal population groups are available. Kostial et al. recommended adequate calcium (940 mg/d) 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 a few recent reports in which it has been reported that coprophagy may be a serious complication in the rat model system as both calcium and lead may be recycled.

With a ligated isolated loop technique it was demonstrated that calcium intake rather than the calcium status of the animals modifies lead absorption. It is also clear that, at least in part, calcium appears to inhibit lead absorption in competition for common binding sites on intestinal binding proteins.

Iron

Iron is a component of heme compounds which transport oxygen, cytochrome that functions in the electron transport chain and metalloprotein. Iron functions mainly in the regulation of oxidative processes. Subjects consuming a low iron diet had a tissue lead concentration significantly higher than subjects consuming adequate iron. Furthermore, excess iron uptake decreased blood, femur and kidney lead concentrations whereas low iron increased the tissue lead concentration. Rapid improvement in the development scores of infants after iron therapy has been reported. 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 utilization for heme synthesis beside the capacity of MT to attenuate the lead-induced inhibition of blood aminolevulinic acid dehydratase has also been reported. The existence of an MT-like protein in erythrocytes that binds lead and possibly protects against lead toxicity by rendering lead unavailable for retention in the target organs.

Zinc

Lead and zinc compete for similar binding sites in vivo, which might partially be responsible for at least part of the protective effect of zinc on lead toxicity. Co-administration of zinc was found to effectively reverse, inhibition of the lead sensitive zinc dependent enzyme δ-aminolevulinic acid dehydratase (ALAD) in male wistar rats. Victery et al. examined the excretion of lead, zinc and calcium in rats exposed to different levels of lead. Furthermore it has been demonstrated that low zinc intake exacerbates lead toxicity. The influence of orally supplemented zinc in preventing lead intoxication in laboratory animals has been reported by us. In concomitant industrial exposure to both zinc and lead, it has been shown that zinc might activate the enzyme which masks the effect of lead. Therefore, 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 has also been shown to induce an increase in MT levels suggesting a possible role of this cysteine rich low molecular weight protein. There are also a few contradictory reports such as that of Kreppel et al. who reported that a zinc induced increase in MT does not seem to be responsible for the role of pre-administered zinc which protects against arsenic induced lethality. On the other hand there have been reports suggesting that zinc pre-treatment afforded an increase in arsenic elimination. We also recently reported that zinc either alone or in combination with monooisoamyl dimercaptosuccinic acid (DMSA) during and after arsenic exposure provided more pronounced elimination of arsenic in male mice. The data from the present study thus provide some new and interesting experimental evidence of a beneficial role of zinc supplementation in guarding against acute arsenic poisoning. Nevertheless, studies with variable doses of zinc particularly during chronic arsenic poisoning have been recommended in order to arrive at a final recommendation.

Selenium

Selenium, a required dietary element for health at low dose is an integral component of ubiquitous enzyme glutathione peroxidase, an antioxidant enzyme. This enzyme helps in neutralisation of reactive oxygen species (ROS). The role of selenium in lead intoxication has rather been controversial. Cerklewski and Forbes investigated the effect of low and high dietary selenium on toxicity of dietary lead in male rats and suggested that low dietary levels mildly protect against toxic effects of lead, but at high levels it exaggerates the lead toxicity. 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. Intramuscular injection of selenium
prior to lead exposure provided prophylactic action against lead effects and we observed that selenium enhances the auto oxidant 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. These 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. Glattre et al. studied the distribution and interaction of arsenic and selenium in rat thyroid and suggested that both arsenic and selenium accumulate in thyroid tissue. Toxic changes were seen during post mortem examination, whereas only minor changes were observed in the selenium or arsenic plus selenium treated groups. We recently reported that selenium administration provided significant protection against the liver injury caused by arsenic.

Competition between selenium and lead or arsenic for binding with the functional proteins and bioligand or active tissue sites or the formation of a reversible compound, metal-selenide thus reducing the availability of “free” concentrations of toxic metals ions in the body might be a possible mechanism for the observed antagonism between lead/arsenic and selenium. The formation of metal-selenide may result from the interaction of metal and active selenium (selenide) released from in vivo reduction of administered sodium selenite. Administration of selenium could provide a detoxifying action on metal ions, in addition to its antioxidant action. The need for selenium substitution in artificial nutrition is suggested and for this use selenomethionine appears to be the most suitable form.

**Copper**

Copper supplementation has also been shown to have some beneficial effects. Copper is a component of the mitochondrial electron transport chain and maintenance of neurotransmitter levels in the brain. Adequate intake of copper provides protection against lead, whereas higher intake of copper increases lead absorption.

The above studies clearly suggest that these micronutrients play a role in preventing toxic metal absorption and could also be co-administered during chelation therapy to maintain essential metal status during chelation treatment and could serve a dual purpose; i) to prevent possible essential metal deficiency syndrome and ii) to accelerate lead elimination due to their own antagonistic/biochemical/pharmacological effects. A beneficial role of zinc supplementation during lead chelation therapy has been reported. A more effective removal of hepatic and renal lead and recovery in the lead sensitive biochemical indices may offer an answer to the problem raised with Ca disodium EDTA (CaNa₂EDTA) therapy. But we have cautioned against the excess and prolonged use of zinc, which may not allow the chelator to bind lead and rather zinc instead of lead. Thomas and Chisolm found that oral supplementation of zinc and copper salts during the course of CaNa₂EDTA treatment did not alter the urinary excretion of lead or zinc but reduced the fall in the plasma zinc concentration. The oral supplementation of zinc during chelation therapy has been found to be beneficial in a patient with plumbism, but simultaneous copper supplementation has a very limited role during chelation of lead.

**Scavengers of Toxicity II: 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 yet not been extensively investigated. Recently we have explored the therapeutic efficacy of an antioxidant along with a chelating agent during the removal of lead in rats. Some groups investigated the ability of some molecules with antioxidant activity to prevent or treat experimental lead toxicity in animals.

The following part mentions some of the forerunners in the list of antioxidants that have been tried in the treatment of metal poisoning, with special reference to lead and arsenic.

**A. Vitamins**

**Thiamine**

Thiamine is one of the important vitamins which have been shown to afford significant protection against short-term lead intoxication. Thiamine administered subcutaneously at a dose of 100 mg/calf, decreased mortality and lead accumulation in various organs of calves, but no beneficial effect on lead sensitive biochemical indices, e.g. blood ALAD, was noted in this study. Further studies suggested that thiamine initially facilitated absorption and an increase in the lead concentration in tissues and that thiamine may also promote a rapid release of lead from tissues.
beneficial effect of dietary thiamine supplement on the tissue accumulation of lead, urinary excretion of ALA and inhibition of blood ALAD activity compared to rats fed a normal thiamine or a thiamine deficient diet was demonstrated by us\textsuperscript{140}. A few other studies reported that thiamine deficiency in the brain might be one of the factors contributing to increased sensitivity to seizure in lead exposed animals\textsuperscript{141}. A protective mechanism, such as thiamine-lead complex formation was proposed. It was suggested that thiamine might facilitate the removal of lead from body fluids and other tissues by the formation of readily excretable complexes\textsuperscript{143, 142–144}. Despite these encouraging reports, there are still no clinical reports available on the effect of dietary thiamine supplementation on lead toxicity. Thiamine as a complementary therapeutic agent or an ‘adjunct’ to a conventional metal chelating agent has been tried\textsuperscript{145, 146}. Thiamine administration concomitantly with CaNa\textsubscript{2}EDTA enhanced the efficacy of a chelator to potentiate urinary lead excretion, to reduce tissue lead including brain lead and restore lead induced biochemical alterations\textsuperscript{147–150}. Thus, thiamine might be utilized to increase the passage of chelating agents through the blood-brain barrier or to decorporate lead from the brain bioligand complexes\textsuperscript{149}. Thiamine might also be participating in chelation as it contains a pyrimidine ring and a thiazole nucleus. The OH group of the side chain and S atom of the thiazole nucleus from 2 moles of thiamine HCl may participate in the chelation of lead\textsuperscript{151}. Furthermore, N atoms or NH\textsubscript{2} groups of the pyrimidine nucleus in the thiamine molecule might also play a role in the chelation of lead. Thiamine supplementations during chelation only slightly augmented lead decorporation but the depletion of brain lead was significant after treatment with thiamine DMSA\textsuperscript{148, 149}. These observations might be significant, as some of the chelators including CaNa\textsubscript{2}EDTA have been shown to be ineffective in removing lead from the brain.

Ascorbic acid is another vitamin which has been studied extensively in modifying lead intoxication\textsuperscript{152, 153}. Co-administration of vitamin C and thiamine greatly enhanced the efficacy of chelating agents to increase urinary lead excretion, to reduce the tissue lead concentration including in the brain, supporting the view that ascorbic acid acts as a detoxifying agent by forming a poorly ionized but soluble complex with lead\textsuperscript{154–156}.

**Vitamin C (Ascorbic acid)**

Vitamin C is a low molecular mass antioxidant that interacts directly with the oxidizing radicals and protects the cells from reactive oxygen species\textsuperscript{157}. Vitamin C scavenges the aqueous reactive oxygen species (ROS) by very rapid electron transfer that thus inhibits lipid peroxidation\textsuperscript{157, 163, 164}. It acts mainly as an antioxidant molecule and its beneficial effects could be attributed to its ability to complex with lead\textsuperscript{165, 166}. 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\textsuperscript{154}, but studies on humans have shown some mixed results. In a study with 78 male workers, 38 received vitamin C and 38 were given a placebo\textsuperscript{167}. They found no effect of ascorbic acid on the absorption or excretion of lead, but 47 psychiatric patients receiving ascorbic acid and zinc had reduced blood lead concentrations\textsuperscript{168}. Simon and Hudes investigated the association between the ascorbic acid concentration and the prevalence of a high blood lead concentration in 19, 578 participants aged 6 yr and older in a National Health and Nutrition Examination Survey 1988–94 (NHANES III)\textsuperscript{169}. In a recently published study we reported some new interesting observations particularly the remarkable effects of combined treatment with vitamin C and succimer (DMSA or MiADMSA) on inhibited blood ALAD activity and in particular its beneficial effect in reducing arsenic induced oxidative stress. Co-administration of vitamin C and MiADMSA in reducing the liver and kidney arsenic burden supports the view that vitamin C acts as a detoxifying agent by forming a poorly ionized but soluble complex\textsuperscript{163}. Recently, a beneficial role of L-ascorbate co-administration against sodium arsenite in maintaining normal ovarian activity and brain monoamines was reported\textsuperscript{170}.

**Vitamin E (α-tocopherol)**

Various vitamins have been found to reduce the toxic manifestation of lead\textsuperscript{154}. 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\textsuperscript{166, 171, 182}. It also appears that the protective effect of vitamin E in lead toxicity is attributed mainly to its antioxidant property\textsuperscript{172–174}. Vitamin E, which is a low molecular mass antioxidant, interacts directly with the oxidizing radicals and protects the cells from reactive oxygen species\textsuperscript{163, 164, 173, 175}. Intramuscular administration of vitamin E prevented inhibition of blood ALAD activity, increase in urinary ALA excretion and was effective in reducing the lead induced altered biogenic amine levels in the brain during concomitant exposure to lead\textsuperscript{175}. Vitamin E supplementation during concomitant lead exposure also prevented lead deposition in liver and blood. Some of the protective effects of vitamin E also emerge directly from its antioxidant property and some through its influence on the drug metabolising enzyme system\textsuperscript{177, 178}. Vitamin E has also been reported to protect against arsenic\textsuperscript{179, 180}. Addition of vitamin E may also alleviate arsenic toxicity. It was observed that vitamin E prevented the arsenite-induced killing of human fibroblasts\textsuperscript{179}. 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. 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. Nevertheless, the group suggest that vitamin C was better in providing clinical recovery and Vitamin E was equally efficient in decreasing the lead burden on the tissues.

We also recently studied whether arsenic induced oxidative stress and the arsenic concentration in soft tissues could be more effectively reduced by some naturally occurring antioxidant such as vitamin C or vitamin E when given alone or in combination with DMSA or one of its analogues MiADMSA. The results lead us to suggest that co-administration of vitamins (vitamin E in particular) may be useful in the restoration of altered biochemical variables (particularly the effects on haem biosynthesis and oxidative injury) although it plays only a limited role in depleting the arsenic burden.

N-Acetylcysteine (NAC)

NAC is a thiol-containing antioxidant that has been used to mitigate various degrees of oxidative stress. Its antioxidant action is believed to originate in its ability to stimulate GSH synthesis, therefore maintaining intracellular GSH levels and scavenging reactive oxygen species (ROS). Besides the antioxidant potential, NAC also has some chelating actions on lead. One of the first reports by Pande et al. suggested that NAC could be used both as a preventive as well as a therapeutic agent along with MiADMSA/DMSA in the prevention and treatment of lead intoxication in rats. Pande et al. 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 also highlighted the favorable response of NAC in post-exposure treatment along with succimer. Combined administration of NAC and succimer after arsenic exposure led to a significant turnover in variables indicative of oxidative stress and removal of arsenic (Fig. 1) from soft organs. 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.

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, modification of immune system activity, and limitation of tumorigenesis and effective inhibition of oxidative stress. One major function of melatonin is to scavenge radicals formed in oxygen metabolism, thereby potentially protecting against free radical induced damage to DNA, proteins and membranes. It has been shown that melatonin stimulates the antioxidative enzyme GPx in the brain, thus providing indirect protection against free radical attack. In animal...
experiments, melatonin prevented the induction of free radical damage caused by a variety of conditions including ingestion of toxins, ionizing radiation, ischemia, reperfusion and excessive exercise. Melatonin has a molecular weight of 232 and is both lipid and water soluble, although its solubility in lipid is clearly greater. Therapeutic efficacy of melatonin either individually or in combination with succimer (DMSA) was recently studied by us in rats. We found very little role of melatonin in the mobilization of lead but it provided significant protection against lead induced oxidative stress in tissues of lead exposed rats.

**α-Lipoic acid (LA)**

α-Lipoic acid is a naturally occurring antioxidant and is able to abate some of the toxic effects of lead. It functions as a cofactor in several multienzyme complexes. Its reduced form, dihydrolipoic acid (DHLA), has two free sulfhydryl groups and the two forms of LA/DHLA possess great antioxidant potential. 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 has an advantage over NAC in opposing GSH loss, since LA is effective in a micro molar range whereas mill molar NAC is needed for a similar effect. The capability of LA to cross the blood brain barrier is an extra advantage because the brain is an important target in lead poisoning. We provided experimental evidence of the beneficial effect of combined LA-succimers administration for treatment of sub-acute lead intoxication in rats. Administration of LA with DMSA or MiADMSA was most effective in reducing lead induced oxidative stress in brain compared to monotherapy. LA administration however, showed no chelating properties in decreasing the lead burden from blood and soft tissues except, interestingly, more pronounced decrease in the brain lead concentration in animals, LA plus thiol chelators, compared to the effect of thiol chelators. The mechanism for the beneficial effect of LA could be attributed to its ability to scavenge some reactive species, to regenerate other antioxidants and also, to some extent its moderate chelating property.

**Taurine**

Taurine a semi essential amino acid has been shown to play a role in maintaining calcium homeostasis, osmoregulation, removal of hypochlorous acid and stabilizing the membranes. Some recent data indicate that taurine can act as a direct antioxidant by scavenging ROS and/or as an indirect antioxidant by preventing changes in membrane permeability due to oxidant injury. The zwitterionic nature of taurine gives it high water solubility and low lipophilicity. Consequently, compared with carboxylic amino acids, diffusion through lipophilic membranes is slow for taurine. In the studies conducted by Gurer and Ercal, taurine was shown to have beneficial effects on lead induced oxidative stress in Chinese Hamster Ovary (CHO) cells and F344 rats. There was increased cell survival in taurine treated lead exposed CHO cells whereas 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 mechanism, rather than a chelating activity, seems to underlie this observed effect of taurine on lead-induced oxidative stress. We recently described the dose dependent effect of taurine, either alone or in combination with meso 2, 3-dimercaptosuccinic acid (DMSA) in the treatment of sub-chronic lead intoxication in male rats. The results suggested a beneficial role of taurine when administered along with DMSA in providing effective reversal of a number of lead sensitive biochemical variables in general, and parameters of oxidative stress in particular, compared to their individual effects. We noted a significant effect of taurine when co-administered with DMSA, in depleting blood and brain lead. It is known that the highest concentrations of taurine are in brain and heart. Perhaps this in part might explain the significant elimination of lead from the brain tissues. This is an interesting and significant observation which requires further exploration. The results thus lead us to conclude a beneficial role of taurine when administered along with a thiol chelator but still it remains to be seen if (i) taurine is a better antioxidant than other available antioxidants in providing significant clinical recovery; (ii) a dose dependent study with a higher dose of taurine needs to be attempted; (iii) the exact mechanism of action of taurine needs to be elucidated.

The use of antioxidants thus brings another novel option to the therapy i.e. the possibility of therapeutic intervention without removing the patient from the source of lead. Antioxidants are recognized as safe molecules and may be given to subjects with low level lead concentrations in their blood even when it is not possible to remove them from exposure to lead.

**3. Combination therapy: A novel approach to the chelation of arsenic and 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 recovery in a number of...
altered biochemical variables. Combination therapy is a new and novel strategy. We have suggested in the earlier paragraphs that combined treatment with a chelating agent having an antioxidant property and a thiol chelator could be a better treatment protocol for lead poisoning than monotherapy with a chelator\(^7, 186\). The potential role of oxidative stress in injury associated with arsenic poisoning suggests that antioxidant may enhance the efficacy of the treatment protocol designed to mitigate arsenic induced toxicity. We have recently reported that combined administration of n-acetylcysteine and succimer led to a rapid mobilization of arsenic and lead, whereas administration of α-lipoic acid and DMSA provided a more pronounced recovery in lead induced altered biochemical variables indicative of oxidative stress\(^7, 186\). An experimental study conducted by us recently provided \textit{in vivo} evidence of arsenic induced oxidative stress in a 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\(^186\). We also reported that co-administration of naturally occurring vitamins such as vitamin E or vitamin C during administration of a thiol chelator such as DMSA or MiADMSA may be more beneficial in the restoration of altered biochemical variables (particularly the effects on haem biosynthesis and oxidative injury) although it has only a limited role in depleting the arsenic burden. The study reports some new interesting observations, particularly the remarkable effects of combined treatment on inhibited blood ALAD activity and in particular its beneficial effect in reducing arsenic induced oxidative stress. Co-administration of vitamin C and MiADMSA in reducing the liver and kidney arsenic burden supports the view that vitamin C acts as a detoxifying agent by forming a poorly ionized but soluble complex\(^169\).

**Combined administration of Two Chelating agents:**

We observed beneficial effects of MiADMSA in mobilizing arsenic from chronically pre-exposed GaAs and in reducing tissue oxidative stress, but it was observed that optimum effects of chelation therapy could be achieved by combined administration of oxalic acid and MiADMSA. Oxalic acid is an effective gallium chelator\(^77\). We also reported that combined administration of DMSA with calcium disodium EDTA decreases lead concentration in the brain\(^22, 51\). Besunder \textit{et al} confirmed these studies in children and combination therapy with DMSA and EDTA for children hospitalized for chelation therapy is recommended instead of monotherapy with either agent\(^160\). After some very positive results obtained with combined DMSA and EDTA administration for chronic lead poisoning, we compared the therapeutic efficacy of calcium disodium ethylenediaminetetraacetic acid (CaNa\(_2\)EDTA) with two different thiol chelators, 2, 3-dimercaptopropane 1-sulfonate (DMPS) and monoisoamyl dimercaptosuccinic acid (MiADMSA). The results of combined treatments with CaNa\(_2\)EDTA and the two thiols (DMPS and MiADMSA) were the more impressive, both in terms of recovery in altered biochemical variables (oxidative stress) and reduction of the body lead burden\(^50\).

There have been recent debates about introducing a combined treatment therapy for lead poisoning\(^22, 88, 89\). The two arguments proposed for promoting this treatment point to the fact that (i) The inefficiency of EDTA to decrease brain lead and the ability of thiol chelators like DMSA for their reported role in promoting soft tissue lead mobilization including the brain (ii) addition of MiADMSA to EDTA caused not only higher lead elimination but also better recovery in altered biochemical variables. The studies done by us thus represent a systematic approach to the development of a new strategy of chelation therapy.
therapeutic protocol for the treatment of lead intoxication.

It is evident from the above that combination therapy is a new and a better approach to treat cases of metal poisoning, as only a little experimental evidence is available and there is a need for in depth investigation in this area.

With some very interesting results obtained with combined administration of two chelators for lead poisoning, it was proposed to investigate the effects of combination therapy for arsenic poisoning, where a strong chelating agent is administered along with another structurally different chelating agent\textsuperscript{22, 158, 159). In a recent study we investigated whether co-administration of thiol chelators such as meso 2, 3-dimercaptosuccinic acid (DMSA) or sodium 2, 3-dimercaptopropane 1-sulfonate (DMPS) along with a newly developed thiol chelator, monoisomyl DMSA, is more beneficial than monotherapy with these chelators in countering chronic arsenic toxicity\textsuperscript{159). Animals were exposed to 10-ppm arsenic in drinking water for 6 months and subsequently treated with two 5-day courses of chelation with DMSA, DMPS (0.3 mg/kg, i.p. once daily), or MiADMSA (0.1 mmole/kg, i.p. once daily) either individually or in combination. The data provided a promising role of combination treatment in potentiating the depletion of blood, liver, kidney and brain arsenic compared to monotherapy with these thiols (Fig. 2). It can be concluded from the study that concomitant administration of DMSA, an extracellularly distributed chelator with a lipophilic chelator such as MiADMSA, could play a significant and important role in abating the number of toxic effects of arsenic in animals compared to treatment with these chelators alone\textsuperscript{159).

The outcomes of such studies, are important, especially in suggesting and developing new safe and highly effective control/therapeutic measures to deal with cases of metal poisoning (particularly arsenic and gallium arsenide).

4. Protective Efficacy of Various Plant Products on the Toxic Effects of Arsenic and Lead

It is clear from the above, that most of the conventional metal chelating agents may have toxic side effects or disadvantages. The possibility of dietary intervention or supplementation of naturally occurring dietary nutrients to prevent the effects of arsenic in a population at risk is of interest\textsuperscript{49). A positive correlation has also been established between dietary supplementation with a number of vegetables and plant parts and the reduction of toxic effects of many toxicants and environmental agents including heavy metals\textsuperscript{212). Concomitant administration of a few plant extracts such as \textit{Hippophae rhamnoides}, \textit{Aloe Vera barbadensis} and \textit{Centella asiatica} either during exposure or during chelation treatment of the arsenic induced hematological, renal and hepatic disorders in laboratory animals have been found to be of some benefit\textsuperscript{213). Blood and liver arsenic concentrations also were determined but generally remained unaltered. Seabuckthorn (\textit{Hippophae rhamnoides}, Elaegnaceae) is a thorny nitrogen fixing deciduous shrub, native to Europe and Asia\textsuperscript{214). All parts of the plant are considered to be a good source of a large number of bioactive substances. The ripe fruit has been considered to be a rich source of Vitamin A, C, E, carotenoids and organic acids\textsuperscript{215, 216). Many medicinal effects of Seabuckthorn for flu, cardiovascular diseases, mucosal injuries and skin disorders have been reported to be due to the high content of antioxidant substances present in this plant\textsuperscript{217).
Recently, Geetha et al. have provided evidence in which the leaf extract of this plant has been found to have a potent oxidative stress action against chromium induced oxidative stress in rat\(^{218,219}\). But no study is available on the protective role of Sea buckthorn against arsenic toxicity.

*Aloe vera* (*Aloe barbadensis*) is a tropical cactus, which has been reported to have a therapeutic potential in a variety of soft tissue injuries\(^{220}\). It has been used in the traditional medicine of many cultures and is said to be beneficial in the treatment of such disorders as arthritis, gout and dermatitis, etc and wounds such as peptic ulcer and burns. The fresh gel, juice or formulated products have been used for medical and cosmetic purposes and general health\(^{221,222}\). In spite of its wide use in folk remedy over a long period, its effects on various heavy metals/ metalloid induced altered biochemical and physiological processes have not yet been described in detail and only a brief recent report suggests that it has some protective effects against arsenic induced oxidative stress.

In the Indian system of medicine Ayurveda, *Centella asiatica* (Umbelliferae) syn Hydrocotyl asiatica has been used in various parts of India for different ailments such as headache, body ache, insanity, asthma, leprosy, ulcers and wound healing\(^{223,224}\). The whole plant of *C. asiatica* has been shown to be beneficial in improving memory and it is reported to improve the general mental ability of mentally retarded children\(^{225,226}\).

It is therefore clear from the above that we are still far away from having a safe, specific and effective chelating agent for the treatment of metal poisoning. Apart from this, still further knowledge is needed in several basic research areas within the field of *in vivo* chelation of metals and there is a call for studies on (a) the molecular mechanism of action of clinically important chelators, (b) Intracellular and extracellular chelation in relation to the 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 (g) Fetotoxic and teratogenic effects of chelators (Figs. 3 and 4).

**Acknowledgments:** The authors thank Mr. K. Sekhar, Director of the establishment for his support and Ms Geetu Saxena, JRF for her efforts during the preparation of the manuscript.

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