Metal Binding Properties of Two Homologues of Razoxane, a Bisdioxopiperazine Antitumour Drug

ZHONG-XIAN HUANG*, ANDREW. M. CREIGHTON** and DAVID R. WILLIAMS

Department of Applied Chemistry, UWIST, Cardiff CF1 3XF, U.K.

Received February 23, 1985

Razoxane (ICRF 159) is an antitumour drug which hydrolyzes *in vivo* to form a polydentate ligand. A wide range of homologues of Razoxane (Fig. 1) have been synthesized and these have been screened for cytotoxic activity. In a previous paper [1], we have investigated an active drug, ICRF 159, and an inactive homologue, ICRF 192, the screening data for which are shown in Table I [2]. Our aim was to study whether metal ion chelation might be involved in the *modus operandi* of the cytotoxic activity of these agents and also whether metal dependent side-effects might be anticipated from their administration. We intentionally studied a pair of homologues, one of which was active and the other inactive (ICRF 159 and 192, respectively) and in order to add more data we now report metal complexing investigations and computer simulation modelling of a pair of stereoisomers, the active agent being ICRF 193, and the inactive one being ICRF 196 (Table I and Fig. 1).

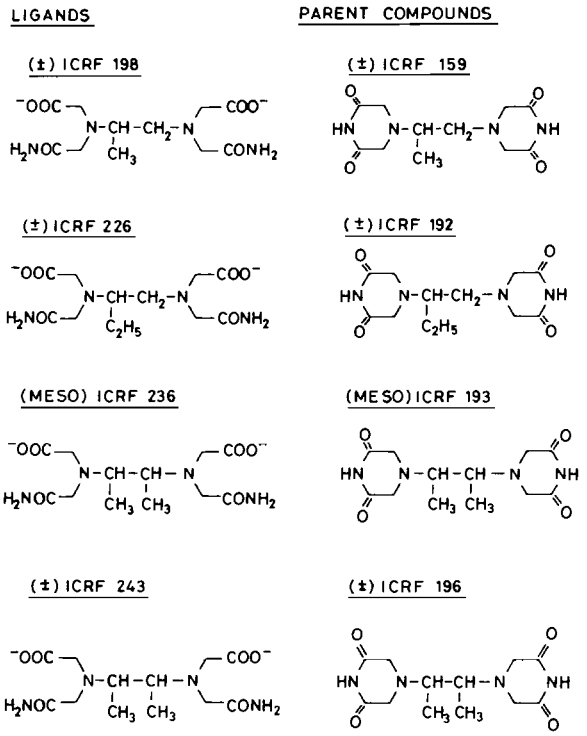

Fig. 1. Ligands and parent cyclic imides referred to in the text and in Table I.

Experimental

ICRF 236 and 243 were prepared from the cyclic imides by reaction with cupric acetate in aqueous dimethyl sulphoxide to give the copper chelates of the ligands from which the metal was then removed with H_2S, essentially by the method described previously [1]. *meso*-NN′-Dicarboxamidomethyl-NN′-dicarboxymethyl-2,3-diaminobutane (ICRF 236) was obtained as a colourless microcrystalline solid, m.p. 215–216 °C (dec). IR (mull) 1580 (infl), 1640 (infl), 1675, 1725 (C=O); 3400 (NH) *Anal.* Found: C, 45.2; H, 7.0; N, 17.4. Calcd. for $C_{11}H_{22}N_4O_6$: C, 45.3; H, 7.0; N, 17.6%. *dl*-NN′-Dicarboxamidomethyl-NN′-dicarboxymethyl-2,3-diaminobutane (ICRF 243) was obtained as a colourless microcrystalline solid, m.p. 246–247 °C (dec)., IR (mull), 1580 (infl), 1625, 1685, 1750 (C=O); 3080, 3160, 3305, 3405 (infl). *Anal.* Found: C, 45.1; H, 6.9; N,

*On leave from Department of Chemistry, Fudan University, Shanghai, China.

**Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, U.K.

TABLE I. Structures, Stereochemistry and Screening Data for Dose Required to Reduce the Colony Forming Ability of Mouse L-cells by 50% (from Ref. 2).

Parent Imides	R	R′	Stereochemistry	ID_{50} (μmol dm^{-3})	Ring opens to produce
ICRF 159	CH_3	H	*dl*	3.0	ICRF 198
ICRF 192	C_2H_5	H	*dl*	720	ICRF 226
ICRF 193	CH_3	CH_3	*meso*	0.09	ICRF 236
ICRF 196	CH_3	CH_3	*dl*	150	ICRF 243

0020-1693/85/$3.30

TABLE II. Formation Constants for ICRF 236 at 37 °C. I = 150 mmol dm^{-3} (NaCl). $\beta_{pqr} = [L_pM_qH_r]/[L]^p[M]^q[H]^r$.

p	*q*	*r*	Lg β_{pqr}	Standard Deviation	Sum of Squares of Residuals	MINIQUAD R Factor	Data Points	No. of Titrations
Proton								
1	0	1	6.870	0.004				
1	0	2	10.503	0.006	3.42×10^{-6}	0.0050	332	6
1	0	3	11.974	0.009				
Calcium(II)								
1	1	0	4.037	0.007	1.12×10^{-6}	0.0034	302	8
Copper(II)								
1	1	0	11.724	0.071	4.18×10^{-6}	0.0073	232	6
1	1	−1	3.569	0.081				
Iron(II)								
1	1	0	7.926	0.010				
1	1	−1	−0.136	0.070	3.28×10^{-6}	0.0067	187	5
2	1	2	20.229	0.044				
Magnesium(II)								
1	1	0	2.912	0.006	8.23×10^{-7}	0.0030	316	9
Manganese(II)								
1	1	0	7.615	0.004	1.76×10^{-6}	0.0043	273	7
1	1	1	9.452	0.046				
Zinc(II)								
1	1	0	9.904	0.011	8.22×10^{-7}	0.0021	338	6
1	1	1	11.349	0.031				

17.5. Calcd. for $C_{11}H_{22}N_4O_6$: C, 45.3; H, 7.0; N, 17.6%. Metal ion and ligand solutions and potentiometric titrations were prepared and carried out at 37 °C and I = 150 mmol dm^{-3} (sodium chloride) as previously described [3]. The data treatment using MAGEC and MINIQUAD cycling, graphical analysis using PSEUDOPLOT, and ECCLES modelling of blood plasma, was described [4].

Results and Discussion

The cyclic diimides which are sufficiently lipophilic to penetrate cells, hydrolyze in order to produce the polydentate ligands referred to as ICRF 236 and 243. The protonation and formation constants for these two ligands are given in Tables II and III. Plasma mobilizing index curves for these two agents with the metal ions concerned are plotted in Fig. 2.

The following conclusions may be drawn:

(*1*). It is not possible to make direct comparisons between the formation constants for metal complexing between ICRF 236 and ICRF 243 because their

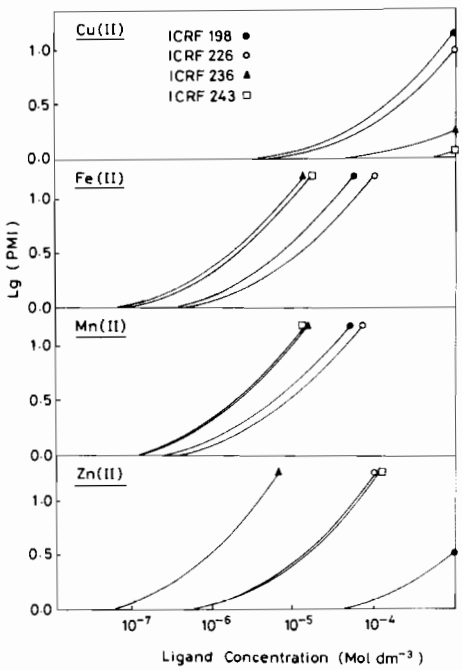

Fig. 2. Plasma mobilizing index curves for copper, iron, manganese and zinc in the presence of varying concentrations of ICRF236 and 243.

TABLE III. Formation Constants for ICRF 243 at 37 °C, I = 150 mmol dm^{-3} [NaCl]. $\beta_{pqr} = [L_pM_qH_r]/[L]^p[M]^q[H]^r$.

p	*q*	*r*	Lg β_{pqr}	Standard Deviation	Sum of Squares of Residuals	MINIQUAD R Factor	Data Points	No. of Titrations
Proton								
1	0	1	8.407	0.007				
1	0	2	12.311	0.011	3.10×10^{-6}	0.0063	352	6
1	0	3	14.149	0.014				
Calcium(II)								
1	1	0	7.256	0.005	1.75×10^{-6}	0.0050	344	7
Copper(II)								
1	1	0	12.626	0.019	1.86×10^{-6}	0.0060	189	6
1	1	−1	4.695	0.065				
Iron(II)								
1	1	0	10.915	0.013				
1	1	−1	2.379	0.046	8.97×10^{-6}	0.0099	223	6
2	1	2	25.214	0.056				
Magnesium(II)								
1	1	0	5.874	0.012	4.20×10^{-6}	0.0078	305	8
Manganese(II)								
1	1	0	10.626	0.008				
1	1	1	12.225	0.060	2.44×10^{-6}	0.0053	311	6
2	1	−1	1.274	0.023				
Zinc(II)								
1	1	0	11.720	0.018	3.13×10^{-6}	0.0058	238	6
2	1	−1	4.254	0.048				

protonation constants are rather dissimilar. A complete consideration requires evaluation using the ECCLES model which takes into account all competing ligands, metal ions and protons in fluids such as blood plasma. When ICRF 236 and 243 formation constants are compared with those of ICRF 198 and 226, it is noteworthy that the ligands studied in this paper display weaker metal binding properties and that the ligand giving rise to cytotoxic activity from this pair, ICRF 236, is two orders of magnitude weaker in terms of its binding power than the less active stereoisomer, ICRF 243. The only possible exception to this observation is Cu(II) which is bound equally by both ligands.

(*2*). Thus, there appears to be no simple correlation between metal binding capabilities and biological activity but such binding may be considerably modified *in vivo* by the presence of other ligands and metal ions. The ECCLES modelling shows the increase in low molecular mass complexes produced by ICRF 198, 226, 236 and 243 (Fig. 2). The divalent metal ions of copper, iron, manganese, and zinc are considerably mobilized ionto l.m.m. species by all the ICRF ligands at concentrations of the drug likely to be found *in vivo* (*i.e.* 10^{-7}–10^{-4} mol dm^{-3}). Calcium and magnesium ions, on the other hand, are not mobilized by the drug under blood plasma conditions. Interestingly, the major species formed tends to be a neutral 1:1 metal: ligand complex which, obviously, should have lipophilic properties.

(*3*). Although we conclude that there is no obvious correlation between metal ion mobilization in blood plasma and the cytotoxicity of the parent bisdioxopiperazines, it is quite conceivable that biological activity arises because of a combination of such metal binding and lipophilicity variations from system to system. For example, this sort of dual effect might be used to explain why the complexing order for Cu(II) ions *in vivo* appears to be ICRF 198 > 226 > 236 > 243, whereas the biological activity reported in Table I is ICRF 236 > 198 > 243 > 226. Further work is clearly necessary in order to identify which metal ion(s) might be the target – it may not be

copper as used in the example above – and to quantify bioavailability factors based upon lipophilicity of parent drug, hydrolysis product, and metal complexes.

(*4*). Over and above the cytotoxic properties of these agents, we have also investigated the ability of such ligands to remove undesirable metal ions, such as cadmium, lead, and nickel, from humans [5].

Acknowledgements

We thank Drs. Peter May, Kevin Quinlan and Gillian Smith for their advice and encouragement.

References

1 Z.-X. Huang, P. M. May, K. M. Quinlan, D. R. Williams and A. M. Creighton, *Agents Actions, 12,* 536 (1982).

2 A. M. Creighton, W. A. Jeffery and J. Long, in M. A. Simkins (ed.), 'Proceedings of 6th International Symposium on Medicinal Chemistry', Cotswold Press, Oxford, 1979, p. 281.

3 M. J. Willes and D. R. Williams, *Inorg. Chim. Acta, 80,* L35 (1983).

4 M. J. Willes and D. R. Williams, *Inorg. Chim. Acta, 106,* L21 (1985).

5 P. M. May, M. J. Willes, D. R. Williams and A. M. Creighton, *Agents Actions, 15,* 486 (1984).