Biological Significance of Cimetidine Sulfoxide Complexes with Copper(II) and Zinc(II) Ions During Cimetidine Treatment

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(Received December 5, 1985; revised February 20, 1986)

Abstract

Following a recent investigation into cimetidine interactions with copper(II) and zinc(II), the present work deals with the study of coordination equilibria relative to the main metabolite of this drug, *i.e.* cimetidine sulfoxide, with the same metal ions under physiological conditions.

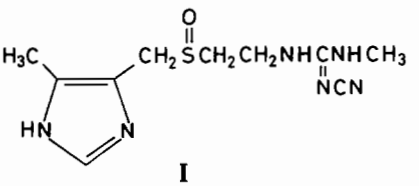
Computer simulations were run on the basis of the corresponding complex stability constants, in order to assess the extent to which cimetidine sulfoxide may affect copper(II) and zinc(II) plasma distributions during long term cimetidine therapy.

No significant effect of this kind can be expected from cimetidine sulfoxide for plasma concentrations corresponding to usual therapeutic levels of the parent molecule, even for patients presenting with impaired renal function.

Introduction

Some side effects of cimetidine may be related to its potential interactions with metal ions. For instance, the disputable role of this drug with regard to rhumatoid arthritis [1–3] could possibly be due to its involvement in copper bioavailability [4–6], whereas sexual dysfunctions would rather derive from its intereference with zinc metabolism [7–9]. Accordingly, cimetidine interactions with copper(II) and zinc(II) ions were recently investigated, and the physiological significance of the corresponding complexes was assessed by means of computer simulations at therapeutic levels of the drug [10].

It was clearly established from these studies that no significant influence could be expected from cimetidine on the distribution of the low-molecular-weight fraction of either of these metals [10]. Nevertheless, several reports have recently pointed out that even though a drug cannot interfere with the metabolism of a given metal ion, some of its degradation products may do so [11–13]. In the present case, the major metabolite of cimetidine has been characterized as its sulfoxide derivative (**I**) in man as well as in rat and



I

dog [14]. The present work thus deals with the quantitative study of the potential copper(II) and zinc(II) cimetidine sulfoxide complexes. Given the role of histidine as the main low-molecular-weight ligand of copper(II) in blood plasma [6, 15] the copper(II)–cimetidine sulfoxide–histidine ternary system has also been investigated. The results are discussed in terms of biological significance for the corresponding complexes, as based on pertinent blood plasma simulations.

Complex Formation Studies

Reagents

A sample of cimetidine sulfoxide was kindly supplied by SK & F Laboratories Ltd, whereas histidine was purchased from Merck as a biochemical grade reagent. Ligand stock solutions, prepared by dissolving these commercial products with double deionised water, were stored under a nitrogen blanket. Stock solutions of copper and zinc chloride in slightly acidic water were prepared and standardized as previously described [10].

Pro analysi Merck sodium chloride 0.15 mol dm^{-3} was used as a background electrolyte to maintain activity coefficients constant and to insure isotonicity with blood plasma. The sodium hydroxide solutions used in the titrations were prepared from BDH concentrated volumetric solutions diluted in freshly boiled double deionised water. They were standardized and proved to be carbonate-free by GRAN titrations [16] against Prolabo RP p.a. potassium phthalate.

Equipment and Technique

Due to the small amount of cimetidine sulfoxide available, potentiometric titrations were carried out

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0020-1693/86/$3.50

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using a combined Metrohm number EA 125 pH glass electrode fitted in a 5 cm^3 Metrohm cell system. The temperature was maintained at 37 ± 0.02 °C by circulating thermostatted water, and a constant bubbling of purified nitrogen was set up in the cell.

Initial solutions to be titrated, whose volume was fixed at 4 cm^3, were made sufficiently acid to make sure that all ligand donor sites were protonated. Successive aliquots of sodium hydroxide 20.06 mmol dm^{-3} were delivered from a Radiometer ABU 12 Autoburette equipped with a 2.5 cm^3 glass cylinder. The corresponding e.m.f. variations were monitored with a BECKMAN 4500 mV-meter, experiments being stopped whenever a steady drift was noted in the readings, due to precipitation problems.

The electrode system was calibrated in terms of concentrations and the ionic product of water was determined as $10^{-13.25}$ under the present experimental conditions [17].

Table I reports the initial total concentrations of the reactants along with the pH ranges investigated. On account of the electrode calibration scale, the pH notation actually stands for $-\log[H]$ throughout this study.

Calculation Procedures

Stoichiometries of the potential complexes as well as rough estimations of their formation constants were deduced from graphical considerations based on (i) protonation curves for protonation and ternary complex equilibria, with

$$\bar{s} = \frac{C_H + C_{His} - C_{OH} - [H] + [OH]}{C_{Cso} + C_{His}} \quad (1)$$

in which C_H represents the total concentration of hydrochloric acid, C_{His} the total concentration of histidine and C_{Cso} the total concentration of cimetidine sulfoxide, (ii) formation curves for binary complex equilibria, with

$$\bar{r} = \frac{C_{Cso} - [Cso\,H] - [Cso]}{C_M} \quad (2)$$

where M alternatively stands for copper or zinc.

The MINIQUAD programme [18] was used to test the existence of these different species by refining the aforementioned estimated constants. Beforehand, the cimetidine sulfoxide protonation constant was optimized together with ligand and acid initial concentrations, by means of the appropriate programmes of the ESTA library [19]. Once the 'best'

TABLE I. Summary of titration data used for calculating stability constants. Initial total concentrations of metal (C_M), cimetidine sulfoxide (C_{Cso}), histidine (C_{His}), strong acid (C_H) expressed in mmol dm^{-3}, and pH range

System	C_M	C_{CSo}	C_{His}	C_H	pH range
Proton–cimetidine sulfoxide		1.25		4.95	2.44–7.47
		2.50		9.91	2.10–8.03
		2.50		9.91	2.12–7.81
		2.50		9.91	2.12–6.91
		2.50		4.95	2.62–7.36
		3.78		4.95	2.93–7.50
Zinc–cimetidine sulfoxide	1.01	5.30		5.06	4.45–7.20
	1.27	5.00		5.09	3.87–7.22
	1.27	2.50		10.04	2.11–6.79
	2.54	5.00		5.23	3.65–7.05
	2.54	2.50		10.18	2.09–6.87
	5.07	2.50		10.45	2.09–6.71
Copper–cimetidine sulfoxide	1.01	5.30		5.06	3.90–7.12
	1.26	5.30		5.09	3.81–7.02
	1.26	2.65		10.05	2.12–6.48
	2.51	5.30		5.23	3.57–6.55
	2.51	2.65		10.18	2.12–5.90
	5.03	2.65		10.46	2.09–5.56
Copper–histidine–cimetidine sulfoxide	1.26	1.25	1.25	10.18	2.13–6.63
	2.51	2.50	2.50	7.71	2.55–6.36
	1.26	2.50	1.25	5.09	2.86–6.78
	1.26	1.25	2.50	5.09	2.83–7.42
	1.26	2.50	2.50	7.67	2.57–7.76
	2.51	1.25	1.25	5.29	2.56–6.09

TABLE II. Formation constants $\beta_{pqrs} = [M_rL_pX_qH_s]/[M]^r[L]^p[X]^q[H]^s$ obtained from these studies. S = sum of squared residuals, R = R factor as defined in ref. 18, n = number of experimental observations. L stands for cimetidine sulfoxide and X for histidine

System	p	q	r	s	log β	S	R	n
Proton–cimetidine sulfoxide	1	0	0	1	5.597 ± 0.001	0.66E – 8	0.0027	117
Zinc–cimetidine sulfoxide	1	0	1	0	1.867 ± 0.013	0.65E – 7	0.0040	168
	1	0	1	–1	–5.439 ± 0.024			
Copper–cimetidine sulfoxide	1	0	1	0	3.472 ± 0.009	0.95E – 7	0.0049	166
	2	0	1	0	6.846 ± 0.008			
	1	0	1	–1	–3.580 ± 0.033			
Copper–histidine–cimetidine sulfoxide	1	1	1	0	12.658 ± 0.051	0.26E – 6	0.0088	158
	1	1	1	–1	5.598 ± 0.079			
Proton–histidine	0	1	0	1	8.650	from ref. 10		
	0	1	0	2	14.417			
	0	1	0	3	15.998			
Copper–histidine	0	1	1	0	9.639	from ref. 10		
	0	2	1	0	17.357			
	0	1	1	1	13.587			
	0	2	1	1	22.841			
	0	2	1	2	26.164			
	0	2	1	–1	6.676			

set of complexes was discriminated, similar constant optimizations were carried out for binary as well as ternary complexation systems. The choice of these 'best' sets was based (i) on numerical grounds (values of goodness-of-fit parameters such as sum of squares, R factors...), (ii) graphical comparisons involving experimental curves and their simulations obtained from the appropriate simulation modules of the ESTA library [20].

Results

Table II reports the formation constants determined in these studies. Constants previously determined for histidine equilibria with proton and copper [10], that were held constant during the calculations, can also be seen in this table. As far as histidine protonation constants are concerned, they were (in accordance with the usual strategy of our group) checked together with histidine concentrations before using this ligand in ternary system investigations. On this occasion, the values determined in a recent study [10], which were at variance with earlier findings by other authors [11], were confirmed.

Zinc cimetidine sulfoxide interactions proved very weak, as was primarily revealed by the small shifts induced on the reference protonation curve by the presence of increasing concentrations of metal in the test solution. Nevertheless, the complexation degree did not appear to be insignificant. In particular, the corresponding formation curves displayed ligand concentration-dependent 'tails' near the neutral pH range (Fig. 1), which is usually regarded as specific to the formation of hydroxo species: MLOH was thus found along with ML in this system (Fig. 2).

In comparison with what was observed for zinc, the effect of copper on the cimetidine sulfoxide protonation curve was indicative of stronger interactions, this being confirmed by the related formation curves (Fig. 3). Both ML and ML_2 species were indeed characterised in this system, but the hydroxo species was of the same stoichiometry as its homologue with zinc, the constant of ML_2OH being made negative during refinement (Fig. 4).

The differences observed between experimental mixed-ligand protonation curves and their simulations on the basis of exclusive binary complexes suggested a significant ternary coordination. This was confirmed by the evidencing of both MLX and MLXOH species.

Discussion

If we compare the present results with those relative to cimetidine [10], a few remarks are in order.

First of all, proton, zinc and copper complexes of cimetidine sulfoxide are found to be less stable than those formed with cimetidine.

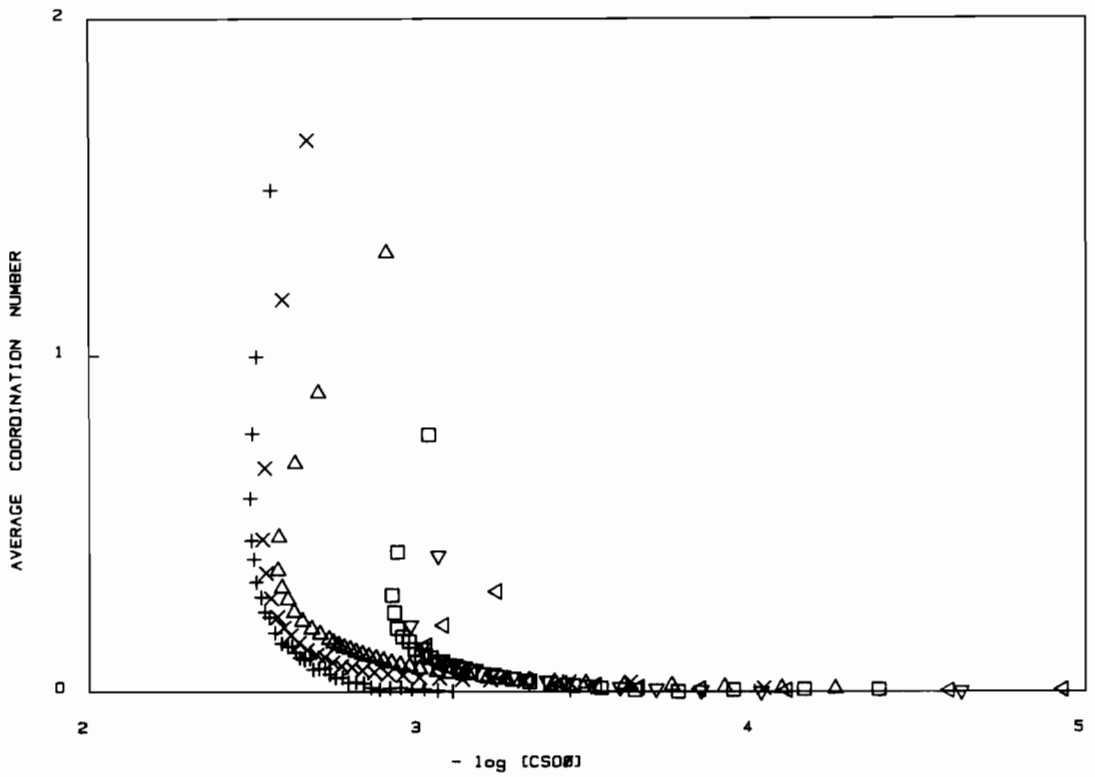

Fig. 1. Experimental formation curves of the zinc–cimetidine sulfoxide system. The following symbols are in the respective order of the experiments summarized in Table I; +, ×, □, △, ▽, ◁.

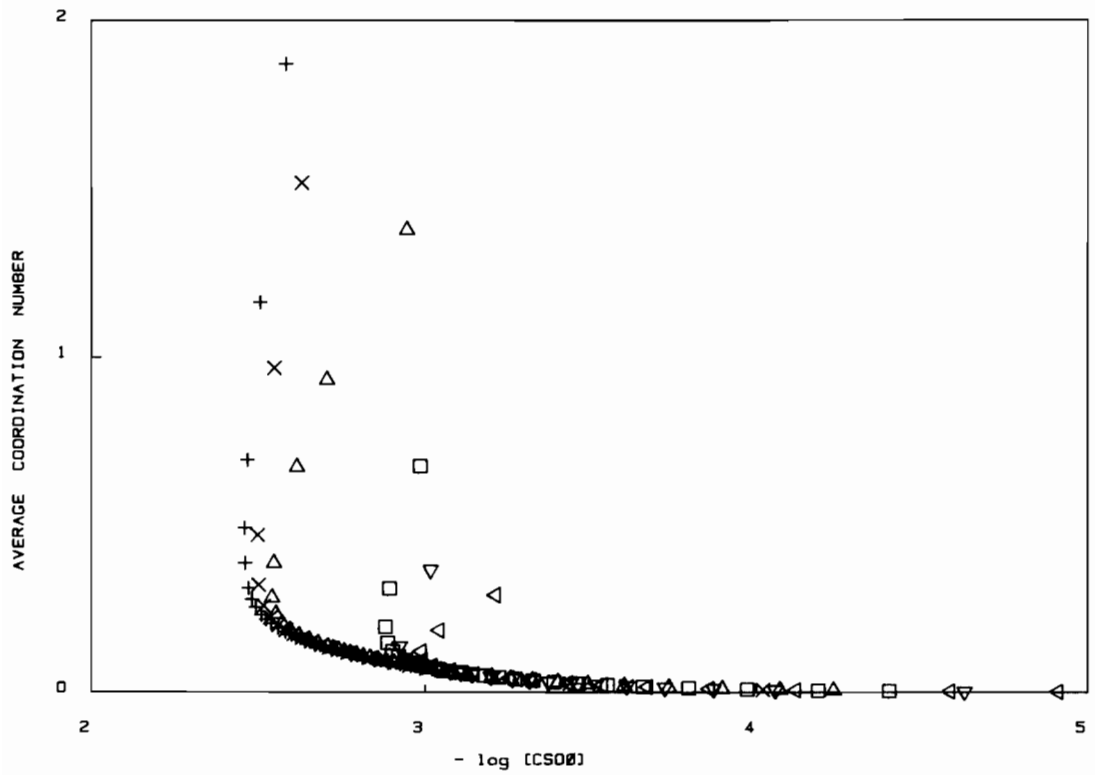

Fig. 2. Simulated formation curves of the zinc–cimetidine sulfoxide system, as based on the results reported in Table II. Symbols are the same as in Fig. 1.

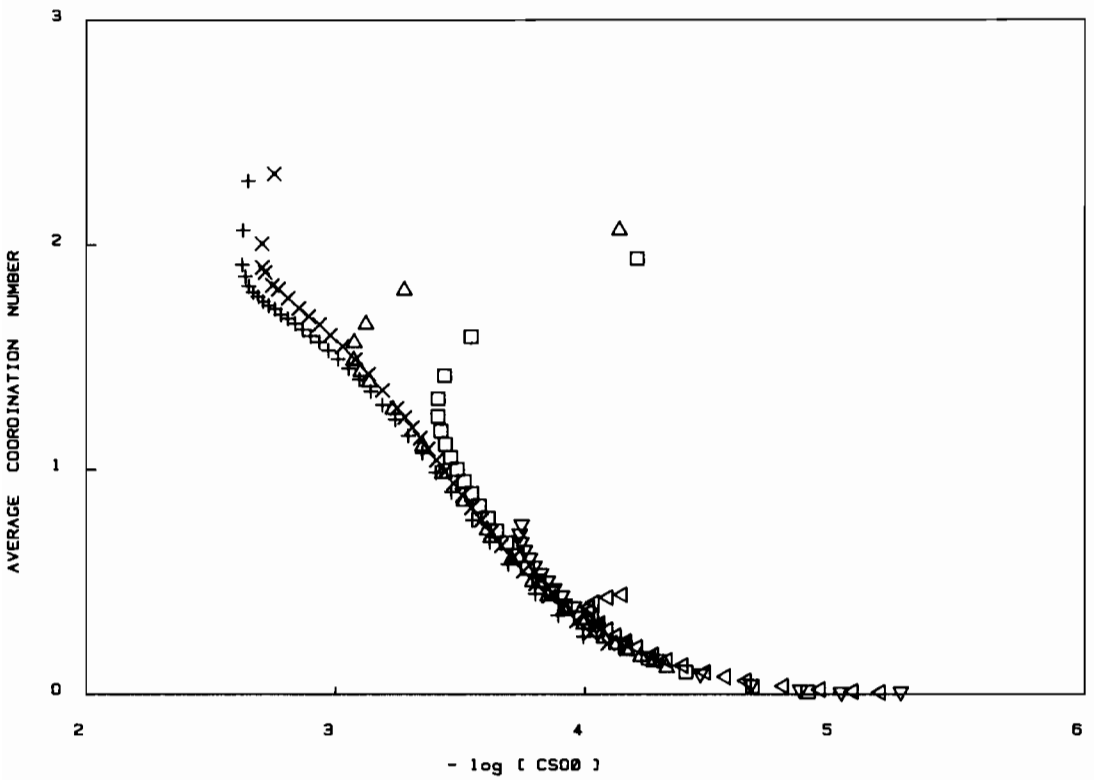

Fig. 3. Experimental formation curves of the copper–cimetidine sulfoxide system. The following symbols are in the respective order of the experiments summarized in Table I: +, ×, □, △, ▽, ◁.

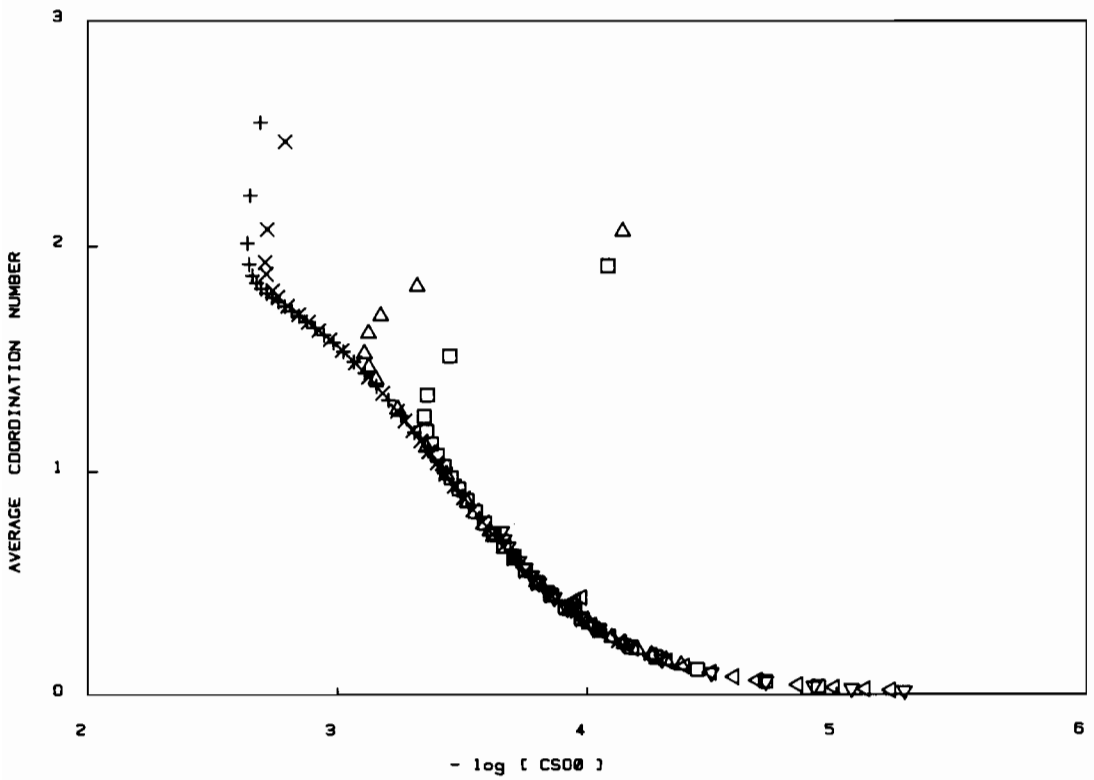

Fig. 4. Simulated formation curves of the copper–cimetidine sulfoxide system, as based on the results reported in Table II. Symbols are the same as in Fig. 3.

Whereas the protonation constant of cimetidine [10] has a value of the same order of magnitude as that of imidazole under similar experimental conditions [21] (respectively 6.70 and 6.78), the protonation constant of cimetidine sulfoxide is inferior to both of these by more than one logarithmic unit. This suggests that (i) no influence is to be expected from the sulphur atom in the aliphatic chain of cimetidine, (ii) due to the large electronegativity of oxygen, the sulfoxide acts as an electron withdrawing group; this results in a clear decrease of basicity for the pyridine nitrogen of the imidazole moiety of cimetidine sulfoxide.

Within the accessible range of free ligand concentrations, copper(II) can accomodate four imidazole molecules [21], but only three molecules of cimetidine [10] and two of cimetidine sulfoxide, presumably for steric reasons. However, if one compares stability constants relative to the first coordination step, it is noticeable that the values relative to cimetidine and imidazole (respectively 4.16 and 4.04) are very close to each other, whereas that corresponding to cimetidine sulfoxide is much lower. Once again, it is likely that the oxygen atom of the sulfoxide group influences the electron density of the imidazole pyridine nitrogen site so that coordination of the latter is made less favourable.

Such a comparison is difficult to establish for zinc since its 1:1 complex with cimetidine could not be found. It may nevertheless be of interest to point out that the ML complex of zinc with cimetidine sulfoxide is significantly less stable than the one formed with imidazole.

Concerning mixed-ligand coordination, two points are worth mentioning: (i) MLXOH has been characterised in the copper–histidine–cimetidine sulfoxide system instead of $M_2LX(OH)_2$ with cimetidine itself [10]; this may again be attributed to a specific interaction of the sulfoxide oxygen atom in the coordination sphere, (ii) MLX of the copper–histidine–cimetidine sulfoxide system was found to be less stable than its homologue with cimetidine (13.24), which seems logical, account being taken of the above considerations. However, when one examines the relative increase of stability of both MLX mixed-ligand species with respect to their parent binary ones, it is clear that cimetidine sulfoxide displays the more stabilising effect (Table III).

Fundamental questions raised by binary and ternary coordinations of the three ligands above with proton, copper and zinc would undoubtedly deserve a closer examination, but they are not pertinent to our present objectives.

Simulation Studies

The main objective of these studies consisted in assessing the extent to which cimetidine sulfoxide may affect the distribution of the low-molecular-weight fraction of copper and zinc in the plasma of patients under cimetidine therapy.

TABLE III. Increments of stability for ternary complexes of copper(II) and histidine with cimetidine and cimetidine sulfoxide

System	$\Delta\log K_M$[a]	$\Delta\log \beta$[a]
Copper–histidine–cimetidine	−0.557	0.114
Copper–histidine–cimetidine sulfoxide	−0.456	0.253

[a]More details about statistical considerations, in particular concerning the following definitions:
$\Delta\log K_M = \log K^{ML}_{MLX} - \log K^{M}_{MX} = \log K^{MX}_{MLX} - \log K^{L}_{ML}$
$\Delta\log \beta = \log \beta_{MLX} - \frac{1}{2}(\log \beta_{ML_2} + \log \beta_{MX_2}) - \log 2$
are available in refs. 10 and 28.

The formation constants relative to cimetidine sulfoxide (Table II) were thus added to the current plasma databank [10, 15]. The ECCLES programme [22] was then used to carry out these simulations, free metal ion concentrations being taken as in our recent studies [10, 15] The plasma concentration of cimetidine sulfoxide was scanned from 10^{-6} to 10^{-3} mol dm^{-3}, which largely exceeds the concentration range resulting from usual therapeutic doses of cimetidine [23, 24], even for those patients with renal failure who are known to present with higher sulfoxide plasma levels [23, 25, 26].

The Plasma Mobilising Index (*PMI*), defined as the ratio by which the low-molecular-weight fraction of a given metal is increased in the presence of drug with respect to the normal state, was monitored for copper and zinc, along with cimetidine sulfoxide distribution.

In spite of the relative increase of stability noted for mixed-ligand complexes of copper with histidine and cimetidine sulfoxide, their absolute formation constants are not high enough for them to represent a significant fraction of this metal, even at the highest cimetidine sulfoxide concentrations. Indeed, copper *PMI* values are not affected at all within the whole range of the latter, and the cimetidine sulfoxide complexes account for only 0.2% of the low-molecular-weight fraction of copper at the upper limit of this range.

For zinc, the influence of cimetidine sulfoxide is still less effective since none of its complexes appears in the 50 most concentrated low-molecular-weight species of this metal in plasma, whatever the concentration of cimetidine sulfoxide may be.

As far as cimetidine sulfoxide itself is concerned, 98.5% is in the form of free base and 1.5% is protonated, regardless of its concentration.

It can thus be concluded that no influence is to be expected from cimetidine sulfoxide on copper

and zinc bioavailabilities in blood plasma, the reverse being also true. Nevertheless, this result does not preclude the possibility for cimetidine sulfoxide to interact with these metals in the intracellular fluid, where it may reach higher levels. This remark also holds for the cerebrospinal fluid, in which a higher degree of penetration has been characterised for the sulfoxide than for cimetidine itself [27].

Acknowledgement

One of us (E.F.) wishes to thank the Universidad de Santiago, Spain, for the award of a maintenance grant.

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