Chelation Therapies: A Chemical and Biochemical Perspective

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Abstract: Chelation therapy occupies a central place in modern medicine and pharmacology, because continuous studies with laboratory animals and extensive clinical experience demonstrate that acute or chronic intoxications with a variety of metals can be considerable improved by administration of a suitable chelating agent. In this review the chemical characteristics, properties and uses of the most common chelating agents as well as those of some new and very promising agents of this type, are discussed. In the second part of the review the biological and biochemical impact of these agents, as well as their use for the treatment of some selected diseases and disorders, are also analyzed and discussed in detail.

Keywords: Toxic metals, chelating agents, chemical characteristics, biological effects.

INTRODUCTION

As a direct consequence of the continuous advances in the field of Bioinorganic Chemistry [1-3], interesting and novel applications in other fields began to emerge and some of them attained rapid development as well as strong impact. Well known and important examples are found in the areas of biolixiviation [4], catalysis [5,6], materials science [7-9], biological environmental monitoring, remediation, decontamination [10-12], and in those of medicine and pharmacology [13-17]. Among these new areas of research, directly related and derived from Bioinorganic Chemistry, Medicinal Inorganic Chemistry plays a very relevant role [17-19].

It is now clearly recognized that a number of metallic elements are essential for the normal functioning of the organism and the correct activity of all its metabolic and physiological processes. Therefore, the correct balance of these essential metal elements is of crucial importance to ensure adequate health conditions. One of the main goals of Medicinal Inorganic Chemistry is to achieve and maintain this correct balance among essential elements thorough manipulation and redistribution of metal ions within the organism or by removal of them from the system. Besides, the development and application of metal-based drugs constitutes a third important aspect of this field [17,18]. This review is focused on the first two goals: binding of metal ions for redistribution or removal.

It is worth recalling the so-called Bertrand diagram, depicted in Fig. (1) [2,20]. For essential elements, very low doses may give rise to deficiency diseases and at absolute deficiency, death may result. With limited intake, the organism survives but may show marginal insufficiency, eventually requiring supplementation [21]. With increasing nutrient a plateau representing optimal function is reached. As the dose is in excess, first marginal toxicity is attained, then mortal toxicity. These are the situations in which removal of the element must be performed. While this diagram may vary quantitatively for each essential nutrient, the basic pattern holds for virtually all the elements [20] and two important conclusions can be derived from this model: each element has an optimal range that maintains optimal tissue concentration and functions. On the other hand, every essential trace element has a toxic range when this optimal range is exceeded [22]. This means that the presence of excessive quantities of an essential element can be as deleterious as insufficient amounts.

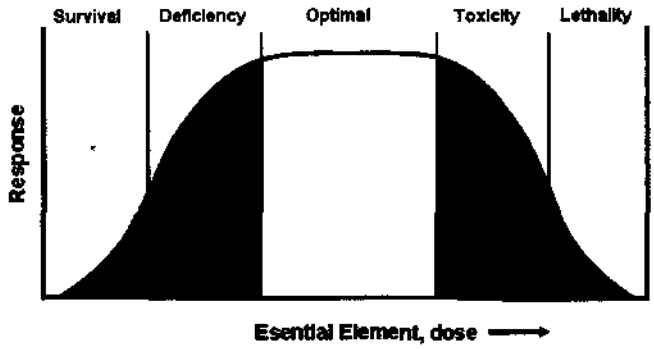

Fig. (1). Dose response range of an essential element (Bertrand's diagram).

An excess of any element can be originated from its accidental ingestion or metabolic disorders leading to the inefficient activity of the biochemical mechanisms that control its uptake, distribution and/or excretion. These possibilities constitute one major class of metal toxicity. The other broad class results from uptake of non-essential metals into the organism through food, skin absorption, or respiration. The toxicities associated with this latter class have received recent attention because of the public health risks derived from environmental pollutants [12,23]. In this review, we present examples of both categories and illustrate ways in which Medicinal Inorganic Chemistry can contribute to the removal of toxic metals and restoration of normal functions. One possible way involves chelation therapy, in which a metal-specific chelating agent is administered as a drug to complex and facilitate the excretion or redistribution of the unwanted excess element. A second way is to identify fundamental biological mechanisms that regulate metal detoxification and to apply these principles to help in the control or minimization of toxic effects.

Chelation therapy occupies a central place in modern medicine and pharmacology, as continuous studies with laboratory animals and extensive clinical experience demon-

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strate that acute or chronic intoxications with a variety of metals (essential or non-essential) can be considerable improved by administration of a suitable chelating agent [2,24,25].

1. CHELATION AND CHELATING AGENTS

1.1. General Aspects

Some ligand molecules have more than one pair of non-bonding electrons that are available for donation both in an electrostatic and a steric sense. Such molecules that can serve as multiple electron-pair donors through several different atoms to a single acceptor ion are called chelating agents and this particular ligand-to-metal interaction is known as chelation. These reactions are entropy-driven and generate very stable ring structures. Besides, the complex involving a chelating donor will be more stable than the equivalent complex involving single-pair donor molecules with the same donor atoms (chelate effect) [26].

Obviously, chelating ligands to be used in biological systems for therapeutic purposes must fulfill a series of important criteria and requirements [24,27-30], which are summarized in Table **1**.

Table 1. Important Criteria for the Selection of a Therapeutically Useful Chelating Agent

Values of the formation constants for the complexes with the metals to be removed, in comparison with those of H^+, Ca(II), Zn(II), Fe(II) and other essential metals.
High water solubility, ensuring an adequate solubility in physiological media.
Partition coefficient of the chelator and its metal complexes, between water and lipid protein cell membranes (hydrophilicity/lipophilicity).
An adequate access to the toxic metal deposits in the organism (for example, intra cellular accumulations). Adequate concentration at the desired site of action.
Net charge of the chelator and its metal complexes at physiological pH.
Chemical and biochemical stability of the chelator and its metal complexes. Both, chelator and complexes, must be non-toxic and should not generate important side effects.
Adequate capacity of remotion of the toxic species from the ligands of its biological environment.
Adequate and rapid of excretion of the generated metallic complexes and minimizing *in vivo* distribution of the unexcreted complexes.
Adequate form for a rapid and effective application. If possible, it should be applicable in oral and/or parenteral form.

The chelating ligand must possess a very low intrinsic toxicity in order to allow its use at relatively high doses. As the complex formation is an equilibrium reaction, it becomes necessary to employ high doses during adequate periods of time to ensure the remotion of all or of the greatest part of the toxic metal (eventually, repeated application of the chelator may be necessary). This also ensures its use in the case of chronic intoxications and long term therapies. On the other hand, the hydrophilicity/lipophilicity concept is also of central importance for the correct chelating agent selection: a greater lipophilicity is related to a most easy penetration of the blood/brain barrier (cf. also Sect. 1.2.4.).

The Pearson hard-soft acid-base concept (HSAB) [1,26] is also very useful in the selection of an adequate chelating ligand [29]. In this classification the term "soft" refers to species that are large and fairly polarizable, whereas "hard" species are small and less easy polarized. Metal ions can be considered as Lewis acids (σ-acids) [31]. Although exceptions exist, the general rule is that hard acids bind preferentially to hard bases and soft acids to soft bases. It is interesting to emphasize that most of the biologically relevant cations are hard (Na^+, K^+, Mg^{2+}, Ca^{2+}, Fe^{3+}) ore borderline (Fe^{2+}, Cu^{2+}, Zn^{2+}, Co^{2+}) acids and show preference for oxygen or nitrogen containing donors. On the contrary, most of the non-essential toxic elements (Cd^{2+}, Hg^{2+}, Tl^+, Pb^{2+}) are soft acids that prefer to interact with sulfur containing donors.

1.2. Chelating Ligands and their General Chemical, Biochemical and Toxicological Properties

In this section the general properties and characteristics of the most common, best known, and widely used chelating agents are briefly discussed. The characteristics of some other chelators, restricted to the treatment of some peculiar problems and disorders shall be discussed in some of the following sections.

The first use of a chelator with clinical purposes seems to be the attempt to reduce acute lead intoxications by means of citric acid, in 1941 [29]. Although the success of these treatments was relatively limited, they started a new era in the treatment of metal intoxications.

1.2.1. 2,3-Dimercaptopropanol (BAL)

During World War II, 2,3-dimercaptopropanol (BAL, British Anti-Lewisite, Fig. (**2**)) was developed as an antidote to the war gas β-chlorovinyldichloroarsine (Lewisite). This gas was fortunately never used during this war and so, immediately after the ending of the conflict, BAL was introduced into the clinical praxis. Its use expanded rapidly, not only for the treatment of arsenic intoxications but also in the cases of cadmium, mercury and lead poisoning [24, 25,32], probably as a consequence of the lack of other adequate chelating agents at this time [29,30].

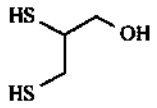

Fig. (2). Schematic structure of 2,3-dimercaptopropanol (BAL).

From the chemical point of view, BAL is a relatively unstable molecule, which is susceptible to oxidation and, therefore, difficult to store [25]. It can only be administered by intramuscular injection of oily solutions, which are very painful and usually accompanied by a number of secondary unpleasant side-effects. Extensive research into the pharmacokinetics of BAL, has shown that absorption from the site of injection is complete and rapid and, apparently, distributed into the intracellular space. The major part of the dose is rapidly excreted by urine. However, BAL is far from being an ideal chelator not only due to its toxicity and low therapeutic efficacy but also to the frequency of numerous unpleasant side effects [24,25,29,30]. On the other hand, it has

been found that BAL causes severe neurotoxic effects [33,34], induces redistribution of mercury from peripheral organs to the brain of experimental animals [35] and in the case of lead, the Pb/BAL complex is almost as toxic as lead [36,37]. Hence, the present knowledge on this agent suggests its use only for brief treatments of acute intoxications [30].

1.2.2. Ethylenediaminetetraacetic acid (EDTA)

The next chelating agent to come into clinical use was EDTA (ethylenediaminetetraacetic acid, Fig. (3)), initially for the treatment of lead intoxications.

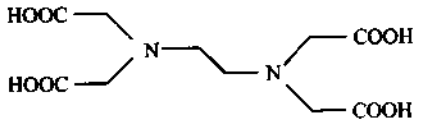

Fig. (3). Schematic structure of ethylenediaminetetraacetic acid (EDTA).

EDTA, is a white crystalline solid, sparingly soluble in water [38]. It is rapidly excreted in urine and is not metabolized in the human body [24,30]. It is a weak tetrabasic acid (the pK values for the ionization equilibria being $pK_1 = 2.00$; $pK_2 = 2.67$; $pK_3 = 6.16$; $pK_4 = 10.26$ [39]) and as each of the nitrogen atoms has an unshared electronic pair, the molecule has six potential sites for metal ion bonding and, as a consequence, it should be described as a hexadentate ligand [24,38]. EDTA forms very stable complexes with most metallic cations and the degree of complex formation depends upon the pH of the environment and the stability of the particular complex.

Table **2** shows the stability constants of EDTA complexes of some essential and toxic metals [39,40]. These data show that the stability constants of the complexes with toxic metals, such as, cadmium, lead or mercury are quite similar to those with some of the essential elements such as zinc, copper or cobalt; however, that of the calcium complex is many orders of magnitude lower. These observations have important implication for chelation therapy. First, the mobilization and excretion of zinc, copper, and other essential trace elements are likely to be increased, along with that of the toxic elements during EDTA treatments, disturbing different biological processes [41-44]; and secondly, the removal of Ca(II) may also affect the bone structure of mammals whereas the chelation of the ionic calcium present in blood, can cause tetany and even death. Therefore, it is very important to control the depletion of calcium during these therapies.

Additionally, this chelating agent has other disadvantages, not only due to the mentioned high stability constants of its complexes with some essential metals, but also due to low intestinal uptake, needing slow intravenous administration, and their exclusively extracellular distribution. Thus, induction of hypocalcemic tetany during intravenous infusion is a potential complication, and zinc depletion is a possible side effect during prolonged use. To avoid some of the mentioned problems the chelator is generally used in the form of $CaNa_2EDTA$, Ca_2EDTA and, in certain cases, also as Zn_2EDTA. For example, the use of $CaNa_2EDTA$ for the treatment of lead poisoning, does not disturb body calcium levels, because Ca(II) is readily exchanged for Pb(II), as suggested by the very different stability constants of both EDTA complexes (cf. again Table **2**).

As mentioned above, the major side effect of EDTA chelation therapy is hypocalcemia, a condition which is enhanced by too rapid administration of the chelator. This effect is usually controlled, not only by the use of $CaNa_2EDTA$ but also by concomitant infusion of calcium gluconate [24,30]. In the same form, teratogenicity, caused by Zn(II) depletion, can be readily reversed by coadministration of zinc [24]. On the other hand, when administered in high doses EDTA is potentially nephrotoxic [24,25,45,46] and nephrotoxicity may be especially important in the case of elderly patients with preexisting impairment of renal function [46]. Another drawback associated with EDTA-chelation is the possible redistribution of some of the metal complexes to the brain and the liver [25,47,48].

On the contrary, the use of chelation therapy with intravenous EDTA for the treatment of atherosclerosis has rapidly increased worldwide and more than one million patients received this treatment during the last forty years [49]. Although controversial in many aspects, it has been used to treat and prevent cardiovascular disease, atherosclerosis, coronary hearth diseases, and other age-related problems. This therapy, when adequately applied, seems to improve metabolic function, increasing blood flow, and favoring the opening of blocked arteries throughout the body [46,49].

Table 2. Stability Constants for EDTA Complexes with Some Essential and Toxic Metals (at 25 °C and Ionic Strength = 0.1) (from [39,40])

Essential metal	log β	Toxic metal	log β
Mg(II)	8.83	Tl(I)	6.41
Ca(II)	10.61	Cd(II)	16.36
Zn(II)	16.44	Hg(II)	21.50
Fe(II)	14.27	Pb(II)	17.88
Fe(III)	25.0	Al(III)	16.50
Mn(II)	13.81	Th(IV)	23.2
Cu(II)	18.70		
Co(II)	16.26		

β defined as: [M(EDTA)]/[M].[EDTA].

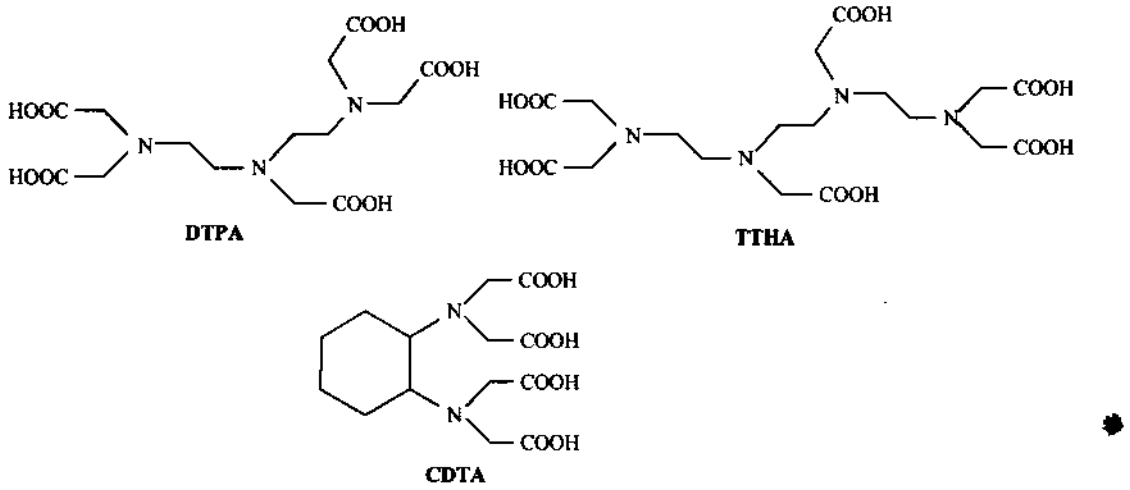

Fig. (4). Schematic structures of diethylenetriaminepentaacetic acid (**DTPA**), triethylenetetraaminehexaacetic acid (**TTHA**) and cyclohexane-1,2-diaminetetraacetic acid (**CDTA**).

1.2.3. Other Polyaminopolycarboxylic Acids

The usefulness and extensive applications of EDTA, encouraged further studies with other polyaminopolycarboxylic acids, which were also gradually introduced in chelation therapies. Three of the most important examples are presented in Fig. (**4**) and selected stability constants of complexes of these chelators are shown in Table **3** [39,40]. As it can be seen, the respective complexes of Cd(II), but specially those of Hg(II), present a higher stability than those of the respective EDTA complexes. After EDTA, DTPA is probably the most used of these polyaminopolycarboxylic chelators. Also in this case the corresponding Ca(II) or Zn(II) salts are often employed [24].

Since the advent of nuclear fission during the 1940s, mankind has been increasingly exposed to the possibility of radionuclide intoxication from experimental tests of nuclear weapons and the expanding use of nuclear fission as a source of energy. Some radionuclides, especially those of strontium, lead, the lanthanides, uranium, thorium and plutonium are removed quite rapidly from the blood and deposited in bone, where they are largely retained. Therefore, it is desirable to remove radionuclides from the body as rapidly as possible, and chelation therapy constitutes also in this case the most adequate option.

The therapeutic removal of plutonium has been particularly well-investigated and DTPA is at the present time the undisputed agent of choice [50]. The stability constant of the Pu(IV)/DTPA complex (log value) is 27.50, compared with the figure of 24.60 for the respective EDTA complex [50]. Besides, and as it can be seen from the stability constants presented in Tables **2** and **3** both, DTPA and CDTA, are also good chelating agents for Th(IV) and CDTA appears also adequate for U(IV) binding.

On the other hand, DTPA and CDTA have been found effective in the mobilization of zinc [32] and CDTA has also

Table 3. Stability Constants for CDTA, DTPA and TTHA Complexes with Some Essential and Toxic Metals (at 25 °C and Ionic Strength = 0.1) (from [39,40])

CDTA-complexes		DTPA-complexes		TTHA-complexes	
Metal	**log β**	**Metal**	**log β**	**Metal**	**log β**
Mg(II)	11.07	Mg(II)	9.34	Mg(II)	8.43
Ca(II)	13.15	Ca(II)	10.75	Ca(II)	9.89
Cu(II)	21.92	Cu(II)	21.38	Cu(II)	20.5
Co(II)	19.58	Co(II)	19.15	Co(II)	18.4
Fe(II)	18.90	Fe(II)	16.4	Fe(II)	17.0
Mn(II)	17.53	Mn(II)	15.51		
Zn(II)	19.35	Zn(II)	18.29	Zn(II)	18.0
Cd(II)	18.94	Cd(II)	19.0	Cd(II)	18.6
Hg(II)	24.79	Hg(II)	26.40	Hg(II)	26.1
Pb(II)	20.24	Pb(II)	18.66	Pb(II)	18.5
Th(IV)	25.6	Th(IV)	28.78		
U(IV)	27.6				

β defined as: [M(EDTA)]/[M].[EDTA].

been assayed for lead and manganese intoxications, whereas its efficacy for strontium removal remains controversial [32].

1.2.4. meso-2,3-dimercaptosuccinic Acid (DMSA) and 2,3-dimercapto-1-propanesulfonate (DMPS)

During the 1950s two new chelating agents, *meso*-2,3-dimercaptosuccinic acid (DMSA) and 2,3-dimercapto-1-propanesulfonate (DMPS) (Fig. (**5**)) were already used in China and the former Soviet Union [25,29,30] but become available in Western countries only during the last three decades.

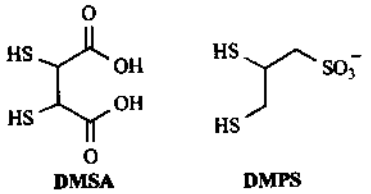

Fig. (5). Schematic structures of *meso*-2,3-dimercaptosuccinic acid (**DMSA**) and 2,3-dimercapto-1-propanesulfonate (**DMPS**).

Due to the presence of the two vicinal –SH groups these chelators have a strong affinity for "soft" metals, forming very stable complexes with many of them. These two thiol groups also generate an important reducing power; which is stronger in the case of DMPS [51]. The polar acid groups influence the overall physicochemical behavior of these compounds and are also responsible for their water solubility.

It is also interesting to compare the octanol/water partition coefficients of these chelators with that of the related BAL. The following values were found: 0.083 for DMPS, 0.047 for DMSA and 5.087 for BAL [52], showing that the two polar compounds (DMPS and DMSA) are highly soluble in water whereas the lesser polar BAL molecule can easily cross the blood/brain barrier.

Both, DMSA and DMPS are very useful antidotes in acute or chronic intoxications with many divalent metals and constitute valuable alternatives to the classic chelators BAL and EDTA [25,53,54]. Besides, DMSA and DMPS have other advantages as they are less toxic and can be administered orally or parenterally and, additionally, DMSA chelation decreases the brain deposition of lead whereas DMPS does not redistribute arsenic, lead or mercury to the brain [25,30,32,55]. Notwithstanding, and interestingly, both chelators as well as its complexes with Cd(II), Hg(II) and Pb(II) inhibited the activity of δ-aminolevulinate dehydratase [37,56,57].

DMSA is usually employed directly in the form of the free acid whereas DMPS is commercialized in the form of its monohydrated sodium salt. Of all the dimercapto chelating agents so far employed as clinical chelators, DMSA is the least toxic [25].

After oral administration, DMSA is rapidly but incompletely absorbed. It is rapidly metabolized and excreted mainly by the urinary tract and the greatest part of the excreted drug is present in the form of a mixed sulfide with L-cysteine [25]. DMPS is also excreted in urine, but over a longer period of time than DMSA and appears transformed in cyclic and acyclic sulfides [25].

1.2.5. Monoalkyl Esters of DMSA

Although, and as discussed in the previous section, DMSA is an effective and potent chelating agent, because of its hydrophilic and lipophobic properties, it is unable to pass through cell membranes, and as a consequence, it cannot capture intracellulary deposited toxic species. Thus, a large number of esters of DMSA have been synthesized to overcome this problem and to enhance tissue uptake. In order to make the compounds more lipophilic, the length of the carbon chain of the parent DMSA was increased by controlled esterification with different alcohols (methyl, ethyl, propyl, isopropyl, butyl, isobutyl, pentyl, isopentyl, and hexyl) [58,59]. Most of these esters have been assayed for the treatment of metal poisoning, and it has been reported that they have better potential for the mobilization of toxic metals than DMSA [58]. Among these new chelators, the monoisoamyl ester of DMSA (MiADMSA, Fig. (**6**)) has been found particularly effective for the detoxification of cadmium, mercury and arsenic [32,58,60-63]. Although the toxicity of DMSA is lower than that of MiADMSA in terms of LD_{50}, the interaction of both compounds with essential elements is similar [32].

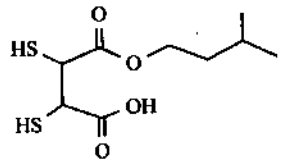

Fig. (6). Schematic structure of the monoisoamyl ester of *meso*-2,3-dimercaptosuccinic acid (MiADMSA).

1.2.6. Lipoic Acid (LA) and Dihydrolipoic Acid (DHLA)

Lipoic acid (1,2-dithiolane-3-pentanoic acid, 1,2-dithiolane-3-valeric acid or thioctic acid, LA, Fig. (**7**)) is a sulfur containing cofactor, and in its reduced form, the dihydrolipoic acid (6,8-dimercaptooctanoic acid or 6,8-thioctic acid, DHLA, Fig. (**7**)), two thiol groups per molecule are present, resembling some of the chelating agents discussed above.

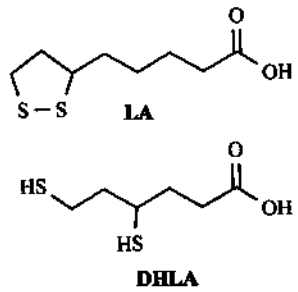

Fig. (7). Schematic structures of lipoic acid (**LA**) and dihydrolipoic acid (**DHLA**).

Lipoic acid is well known as a powerful antioxidant and is commonly available as a nutritional supplement [64], being absorbed intact from dietary sources, and transiently accumulated in many tissues [65]. Typical dietary sources are muscle meats, hearth, kidney and liver, and to a lesser degree fruits and vegetables [65,66]. It is an important cofactor for mitochondrial α-ketoacid dehydrogenases and there is a growing evidence that orally supplied LA may not be used as

a metabolic cofactor but instead, elicits a unique set of biochemical activities with potential pharmacotherapeutic value [65]. Notwithstanding, while the direct biochemical roles of LA as a cofactor are relatively well understood, less is known about the precise metabolic functions of orally supplied LA.

In 1966, German physicians started to administer LA to patients with liver cirrhosis, mushroom poisoning, heavy metal intoxication and diabetic polyneuropathy [67], initiating the clinical use of this compound.

The chemical reactivity of LA is mainly conferred by its dithiolane ring. The oxidized (LA) and reduced (DHLA) forms generate a very potent redox-couple, possessing a standard reduction potential of – 0.32 V. This makes DHLA one of the most potent naturally occurring antioxidants and there is evidence that both LA and DHLA are capable of scavenging a variety of reactive oxygen species [65].

From the point of view of this review it is of special interest to mention that both, LA and DHLA chelate different metals *in vitro* and *in vivo* [65,67-69]. In polar but non-aqueous solvents LA forms complexes with Mn(II), Cu(II), Zn(II), Cd(II), Pb(II) and Hg(II) [70-72] whereas DHLA chelates Co(II), Ni(II), Cu(II), Zn(II), Hg(II) and Pb(II) [71,72]. Besides, a series of DHLA complexes with Ni(II), Co(II), Hg(II) an Cu(I) were obtained also in non-aqueous media and its spectroscopic characterization confirmed the metal binding to the pair of deprotonated thiol groups [73].

The complexation of metals by DHLA may also result in antioxidant activity [65], as suggested by lipid peroxidation induced by Cd(II) [67,74] or by the inhibitory effect of LA on the Cu(II)-catalyzed ascorbic acid oxidation [75]. DHLA is also an effective reducing agent for some transition metals. For example, it can easily reduce Fe(III) to Fe(II) [67] and *in vitro* it was shown that excess of DHLA could effectively compete with ferritine, liberating Fe(III) from this iron-storage protein [76], but it is not known if this process occurs *in vivo*, too [67]. DHLA also interacts with Cu(II) in a dose dependent manner, generating pro-oxidant or anti-oxidant properties, depending upon the Cu(II):DHLA ratio and the pH-value [77].

Taking into account that the oxidative stress may be a possible mechanism of lead toxicity [78,79], it has also been suggested that LA in combination with DMSA, should be an adequate therapeutic combination in the treatment of lead poisoning [78]. On the other hand it has been found in animal experiences that biliary excretion of inorganic Hg(II) was strongly enhanced by LA administration, whereas the excretion of Zn(II), Cd(II) and Cu(II) diminishes, suggesting different interaction mechanisms for these metals and, apparently, the formation of a relatively stable DHLA/Hg(II) complex [80]. Therefore, a grown body of evidence suggests that DHLA chelates transition metals and mitigates metal-catalyzed free radical reactions. The way in which LA/DHLA effectively chelates and removes transition metals *in vivo* is still to be fully elucidated [65], but it is evident that their action depends on dosage size and, eventually, on the spacing of dosages in time [69]. It is also very important to emphasize that DHLA also has the capacity to regenerate the endogenous antioxidants vitamin E, vitamin C and glutathione [67]. Besides, the usefulness of LA in the treatment of diabetes [81] and as an effective protector in numerous neurodegenerative disorders [82] has been increasingly accepted.

It is evident, that there are only few compounds as multifaceted as LA as bioactive agents and it will be important to define the precise cause-and-effect relationship between LA and its cellular targets of immediate action. More research is significantly necessary to understand physiological uptake, accumulation and metabolism of LA and its metabolites to define appropriate cell-based models to examine LA action [65].

1.3. Metal Intoxications and its Treatment

1.3.1. Brief Overview of Chelation Therapies Applied to Some Toxic Elements

Different elements, such as aluminum, antimony, arsenic, bismuth, cadmium, lead, mercury, nickel, and thallium can be toxic upon adventitious exposure, caused by overingestion, pesticide exposure, or other environmental or occupational exposures [2]. Although at present such intoxications are relatively rare, most of them can be adequately treated by the different chelating agents discussed in the previous sections. As extensive reviews on this subject matter have been provided recently by Andersen [29,30], in this section only a brief overview, including some recent references, is presented and the readers are referred to the two mentioned articles for a more detailed information and bibliography. Moreover, the producers of DMPS have also prepared a very complete and outstanding monograph covering all aspects of the chemistry, biochemistry, toxicology and applications of this chelating agent, containing more than 1600 references [83]. In general, it appears that DMPS is the best antidote for the treatment of heavy metal poisoning, which can even be applied preventively if such intoxication is suspected [83].

Although BAL has been used in the treatment of lead, mercury and arsenic intoxications, its future use is questionable, for the reasons discussed above and by the development of newer and more efficient chelating agents.

DMSA was approved by the US FDA for the treatment of childhood lead poisoning [25,32] and is also very effective in the treatment of cadmium, mercury and arsenic intoxications. One of the major drawbacks with the use of DMSA in the treatment of lead poisoning is that it is basically a soft tissue lead mobilizer which cannot remove lead from bone and other hard tissues. Combined treatment with DMSA and $CaNa_2EDTA$ seems useful to overcome this problem [84], as is also the use of the esters of DMSA [85], in particular the previously mentioned MiADMSA [32]. This ester is also useful for the treatment of mercury and cadmium intoxications [32,60,61].

On the other hand, DMPS has shown to be effective for the removal of toxic forms of arsenic, lead and mercury, and is registered in Germany for the treatment of mercury intoxications [25] and most recently in Germany and Austria for treatment of lead intoxications [83]. In addition, the World Health Organization Expert committee has considered it as the first-line drug for ascertained inorganic mercury acute or chronic poisoning [86,87]. This drug appears also as very

effective in the case of beryllium and bismuth intoxications [83].

Recently, it has been pointed out that the combined use of DMSA or MiADMSA and some natural antioxidants, such as vitamin C or vitamin E, may improve the action of these chelating agents in the treatment of chronic lead intoxications [88].

The case of cadmium is relatively complex. BAL was shown to potentiate cadmium toxicity. On the contrary, oral administration of DMSA is relatively efficient. DTPA and TTHA (see Fig. (**4**)) are also very efficient antidotes for oral cadmium toxicity. On the basis of numerous studies, the optimum chelation treatment for acute oral human cadmium intoxication using chelators would be oral administration of DMSA combined with parenteral administration of Ca-DTPA [29]. For the mobilization of aged cadmium deposits MiADMSA seems to be particularly useful.

From this brief analysis it becomes evident that DMSA and DMPS are evidently superior, in most aspects, to other chelators, but their potential have not yet fully recognized within clinicians. On the hand, they are often forced to choose less effective therapies for their patients because the unavailability of DMSA and DMPS in many countries.

1.3.2. Treatment of Vanadium Toxicity

Environmental contamination by vanadium has dramatically increased during the last decades, especially in the most developed countries due to the widespread use of fossil fuels, many of which liberate finely particulate V_2O_5 to the atmosphere during combustion [89,90]. Therefore, and also owing to the emerging interest in the pharmacological effects of some of its compounds [91-94, and cf. also the contributions of Barrio & Etcheverry and Costa Pessoa & Tomaz, in the present issue] the toxicology and detoxification of vanadium constitute areas of increasing interest [95]. As chelation therapies applied to vanadium detoxification has never been discussed in detail in previous reviews on this subject matter, we have now analyzed this aspect in a more extended way.

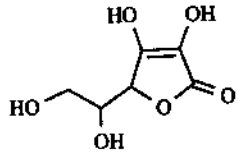

Fig. (8). Schematic structure of L-ascorbic acid.

Most of the so far known and previously discussed chelating agents have been tested, with varying success, for vanadium detoxification, generally with laboratory animal experiments. Reported systems include EDTA and related polyaminopolyarboxylic acids, BAL, D-penicillamine, as well as different phosphonic acids [95,96].

From all the vanadium detoxification agents investigated so far, ascorbic acid (Fig. (**8**)) appears the most effective for human use, as shown by systematic and comparative studies of a wide number of antidotes of very different chemical characteristics and properties [95,96]. It is probably the least toxic of all the so far examined drugs and can be administered orally in relatively large doses. Its strong detoxification activity can surely be related to the facility with which it reduces vanadium(V) to VO^{2+} [95,97,98]. This oxocation could be then complexed by an excess of the vitamin but, as it is known from the general behavior of metallic ascorbate complexes [97] and confirmed by detailed studies on the oxovanadium(IV)/ascorbate system [99], the stability of these complexes is relatively low, which, in the case of VO^{2+} species, is also in agreement with the absence of chelate binding [99]. This fact suggests that this type of complexes would not be useful for the stabilization and excretion of reduced vanadium. Consequently, it can be suggested that a better way for the elimination of the generated oxovanadium(IV) may be its chelation to some of the oxidation products of vitamin C.

As it is known [95], dehydroascorbic acid, generated as the primary oxidation product is also very unstable and undergoes a rapid series of transformations as shown schematically in Fig. (**9**). It is degraded first to 2,3-diketogulonic acid which can further be degraded to a mixture of oxalic and L-threonic acids. At higher pH-values, the latter acid is finally oxidized to tartaric acid. Although all these species could interact with the VO^{2+} cation, a detailed investigation of these ligand systems showed that the primary complex generated by interaction of the oxocation with dehydroascorbic acid is very unstable towards oxidation. It is hydrolyzed irreversibly with opening of the lactone ring generating 2,3-diketogulonic acid producing a 2:1 ligand to metal complex [99], in which the enolized form of the mentioned acid acts as a bidentate chelator of the cation, as shown in Fig. (**10**). This species seems to be very stable, as we could obtain different complexes of this type not only starting with the system VO^{2+}/dehydroascorbic acid [99] but also by direct reduction of vanadate(V) with ascorbic acid [100].

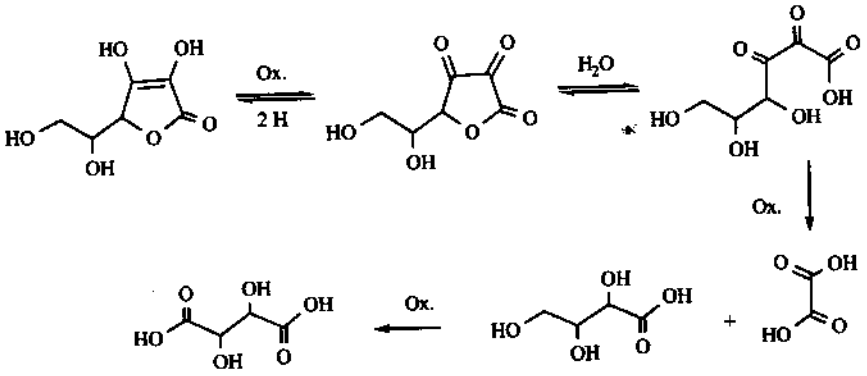

Fig. (9). Schematic representation of the stepwise oxidation of L-ascorbic acid.

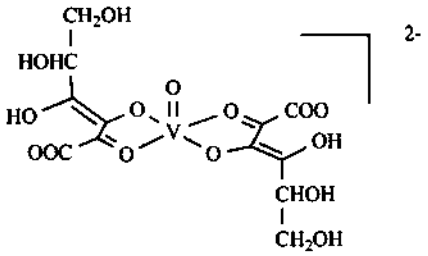

Fig. (10). Structural model proposed for the chelate species generated by interaction of the VO^{2+} cation with 2,3-diketogulonic acid (from [99]).

The interaction of different vanadium species with DMSA and DMPS (Fig. (**5**)) was also investigated. The interaction of the VO^{2+} cation with both chelating agents, investigated by electron absorption spectroscopy, in aqueous solutions at different pH-values, shows the generation of the chelated species $[VO(DMSA)_2]^{2-}$[101] and $[VO(DMPS)_2]^{4-}$ [102], respectively, in which the oxocation interacts with two pairs of deprotonated –SH groups of the ligands. It was also found that both, DMSA and DMPS, rapidly reduces VO_3^- to VO^{2+} and also produce the partial reduction of V_2O_5 suspensions at neutral or slightly acidic pH-values [101,102].

Most recently, the interaction of vanadium species with the monoisoamyl ester of DMSA (Fig. (**6**)) was investigated too [103]. Also in this case, the interaction with the oxovanadium(IV) cation generates a complex of $[VO(MiADMSA)_2]^{4-}$ stoichiometry. This chelating agent also reduces rapidly vanadium(V) to VO^{2+} and produces a relatively rapid reduction of V_2O_5 suspensions at pH = 6.5, suggesting that MiADMSA may be a better reducing agent for vanadium(V) than DMSA and DMPS [103].

To conclude, the results obtained with the three mentioned chelators clearly show that they appear as very interesting and promising agents for the detoxification of vanadium(V), and merit to be further explored.

2. CHELATING AGENTS IN THE TREATMENT OF DISEASES

2.1. General Aspects

It is well-known that a number of diseases and physiological disorders are often related with the presence of an excess or accumulation of certain essential elements in different parts of the body [2,3,104]. Therefore, chelation therapies may be especially useful and adequate for the treatment of such disorders. As the knowledge on metal transport, of the relation between metal accumulation and disease as well as on the origins and reasons of these accumulations have advanced, the design of better and more efficient and specific chelating agents has advanced concomitantly.

In this section three selected problems shall be discussed: Wilson disease, a genetic disorder which produces copper accumulation, the use of chelating agents for the treatment of iron overload, and Alzheimer disease and some other neurological disorders strongly related to the presence and activity of certain metal species present in the brain.

2.2. Wilson Disease

Wilson disease is an autosomal recessive disorder of copper transport resulting in the accumulation of this element in many organs and tissues of the body, especially in the liver and brain [2,104-109]. Wilson disease displays extensive clinical heterogeneity, with symptoms that are largely non-specific, difficulting diagnosis. Patients with this disease can be broadly divided into three groups: those displaying hepatic symptoms, those displaying neurological symptoms, and those displaying both neurological and hepatic symptoms [105,110]. Deposition of copper in the limbus of the cornea gives rise to the dull-yellow Kayser-Fleischer rings which are a hallmark of the disease [2,104,106,110].

The progression of the disease can also be roughly divided into three stages: 1. copper accumulation in the cytosol of hepatocytes; 2. increase of cytosolic copper levels cause the cells to begin to move some of the copper into lysosomes, probably in the form of Cu/metallothionein; 3. the high levels of copper lead to hepatic necrosis and to the release of copper into the bloodstream, causing damage to erythrocyte membranes leading to hemolytic anemia. The final stage of the disease involves accumulation in other organs such as the brain and kidneys. Defective biliary excretion of copper may be the single most important cause of copper accumulation [105,110]. The neurological symptoms associated with Wilson disease are highly varied and may include deteriorating coordination, rigidity, tremors, dementia, personality changes, slurry of speech, and behavioral problems [110].

Treatments for Wilson disease have, obviously, been centered on chelation therapy, with the aim of mobilizing copper from the affected organs and promoting its excretion. The first assayed chelating agent was BAL (Fig. (**2**)) [104,105] and, although it shows significant clinical improvement of patients after long-term use [110], it was rapidly abandoned, due to the different inconveniencies and problems mentioned above (Sect. 2.1.1).

The next most frequently used chelating agent was the orally active D-.penicillamine ((2S)-2-amino-3-methyl-3-sulfanyl-butanoic acid, Fig. (**11**)) [104,105,110]. The selection of this chelator was based on an observation that patients receiving parenteral administration of penicillin excreted penicillamine in their urine, suggesting that the compound may be bioavailable in its reduced state and, therefore, useful for metal chelation [104]. Besides, it is less toxic and more effective than BAL [110].

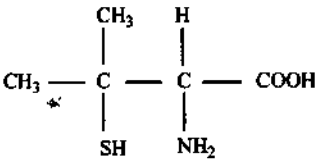

Fig. (11). Schematic structure of D-penicillamine.

Different authors have pointed out that the chelating properties of D-penicillamine alone cannot be responsible for the mobilization of toxic copper, and usually a mechanism called reductive chelation has been proposed, in which ini-

tially unstable Cu(II) complexes are formed that finally yield Cu(I) and the oxidized chelator [109]. Different studies, focused on this possibility have been performed, suggesting the formation of mixed valence Cu(I)/Cu(II) complexes as the responsible species for copper elimination [109]. Birker and Freeman isolated a purple mixed valence cluster complex of stoichiometry $[Cu(II)_6Cu(I)_8(penicillamine)_{12}Cl]^{5-}$ and determined the structure of its hydrated thallium salt [111]. The complex is very stable at physiological pH and saline concentration, but decomposes relatively rapid in urine under aerobic conditions. This structure determination and the detected properties of this complex give some insights into the chelating action of D-penicillamine [109,111]:

- Cu(II) is in equilibrium with the aqueous medium strongly coordinated by N and S atoms, while Cu(I) is removed from equilibrium.
- The CH_3-groups of the chelator are essential in preventing Cu(I) oxidation.
- The high aqueous solubility of the complex derives from the 12 negatively charged $-COO^-$ groups on the cluster "surface".
- Chloride is essential for the formation of the complex, playing an important structural role

The peculiar stability of this cluster, together with the ability of D-penicillamine to act in the dual capacity of reducing and chelating agent, makes this chelator a very effective agent for copper elimination [111].

Despite these very good characteristics for the treatment of Wilson disease, producing significant clinical and biological improvements [105,109,110] D-penicillamine is not totally free from side-effects which appear in a considerable fraction of patients during continued use, mainly hypertension, nephritic syndrome and various autoimmune reactions [29,30]. Besides, and due to the facility with which it binds copper, it removes a significant amount of this element from the body and induces a negative copper balance over time [104].

Another possible disadvantage of D-penicillamine is its high hydrophilicity, which would prevent the drug from being able to passively permeate the blood/brain barrier. For this reason, and following the same ideas as in the case of DMSA (Sect. 1.2.5.) more lipophilic ester derivatives, using methyl-, hexyl- or benzyl-alcohols, have been recently prepared, in order to increase the cellular uptake of the chelator [112].

D-penicillamine has also been used, with variable success, for the treatment of intoxications with some heavy metals, for example lead or mercury [29,30,113].

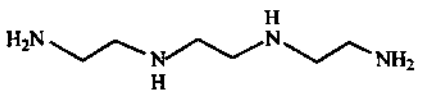

Fig. (12). Schematic structure of triethylenetetramine (TETA).

Triethylenetetraamine (TETA, Fig. (**12**)) was introduced in 1982 to be used in patients intolerant to D-penicillamine, although it is considered less potent [104]. It can also be orally administered but shows a poor intestinal absorption [30, 109]. So far, there is less experience with this drug than with D-penicillamine and, therefore, its toxicity is relatively unexplored [105] although, apparently, its acute toxicity is low and it is also free of some of the side-effects reported for D-penicillamine [30].

Another interesting chelator assayed is the tetrathiomolybdate anion, usually administered as $(NH_4)_2MoS_4$ [104,105]. It is well known that molybdate induces copper deficiency in ruminants [2]; the MoO_4^{2-} is converted to MoS_4^{2-} by bacterial action in the animal's rumen and can interact with copper. This process appears to follow two pathways; one operates in the gastrointestinal (GI) tract and the other in the blood. In the GI, MoS_4^{2-} forms complexes with copper and food proteins, i.e., when administered orally MoS_4^{2-} may block absorption of food and endogenously secreted copper. In the blood, the tetrathioanion forms a ternary complex with copper and serum albumin. In both cases, the complexed copper is rendered unavailable for cellular uptake [114, 115]. In short term experiments it was found that this compound may be useful for an initial treatment of Wilson disease [114]. Notwithstanding, taking into account the possible toxicity of tetrahiomolybdate [109], one must be very careful in administering this compound [105].

It is also interesting to mention that in the last years increasing attention has been given to the use of Zn(II) salts for the treatment of Wilson's disease [104,110,116]. Whereas all the previous mentioned chelation therapies promote the excretion of copper through urine, zinc therapy promotes its fecal excretion. It is admitted that Zn(II) favors copper excretion by inducing the synthesis of metallothionein in the intestine and thereby blocking the absorption of copper. In spite of the fact that zinc has a greater ability to induce metallothionein synthesis than does copper, metallothionein has a greater affinity for copper than for zinc [117]. In this way Zn(II) inhibits absorption of copper from the intestine and increases the fecal elimination of copper. It inhibits not only the absorption of copper from food, but also blocks the reabsorption of endogenously secreted copper from saliva and gastric juice [105]. Another possible mechanism for the efficacy of Zn(II) salts may be the induction of hepatic metallothioneins to bind hepatic copper and reduce further damage of liver [104]. Today, zinc salts are often administered together with chelating agents and it has also been suggested that chelators may be discontinued after initial treatment, leaving only zinc supplementation for maintenance therapy [104, 116].

Finally it should be mentioned that in China, hundred of patients with Wilson's disease, have been successfully treated with oral DMSA (Sect. 1.2.4.) during the last 40 years [29,104]. On the other hand, recent studies with DMPS suggest that also this chelating agent may be potentially useful for the treatment of this disease [83].

2.3. Iron Overload

The importance of well-defined amounts of iron for the survival, replication and differentiation of the cells of animals, plants and almost all microorganisms is well established [1-3,118,119]. Iron deficiency is the most frequent nutritional problem in the world [21] but excess of iron is toxic, particularly in man. In healthy human subjects iron

metabolism is conservative, the amount of iron absorbed from the diet being balanced by that lost [2,118]. Disorders in iron metabolism, characterized by iron deficiency or overload, are relatively common.

Genetic hemochromatosis is a very frequent disorder in humans, characterized by a defect in the regulation of iron absorption which results in a progressive accumulation of this element in parenchymal tissues generating liver, pancreas and hearth dysfunction [118]. Secondary iron overload is found in disorders such as β-thalassemia, sickle-cell disease and a number of other disorders in hemoglobin synthesis which are characterized by anemia and require frequent blood transfusions. These transfusions solve the problem in the short term, but, in view of the almost non-existent iron excretion from man, iron accumulation is inevitable. Without treatment, iron accumulates in liver, hearth and joints, among other tissues, and can finally lead to organ failure and death [104,120,121].

Since the mid-1960s, desferrioxamine B is almost the only drug currently available for the transfusion-related iron overload [104,119-122].

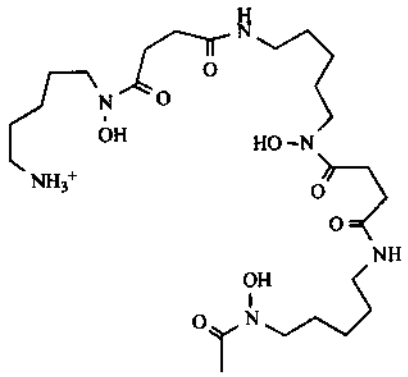

Fig. (13). Schematic structure of desferrioxamine B.

Desferrioxamine B (Fig. (**13**)) a fungal siderophore, isolated from *Streptomyces pilosus*, is an hydroxamate-based hexadentate trivalent metal ion chelator. Because of its high hydrophilicity and peptidic nature, it displays very poor oral bioavailability and must be administered by slow subcutaneous infusions over 8-12 hours, 5-7 days per week for life [121,123]. This protocol leads to high cost of treatment and patient compliance and, additionally, the chelator can also induce allergic reactions and other side effects, such as ophthalmic, pulmonary, renal and auditory toxicity, leucopenia and skin rashes [124]. For these reasons, the need for an orally active iron chelator was apparent from the very beginning of chelation therapy [121,122] and, consequently, hundreds of compounds have been tested both *in vitro* and in iron-overload animal models [104,121,122,125].

EDTA and other of the polyaminopolicarboxylic acids discussed above (Sect.1.2.2. and 1.2.3.) may be potentially useful, due to the "hard" nature of Fe(III), which favors interaction with oxygen-donors. Like desferrioxamine B, these chelating agents are not orally active and, additionally, present a low selectivity, and can easily deplete other essential metals. Notwithstanding, DTPA has been successfully used with some patients, provided that Zn(II) supplementation was administered simultaneously [119].

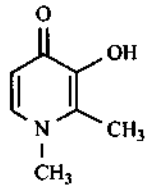

Fig. (14). Schematic structure of deferiprone.

A new advance in the field was generated by the introduction of deferiprone (1,2-dimethyl-3-hydroxypyridin-4-one, also known as L1, CP20 or Ferriprox; Fig. (**14**)), a low molecular weight, bidentate orally active iron chelator, belonging to the 3-hydroxy-4-pyridinone group compounds [104,121,122,126]. These compounds have a higher affinity for Fe(III) than similar oxygen-donating bidentate ligands do, due to aromatic resonance effects, which place additional electron density on the 4-position oxygen donor, making the deprotonated pyridinone pro-ligand a double oxo donor for metal cations [127].

A great number of 3-hydroxy-4-pyridinone derivatives has been prepared and investigated as possible iron chelators [104,119,122,127]. Variations in the N-substituents modulate the physicochemical properties and lipophilicity of these molecules. However, only few of these compounds have progressed to the clinical trial stage.

Notwithstanding its good chelating characteristics and high iron affinity, deferiprone remains controversial due to concerns about side-effects, although it is possible that lower and more frequent dosing of the chelator could attenuate these effects [124]. Because of these side-effects concerns, the drug is approved for use only as a second-line treatment after desferrioxamine B in the EU and is not FDA- approved [104,121]. Nevertheless, deferiprone offers a significant advantage over desferrioxamine B in its ability to remove cardiac iron [128].

The newest available iron chelator is deferasirox (4-[3,5-bis-(2-hydroxyphenly)-1,2,4,)triazol-1-yl]-benzoic acid), which emerged as the compound best combining high iron affinity, and selectivity, oral activity and tolerability. Following extensive clinical studies, it was approved by FDA in 2005 and it has since that moment been registered in more than 75 countries [121].

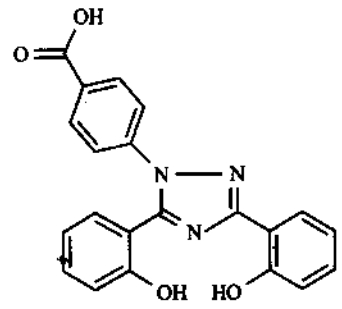

Fig. (15). Schematic structure of deferasirox.

As seen from Fig. (**15**), deferasirox is a tridentate chelator with NO_2 donor groups, involving one triazole nitrogen and two phenolate oxygens. It selectively binds Fe(II) and Fe(III) and shows little affinity for other essential divalent metals. Its relatively long half-life before excretion allows

once-daily dosage and good overall patient compliance. Besides, and because of its significant aromatic structure, it is able to permeate biological membranes [104].

A possibility to potentially expand the so far limited chelating possibilities for transfusional iron overload is to combine chelators [104,121,122,129]. These combinations could increase iron mobilization and excretion as a result of differential access to iron pools and may also allow dose reduction while maintaining efficacy. The desferrioxamine B/deferiprone combination [121,122,130] is increasingly used as is also that of desferrioxamine B/deferasirox [104,129]. In these cases, it has also been suggested the smaller bidentate or tridentate chelator functions as a "shuttle" to first bind the cation and then transfer it to the multidentate chelator which acts as a "sink" for the cation [104].

In the case of hemochromatosis, patients are not anemic and iron overload is usually treated by phlebotomy. Although this mode of iron removal is very effective and generally safe, it can not been applied in all patients suffering this disease, for example in cases of underlying anemia, heart problems, or poor venous access [121]. Thus, there is a need for an alternative [131], and this need may be satisfied again by the use of iron chelators.

In view of the differing iron loading patterns of transfusional iron overload (macrophages and hepatocytes) and hemochromatosis (mainly hepatocytes), the side-effect profile of any one chelator may differ in the two indications, mandating additional safety evaluation. As deferasirox has proved to be very efficient in removing iron deposits in liver [121], it constitutes a viable candidate to explore the value of chelation therapy in the hemochromatosis indication, which should be explored.

Finally, it is also interesting to mention that most of the assayed iron chelators are also good options for the removal of aluminum. Both, desferrioxamine B and deferiprone have been used [29,30,122]. Recent experiments with laboratory animals showed that the oral administration of deferiprone was very effective in enhancing urinary aluminum excretion [132].

2.4. Alzheimer's Disease

Alzheimer's disease (AD) is the main form of dementia, characterized by the loss of cholinergic neurons and the progressive deterioration of cognitive function, memory and self-care. It has long been associated with the accumulation of insoluble amyloid "plaques" in the brain. These plaques are formed by a process called *amyloidosis*, whereby a 40 to 43-residue peptide called β-amyloid (Aβ) aggregates into insoluble fibers [104,133-135]. Many other neurodegenerative diseases have been associated with the aggregation of specific proteins or peptides in different parts of the brain, including Parkinson's disease, Huntington's disease and prion diseases [133].

On the other hand, increasing evidence indicates that metal ion homeostasis is altered in AD and a number of metal cations such as Zn(II), Cu(II) and Fe(III), accumulate in the neuropil of the AD brain and are further enriched within amyloid deposits. In particular, Aβ binds these metal ions very avidly and this may explain their enrichment in plaque pathology. Plaque deposits also generate oxidative stress, which may originate in the formation of free radicals in the presence of redox-active metal cations [133,136-139].

It has been established that Cu(II) and Fe(III) interactions with Aβ mediate the toxicity of the peptide in cell culture. Aβ catalyses H_2O_2 generation through the reduction of Cu(II) and Fe(III), using O_2 and biological reducing agents such as vitamin C, cholesterol or catecholamines, as substrates. H_2O_2 is freely permeable across cell tissue boundaries and, unless scavenged by defense systems (catalase, glutathione peroxidase), it will react with reduced metal species (Cu(I), Fe(II)) to generate $OH^{\cdot}$ (Fenton reaction), which in turn generates lipid peroxidation adducts in various cellular compartments. Such oxidative damage typifies AD neuropathology and precedes Aβ deposition in this disease [136,140]. Oxidative stress is considered one of the main triggers of aging in the brain. Oxidative damage of macromolecules increases with age leading to a progressive decline in cell and tissue function [141].

As a result, if one admits that metal ions play a central role in AD, chelation therapy would be again an interesting option for its treatment. Obviously, a logical property of an adequate chlelating agent is to target Aβ oligomerization and Aβ-related generation of free radicals. Another important property of a potentially useful agent is its possibility to cross the blood-brain-barrier. This excludes a large number of the previously discussed systems, due to their hydrophilic nature [137]. At the present time, there is no obvious solution to the design of a non-toxic ligand with high affinities for iron, copper and zinc and the ability to mobilize such metals from intracellular sites [140]. The term metal-protein attenuating compound (MPAC) was coined to describe the approach of chelator introduction to disrupt specific, abnormal metal-protein interaction [104, 137], and it is very different from the process of chelation and excretion of bulk metal cations, as described in previous sections.

Some of the chelating agents discussed previously, such as desferrioxamine B, D-penicillamine, triethylenetetraamine and deferiprone, have been assayed, with variable success, for the treatment of AD [104,140]. Besides, some other chelating agents have been proposed on the basis of the MPAC concept. Some of them are briefly discussed in the following paragraphs.

One of the most interesting and recently explored chelators of this type is clioquinol (5-chloro-7-iodo-8-hydroxyquinoline, CQ, Fig. (**16**)). It is a now retired United States Pharmacopoeia antibiotic, which was formerly used as an anti-infective and antiamoebic agent. Studies with a transgenic mouse model of AD (Tg2576) have shown that CQ markedly reduces Aβ precipitation in the brain and, consequently, could be clinically useful [108,136,142-144]. Its favorable pharmacological effects are probably related to its high lipophilicity, which facilitates its penetration through the blood-brain barrier [136].

Unfortunately, the long-term use of CQ is limited by different adverse side-effects, and even though it is not currently used as a therapeutic agent in AD treatment, its *in vivo* studies demonstrate that this type of chelating agents consti-

tute a promising route for AD therapies, as it is able to chelate metal ions from metal-Aβ species and to assist, in part in the disaggregation of Aβ aggregates [144]. Therefore, its possible use in humans in the future cannot be totally excluded [104].

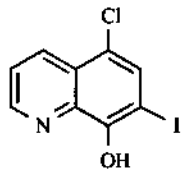

Fig. (16). Schematic structure of clioquinol (CQ).

Structural characterization of the clioquinol complexes with Zn(II) and Cu(II) reveals a 1:2 metal-to-ligand ratio showing a trigonal-bipyramidal geometry for $[Zn(CQ)_2(H_2O)]$ and a square planar geometry for $[Cu(CQ)_2]$ [145]. The vibrational spectra of CQ and of its Cu(II) complex have also recently been investigated in detail [146].

On the basis of the promising properties of clioquinol in the generation of stable $\mathbf{ML_2}$ complexes with Cu(II) and Zn(II) it was suggested that similar, structurally related, tetradentate ligands could even form more stable **ML** tetradentate complexes. The first investigated ligand of this type was 2,2'-methilenedi-8-quinolinol (Fig. (**17**)) [147], which structural and spectroscopic characteristics were also recently investigated [148]. This new chelator appears as significantly more effective than CQ in restoring Aβ solubility after metal-ion induced precipitation, particularly at low metal ion concentrations, and is also able to inhibit Aβ/Cu(II) mediated production of H_2O_2 *in vitro* [147].

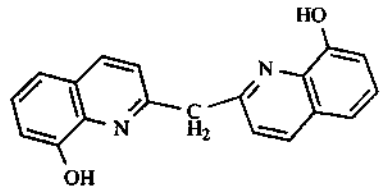

Fig. (17). Schematic structure of 2-2'-methilenedi-8-quinolinol.

Extending this study, different substitutions on the CH_2 linker were performed, introducing for example $>C(CH_3)_2$, $-CH_2-CH_2-$, $>CF_2$, $>CO$, $-CH_2-NH-$, -NH-C(O)- and other moieties as attachment of the two hydroxyquinoline moieties. These new chelating agents also complex Cu(II) and Zn(II) generating 1:1 metal complexes and inhibit the toxic formation of H_2O_2 due to the copper complexes of Aβ [149].

Some other metal chelating agents have also been proposed, for example different type of derivatives of deferiprone and some structurally related molecules [104,137], but most of them have been scarcely explored. Most recently a bicyclam compound, 1,1'-xylyl bis-1,4,8,11-tetraaza cyclotetradecane, also called JKL 169, which has also entered in clinical phases for other applications such as HIV infection, cancer or inflammatory diseases, has been proposed as a very safe drug with only weak toxicity for copper complexation. It affects body distribution of copper and may be an interesting candidate for further studies of new therapeutic routes in AD treatment [150].

3. CONCLUSIONS

It is evident that chelation therapies offer valuable and often unique routes for the treatments of heavy metal intoxications and of different disorders and diseases. The increasing acceptance of drugs such as DMSA and DMPS and the utilization of DMSA esters have notably widened the possibilities of these therapies. Also, the use of a combination of chelating agents has been shown its usefulness in many cases and is an aspect which must be further explored. The concept of metal-protein attenuating compounds (MPAC) appears also as very useful in the treatment of neurodegenerative diseases, inhibiting abnormal metal-protein interactions and controlling local deficiencies or elevations in metal ion concentrations.

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ABBREVIATIONS

BAL	=	British Antilewisite, 2,3-dimercaptopropanol
CDTA	=	cyclohexane-1,2-diaminetetraacetic acid
CQ	=	clioquinol, 5-chloro-7-iodo-8-hydroxyquinoline
DHLA	=	dihydrolipoic acid
DMPS	=	2,3-dimercapto-1-propanesulfonate
DMSA	=	*meso*-2,3-dimercaptosuccinic acid
DTPA	=	diethylenetriaminepentaacetic acid
EDTA	=	ethylenediaminetetraacetic acid
HSAB	=	hard-soft acid-base theory
LA	=	lipoic acid
MiADMSA	=	monoisoamyl ester of DMSA
MPAC	=	metal-protein attenuating-compounds
TETA	=	triethylenetetramine
TTHA	=	triethylenetetraaaminehexaacetic acid

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