Chemical and Biological Considerations in the Treatment of Metal Intoxications by Chelating Agents

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Abstract: Effective chelation treatment of metal intoxications requires that the pharmacokinetics of the administered chelator in fact leads to chelation of the toxic metal, preferably forming a less toxic species which is effectively excreted. This depends on physical and chemical characteristics of metals and chelators as e.g. ionic diameter, ring size and deformability, hardness/softness of electron donors and acceptors, administration route, bioavailability, metabolism, organ and intra/extra cellular compartmentalization, and excretion. *In vivo* chelation is unlikely to reach equilibrium determined by the standard stability constant, as rate effects and ligand exchange reactions as well as the pharmacokinetics of the chelator considerably influence complex formation. Hydrophilic chelators enhance renal metal excretion, but mainly their extracellular distribution limit their effect to mainly extracellular metal pools. Lipophilic chelators can decrease intracellular stores, but may redistribute toxic metals to e.g. the brain. In chronic metal induced disease, necessitating life-long chelation, toxicity and side effects of the chelator may limit the treatment. The metal selectivity of chelators is important, due to the risk of essential metals depletion. Dimercaptosuccinic acid and dimercaptopropionic sulfonate are presently gaining increased acceptance among clinicians, undoubtedly improving the management of human metal intoxications including lead, arsenic and mercury compounds. Still, development of new safer chelators suited for long-term oral administration for chelation of metal deposits, mainly iron, is an important challenge to the future research.

Keywords: Chemical chelation, Metal intoxication, BAL, DMSA, DMPS.

INTRODUCTION

Extensive experience demonstrates that acute and chronic human intoxications with a range of metals can be treated with considerable efficiency by the administration of a relevant chelating agent. However, the success of clinical use of a chelating agent in the human organism depends on a number of conditions: competing metals and ligands, perfusion, compartmentalization of metal and chelating agent, metabolism and/or excretion of the chelating agent, and changes in toxicity of the metal either "free" or chelated as well as the toxicity of the chelating agent. Development of efficient clinical chelation schedules is therefore based on combinations of chemical considerations and experiments in test tubes, whole animal experimentation on the toxicokinetics and toxicodynamics of metal and chelating agents, and clinical experience, from single cases occurring at low frequency all over the world, and treated quite differently with regard to monitoring metal excretion and status.

This review briefly summarizes the chemical and biological background for treatment with chelating agents of poisonings and diseases caused by acute or chronic overexposure to metals, describes advantages and limitations in the use of the most important presently employed clinical chelators, and outlines the recent development in knowledge about the new chelating agents meso-2,3-dimercaptosuccinic acid (DMSA) and D,L-2,3-dimercapto-1-propanesulfonic acid (DMPS) compared with that of classical chelators, mainly 2,3-dimercaptopropanol (British Anti Lewisite, BAL) and ethylenediamminetetraacetic acid (EDTA). Structures of the chelators described can be seen in Fig. (**1**). Finally, important research needs and avenues for future

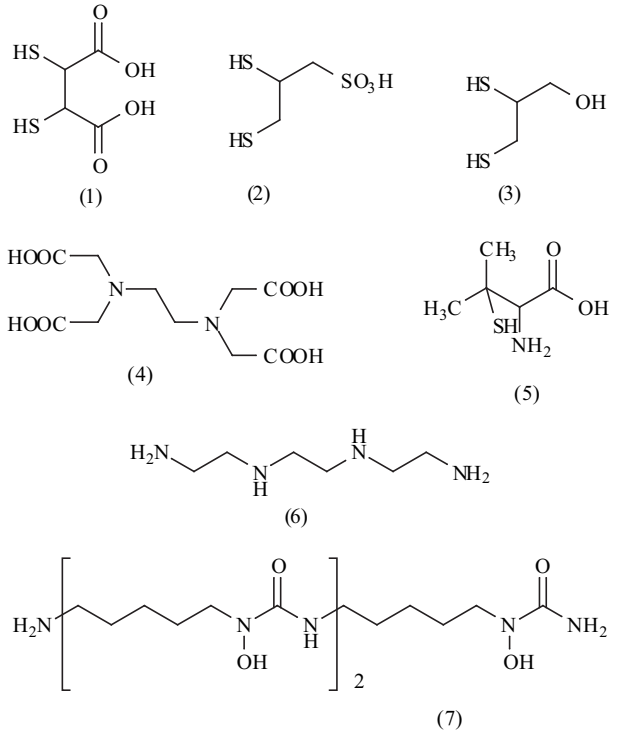

Fig. (1). Chemical structures of chelators: (**1**) DMSA, (**2**) DMPS, (**3**) BAL, (**4**) EDTA, (**5**) DPA, (**6**) TETA, (**7**) DFOA.

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development will be discussed. Experimental and clinical use of chelating agents has previously been reviewed by Aposhian [1] and Andersen [2, 3].

DECREASING METAL TOXICITY BY CHELATION

Historically, chelating agents have been used to lower the toxicity of metal or metalloid based drugs as arsenic compounds for syphilis and trypanosomiasis treatment and antimony drugs for schistosomiasis treatment. The first experimental use of a chelator against metal poisoning was Kety and Letonoff's attempt to use citrate as an antidote towards acute lead intoxication in 1941 [4]. Due to the metabolic instability of citrate the success was limited, but this experiment signaled a new way of thinking in the treatment of acute and chronic metal intoxication.

During the Second World War, 2,3-dimercaptopropanol (BAL) was developed as an experimental antidote against arsenic based war gases [5]. Subsequently, BAL came into clinical use against intoxications with organic arsenical drugs for syphilis treatment [6-8]. BAL soon became a general antidote in metal poisoning due to its apparently high efficacy in human arsenic and inorganic mercury intoxications [8-11], and the limited number of alternatives available until DMSA and DMPS became available in the western countries in the 70'ties. Thus, BAL has been used in a variety of human metal intoxications, including the recessive Wilson's disease [12, 13]. This disease is due to a mutation in an ATPase involved in transmembrane transport of Cu, leading to decreased Cu excretion in homozygotes and Cu accumulation mainly in liver and central nervous system [14]. However, BAL is far from being an ideal chelator due to its high toxicity and the high frequency of various unpleasant side effects and because increased brain deposition due to BAL administration has been reported for arsenite and organic mercury compounds, and BAL increased the toxicity of cadmium and lead in animal experiments [15-18].

D-penicillamine (DPA) treatment of Wilson's disease was initiated by Walshe (1956) [19]. The initial effect of DPA administration on urinary copper excretion is dramatic, and as DPA can conveniently be administered orally, BAL was fortunately put aside for the treatment of Wilson's disease. Due to frequent development of penicillamine intolerance among patients, Walshe used triethylenetetramine (TETA) as an alternative [20, 21]. TETA is however a less efficient Cu mobilizer than DPA, and its toxicity is not extensively studied [22]. In China, numerous Wilson's disease patients are claimed to have been treated with DMSA with good results [23]. An alternative to chelation treatment of Wilson's disease cases is oral administration of a Zn salt, most often zinc sulfate [24-26]. The Zn ion induces intestinal epithelial metallothionein, thereby sequestering Cu absorbed over the mucosal membrane and reducing systemic uptake [27]. An alternative treatment in patients not tolerating DPA or TETA is oral administration of tetrathiomolybdate, which chelates Cu and reduces the intestinal Cu uptake [28].

Also EDTA came into clinical use soon after the Second World War to treat lead intoxication and for elimination of radionuclides [29], which is done more efficiently by diethylenetriaminepentaacetic acid (DTPA) or Prussian Blue. EDTA and DTPA are problematic clinical chelating agents due to low intestinal uptake necessitating slow intravenous administration, their exclusively extracellular distribution, and high stability constants with some essential metals. Therefore, induction of hypocalcemic tetani during intravenous infusion is a possible complication, and zinc depletion is a possible side effect during prolonged use. Accordingly, Ca and/or Zinc salts of EDTA and DTPA are used in the clinic. Besides its use to treat cases of childhood and occupational lead exposure, EDTA is used for a challenge test to estimate lead burden, however, EDTA may redistribute lead to the brain both after chronic or acute lead exposure [20-32], making this diagnostic use of chelation highly problematic. Further, while the most efficient iron chelator desferrioxamine (DFOA) completely covers the surface of Fe(III) during complex formation, thereby preventing iron catalyzed free radical reactions [33,34], EDTA is not able to shield the surface of the Fe(III) ion but forms an open complex ("basket complex"), thereby increasing the possibility for iron reduction and thereby the catalytic capacity of Fe(II) for generating Fenton reaction mediated oxidative stress [35]. As chronic lead intoxication often leads to iron deficiency as a side effect, there is a need for iron supplementation along with chelation therapy, which is quite dangerous during EDTA chelation, while it is efficient and safe during chelation with DMSA [36].

Various mutations in hemoglobin genes affect the oxygen transporting capacity of erythrocytes, decrease the stability of erythrocytes, and lead to progressing organ damage due to multiple micro bleedings. The only available treatment of homozygous patients for the more severe mutations is multiple blood transfusions, leading to iron overload and thereby mental degeneration and hepatic necrosis. Inherited hemoglobin diseases are very frequent in Mediterranian (beta thalassemias) and African (sickle cell anemia) countries, due to selection for the heterozygous genotype, conferring resistance against malaria. In 1962, DFOA was demonstrated to increase urinary iron excretion in Thalassaemia major patients [37], offering treatment of infusion related iron toxicity in these patients for the first time.

The new chelators DMSA and DMPS have been used in China [23, 25, 38] and the Soviet Union [39, 40] for almost 50 years. These drugs have now been available in Western countries as experimental drugs for a few decades, DMSA as a registered drug for treatment of lead intoxication in USA and DMPS in Germany for treatment of mercury intoxications for about 10 years. Today, they hold promise as antidotes in acute or chronic intoxications with many divalent metal salts and also some other metal or metalloid compounds, as extensively demonstrated in a large number of animal studies. Their use in various intoxications was previously reviewed by Aposhian *et al.* [1] and Andersen [2]. Unfortunately, the western clinicians have not yet fully realized their value as alternatives to the classical chelators BAL and EDTA, however, DMSA and DMPS have major advantages; they are less toxic and suited for long-term oral as well as parenteral administration. Also, DMPS does not redistribute arsenic, lead or inorganic mercury to the brain [41, 42] and DMSA chelation decreases the brain deposition of lead [43] and methylmercury [44].

Fortunately, metal intoxications presently occur at lower rates than formerly. Notwithstanding, acute iron intoxications in children ingesting iron tablets, intoxications by bismuth, gold and platinum compounds due to medical uses, intoxications with thallium and arsenic based pesticides, intoxications due to various forms of mercury, chronic cadmium intoxication due to environmental or occupational exposure occur at an appreciable frequency. Most widely distributed are acute or chronic childhood or occupational lead intoxication. This raises need for further development of clinical chelation schemes: The number of chelating agents in actual use as experimental drugs or clinically established drugs is small, and there is a need for better chelators for several applications. Several important questions in chelation treatment of metal toxicity, e.g. effects of chelation on brain deposition or mobilization or effects on intestinal uptake, still remaining can be answered only by animal experimental studies. Oral chelation with efficient chelators of low toxicity is still not available or clinically accepted for some important intoxications to be discussed below.

CHEMICAL CONSIDERATIONS

During complexation of metals with i molecules of the monodentate ligand L or with one molecule of the *i* dentate ligand λ:

$$M + iL \rightarrow ML_i \quad (1)$$

$$M + \lambda \rightarrow M\lambda \quad (2)$$

the overall stability constants can be expressed by

$$\beta_L = \frac{[ML_i]}{[M][L]^i} \quad (3)$$

$$\beta_\lambda = \frac{[M\lambda]}{[M][\lambda]^i} \quad (4)$$

The stability of a complex depends on

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 = -RT \ln \beta \quad (5)$$

During complex formation of a metal with *i* monodentate ligands, enthalpy changes related to bonding often contributes considerably to the free energy, while entropy changes associated with ordering ligands around the ion counteracts the entropy effect of desolvation. If the *i* ligands are introduced into one molecule to form a multidentate ligand, the entropy contribution from desolvation is fully available, and the complex stability is greatly increased. Based on these considerations and his experimental data, Schwarzenbach [45] defined the chelate effect as the logarithm of the equilibrium constant for a displacement reaction where i independent donors are exchanged by i identical donors present in one ligand, thereby expressing the increased stability of the chelate as related to the free energy of the reaction. If the enthalpy change due to complex formation does not depend on whether the donor groups are independent or joined in a multidentate ligand, the chelate effect should be entirely due to the entropy change.

The formation of the ML_i complex depends much more on the concentration of the ligand (*L* is in the i'th power in β_{ML}) than does the formation of the $M\lambda$ complex (λ is in the first power in $\beta_{M\lambda}$). Especially at low ligand concentration, chelates are far more stable than the corresponding complexes with unidentate ligands. While entropy change most often is the main contribution to the stability of metal complexes with multidentate ligands, a considerable enthalpy contribution may result when repulsive forces between charged groups are overcome by introducing them into one molecule. Steric conditions, e.g. ion size and ring size, considerably influence the stability, mainly through changes in ΔH.

The size of the chelate effect can be visualized from the change in log β for complexes with multidentate ligands with increasing numbers of identical donor groups. Thus, the stability of the Cd complexes with the polyaminopolycarboxylic acids increases in the series iminodiacetic acid with 3 donor groups and log β = 5.71; nitrilotriacetic acid with 4 donor groups and log β = 9.78; EDTA with 6 donor groups and log β = 16.36; and DTPA with 8 donor groups and log β = 19.00 [46]. Similar effects are seen with the series of homologous polyamines, where log β for the Cd complexes increases from 5.45 to 16.10 when the number of donors increases from 2 to 5 [46].

Hardness/softness (H/S) characteristics of electron donors and acceptors determine complex formation. The HS characteristics determine not only the stability of the formed complex, but also the selectivity of the chelator and the toxic metal relative to competing essential metals and biological ligands. The softness character is related to the ability of the empty frontier orbital of metal ions for accepting electrons, and to the deformability of the outermost occupied electron orbital of donor groups, i.e. the propensity of metals and donors for forming covalent bonds. The HS character of metal ions has been quantified in the literature by various descriptors related to the bonding preference of ions: The ionic index, Z^2/r is positively related to degree of ionic bonding in an ion's complex. The softness of an ion increases with the sum of the ionization energies divided by the ionic index, $r\ I/Z^2$ [47]. Softness is related to the covalent index, $\sigma X_m^2 r$, where X_m is the electronegativity, because X_m is related to the ion's empty frontier orbital energy and thereby to the ion's ability to accept electrons and form covalent bonds [48]. For practical uses, metals and donors are divided into 3 groups, hard (H), intermediate (I) and soft (S).

Metal ions and donor groups prefer to form complexes with partners having similar HS character, however, the stability of complexes increases with the softness degree of both metal and donor. For a series of cadmium complexes with simple tridentate ligands, made by substituting the imino H in iminodiacetic acid with different functional groups, log β increases from 5.71 (R = H) or 6.75 (R = CH_3) to 9.78 (R = COO^-), 10.53 (R = NH_2) or even 16.72 (R = SH) [46]:

$$RN(COO^-)_2 + Cd(II) \rightarrow Cd^{II}RN(COO^-)_2 \quad (6)$$

For another series of cadmium complexes, log β varies between 12.43 (R = CH_3) and 22.33 (R = SH) [46]:

$$2\ RN(COO^-)_2 + Cd(II) \rightarrow Cd^{II}(RN(COO^-)_2)_2 \quad (7)$$

Solvent exchange and ligand exchange rates depend among others on the HS character of electron donors and acceptors. Also, the rate of complex formation depends on

the very first step, that is whether the chelator can easily get a grip on the metal ion by displacing a solvent molecule or a monodentate ligand to obtain the initial coordination site. This initial ligand exchange reaction determines the stability of the formed mixed complex. If a more stable complex than the disrupted complex is formed, further ligand exchange reactions are thermodynamically facilitated, some times even when subsequent ring opening is necessary.

The next step is formation of the first ring in the new complex by coordinating a second donor group of the multidentate ligand to the metal ion, whereby the chelate effect decreases the rate of dissociation of the complex. Such processes may be quite rapid. If a preexisting chelate ring formed with a biological multidentate ligand has to be broken in the initial complexation reaction, the process is often much slower. Besides the number of donor groups available for electron pair donation, i.e. the maximum number of rings formed contributing to the chelate effect, the HS character of these donors, and steric conditions for simultaneous access of ligands to coordination positions on the metal ion determine the formation rate and overall stability. Also, lipophilicity, metabolic stability and rate of (most often urinary) clearance are important.

CHELATION IN BIOLOGICAL SYSTEMS

The efficiency of clinical chelation depends on chemical and biological characteristics of metal, chelator and the organism. Metal associated, chelator associated and organism associated characteristics interplay to determine the degree of complexation of a metal by a chelator. Focus in most clinical uses has been put on mobilization (mainly due to renal excretion) of the toxic metal. However, an important effect of chelating agents is the reduction of metal toxicity. Thus, a chelating agent forming a stable complex with a toxic metal may shield the metal ion from biological targets, thereby reducing the toxicity, even at times after administration where mobilization has not yet occurred, or it may expose the metal to the biological environment and prevent the metal from being scavenged by biological protective mechanisms, and thereby increase the toxicity of the metal, as described above for iron complexes with EDTA and DFOA. In most studies of the effects of chelators on acute metal toxicity, metal excretion or organ distribution was not quantified accordingly, so it is unclear to what extent increased excretion and decreased toxicity contributed to the observed alleviating effect of chelation treatment.

Important chelator characteristics are:

Toxicity of chelator and chelator-metal complex and side effects of the chelator hydrophilicity/lipophilicity of the chelating agent and hydrophilicity/lipophilicity of the resulting metal complex,

Stability of metal-chelator complex, mainly determined by hardness-softness character and the chelating effect.

Toxicity of the chelator itself and of the resulting complex (see Table (**1**) may be an important limitation for treatment. Development of side effects most often occurs after a period of use and necessitates cessation and institution of another chelator, if possible.

The toxicokinetics of a chelating agent depends on hydrophilicity/lipophilicity of the compound, mainly whether it has an extracellular distribution or can chelate intracellular metal deposits, and whether oral administration is an option or intravenous infusion is necessary. Also it determines the hydrophilicity/lipophilicity of the formed metal complex. The metal-chelator complex formed should

Table 1. Clinically Important Chelating Agents. The Acute Toxicity is Illustrated by Representative LD_{50}-Values Selected from the Large Published Database

Compound	Species	Administration route	LD_{50}	Ref.
$CaNa_2EDTA$	Mouse, Rat	ip	4-6 g/kg	49, 50
$CaNa_3DTPA$	Mouse, Rat	ip	2-4 g/kg	49, 50
BAL	Mouse	ip	90-180 mg/kg	51, 52
DPA	Mouse	ip	337 mg/kg	50
DPA	Rat	oral	> 1.2 g/kg	53
DMSA	Mouse	oral	4.34 g/kg	54
DMSA	Mouse	ip	2.48 g/kg	55
DMPS	Mouse, Rat	ip	1.1-1.4 g/kg	55, 56
DFOA	Rat	oral	> 1 g/kg	57
DFOA	Rat	iv	520 mg/kg	57
L1	Mouse, Rat	ip	0.6-1 g/kg	58, 59
TETA	Mouse, Rat	oral	1.6-2.5 g/kg	60, 61
PB	Mouse	oral	> 5 g/kg	62
PB	Rat	ip	1.13 g/kg	63

preferably be rapidly excreted by the kidneys. The urinary excretion of highly lipophilic complexes is decreased due to reabsorption, thereby the metal's organ distribution is extensively changed, also, chelators forming lipophilic metal complexes may enhance the intestinal metal uptake, thereby potentially enhancing the toxicity of the metal, which has been extensively demonstrated for diethyldithiocarbamate (DDC) and Cd [64-67]. DDC forms lipophilic complexes with and increases the brain deposition of most divalent metal ions besides Cd, including inorganic and organic Hg, Tl, Pb, Ni, Cu and Zn [68-75].

Tetraethylthiuram disulfide (TTD, disulfiram, antabuse) is used for alcohol avoidance therapy in alcoholics. TTD is metabolized to two molecules of DDC and thereby increases the intestinal uptake and brain deposition of orally administered Cd, Ni and Pb [76-78]. Also carbamate-based pesticides as Thiram, the tetramethyl analog of TTD, enhance intestinal metal uptake and brain deposition of Ni [79]. The effect of dithiocarbamates on Cd biokinetics depends on their lipophilicity, thus the degree of enhancement of intestinal uptake and brain deposition correlates with the octanol-water partitioning coefficient [80].

The oral use of chelating agents is considered to require preceding emptying of the GI system for the toxic metal and removal from further exposure to avoid increased intestinal metal absorption. However, orally administered chelating agents forming hydrophilic metal complexes may efficiently reduce intestinal metal uptake and local toxicity at times after oral intoxication where extensive amounts of metal is still in the GI tract, and thereby decrease both local and systemic toxicity [81, 82]. Oral administration of EDTA or DMSA reduced the intestinal uptake and toxicity of oral Cd [81-83], and chelation of Hg(II) with DMSA or DMPS [84] and Ni(II) with EDTA (Nielsen and Andersen unpublished) reduced intestinal uptake. In conclusion, oral administration of chelating agents may in some cases be a very efficient treatment of oral metal intoxication, and should be studied experimentally in much more detail.

IN VIVO EFFICACY OF CHELATING AGENTS

In biological systems, toxic metals are present at very low "free"concentrations, due to the availability of numerous small biological ligands forming mixed aquo-bioligand complexes with metals. Therefore, complexation reactions *in vivo* between toxic metals and antidotal chelating agents most often occur as a series of ligand and/or metal exchange reactions. Even in situations where the equilibrium constant is highly favorable, complex formation may be limited due to rate effects, competition by other ligands/metals, and systemic transport kinetics of the chelator. Under physiological conditions, numerous small mono and bidentate ligands as well as functional groups in proteins participate in chelation reactions and compete for chelating agents. Ca(II), present at a concentration of about 1 mM, is the most important metal species competing for clinical chelating agents.

Assuming equilibrium between chelator and toxic metal and quantitative urinary excretion of the ML complex, the efficiency, E, of a chelating agent for mobilizing a toxic metal can be described as

$$E = \frac{[ML]}{[M]} \tag{8}$$

since the potential for mobilizing the metal depends on the degree of formation of the ML complex. In the simple situation of one major biological competing metal, Ca(II), and a total chelator concentration L_t, the conditions for a large E can be visualized from the standard stability constants:

$$E = \frac{[ML]}{[M]} = \beta_{ML}\,[L] \tag{9}$$

By introducing the stability constants for the metal and calcium complexes into this expression and defining $[L_t]$ as the sum of all forms of the chelator in plasma, Shubert (85) derived:

$$E = \frac{\beta_{ML}}{\beta_{CaL}} \frac{[L_t]}{[Ca^{2+}]} \tag{10}$$

As biological systems are highly complex, the efficacy of chelating agents is often better described from animal experimental or clinical treatments than by theoretical calculations of, e.g. E. In practice, major endpoints are increased mobilization of the toxic metal in experimental animals or humans evaluated from urinary and some times fecal output, as well as effects on mortality and signs of toxicity. The mobilizing effectiveness (ME) may be calculated as the factorial increase, MEF, in urinary and fecal excretion between treated and un- or pretreated animals or humans. Alternatively, the fractional retention, MEQ, of the metal in organs of treated animals relative to controls is calculated [49]. The therapeutic effectiveness, TE, for acute metal intoxication can be calculated as the factorial change, TEF, in LD_{50} due to the chelation treatment [49]. The efficiency of two chelators may be compared by calculating their relative potency RP in animal experiments, that is the ratio between equally effective doses, or by their relative efficiency, RE, the ratio of effects at equimolar doses [49]. Since different chelators have very different efficacy towards acute metal toxicity, in some combinations allowing 100% survival even after doses considerably higher than LD_{99} [81, 82] the RE method has limited applicability.

TOXICITY AND SIDE EFFECTS OF CLINICAL CHELATORS

BAL

Based on its lower LD_{50} value, BAL is considered more toxic than the chelators available today as alternatives (Table (**1**)). BAL has a low therapeutic efficacy in most cases, and due to its high toxicity, BAL is suited only for brief treatment of acute intoxications. BAL is unstable, susceptible to oxidation, and therefore difficult to store as a ready-for-use preparation. It can be administered only by intramuscular injection, which is very painful and requires local anesthesia. Due to BAL's lipophilicity, it is normally injected IM in peanut oil. A considerable fraction of treated individuals experiences unpleasant side effects including nausea, vomiting, sweating, high fever, hypertension and tachycardia. Due to the advent of more efficient and safe

drugs-DMSA and DMPS, the clinical uses of BAL could now be phased out.

The pharmacokinetics of BAL is comprehensively described by Catsch and Harmuth-Hoene [49]. The absorption from the site of injection is rapid and complete, and BAL is apparently distributed into the intracellular space. The major fraction of the dose is rapidly excreted in urine as dithiols and glucuronides. BAL administration has been demonstrated to increase the brain deposition of arsenite [15] and organic mercury compounds [16], and increased the toxicity of cadmium [17] and lead [18] in animal experiments.

DPA and N-Acetyl-D-Penicillamine (NAPA)

Both these compounds can be administered orally, and DPA can also be administered by intravenous infusion. The intestinal absorption of DPA in rats and humans is about 50%. The volume of distribution is close to that of extracellular water, and formation of mixed disufides with serum albumin is extensive. The majority of the absorbed dose is rapidly excreted in urine as free DPA or the oxidized dimer without significant metabolism [86-89]. The metabolic behavior of NAPA is similar to that of DPA [90]. The toxicity of DPA is very low (Table (**1**)). The use of DPA is however limited due to the development of side effects which appear in a considerable fraction of patients during continued use, mainly hypertension, nephrotic syndrome, and various autoimmune reactions.

EDTA and DTPA

Catsch and Harmuth-Hoehne [49] offer a detailed review of the toxicity and pharmacokinetics of EDTA and DTPA. Both compounds are poorly absorbed in the GI tract (< 5%), and are administered by slow iv infusion of their calcium or zinc complexes. Their volumes of distribution are close to that of extracellular water, and both chelators are rapidly excreted in the urine without significant metabolism. EDTA and DTPA form complexes with a variety of metal ions, including most essential metals. Accordingly, continued exposure may induce trace element depletion, especially for Zn, Cu and Mn [91]. The teratogenicity of high EDTA doses is due to Zn depletion, which is readily reversed by coadministration of zinc [92]. In chelation treatment, the monocalcium salts of EDTA and DTPA are used to avoid hypocalcemic tetani. $ZnNa_3$DTPA may alternatively be used. Extensive zinc binding is most likely involved in the acute toxicity of $CaNa_2$EDTA, thus Zn_2EDTA is more than one order of magnitude less toxic than Ca_2EDTA, which is a factor of 20 times less toxic than the tetrasodium salt.

TETA

It is administered orally, however, its absorption is poor, as less than 20% of an oral dose of ^{14}C-labelled TETA to rats was recovered in carcass and urine. After iv administration, half the dose was rapidly excreted in urine, and the cumulative fecal elimination was about 20%, indicating biliary excretion [93]. Kodama *et al.* [94] recovered only about 1% free TETA in the urine after an oral dose of TETA given to human volunteers. The major part was excreted as 1-acetyl-TETA [95].

The acute toxicity of TETA is low (Table (**1**)). Yanagisawa *et al.* [96] calculated the threshold of toxicity to be close to 50 mg/kg/day in the female rat and less in the male rat. The recommended dosage to Wilson's disease patients is 0.75 - 2 g/day, which is quite close to a potentially toxic dose. Based on experience with the long-term use of TETA in Wilson's disease patients, this chelator is remarkably free of side effects compared to DPA [97].

DFOA

The absorption of DFOA in the gastrointestinal tract is low. DFOA is therefore administered by IV infusion or injection. Its distribution volume is extracellular, and the protein binding in plasma is low, less than 10%. Its renal excretion is biphasic with the slow half-life being about 6 h. The acute toxicity is rather low (Table (**1**)), and IV infusion is safe if care is taken not to administer the dose rapidly which can result in hypotension. However, a wide range of side effects have been noted during continued use in iron overload patients including opthalmic and auditory toxicity, bacterial and fungal infections, changes in blood histology, allergic and skin reactions, and pulmonary, renal and neurological effects [98].

DMSA and DMPS

As mentioned, these two chelators have very low toxicity. As opposed to effects of BAL on the toxicokinetics of metals, DMPS and DMSA have been shown to decrease the brain deposition of several toxic metals [41-44]. DMPS is slightly more toxic than DMSA, and both compounds are much less toxic than BAL.

Dry preparations of these chelators are highly stable at room temperature. They are available as tablets for oral administration, and they both are suited for parenteral administration as well. In China, DMSA has been administered parenterally to hundreds of patients [23]. Both these drugs are absorbed to some degree in the intestinal tract (DMPS: 50-60% in dogs [99], DMSA: Up to 40% urinary excretion within 16 h of an oral dose of DMSA in humans [100]). The extensive work of Aposhian's group has added significantly to our knowledge about the pharmacokinetics and metabolism of these two compounds. The distribution of both drugs is predominantly extracellular, however, DMPS has also some intracellular distribution [101, 102]. The primary route of excretion is urinary with plasma and whole blood half-lives and urinary elimination half-time of less than 4 h in humans for DMSA [100, 103] and slower excretion of DMPS, with blood and plasma half-lives of 9-10 h [104].

After an oral dose of DMSA to humans, more than 95% of the blood content is covalently bound to proteins, mainly to albumin [103]. More than 90% of urinary DMSA is excreted as the DMSA-cysteine mixed disulfide [105]. Also DMPS is mainly bound to albumin in serum, however, as opposed to DMSA, the urinary excretion products after oral administration of DMPS to humans are various acyclic and

cyclic homopolymers of DMPS, whereas a mixed disulfide with cysteine is almost completely absent [106].

A case of DMSA overdose occurred in a 3-year-old girl, who ingested ca. 2.4 g DMSA or 185 mg/kg b.w. without clinical signs of intoxication [107]. Many patients have been treated with DMSA in the USA and with DMPS in Europe during the last 20 years. The frequency of toxic side effects necessitating discontinued treatment has been very low, much lower than with the other established clinical chelators. Adverse reactions during treatment with DMSA or DMPS include gastrointestinal discomfort, skin reactions, mild neutropenia and elevated liver enzymes. For both compounds, symptoms may subside allowing continued therapy. DMPS seems to be better tolerated than DMSA with respect to gastrointestinal symptoms, but may cause hypotension, especially after rapid iv infusion. Some patients, especially those with allergic asthma symptoms, may develop hypersensitivity to DMPS [108, 109].

Two serious reactions to DMSA therapy have been reported: DMSA chelation of a man with chronic lead intoxication was discontinued due to a strong mucucutaneous reaction to the drug [110]. A 45-year-old black male developed hemolytic anemia during DMSA chelation for occupational lead intoxication. After cessation of treatment, the hematological values normalized. The patient was glucose-6-phosphate dehydrogenase deficient, a genetic trait known to contraindicate BAL chelation due to risk of hemolysis [111]. For DMPS, severe toxicity has not been reported in peer reviewed literature except for a case of Stevens-Johnson syndrome in a lead intoxicated patient after 8 daily oral doses of 200 mg/m2 DMPS [112]. DMSA is registered in the USA as a drug for treatment of lead intoxication. DMPS is registered in Germany for the treatment of mercury intoxication; however, it is not approved in the USA.

L1

L1 offers an alternative to DFOA in the treatment of transfusional Fe overload in hemoglobinopathies due to its low price compared to DFOA and to the possibility of treatment of patients not tolerating DFOA. Further, L1 can be administered orally. L1 is rapidly absorbed in the gastrointestinal tract. The main excretion route is via kidneys, with a half-life of 47-134 min [113,114]. The recovery from urine is close to 100%, the main species are free L1, the Fe and Cu complexes and the glucuronide.

The acute toxicity of L1 is somewhat lower than that of DFOA (Table (**1**)). Clinical experience with L1 indicates various side effect, e.g. gastric discomfort, increase in antinuclear antibodies and rheumatoid factors, zinc depletion, transient agranulocytosis or transient musculoskeletal and joint pain. Unfortunately, we still lack an ideal chelator for life-long chelation of chronic transfusional Fe overload.

BRIEF SUMMARY OF IMPORTANT METAL INTOXICATIONS

Several metals cause acute and chronic intoxications in humans. A complete treatment is outside the scope of this review; the reader is referred to a recent extensive review [2]. Here, the most important human metal intoxications are briefly summarized.

Iatrogenic treatment of renal failure or total parenteral nutrition previously resulted in a number of cases of severe chronic aluminum intoxication, leading to serious neurodegeneration and osteomalacia [115]. Despite subsequently improved medical treatment, end-stage renal disease patients today still are at risk of developing chronical intoxication. The present optimal treatment is intravenous infusion of DFOA, but due to its side effects, this treatment is far from optimal [116]. An alternative treatment is oral administration of 1,2-dimethyl-3-hydroxypyrid-4-one (L1) [117], which was originally developed as an alternative to DFOA for iron chelation. Unfortunately, also L1 frequently has severe side effects, limiting its use [98].

Arsenic based medications have been used for centuries, but have now been phased out in most countries. Highly toxic arsenic based pesticides are still used, mainly in the developing countries, and are thereby available for homicidal or suicidal purposes. Salts of arsenate and arsenite have always been popular poisons. BAL and DPA have been used as antidotes in human arsenic intoxications, but based on extensive animal experimental evidence, DMSA and DMPS are both superior, at least a factor of 10 more efficacious than BAL [55, 56, 118]. DMSA and DMPS both now are slowly coming into clinical use instead of BAL.

Bismuth salts have been extensively used in various drugs. DeNol (tripotassium dicitratobismuthate, colloidal bismuth subcitrate) and related drugs have led to numerous cases of severe encephalopathy after daily intakes of several grams [119]. In experimental animals, DMSA and DMPS are effective antidotes and mobilizers of tissue Bi, while EDTA, DPA and DFOA are rather inefficient. Even though BAL efficiently mobilizes tissue Bi, DMPS is considered the antidote of choice due to BALs extensive toxicity [120, 121].

Very few acute human intoxications with cadmium salts have been reported, however, chronic occupational or dietary exposures have led to numerous cases of renal damage, eventually leading to severe bone disease [122]. BAL and DDC are contraindicated in acute cadmium intoxication due to their potentiating effect [17, 66]. In animal experiments, EDTA, DTPA, DMSA and DMPS were all efficient antidotes towards a highly toxic oral cadmium dose [81, 82]. Presently, there is no chelator available for mobilization of aged body burdens of cadmium. Development of chelators able to mobilize hepatic and renal cadmium burdens has been attempted by researchers for many years, and several efficacious experimental compounds are available, yet, due to their toxicity, there is still a long way before humans can be treated [2].

The major cause for chronic copper intoxication is Wilson disease. As mentioned, several different treatments (DPA, TETA, Zinc salts, tetrathiomolybdate) are available, yet none are perfect. In acute Cu intoxication in experimental animals, DPA, TETA, DMSA and DMPS are efficacious antidotes, DMPS having the highest effect; also, TETA, DPA and DMSA increased the urinary Cu excretion in experimental animals [23, 123, 124].

Gold salts have medical importance for treatment of rheumatoid arthritis [125]. The most used compound, gold thiomalate, requires weekly injections and has numerous side effects. In animal studies, DMSA and DMPS are efficient antidotes and enhancers of gold excretion [126, 127]. In human gold intoxication, BAL and DPA have been used. Unfortunately a direct animal experimental comparison of these 4 chelators is not available.

Acute iron intoxication is one of the most common child poisonings, reaching about 20,000 cases per year in the US, according to annual reports of the American Association of Poison Control Centers. The severe cases due to ingestion of concentrated iron supplements lead to corrosion of the gastric mucosa, metabolic acidosis, coagulopathies, multiorgan failure. The treatment is mechanical removal of residual tablets, extensive supportive care and chelation with DFOA. Clinical studies of milder cases due to multivitamin tablets have failed to demonstrate a beneficial effect of DFOA chelation [128].

Childhood lead poisoning has been and is still a significant problem in poor population subgroups living in low-standard housing. Despite extensive efforts by the US CDC to reduce childhood lead exposure, about 1 million children below 7 years of age is still at risk in the US, mainly due to old lead based house paint [129]. This may lead to severe acute intoxication due to oral intake of paint flakes. Insidious exposure to house dust may lead to elevated body burden reflected in blood lead values, which is demonstrated in epidemiological studies to affect cognitive development [130-133].

In acute lead intoxication of experimental animals, BAL, DPA, EDTA and DMSA among several other chelating agents have been used. Based on an extensive database, DMSA seems to be the most efficacious antidote both in reducing mortality, preventing intestinal uptake, enhancing excretion and decreasing brain lead levels [31, 32, 42, 43, 134-138]. DMSA chelation of acute and chronic human lead intoxications has alleviated toxic symptoms and enhanced lead excretion in a number of case and small cohort studies [110, 139-146].

Inorganic and organic mercury compounds and metallic mercury are extensively used for numerous purposes, leading to acute and chronic intoxications. Human poisonings with various mercury compounds have been treated with BAL, DPA, NAPA, DMSA and DMPS. In experimental animal studies, DMSA and DMPS are effective antidotes against inorganic and organic mercury compounds and effectively enhance mercury excretion. Based on a large database, DMPS is the antidote of choice for oral intoxication with inorganic mercury compounds, while DMSA is the agent of choice for organic mercury intoxication [44, 147-151].

The highly efficient antitumor drug cis-dichlorodiammine platinum (II) (cis-Pt) has various side effects, especially nephrotoxicity [152]. DDC [153] and derivatives of DDC [154-157] protected against nephrotoxicity, decreased renal and hepatic Pt levels and increased the biliary excretion of Pt conceivably protecting the kidney by changing the excretion route from preferentially renal to biliary. In acute toxicity studies, sc DMSA reduced mortality after IP administration of 50 mg/kg H_2PtCl_6 to mice. Also, repeated IP injections of DMSA after IV administration of cis-PT to rats reduced the renal and hepatic Pt levels and increased the urinary Pt excretion in rats [158]. However, DMSA was unable to alleviate Pt induced renal damage.

Formerly, thallium salts were extensively used as rodenticides, leading to much severe intoxications. Even though thallium compounds have been phased out as rodenticides in most countries, thallium poisonings still occur. The clinical treatment is oral administration of various forms of hexacyanoferrate complex (Prussian Blue). Based on comparative animal experiments, this treatment seems superior to the use of other chelating agents [71, 159-164].

STATUS AND FUTURE FOR CLINICAL USE OF CHELATING AGENTS IN METAL INTOXICATION

Regarding antidotes for acute and chronic lead, arsenic and inorganic or organic mercury intoxications, evidence has accumulated during the last two decades that BAL is no longer necessary as an antidote. DMSA and DMPS are less toxic with less side effects, more efficacious, cheaper, more easily stored, suited for both oral and parenteral administration, and can be administered for extended time periods. Also in several other intoxications, DMPS and DMSA are superior to the alternatives. Fortunately, DMSA and DMPS have gained more general acceptance among clinicians during the last 15 years, undoubtedly improving the management of many human metal intoxications. Still, knowledge is needed in several basic research areas of *in vivo* chelation of metals, e.g. on the molecular mechanisms of action of clinically important chelators. It should be remembered that the chronic treatment of genetic metal storage diseases is associated with severe side effects. Development of less toxic chelators for these conditions has very high research priority.

An important research theme is interaction between intracellular and extracellular chelation in relation to mobilization of aged metal deposits and the possible redistribution of toxic metal to sensitive organs as e.g. the brain. Here, combined chelation treatment with lipophilic and hydrophilic chelators, which presently has a minimal clinical role, needs investigation.

Effects of chelators on metal biokinetics during continued exposure to the metal, especially possible enhancement or reduction of intestinal metal uptake should be studied, as should development of orally administrable chelators.

Especially the development of orally administrable chelating agents for efficient, non-toxic mobilization on home-patient basis over extended time periods (even life-long chelation) of aged deposits of toxic metal as e.g. Al, Cd, Fe, Hg, and Cu will probably be a main future research issue.

ABBREVIATIONS

BAL = 2,3-dimercaptopropanol

DDC = Diethyldithiocarbamate

DFOA = Desferrioxamine

DMPS = Dimercaptopropanesulfonic acid

DMSA = Dimercaptosuccinic acid

DPA = D-penicillamine

DTPA = Diethylenetriaminepentaacetic acid

E = Efficiency (of a chelator for mobilizing a metal)

EDTA = Ethylenediaminetetraacetic acid

iv = Intravenous

L1 = 1,2-dimethyl-3-hydroxypyrid-4-one

LD = Lethal Dose (Subscript indicates percent mortality)

ME = (Metal) mobilizing effectiveness of a chelator (see MEF and MEQ)

MEF = Factorially increased (metal) output

MEQ = Factorially decreased (metal) retention

NAPA = N-acetyl-D-penicillamine

PB = Prussian blue

RE = Relative efficiency of two chelators, ratio between effects at same dose

RP = Relative potency of two chelators, ratio between equally effective doses

TE = Therapeutic effectiveness (see TEF, RP and RE)

TEF = Factorially increased LD (LD_{50} or LD_{99})

TETA = Triethylenetetramine

TTD = Tetratethythiuramdisulfide

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