COMPUTER SIMULATION OF CHELATION THERAPY

Plasma mobilizing index as a replacement for effective stability constant

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1. Introduction

When Schubert introduced [1] the concept of 'effective' or 'conditional' stability constants he did so in order to represent the ability of ligands to bind metal ions in vivo. He recognised that the extent of this binding would be determined not only by the metal–ligand formation constant but also by other parameters, in particular the ligand's protonation constants and the equilibrium constants of any other competitive metal-ion–ligand interactions in the physiological medium. This approach was successful in showing how the calcium complex of polyaminocarboxylic acids could modify their therapeutic capacity for binding other metal ions [1].

Nevertheless, many research workers still attempt to correlate the biological response to chelating agents simply with the 1 : 1 complex formation constant. One reason is that the calculation of effective stability constants is not straightforward, particularly when many side-reactions need to be considered. There are also other difficulties with Schubert's approach; for example, the relative effects of concentration are obscure. A more fundamental issue is that in vivo the ligand may form more than one complex species in significant concentrations. In addition to the complexes which might be protonated to differing extents, there may be ternary complexes formed in conjunction with naturally occurring ligands.

Our recent publications of computer models involving many thousand complexing reactions permit a more basic and comprehensive study of metal-ion equilibria in biofluids [2]. Because important metal–protein interactions are still too poorly characterised to be included satisfactorily in such simulations, methods of establishing and depicting results in a manner independent of these protein equilibria must be found. The large difference in the concentrations of proteins and of low molecular weight ligands compared with the concentrations of most metals in blood plasma makes this possible. Thus, it has been shown that although the absolute concentrations of metal complexes are controlled by the extent of protein binding, the percentage distribution of transition metal ions amongst low molecular weight ligands is not [2].

2. Methods

2.1. *Plasma Mobilizing Index (PMI)*

We now report an extension of this approach to include a number of therapeuticals in current use which promote the excretion of Cu, Fe, Mn, Pb, or Zn. The relative ability of each agent to compete for these metals in the biofluids has been obtained by calculating a 'Plasma Mobilizing Index' (PMI) from the computer simulation results. For each metal ion, we define

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

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Considering the blood plasma compartment as a closed system, this index is a convenient measure of the ability of the chelating agent, at a given concentration, to mobilize metal-ions from the labile protein-bound fraction. This arises because the free metal-ion concentration is buffered by the metal–protein complex until the agent has bound an appreciable fraction of this exchangeable metal in plasma. The effect of errors associated with protein binding, as previously discussed, is eliminated because the uncertain absolute value of the free metal-ion concentration appears as a factor in all terms in both the numerator and the denominator of the PMI expression.

3. Results and discussion

Our studies reveal the considerable importance and general applicability of two factors in determining the physiological response towards a chelating agent.

(1) The agent must be capable of competing effectively for exchangeable protein-bound metal-ions in blood plasma.

(2) The lipophilicity of the predominant complexes in plasma as determined by their charge, controls the metal-ion distribution between body compartments.

Whilst other contributions to the pharmacokinetics of the complexes can obviously be expected, the results of computer models, interpreted in accordance with the above two concepts, rationalise, on a chemical basis, a substantial number of observations concerning chelation therapy, as will now be illustrated.

The PMI-curves calculated for ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), cyclohexylenedinitrilotetraacetic acid (CDTA), desferrioxamine (DFO), penicillamine (Pen), 2,3-dimercaptopropanol (BAL) and triethylenetetramine (Trien) are shown in fig.1. Their order correlates strikingly well with the ability of these agents to promote trace-metal excretion. Although direct comparisons in the literature are sparse, the relative physiological efficacies appear to be as follows. For Cu, Trien > DTPA > EDTA [3,4]. For Fe, DFO, CDTA > DTPA > EDTA > no effect [5]. For Mn, CDTA ≈ DTPA > EDTA > Pen = DFO = no effect [6,7]. For Pb, DTPA ≥ EDTA ≥ CDTA > Pen ≈ BAL > DFO = no effect [8,9]. For Zn, DTPA, CDTA > EDTA > Pen > DFO = no effect [10,11].

The good correlation between calculated and observed parameters is most significant when one

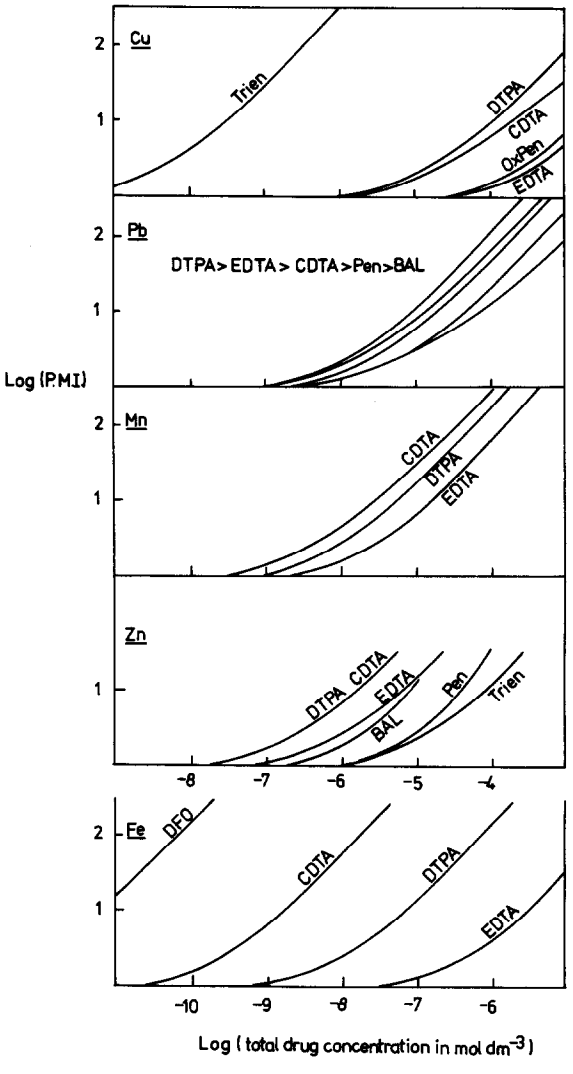

Fig.1. PMI Curves with all seven chelating agents have been calculated for each metal-ion. Where these are omitted from fig.1 the agent is unable to compete in vivo for the metal in question. Note that the exceptionally high PMI-values computed for DFO are in practice offset by the rapid metabolic degradation of this agent leading to relatively low total concentrations in the plasma.

considers the relative differences in biological response elicited by the chelating agents rather than their exact positions in the comparative series just given. In this regard the PMI-values, unlike the formation constants (see table 1) accurately portray the physiological properties. For example, if the excretion of Pb and Zn caused by DTPA, EDTA and Pen is considered, both the similarity of these ligands in their effect on Pb [8] and the systematic difference they display towards Zn [10,11] are reflected in fig.1.

The one noticeable anomaly in this study is the cupresis induced by penicillamine. The figure indicates that Pen-disulphide is unable to compete successfully for Cu(II) in plasma. Hence, we have recently challenged the view that Pen exerts its very considerable effect by acting as a chelating agent [12]. Instead, it was proposed that copper may be liberated from (inert) metalloproteins by reduction to Cu(I). This hypothesis is in accord with several experimental studies [11,13].

Table 1
Important species formed by chelating agents in blood plasma as found by computer simulation

Chelating agent administered[a]	Species formed	Formation constant[b] (log values)	Percentage total ligand[c]	Percentage total metal in low mol. wt fraction[c]
EDTA	$Ca.EDTA^{2-}$	10.4	73	–
	$Zn.EDTA^{2-}$	16.0	26	100
	$Cu.EDTA^{2-}$	18.5	~0	84
	$Fe.EDTA^{-}$	24.8	~0	77
	$Fe.EDTA.OH^{2-}$	30.5	~0	23
	$Pb.EDTA^{2-}$	17.1	~0	100
	$Mn.EDTA^{2-}$	13.6	~0	100
DTPA	$Zn.DTPA^{3-}$	17.8	91	98
	$Ca.DTPA^{3-}$	10.6	7	–
	$Cu.DTPA^{3-}$	21.0	~0	99
	$Fe.DTPA^{2-}$	27.8	~0	99
	$Pb.DTPA^{3-}$	18.6	~0	100
	$Mn.DTPA^{3-}$	15.3	~0	100
CDTA	$Zn.CDTA^{2-}$	18.6	77	100
	$Ca.CDTA^{2-}$	12.0	22	–
	$Cu.CDTA^{2-}$	21.7	~0	98
	$Fe.CDTA.OH^{2-}$	36.5[d]	~0	99
	$Fe.CDTA^{-}$	28.0	~0	1
	$Pb.CDTA^{2-}$	19.1	~0	100
	$Mn.CDTA^{2-}$	16.4	~0	100
DFO	$Fe.DFO^{+}$	29.8	98	99
	Fe.DFO.OH	34.0	1	1
	$H_3.DFO^{+}$	26.8	1	–
	Zn.DFO	11.0	~0	8
Pen	$H_2.Pen$	18.1	40	–
	$Zn.Pen_2^{2-}$	18.7	20	89
	$H.Pen^{-}$	10.3	16	–
	$Zn.H.Pen_2^{-}$	25.0	2	7
	Cu.H.OxPen.His	25.3	~0	54
	$Cu.OxPen.His^{-}$	17.6	~0	27
	Pb.Pen	12.5	~0	56
	$Pb.H.Pen_2^{-}$	26.4	~0	35
	$Pb.Pen.Cit^{3-}$	16.2	~0	8
	Mn.Pen	5.0	~0	1

Table 1 (continued)

Chelating agent administered[a]	Species formed	Formation constant[b] (log values)	Percentage total ligand[c]	Percentage total metal in low mol. wt fraction[c]
BAL	$Zn.BAL^{2-}$	22.0	38	84
	H_2BAL	19.2	16	–
	Zn.BAL	13.0	6	14
	Pb.BAL	14.0	~0	97
	$Pb.BAL_2^{2-}$	20.5[d]	~0	2
Trien	$H_2.Trien^{2+}$	18.5	86	–
	$H_3.Trien^{3+}$	24.9	9	–
	$Zn.Trien^{2+}$	11.2	3	99
	$H.Trien^{+}$	9.6	2	–
	$Cu.Trien^{2+}$	19.4	~0	100
	$Fe.Trien^{3+}$	21.3	~0	100
	$Pb.Trien^{2+}$	9.8	~0	15
	$Mn.Trien^{2+}$	4.7	~0	0.5

[a] Symbols represent anionic forms of the agents defined in the text. In addition, OxPen = oxidized Pen, His = histidinate and Cit = citrate.

[b] Formation constants have been selected from the literature [17,18] and corrected to conform with the temperature and ionic strength of blood plasma as previously described [2].

[c] The percentages refer to a total ligand concentration = 10^{-3} mol.dm^{-3} In general, lower total ligand concentrations will cause the figure for the percentage of total metal to decrease, finally becoming zero, whereas there will be little change reflected in the percentage distribution of the ligand itself.

[d] This formation constant is somewhat uncertain. The value used in the computer simulations is probably too low in which case, a correspondingly low set of PMI-values are shown in the figure.

The value of computer models in this field of research is well illustrated by the poor Cu-excretion caused by EDTA in spite of a high equilibrium constant [4]. The response is predictable, however, in view of the low PMI computed for this ligand. In contrast, any consideration based on the effective stability constant calculated by Schubert would lead to an erroneous conclusion [1].

The other important aspect of chelation therapy which the computer simulations have illuminated concerns the distribution of metal-ions amongst different body compartments. In general, those chelating agents which form charged species are confined to the plasma until eliminated by the kidneys. Neutral complexes on the other hand passively diffuse through membranes so that the metal tends to spread throughout the body tissue [14].

The models are thus able to explain the medical observation that plumbism is best treated in two stages – initial EDTA intravenous-infusions are followed by longer term oral-therapy with Pen [15]. The negatively charged $Pb–EDTA^{2-}$ complex is strongly formed in plasma and quickly removed. However, lead which has dispersed into the tissues is inefficiently leached back into plasma by the polyaminocarboxylic acid. Pen forms a neutral complex which is easily returned to plasma once a favourable concentration gradient is established. This also explains the experimental finding that early treatment with Pen can be detrimental because it actually increases lead deposition in essential organs [16]. Moreover, the serious side-effects caused by Zn-deficiency sometimes associated with Pen-therapy arise because the $Zn–Pen_2^{2-}$ complex predominates. This species is negatively charged and hence eliminated into the urine [11].

4. Conclusions

Computationalised approaches can clearly facilitate the search for chelating therapeutical replacements. Indeed, the design of drugs which can selectively

alter the concentration of a metal-ion in a specific body compartment can now be envisaged. Further, the systematic evaluation of proposed new drugs by computer simulation would indicate whether physiological screens should focus upon trace-metal concentrations more than is customary. In this manner, a substantial improvement in drug safety procedures could be easily and cheaply accomplished.

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