Assessment of Pharmaceutical Agents for Removing Cadmium from Humans Using Chemical Speciation Models

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Received May 26, 1984

Abstract

The chelating agents, 2,3-dimercaptopropanol (BAL), D-penicillamine, diethyldithiocarbamate (DDC), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), 2,3-dimercaptosuccinate (DMSA) and 2,3-dimercaptopropane-1-sulphonate (Unithiol), are assessed for their ability to mobilise protein-bound cadmium in blood plasma. Formation constants for the interaction of protons, Cd(II) and Zn(II) ions with DMSA, Unithiol and D-penicillamine are experimentally determined at 37 °C, I = 150 mmol dm^{-3} NaCl. These data, together with previously measured formation constants for the other ligands are used to compute the relative effectiveness of these drugs in binding cadmium in human blood plasma.

Introduction

At the present time there is no recommended chelating agent for the clinical therapy of cadmium poisoning and 'the treatment of acute or chronic intoxication by cadmium can only be symptomatic' [1]. Although there have been many investigations into the ability of various chelating agents to enhance cadmium excretion in the rat, very little is known concerning the chemical interaction of these ligands with the toxic metal *in vivo*. In this paper we discuss the computed efficacy of a range of potential chelating drugs for cadmium and thereby identify the agents which are likely to be the most effective in the treatment of intoxicated patients.

The liver and kidney are the two major deposition sites of cadmium *in vivo* [2]. The relative concentrations of metal in these organs depends on the magnitude and duration of exposure. Gunn and Gould [2] demonstrated that hepatic cadmium levels are initially very high but gradually decline and are accompanied by a concomitant rise in renal concentrations due to redistribution from the liver to the kidney. Metal deposited in the liver will show a propensity to be excreted as a lipophilic complex in the bile, whereas renal cadmium may be mobilised as a hydrophilic species in the urine. The different excretory routes necessitate specific approaches to cadmium chelation therapy depending on which is the major depository site. Treatment thus falls into three categories.

1. Immediate therapy with a hydrophilic drug which will complex extracellular cadmium and promote its excretion in the urine.

2. Administration of a lipophilic agent to patients with a high liver burden, mobilising hepatically deposited cadmium in the bile.

3. Treatment of cases of chronic intoxication, where the kidney is the major depository site, when a synergistic combination of drugs is required. A lipophilic drug is first administered to mobilise the metal from the kidney into plasma; a secondary hydrophilic agent is then given which effectively competes with the primary drug for the metal forming a hydrophilic complex which is excreted in the urine.

In this paper, the relative abilities of the ligands, 2,3-dimercaptopropanol (British Anti-Lewisite, BAL), 2,3-dimercaptopropane-1-sulphonic acid (Unithiol), 2,3-dimercaptosuccinic acid (DMSA), D-penicillamine, diethyldithiocarbamate (DDC), ethylenedinitrilotetraacetic acid (EDTA) and [N-carboxymethyl-2,2′-iminoethylenedinitrilo] tetraacetic acid (DTPA) are assessed and the usefulness of these agents in the categories of treatment outlined above are discussed.

The ability of each of the agents to promote excretion of cadmium in animals has previously been studied. BAL appears to be effective in reducing the mortality of acute exposure to cadmium by directing the metal away from certain sensitive loci [3]. However, the neutrality of the resulting $Cd(BAL)^0$ complex causes the metal to preferentially accumulate in the kidney where it enhances the nephrotoxicity of cadmium causing severe renal damage [3–6]. Cherian has shown BAL to be effective in chronic cadmium poisoning, mobilising both hepatically and renally deposited cadmium even after the induction of metallothionein synthesis [7, 8]. However, the toxicity of this drug, LD_{50} = 0.9 mmol kg^{-1} [9], usually restricts its clinical application.

0020-1693/84/$3.00

The toxicity of the drug has been reduced in the two analogues of BAL, DMSA and Unithiol by the introduction of hydrophilic grouping into the molecule to increase its water solubility. In DMSA two carboxylate groupings have been introduced into the molecule. DMSA has been shown to be effective in the treatment of mercury [10–12] and lead [13, 14] poisoning. *In vivo* investigations into the efficacy of DMSA in the treatment of cadmium poisoning have shown the drug to effectively mobilise hepatic deposits and to reduce renal uptake of the cadmium when given immediately after the metal [15, 16]. In the second derivative, Unithiol, the hydroxide moiety of its precursor has been replaced by a sulphonate grouping, producing a hydrophilic ligand of low toxicity [17] which is effective in the treatment of mercury poisoning [18–20]. Unithiol has been shown to promote cadmium excretion *in vivo* provided therapy is administered within a very short period after intoxication [21, 22].

The polyaminopolycarboxylic acids, EDTA and DTPA, have been shown to effectively mobilise cadmium *in vivo* [15, 23–25] but since they are hydrophilic, they are only capable of removing extracellular cadmium. Their usefulness in the treatment of cadmium poisoning is further limited by their potential renal toxicity [15, 26] as EDTA appears to enhance the nephrotoxic effects of cadmium [23]. Similarly, D-penicillamine has been shown to exacerbate cadmium's nephrotoxicity and thus appears to have limited clinical applications [15, 27].

DDC is the accepted drug for the treatment of nickel carbonyl poisoning [28]. It is lipid soluble and forms a liphophilic complex with cadmium [29, 30]. As this agent has been shown to chelate extracellular cadmium and cause its redistribution to the brain [29], it is important that its therapeutic use should be restricted to cases of chronic intoxication.

Results and Discussion

Assessment of Chelating Agents for Complexing and Removing Cadmium in vivo

The efficacies of chelating agents for complexing and removing cadmium *in vivo* may be compared by means of computer simulation. The ECCLES program [31] can be used to evaluate the effect on the low-molecular-weight (LMW) metal-ion distribution of the administration of exogenous chelating agents. The efficacy of a chelating agent for mobilising a metal-ion from the labile metal-protein complex in blood plasma is expressed in terms of a Plasma Mobilising Index (PMI) [32]:

$$\text{PMI} = \frac{\text{concentration of low-molecular-weight metal complex species in presence of drug}}{\text{concentration of low-molecular-weight metal complex species in normal plasma}}$$

PMI values for a particular ligand are computed over a wide range of drug concentrations and the results plotted as log PMI *versus* minus log drug concentration. These PMI curves allow easy visual comparison of the ability of a range of drugs to mobilise a toxic metal in plasma and also indicate any depletion of essential metals that is likely to occur. These PMI calculations require accurate values for formation constants for the appropriate metal–ligand interactions, determined under conditions applicable to blood plasma – 37 °C, I = 150 mmol dm^{-3} sodium chloride.

Formation constants for the interaction of Cd(II) ions with EDTA and DTPA have been the subject of previous investigations in this laboratory [33] and these values were used in the present simulations (Table I). Values for the stability constants for Cd(II)–DDC and Cd(II)–BAL interactions were

TABLE I. Formation Constants for Interaction of Protons, Cd(II) and Zn(II) Ions with EDTA, DTPA, BAL and DDC. $\beta_{pqr} = [M_qL_pH_r]/[M]^q[L]^p[H]^r$.

Interaction	Species			lg β_{pqr}
	p	*q*	*r*	
EDTA	1	0	1	9.120
Protonation	1	0	2	15.033
Cd(II)-EDTA	1	1	0	13.82
	1	1	1	16.55
Zn(II)-EDTA	1	1	0	14.61
	1	1	1	17.67
DTPA	1	0	1	9.673
Protonation	1	0	2	17.941
	1	0	3	22.095
	1	0	4	24.776
	1	0	5	26.908
Cd(II)–DTPA	1	1	0	17.03
	1	1	1	20.80
	1	1	2	23.59
Zn(II)–DTPA	1	1	0	17.45
	1	2	0	21.78
	1	1	1	22.53
	1	1	2	24.88
BAL Protonation[a]	1	0	1	12.30
	1	0	2	20.80
Cd(II)–BAL[a]	1	1	0	16.00
	2	1	0	27.00
Zn(II)–BAL[a]	2	2	0	32.5
	2	1	0	24.0
DDC Protonation[a]	1	0	1	3.0
Cd(II)–DDC[a]	2	1	0	12.87
Zn(II)–DDC[a]	2	1	0	10.60

[a]Estimated from the literature.

TABLE II. Formation Constants for Proton-, Cd(II)-, and Zn(II)-DMSA Interaction at 37 °C. I = 150 mmol dm^{-3} NaCl. $\beta_{pqr} = [M_qL_pH_r]/[M]^q[L]^p[H]^r$.

Interaction	Species			lg β_{pqr}	Standard Deviation	Sum of Squared Residuals	MINIQUAD *R* Factor	No. of points	No. of titrations
	p	*q*	*r*						
DMSA	1	0	1	12.00		9.85×10^{-6}	0.002	306	8
Protonation	1	0	2	21.424	0.002				
	1	0	3	24.928	0.004				
	1	0	4	27.376	0.005				
Cd(II)–DMSA[a]	1	1	0	17.11	0.03	4.11×10^{-7}	0.003	334	6
	1	1	1	23.50	0.04				
	1	1	3	28.73	0.04				
Zn(II)–DMSA	2	2	0	34.08	0.03	2.41×10^{-6}	0.004	342	5
	2	2	1	40.07	0.02				
	2	2	2	44.57	0.09				
	2	1	0	19.46	0.06				
	3	1	1	34.35	0.03				
	3	1	2	43.87	0.07				

[a]Determined in the presence of DTPA as a competing ligand.

TABLE III. Formation Constants for Proton-, Cd(II)-, and Zn(II)-Unithiol Interaction at 37 °C. I = 150 mmol dm^{-3} NaCl. $\beta_{pqr} = [M_qL_pH_r]/[M]^q[L]^p[H]^r$.

Interaction	Species			lg β_{pqr}	Standard Deviation	Sum of Squared Residuals	MINIQUAD *R* Factor	No. of points	No. of titrations
	p	*q*	*r*						
Unithiol	1	0	1	12.50		7.10×10^{-7}	0.003	213	5
Protonation	1	0	2	21.058	0.002				
Cd(II)–Unithiol	3	3	1	61.91	0.02	2.44×10^{-6}	0.008	287	8
	2	2	0	37.72	0.04				
	2	1	0	28.19	0.03				
	2	1	1	35.19	0.04				
Cd(II)–Unithiol[a]	1	1	0	17.32	0.06	2.18×10^{-6}	0.007	205	5
	2	1	0	28.22	0.09				
Zn(II)–Unithiol	2	2	0	33.58	0.01	4.12×10^{-6}	0.007	349	9
	2	1	0	27.56	0.03				
	3	2	1	52.63	0.04				

[a]Determined in the presence of DTPA as a competing ligand.

estimated from the literature. The complexation of Cd(II) ions with DMSA and Unithiol have not previously been investigated. Hence, these formation constants were experimentally determined. Constants for D-penicillamine were also measured under biological conditions.

In view of the chemical similarities between cadmium and zinc, any agent capable of removing cadmium is also likely to deplete the body of zinc. Therefore, the ability of each of the agents studied to mobilise zinc in plasma was also assessed. The formation constants used in these simulations are given in Tables I–III.

Metal–Ligand Complexation by DMSA

DMSA has two sulphydryl and two carboxylate groups comprising four possible protonation sites. Although previous studies [34, 35] have reported values for all four protonation constants, the magnitude of the lg of first association constant (11.82 [34] and 10.79 [35]) indicates the second sulphydryl group only deprotonates at very high pH and thus is beyond the scope of the glass electrode. In view of this, an estimated value of 12.00 for the first protonation constant was assumed. Formation constants derived from MAGEC-MINIQUAD [36, 37] optimisation of the experimental data are given

in Table II. Note that the arbitrary choice of 12.0 for lg β_{101} has no effect on the computed species concentrations.

The interaction of Cd(II) ions with DMSA in acidic solution results in the formation of an insoluble white complex. On addition of alkali, the precipitate redissolves, complete dissolution occurring at approximately pH = 7. To overcome this difficulty, the system was studied using DTPA as a competing ligand [38]. The competition titrations were carried out using an acidic solution of DTPA and cadmium in the vessel and an alkaline solution of DMSA in the burette. The presence of DTPA precluded the formation of the insoluble Cd(II)–DMSA species, the statistically most favourable model obtained from MINIQUAD [37] optimisation of the data obtained being given in Table II.

Zinc interacts with DMSA to form a variety of polynuclear complexes, indicated by the crossing-over and non-superimposability of the formation curves. MINIQUAD analysis of the data (Table II) shows M_2L_2, M_2L_2H, $M_2L_2H_2$, ML_2, ML_3H and ML_3H_2 to predominate in solution. The results of these studies differ from those of Schwarzenbach [34] who characterised the Zn(II)–DMSA in terms of ML, MLH, MLH_2, ML_2, M_2L, MLOH, and ML_2OH. The reported absence of any polynuclear complexes may arise from the different method of optimisation used in the early studies. Contrary to Schwarzenbach's results, the formation of hydroxy complexes was not detected in the present study, despite an extensive search for such complexes. Comparing the values of the formation constants for the ML_2 complex obtained from the two studies, Schwarzenbach measured log β_{210} as 19.76 compared with a value of 19.46 obtained in the present study. Allowing for differences in temperature and ionic strength, this agreement is good.

Metal–Ligand Complexation by Unithiol

2,3-Dimercaptopropane-1-sulphonic acid has two sulphydryl groups comprising two possible protonation sites. Like DMSA, the second sulphydryl group only deprotonates at very high pH and is thus beyond the range of the glass electrode. The presence of the two electron-withdrawing carboxylate groupings on DMSA means the magnitude of the first protonation constants for Unithiol is likely to be greater than the value of lg β_{101} = 12.0, estimated for DMSA. Consequently, a value of 12.50 for lg β_{101} was employed for Unithiol. The results of the MAGEC-MINIQUAD optimisation of the protonation data are shown in Table III.

Cd(II) ions interact with Unithiol to form a range of polynuclear and protonated species. A wide range of possible models were optimised using MINIQUAD. Of these, the model containing the M_3L_3H, M_2L_2, ML_2 and ML_2H species (Table III) was considered to most accurately represent the experimental data. The formation of these polynuclear complexes precludes the measurement of the formation constant of the mononuclear species such as ML. At the low metal-ligand concentrations encountered in plasma, the formation of polynuclear complexes is unlikely and therefore it is important to have values for the mononuclear formation constants. These constants may be determined by use of a secondary competing ligand [38]. In such a system the competing ligand reduces the free metal ion concentrations, thus favouring the formation of mononuclear over polynuclear complexes. DTPA was chosen as the secondary ligand since the formation constants for its interaction with Cd(II) ions under biological conditions are known [33], its affinity for cadmium is comparable to Unithiol, it binds strongly in acid solution (the region where polynuclear complex formation is most likely) and ternary complex formation with the polyaminopolycarboxylic acids are unlikely. The results of MINIQUAD optimisation of the competition data is given in Table III. Comparison of the magnitude of lg β_{210} derived from these investigations with that obtained from the binary data (28.22 and 28.19, respectively) reveals a close correlation between the two studies.

Zn(II) ions also interact with Unithiol to form polynuclear and protonated complexes. The constants for the 220, 210, 321 species which are considered most likely to form in solution are given in Table III.

Metal–Ligand Complexation by D-Penicillamine

To obtain constants applicable to biological conditions, D-penicillamine was included in these experimental investigations. The results for the interaction of this ligand with protons, Cd(II) and Zn(II) ions, are given in Table IV. D-Penicillamine has three possible protonation sites – the sulphydryl, amino and carboxylate groupings. In metal-ligand complexation it has been proposed that the D-penicillaminate molecule binds to the central metal ion *via* both the sulphur and amino groups [39], leaving the carboxylate group free to form protonated species such as the ML_2H complex formed by cadmium.

Mobilisation of Cd(II) ions by Chelating Agents

The relative abilities of the chelating agents to mobilise cadmium from the labile protein complexes in plasma was evaluated using the ECCLES program [31]. The resulting PMI curves are depicted in Fig. 1 and a list of the major cadmium-containing species at a drug concentration of 10^{-5} mol dm^{-3} is given in Table V.

From the results of the simulations it is possible to assess the efficacy of each ligand in the treatment of the various categories of cadmium intoxication.

TABLE IV. Formation Constants for Proton-, Cd(II)-, and Zn(II)-D-penicillaminate (D-Pen) Interaction at 37 °C. I = 150 mmol dm^{-3} NaCl. $\beta_{pqr} = [M_qL_pH_r]/[M]^q[L]^p[H]^r$.

Interaction	Species			$\lg \beta_{pqr}$	Standard Deviation	Sum of Squared Residuals	MINIQUAD R Factor	No. of points	No. of titrations
	p	q	r						
D-Pen	1	0	1	10.244	0.003	4.49×10^{-6}	0.004	323	5
Protonation	1	0	2	17.921	0.005				
	1	0	3	19.827	0.007				
Cd(II)–D-Pen	1	1	0	10.742	0.005	1.27×10^{-6}	0.003	306	6
	2	1	0	17.68	0.02				
	2	1	1	24.67	0.03				
Zn(II)–D-Pen	1	1	0	10.017	0.006	4.22×10^{-7}	0.002	221	5
	2	1	0	18.809	0.009				

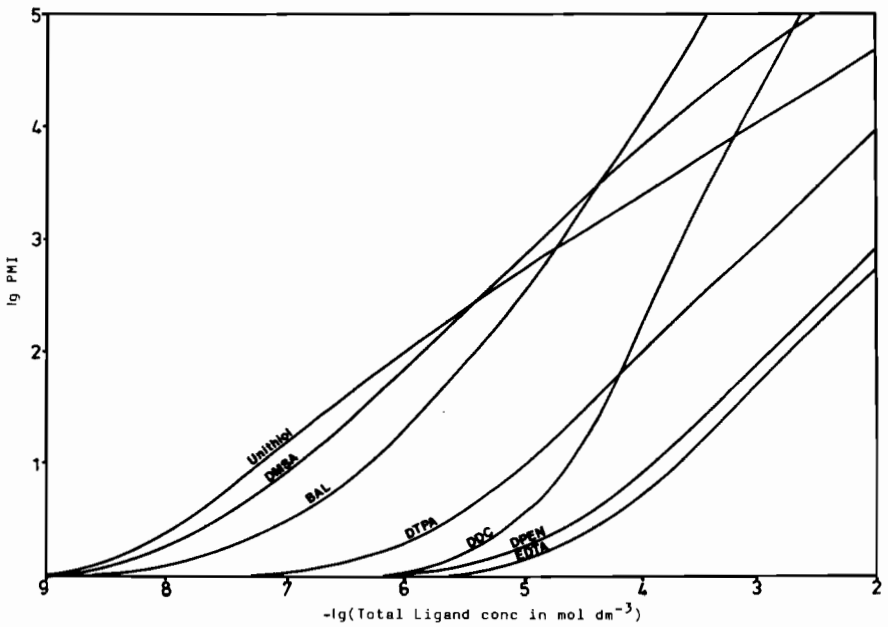

Fig. 1. Curves for lg of Cd(II) Plasma Mobilising Index (PMI) *versus* –log drug concentration.

TABLE V. Major LMW Cadmium Complexes Produced by the Administration of Chelating Agents at a Concentration of 10^{-5} mol dm^{-3}. [a]

Ligand	Species formed	lg β	% LMW Cadmium
BAL	$Cd(BAL)^0$	16.00	52.2
	$Cd(BAL)_2^{2-}$	27.00	45.9
D-Penicillamine	$Cd(PEN)^0$	10.74	33.6
	$Cd(CYS)^0$	10.33	28.6
	$Cd(CIS)^0$	8.25	16.7
DDC	$Cd(DDC)_2^0$	12.87	50.1
	$Cd(CYS)^0$	10.33	14.9
	$Cd(DDC)(CYS)^-$	15.24	12.2

(continued overleaf)

TABLE V. *(continued)*

Ligand	Species formed	lg β	% LMW Cadmium
EDTA	$Cd(CYS)^0$	10.33	32.0
	$Cd(EDT)^{2-}$	13.82	31.9
	$Cd(CIS)^0$	8.25	18.6
DTPA	$Cd(DTP)^{3-}$	17.03	88.7
DMSA	$Cd(DMS)^{2-}$	17.11	90.7
	$Cd(DMS)H^-$	23.50	9.0
Unithiol	$Cd(DMP)^-$	17.32	90.3
	$Cd(DMP)_2^{4-}$	28.19	6.4

[a]Abbreviations: CYS = Cysteinate, CIS = Cystinate, PEN = D-Penicillaminate, EDT = EDTA, DTP = DTPA, DMS = 2,3-dimercaptosuccinate (DMSA), DMP = 2,3-dimercaptopropane-1-sulphonate (Unithiol).

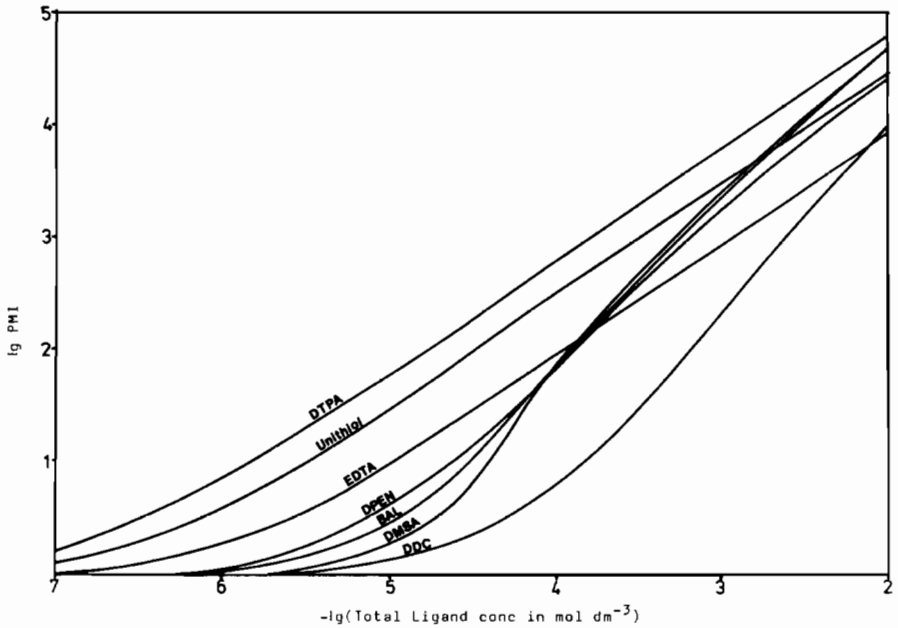

Fig. 2. Curves for lg Zn(II) Plasma Mobilising Index (PMI) *versus* –log drug concentration.

DTPA, EDTA, Unithiol and DMSA are all hydrophilic in nature and are thus likely to be most effective in the treatment of cadmium poisoning if administered immediately after intoxication. The simulations show that these ligands form charged complexes with cadmium (Table V) and are thus likely to promote urinary excretion of the metal. The PMI curves indicate that DMSA and Unithiol are the most effective mobilisers of cadmium in plasma. However, recent studies have shown that this efficacy may be reduced by the interaction of these ligands with the organic constituents of plasma [40], thus reducing the amount of drug available to bind the toxic metal. DTPA may be an effective hydrophilic drug for cadmium poisoning providing its nephrotoxic effects [15, 23, 26] can be restricted, possibly by simultaneous zinc supplementation.

Of the agents studied, only BAL, DDC and D-penicillamine possess any degree of liphophilicity. These ligands form neutral complexes with Cd(II) ions (Table V). They, therefore, constitute potential drugs for the treatment of fairly recent exposure to cadmium where the metal is concentrated in the liver. However, the use of BAL and D-penicillamine appears to be restricted by their toxicities [9, 27].

The possible treatment of chronic intoxication using a synergistic pair of drugs is more complicated. Both drugs must be effective mobilisers of cadmium *in vivo*, but for an exchange of the metal between the two ligands in plasma, the secondary ligand must have the greater affinity. In other words, the PMI value for the hydrophilic agent must exceed that of the lipophilic drug.

TABLE VI. Major LMW Zinc Complexes Produced by the Administration of Chelating Agents at a Concentration of 10^{-5} mol dm^{-3}.[a]

Ligand	Species formed	log β	% LMW Zinc
BAL	$Zn(BAL)(CYS)^{2-}$	21.24	30.4
	$Zn(BAL)_2^{2-}$	24.00	15.4
	$Zn(CYS)_2^{2-}$	17.77	12.0
D-Penicillamine	$Zn(PEN)(CYS)^{2-}$	18.64	33.3
	$Zn(PEN)_2^{2-}$	18.81	22.5
DDC	$Zn(CYS)_2^{2-}$	17.77	28.7
	$Zn(DDC)(CYS)^{-}$	14.53	16.3
	$Zn(CYS)(HIS)^{-}$	14.93	13.3
EDTA	$Zn(EDT)^{2-}$	14.62	89.6
DTPA	$Zn(DTP)^{3-}$	17.45	96.4
DMSA	$Zn_2(DMS)_2^{4-}$	34.08	44.0
	$Zn(CYS)_2^{2-}$	17.77	20.8
Unithiol	$Zn(DMP)_2^{4-}$	27.56	68.2
	$Zn(DMP)(CYS)^{3-}$	23.01	19.8

[a]Abbreviations: HIS = Histidinate, CYS = Cysteinate, CIS = Cystinate, PEN = D-Penicillaminate, EDT = EDTA, DTP = DTPA, DMS = 2,3-dimercaptosuccinate (DMSA), DMP = 2,3-dimercaptopropane-1-sulphonate (Unithiol).

Thus, regardless of its toxicity, BAL seems an unsuitable lipophilic ligand because it binds the metal too strongly. A combination of DDC, or some other suitable lipophilic agent, with DMSA, Unithiol or DTPA may prove effective in the treatment of chronic intoxication.

Mobilisation of Zn(II) Ions by Chelating Agents

The PMI curves given in Fig. 2 illustrate the computed effectiveness of each of the sequestering agents studied at mobilising Zn(II) ions from the plasma proteins. From this it is apparent that most of the ligands studied are likely to bind to the metal *in vivo*, the greatest depletion being caused by DTPA administration. These postulates are in accord with *in vivo* observations [41, 42].

Table VI lists the major LMW zinc-containing species formed at a drug concentration of 10^{-5} mol dm^{-3}. Since these species are all negatively charged, each of the agents are likely to promote an increased urinary excretion of the metal.

Conclusions

An assessment of chelating drugs in the treatment of cadmium poisoning reveals that DMSA or Unithiol are likely to be the most efficacious ligands in the immediate treatment of intoxication. The likely possibility of side effects arising from the depletion of zinc may be greatly reduced by the simultaneous administration of this essential metal with the therapeutic agent.

A synergistic therapy is proposed for the treatment of chronic intoxication involving a lipophilic drug to mobilise intracellular cadmium followed by a hydrophilic agent (such as DMSA or Unithiol) to promote urinary excretion of the metal. However, the use of currently available lipophilic drugs appears to be restricted by their toxicity. In view of this, future research should concentrate on the development of lipophilic drugs for mobilisation of cadmium from the tissues which have a sufficiently low toxicity to be used in the clinical situation.

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