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Chelation Therapy: Types of Chelating Agents, Limitations and Utility

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Abstract

Chelation therapy plays a central role in molecular medicine and pharmacology. It works when acute or chronic intoxication due to the variety of metals occurs which can be improved by the administration of suitable chelating agent. This technique uses remedies to remove the metals, so they do not make any negative effect. As per the FDA approval, intravenous injection or oral pills are used to remove harmful metals from the body via kidney. Chelating agents bind to the toxic metals via chelation to neutralize the toxic nature of metals. Chelates are more stable than non-chelated compounds of comparable composition. Some chelates undergo dentation with a metal and form co-ordination complex while some are used as to contract poisoning like antidotes. Chelates also prevent loss of nutrients and help nutrient mobility inside the body. Moreover, it is the form of therapy with application in conventional as well as alternative medicine. In this review, the chemical characteristics, properties, and uses of some chelating agents are hereby described.

Keywords

Chelation, Chronic intoxication, Dentation, Poisoning, Dentation, Poisoning

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INTRODUCTION

Metals form an integral part of many structural and functional components in the body and researchers have always paid a considerable interest in studying the critical role of metals in physiological and pathological processes. The metals are used to restore the normal physiology of the biosystems either by direct implementation of essential metals, by chelating out excess toxic metals, or using them as carriers for targeted drug delivery, or for tagging biomolecules for diagnostics, all come under the heading of *heavy metal pharmacology*. Chelation therapy is the implementation of certain chemical agents so as to remove the heavy metal concentration from the biological systems. They were introduced in pharma biosystems after the use of poison gas in World War I. Chelation has its origin in the Greek word *chele* that means *claw* of a lobster, thus depicting the concept of clinging or holding with a strong grip hence being the second alternative for prevention from toxic metals exposure. The term *chelate* was first applied by Sir Gilbert T. Morgan and H. D. K. Drew in 1920. They proposed the term for the *caliper*-like groups which function as two associating units and fasten to a central atom so as to produce heterocyclic rings [1].

It is pertinent to mention here that Chelation occurs and the bidentate ligand form ring structure between the metal ion and the two-ligand atom. Metal may react with O^-, S^-, and N^- containing ligands present in the form of OH, COOH, SH, NH_2, NH and N and the resultant metal-complex is formed by coordinate bond in which both electrons are contributed by the ligand [1]. The first experimental use of a chelating agent namely citrates to overcome the metal poisoning i.e. lead intoxication was carried by Kety and Letonoff in 1941 [2]. After chelation, the heavy metal is mobilized after enabling it to pass through renal excretion.

Chelating agents vary in their specificity for toxic metals. Ideal chelating agents should (1) retain their chelating property in body fluids and their distribution should remain same as that of toxic metals, (2) water-soluble, (3) high lipophilicity and low molecular size in neurotoxicity is preferred as it can easily pass via renal excretion (4) unable to bio transform, (5) reaches to a particular site of metal storage without any side effect, (6) form non-toxic complexes excreted from the body and (7) also have a low affinity for essential metals particularly calcium and zinc. Common chelating agents include *alphalipoic acid* (ALA), *Betaamino phenoxyethane tetracetic acid acid* (BAPTA), *deferiprone*, *deferoxamine*, *diethylenetriamine pentaacetic acid* (DTPA), *dimercaprol* (BAL), *dimercaptopropane sulfonate* (DMPS), *dimercaptosuccinic acid* (DMSA), *ethylenediaminetetraacetic acid* (EDTA), *calcium disodium ethylenediaminetetraacetic acid* ($CaNa_2$-EDTA), *ethylene glycol tetraacetic acid* (EGTA), *Triethylenetetramine*, *Nitrilotriacetic acid*, *D-penicillamine*, etc. In the present review shall discuss chelation therapy which is an important tool for overcoming heavy metal concentrations in the body.

1. BAL (British anti-lewisite/2, 3-dimercaprol)

BAL (2,3-dimercaptopropanol/) is the first clinically useful chelating agent, developed during World War II as a specific antagonist to vesicant arsenical war gases, based on the observation that arsenic has an affinity for sulfhydryl containing substances. BAL, a dithiol compound with two sulfur atoms on adjacent carbon atoms, competes with the toxic effects. It was developed secretly as an antidote for Lewisite, the now-obsolete arsenic-based chemical warfare agent. Today, it is used medically in treatment of arsenic, mercury, lead, and other toxic metal poisoning. In addition, it has in the past been used for the treatment of Wilson's disease, a genetic disorder in which the body tends to retain copper.

In humans and experimental models, BAL has been shown to be the most effective antidote when administered soon after exposure. Dimercaprol is itself toxic, with a narrow therapeutic range and a tendency to concentrate arsenic in some organs like brain [3]. Other drawbacks include the need to administer it by painful intramuscular injection because of its oily nature. Serious side effects include nephrotoxicity and hypertension.

BAL has been found to form stable chelates *in vivo* with many toxic metals including inorganic mercury, antimony, bismuth, cadmium, chromium, cobalt, gold and nickel. However, it is not necessarily the treatment of choice for toxicity to these metals. BAL has been used as an adjunct in the treatment of the acute encephalopathy of lead toxicity. It is a potentially toxic drug, and its use may be accompanied by multiple side effects. Although treatment with BAL will increase the excretion of cadmium, there is a concomitant increase in renal cadmium concentration, so that its use in case of cadmium toxicity is to be avoided. It does, however, remove inorganic mercury from the kidneys, but it is not useful in the treatment of alkyl mercury or phenyl mercury toxicity. BAL also enhances the toxicity of selenium and tellurium, so it is not to be used to remove these metals from the body. Other side effects of BAL include vomiting, headache, lachrymation, rhinorrhea and salivation, profuse sweating, intense pain in the chest and abdomen, and anxiety [3, 4].

2. DMPS (2, 3-Dimercapto-1-propanesulfonic acid)

DMPS and its sodium salt (known as unithiol) are chelating agents that form complexes with various heavy metals. DMPS is a water-soluble derivative of BAL developed in response to BAL's toxicity and unpleasant side effects. DPMS has been shown to reduce lead levels in blood in children [5]. It has an advantage over ethylenediaminetetraacetic acid (EDTA) which is that it is administered orally and does not appear to have toxic effects. It has been widely used in the former Soviet Union to treat many different metal intoxication cases. DMPS has been used experimentally to estimate the real burden of lead and inorganic mercury [6]. Its effectiveness in mobilizing metals from the kidney may be because it is transported into kidney cells on the organic anion transport system. It increases the urinary excretion of mercury in persons with an increased body burden from industrial exposure, dentists and dental technicians, persons with dental amalgams and those exposed to mercurous chloride in skin creams [7].

3. DMSA (*meso*-2, 3-Dimercaptosuccinic Acid)

DMSA is a chemical analog of BAL. More than 90 percent of DMSA is in the form of a mixed disulfide in which each of the sulfur atoms is in disulfide linkage with a cysteine molecule. The drug is of current interest clinically because of its ability to lower lead levels in blood. It has advantages over EDTA because it is given orally and has greater specificity for lead. It may be safer than EDTA in that it does not enhance excretion of calcium and zinc to the same degree. Studies in rodents showed that a single dose of DMSA primarily removes lead from soft tissues [8]. DMSA possesses low toxicity upon oral administration and no redistribution of metal from one organ to another [9, 10]. Animal studies suggested that DMSA is an effective chelator of soft tissues, but it is unable to chelate lead from bones [9]. DMSA is not known to cause elevations in the

excretion of calcium, zinc, or iron, although zinc excretion increases to 1.8 times baseline during treatment.

4. $CaNa_2EDTA$ (Calcium Disodium Ethylenediamine tetra acetic Acid)

The chelating nature of $CaNa_2EDTA$ arises from its ability to "sequester" di- and tricationic metal ions such as Ca^{2+} and Fe^{3+}. After EDTA binds, metal ions remain in solution but exhibit diminished reactivity. Calcium salt of ethylenediaminetetraacetic acid (EDTA) must be used for clinical purposes because the sodium salt has greater affinity for calcium and will produce hypocalcemic tetany. $CaNa_2EDTA$ is a derivative of ethylenediaminetetraacetic acid (EDTA), synthetic polyaminocarboxylic acid [11]. EDTA exhibits low toxicity with LD_{50} (rat) of 2.0-2.2 g kg^{-1}. Oral exposures have been noted to cause reproductive and developmental effects. $CaNa_2EDTA$ cannot pass through cellular membrane so its application can help remove the metal ions present in extracellular fluid. Further, EDTA treatment can result in mobilization of lead from hard tissue deposit to soft organs [12, 13]. The calcium salt of EDTA has been shown to cause necrosis of renal tubular cells. These drawbacks preclude application of $CaNa_2EDTA$ as a suitable drug to treat lead poisoning.

5. Vitamins C

Vitamin C or L-ascorbic acid is an essential nutrient and a potential antioxidant for humans. It is made internally by almost all organisms, with humans being a notable exception. The pharmacophore of this vitamin is an ascorbic anion. In humans, vitamin C is essential to a healthy diet as well as being a highly effective antioxidant, acting to lessen oxidative stress, and a substrate for ascorbate peroxidase and an enzyme cofactor for the biosynthesis of many important biochemicals. Vitamin C acts as an electron donor for eight different enzymes. It also acts as a scavenger of free radical and plays an important role in regeneration of α-tocopherol [14]. The plasma ascorbate concentration in oxidative stress patient (less than 45 μmol L^{-1}) measured is lower than healthy individual (61-80 μmol L^{-1}). The increasing plasma ascorbate level may have therapeutic effects in oxidative stress individual. Individual with oxidative stress and healthy individual have different pharmacokinetics of ascorbate.

Ascorbic acid behaves not only as antioxidant but also as a prooxidant. Ascorbic acid reduced transition metals, such as cupric ions (Cu^{2+}) to cuprous (Cu^{+}) and ferric ions (Fe^{3+}) to ferrous (Fe^{2+}) during conversion from ascorbate to dehydroxyascorbate. This reaction *in vitro* can generate superoxide and other ROS. However, in the body, free transition elements are unlikely to be present while iron and copper are bound to diverse proteins. Recent studies show that intravenous injection of 7.5 g of ascorbate daily for 6 days did not increase prooxidant markers. Thus, ascorbate as a prooxidant is unlikely to convert metals to create ROS *in vivo*. There are few side effects of vitamin C when taken in excess such as diarrhoea, iron poisoning, hemolytic anaemia, kidney stone formation and suppression of progesterone in pregnant ladies.

6. Vitamin E (α-tocopherol)

Vitamin E (α-tocopherol) is the collective name for a set of 8 related α-, β-, γ-, and δ-tocopherols and the corresponding four tocotrienols, which are fat-soluble vitamins with antioxidant properties. Vitamin E also provides protection against silver induced lesions. When cadmium was co-administered with vitamin E, the results showed reduced accumulation of cadmium in the kidney, liver and blood. The antioxidant levels enhanced by Cd induction came down to near normal levels, which indicate that antioxidant properties of vitamin E may be responsible for providing protection from cadmium toxicity [15]. Vitamin E has been shown to reduce lipid peroxidation and to increase the cell viability. The supplementation of vitamin E to Pb-treated erythrocytes has been shown to prevent the inhibition of δ-aminolevulinic dehydratase activity and lipid oxidation [16] *in vivo* and *in vitro*. Vitamin E could also be useful to protect membrane lipids and proteins from oxidation due to lead intoxication.

7. β-Carotene

β-Carotene is fat-soluble organic compound, a terpenoid, a red-orange pigment abundant in plants and fruits. As a carotene with β-rings at both ends, it is the most common form of carotene. It is a precursor (inactive form) of vitamin A (retinol). The most common side effect of high doses of β-carotene consumption is carotenodermia, a harmless condition that presents as a conspicuous orange skin tint arising from deposition of the carotenoid in the outermost layer of the epidermis. It has also been associated with increased rate of lung cancer among those who smoke [17].

8. N-Acetylcysteine (NAC)

N-acetylcysteine or N-acetyl-L-cysteine (NAC), a pharmaceutical drug marketed with various trade names, is used mainly as a mucolytic agent and in the management of paracetamol (acetaminophen) overdose. Out of the three sulfur-containing amino acids, L-cysteine is an essential one as the other two may be synthesized by using it. L-cysteine has been shown to act as an antioxidant and has a pivotal role in endogenous free radicals induced intoxication in the body. The exposure to heavy metals has been

found to reduce level of cysteine [18]. Recently it has been reported that a high oral dose of NAC modulates inflammation in cystic fibrosis (CF) and has the potential to counter the intertwined redox and inflammatory imbalances in CF [19]. The supplementation of NAC may attenuate Cd induced nephrotoxicity via chelation [20]. The co-administration of NAC and DMSO after arsenic exposure leads to removal of arsenic from soft organs. NAC was metabolized to S-nitroso-N-acetylcysteine (SNOAC), which increased blood pressure in mice treated with acetylcysteine. So, its large doses can damage the heart and lungs.

9. α-Lipoic Acid

Lipoic acid is an organosulfur compound (yellow solid). Its R-enantiomer is an essential cofactor for many enzyme complexes. This is a carboxylic acid and features a cyclic disulfide or ditholane ring, functional group. It exists as dihydrolipoic acid, the reduced form of lipoic acid intracellularly. Lipoic acid was first postulated to be an effective endogenous thiol antioxidant when it was found to prevent the symptoms of vitamin C and vitamin E deficiency. Dihydrolipoic acid is able to regenerate (reduce) antioxidants, such as glutathione,
vitamin C, and vitamin E. It is equipped with properties to quench ROS *in vitro*. The relatively good scavenging activity of lipoic acid is due to the strained conformation of the 5-membered ring in the intramolecular disulfide. Owing to the presence of two thiol groups, it can chelate metals such as iron, copper, mercury, and cadmium and remediate free radical damage in biological system [21]. It is readily available from the diet and absorbed through the gut and can easily enter BBB in humans unlike DMSA and DMPS. However, its effectiveness is heavily dependent on the dosage and frequency of application. The exogenous supplementation of LA has been reported to act as an efficient antioxidant thereby reducing oxidative stress both in vitro and in vivo [22]. Its intake can change the redox level of tissues directly by quenching the free radicals and indirectly by enhancing the levels of other antioxidants/antioxidant enzymes. Among the mono- and dithiols (glutathione, cysteine, dithiothreitol, and lipoic acid), LA has been reported in vitro to be the most potent scavenger of free radicals generated during cadmium hepatotoxicity [23].

10. Melatonin

Melatonin, chemically known as N-acetyl-5-methoxy tryptamine, is a naturally occurring hormone found in most animals, including humans, and some other living organisms, including algae. It is produced by the pineal gland (outside BBB) under the influence of the suprachiasmatic nuclei (SCN) of the hypothalamus, which receives information from the retina about the daily pattern of light and darkness. Its circulating levels vary in a daily cycle. Despite its role in regulating circadian rhythm (synchronization of the biological clock) in humans, it also acts as powerful endogenous scavenger of ROS and RNS such as OH, O_2^-, and NO radicals. Melatonin is an antioxidant that can easily cross cell membranes and BBB. Melatonin is a direct scavenger of ROS. Unlike other antioxidants, melatonin does not undergo redox cycling. Redox cycling may allow other antioxidants (such as vitamin C) to regain their antioxidant properties. Melatonin, on the other hand, once oxidized, cannot be reduced to its former state because it forms several stable end-products upon reacting with free radicals. Therefore, it has been referred to as a terminal (or suicidal) antioxidant.

Melatonin helps maintain membrane fluidity by preventing LPO and scavenging ROS and RNS [24]. Its capacity to absorb free radicals extends at least to the quaternary metabolites of melatonin, a process referred to as “the free radical scavenging cascade.” Due to antioxidant potential attached to melatonin, it has been found to protect haematopoietic cells from the lead-induced damage [25]. The indole group present in the melatonin has been implicated in protection of thiol groups of membrane proteins from lead toxicity [26, 27].

As reported, melatonin stimulates antioxidative enzymes such as GPx and SOD and quickly eliminates H_2O_2 from rat brain cortical cells [28, 29]. It also enhances the production of the enzymes involved in glutathione synthesis [30]. The circulating melatonin decreases with age and in recent years much interest has been focused on its immunomodulatory effect. Melatonin stimulates the production of progenitor cells for granulocytes macrophages. It also stimulates the production of NK cells and CD^{4+} cells and inhibits CD^{8+} cells. The production and release of various cytokines from NK cells and T-helper lymphocytes also are enhanced by melatonin. Melatonin presumably regulates immune function by acting on the immune-opioid network by affecting G-protein-cAMP signal pathway and by regulating intracellular glutathione levels [31]. Melatonin’s antioxidative property may offer protection against carcinogenesis, neurodegeneration, and aging [32, 33].

Possibly there are three different mechanisms of actions of melatonin: (1) melatonin could trigger antioxidant cellular mechanisms, (2) melatonin could form a complex joining the cadmium and thus avoid the uptake of the metal and its harmful effects, a fact

shown at the brain and pituitary level, and (3) melatonin is very liposoluble so it can diffuse freely and cross all cellular barriers, facilitating the elimination of cadmium from tissues. In conclusion, melatonin is a natural antioxidant, with high power of scavenger of free radicals, so it can play an important role in protecting cells against the oxidative damage induced by cadmium. Thus, melatonin may be a good tool to palliate, at least in part, the toxicity of this metal [34].

11. Triethylenetetramine (TETA)

TETA is a Cu(II)-selective chelator which is commonly used for the treatment of Wilson's disease. Recently, it has been shown that TETA can be used in the treatment of cancer due to its telomerase inhibition and anti-angiogenesis properties. TETA has poor absorption with a bioavailability of 8 to 30% and has a relatively short half-life (2-4 h) in humans. It is widely distributed in tissues with relatively high concentrations measured in liver, heart and kidney. It is mainly metabolized via acetylation and is mainly excreted in urine. The most recent discoveries in TETA pharmacology show that the actual TETA-metabolizing enzyme is spermidine/spermine acetyltransferase [35]. The TETA exposure causes corrosion to the eyes, skin, and respiratory tract. Inhalation may cause lung oedema, but only after initial corrosive effects on eyes and/or airways have become manifest. Repeated or prolonged contact may cause skin sensitization.

12. Nitrilotriacetic acid (NTA)

NTA is a tripodal tetradentate trianionic ligand. It is used for water softening and as a replacement to sodium and potassium triphosphate in detergents and cleansers. In one application, NTA as a chelating agent removes Cr, Cu, and As from wood that had been treated with chromated copper arsenate. In the laboratory, this compound is used in complexometric titrations. Also, a variant of NTA is used for protein isolation and purification in the His-tag method. The modified NTA is used to immobilize nickel on a solid support. This allows purification of proteins containing a tag consisting of six histidine residues at either terminus. Nitriloacetic acid can cause eye, skin, and respiratory tract irritation; and can cause kidney and bladder damage. The compound is anticipated to have the potential to cause human cancers. The uses of NTA are like those of EDTA, both being chelating agents but in contrast to EDTA, NTA is easily biodegradable and is almost completely removed during wastewater treatment. The environmental impacts of NTA are minimal. Despite widespread use in cleaning products, the concentration in the water supply is too low to have a sizeable impact on human health or environmental quality [36].

13. Deferoxamine (DFOA)

Deferoxamine (desferrioxamine or Desferal) is used to bind iron and aluminium. It is specifically used to treat acute iron poisoning in children, hemochromatosis either due to multiple blood transfusions or an underlying genetic condition, and aluminium toxicity in people on dialysis. Administration for chronic conditions is generally accomplished by subcutaneous injection over a period of 8-12 h each day. Administration of deferoxamine after acute intoxication may colour the urine a pinkish red, a phenomenon termed "*vin rosé urine*". Apart from iron toxicity, deferoxamine can be used to treat aluminium toxicity (an excess of aluminium in the body) in select patients. In US, the drug is not FDA-approved for this use. Deferoxamine is also used to minimize doxorubicin's cardiotoxic side effects and in the treatment of a patient with aceruloplasminemia [37]. Deferoxamine may be effective for improving neurologic outcomes in persons with intracranial haemorrhage, although there are no strong evidence supporting the efficacy and safety for this indication.

14. Penicillamine

It is also called 3-mercaptovaline. It is a chelating agent for lead, copper, iron and mercury. When used to treat copper toxicity, it helps to solubilize copper in the cells to allow for more rapid urinary excretion. Treated animals should have increased copper urinary excretion. It also can combine and form disulfide bonds with cysteine resulting in a more soluble compound that facilitates the excretion in urine; this also helps in prevention of the formation of cystine calculi.

It has a plasma peak time of 1-3 h, peak plasma concentration of 1-2 mg/L for the 250 mg dose and half-life of 4-6 days. More than 80% is protein-bound, and it is excreted in the urine. It can be a treatment for lead poisoning if no other preferred chelating agents are available. For adults, the daily oral dose is between 1000 and 1500 mg daily in divided doses until the urinary lead stabilizes at less than 0.5 mg/day [38].

There are various side-effects of penicillamine administration. Its use is prevented where patients are with a previous history of penicillamine-related aplastic anaemia or Penicillin allergy. Patients should also discontinue its use if there is an immune reaction, renal insufficiency, rheumatoid arthritis, Pregnancy risk factor D, Concurrency with antimalarials and immunosuppressants.

15. Deferiprone

Deferiprone is a bidentate hydroxypyridinone that binds with iron to form a stable 1:3 iron-chelator complex. It has excellent oral bioavailability. It is rapidly absorbed after oral ingestion, with peak plasma levels at 45-60 min after ingestion. Ingestion with meals does not alter the absorption. It is metabolized in the body by hepatic glucuronidation to an inactive metabolite. The chelator complex and free drug metabolite are mainly excreted in the urine. Deferoxamine has been the standard iron chelator in clinical practice since the 1970s and has helped to improve the prognosis of patients with thalassemia major [39].

It is given orally as well as subcutaneous infusion over 8-10 h and 5-7 days per week and is well tolerated by patients. Agranulocytosis, neutropenia, arthropathy and thrombocytopenia are the most important side effects. Careful monitoring of blood counts remains a critical component of therapy with deferiprone.

16. Combination Therapy

Combination therapy most often not only is used to refer to the simultaneous administration of two or more medications to treat a single disease but also is used when other types of therapy are employed at the same time. Here in chelation therapy, it refers to the administration of two chelators differently administered with the notion that various chelating agents may immobilize (trap) the toxic metals from different tissues to render better protection from their toxicity [40]. In combination chelation therapy, also the co-administration of dietary nutrients like vitamins, for example, thiamine, an essential metal such as zinc [41], or an amino acid like methionine with a chelating agent, has been found to render better clinical recoveries as well as mobilization of lead from the tissues [42].

CONCLUSION

The structures of chelating agents permit the attachment of their two or more donor atoms (or sites) to the same metal simultaneously and produce one or more rings with potential non-toxicity which are excreted from the body through urine. The toxicokinetic and toxicodynamic of metal and chelating agents are an integral part of an effective chelation therapy in addition to the following criteria like high affinity for the toxic metal, low affinity for essential metals, minimal toxicity, lipid solubility and good absorbability from the gastrointestinal tract. Moreover, chelated minerals are bound to amino or organic acid, they do not require as much as stomach acid to be effectively digested. Detoxification through chelating agents are effective at removing heavy metals from the blood stream and periphery but may not produce benefits for long-term behavioural, cognitive or neuromotor outcomes. An ideal chelating agent is therefore highly soluble in water, can transversely cell membranes, resistant to biotransformation, non-toxic and form complexes with metals at different pH.

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