Copper Anti-inflammatory Drugs in Rheumatoid Arthritis. Part 1. Computer Aided Drug Design

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Abstract

Based on the premise that low molecular weight copper complexes are important in the reduction of inflammation associated with rheumatoid arthritis, a computer model of blood plasma has been used to evaluate the factors affecting the ability of a ligand to increase this low molecular weight copper pool *in vivo*. The results suggest that linear quadradentate polyamines, which give rise to a 5, 6, 5 ring system when complexed, are best. Neutral complexes can be achieved using two carboxylate, phenolate or methyl phosphate groups.

Experimental

All calculations were carried out using the ECCLES program and data base [1]. Additional equilibrium constants were extracted from the literature [2] and, where necessary, adjusted to 37 °C and an ionic strength of 0.15. In certain cases, where equilibrium constants were not available in the literature, they were estimated from analogous model compounds. A complete list of constants is available upon request.

Introduction

It has been said "...everything is known about rheumatoid arthritis except its cause, natural history and treatment. It is one of the perversities of the human condition that the rarer the disease, the more clearly it seems to be understood" [3].

Rheumatoid arthritis is a debilitating disease, afflicting about 5% of the Western world population [4]. There is no cure. Although the disease may be controlled with immunosuppressive drugs or the symptoms treated with anti-inflammatory drugs, more efficient therapeuticals are needed.

The role of copper in the treatment of rheumatoid arthritis (RA) is unclear but its use is gaining acceptance [5]. Certainly, copper has been linked to RA treatment for centuries via copper-rich diets or copper jewelry. More scientifically, an Australian group [6, 7] has measured the dermal assimilation of copper from copper bracelets and found it to be significant. In 1976, Sorrenson [8, 9] tested a variety of copper complexes on rats with carrageenan foot edema and reported substantial reduction in inflammation. We have repeated [10] some of these experiments and found that, irrespective of the complex administered, at a moderate dose of 9.3 mg copper/kg, a 60% reduction in inflammation is obtained. Reducing the dose reduces the effect.

Copper is a biologically essential metal ion. In blood plasma, 90% of the copper is irreversibly bound to ceruloplasmin, 10% is reversibly bound to serum albumen, and a small amount, $<1\%$, is distributed amongst low molecular weight complexes, predominantly [Cu(histidinate)(cystinate)] [1]. In patients with inflammatory disease, elevated levels of plasma copper are found [11, 12]. These levels return to normal upon remission. While the role of copper is unclear, it is thought that it is the low molecular weight fraction which is responsible for the anti-inflammatory activity, possibly by making the copper available to superoxide dismutase [13].

Our objective, then, in designing copper-based anti-inflammatory drugs, has been to increase the concentration of the low molecular weight, membrane-penetrable plasma fraction of copper. This may be achieved in three ways:

(a) Copper may be released from ceruloplasmin or some other inert copper store. This approach would be the most difficult as it would involve chemical degradation of the protein or reductive chelation of the metal. The latter is thought to be the mode of action of penicillamine, which is commonly used in the treatment of rheumatoid arthritis [14].

(b) The copper reversibly bound to serum albumen may be removed using a powerful low molecular weight copper complexing ligand.

(c) Copper could be administered orally or topically as a neutral, membrane-penetrable, low molecular weight complex.

The same ligand could be used in both (b) and (c), and this is the approach we have used.

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In thermodynamic terms, what is required is a strong dianionic chelator which is specific for copper, as it must be effective in competition with *in vivo* metal ions and ligands. Ultimately, the only way of testing drug efficiency is with animal screens. We can, however, carry out preliminary *in vitro* assessment using computer modelling. To this end extensive use of the blood simulation model of May *et al.* [1] has been made. Using the thermodynamic data for the possible equilibria occurring in blood plasma, the model allows the change in low molecular weight copper concentration to be calculated as a function of administered ligand under physiological conditions. It is not sufficient just to compare equilibrium constants of different possible drugs, as this does not make allowance for competition from other ligands and metal ions. The danger of ignoring this competition is illustrated in Fig. 1 by EDTA. A concentration of 10^{-8} EDTA causes a 10-fold increase in low molecular weight copper. The introduction of zinc into the model, at its physiological concentration, decreases the mobilizing ability of EDTA by a factor of 10^5. The effect of Ca^{2+} is even more dramatic. The reason for these effects is that, although the $[Cu(EDTA)]^{2-}$ complex is more stable than the Zn^{2+} and Ca^{2+} complexes, the *in vivo* concentration of these two metal ions is much higher than the *in vivo* concentration of Cu^{2+}.

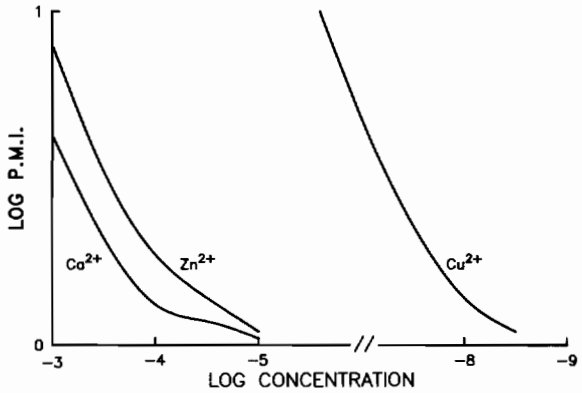

Fig. 1. The effect of Ca^{2+} and Zn^{2+} upon the Cu^{2+} plasma mobilizing index of EDTA.

Results and Discussion

The first structural feature considered in the design of a ligand is the donor atom (Fig. 2a). As the

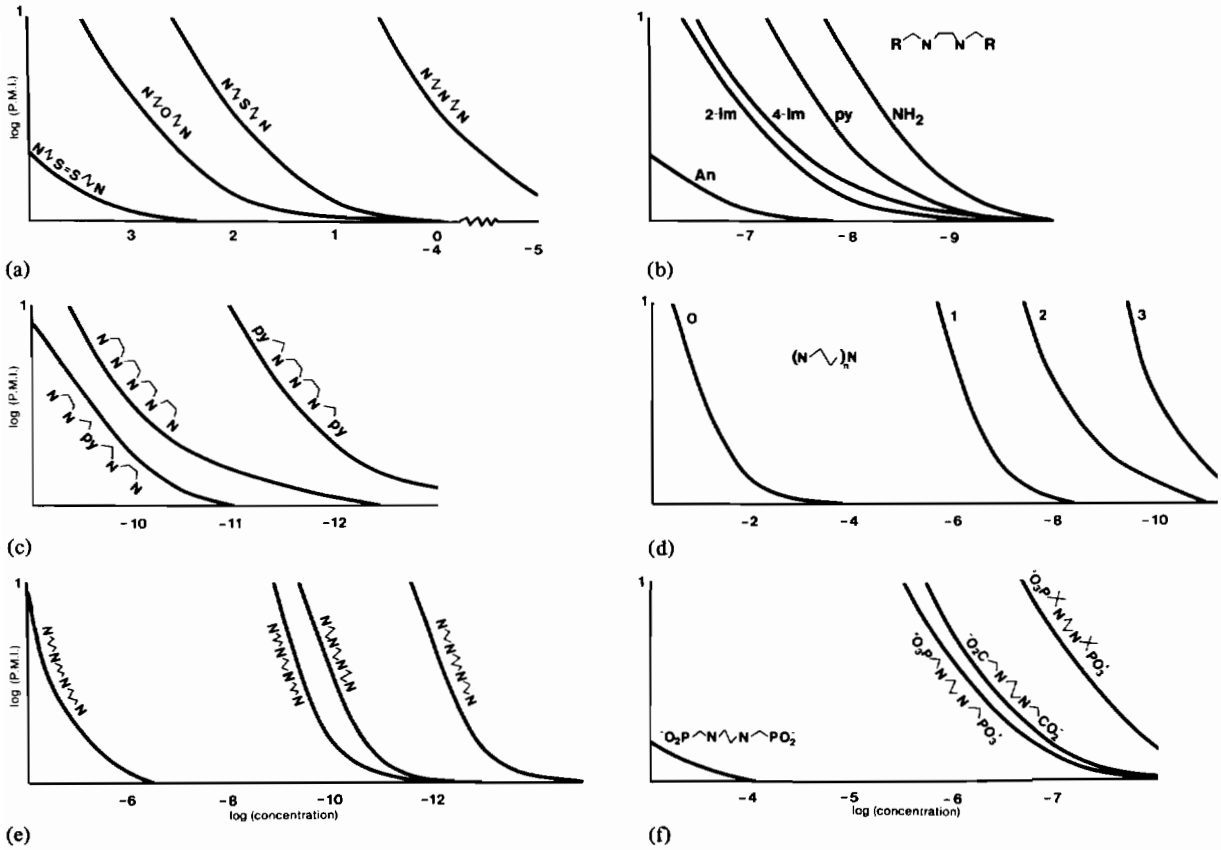

Fig. 2. The effect of different ligand features upon plasma mobilization of Cu^{2+}: (a) donor atom; (b) nitrogen ligands; (c) site of substitution; (d) coordination number; (e) ring size; (f) anionic substituents.

atom changes from O to S to N, the mobilizing efficiency of the ligand increases dramatically, the nitrogen analog being 10^6 times more efficient than its sulfur counterpart. Note that the thiol group, which is known to form very stable copper complexes, has not been considered. There are two reasons for this. First, the copper undergoes reductive chelation with thiols, and secondly the thiol group is notoriously toxic.

Several *N,N'*-disubstituted ethylenediamine compounds were used to examine the effect of the nitrogen ligand used. A comparison of 2-aminobenzyl-, 2-methyleneimidazolyl-, 4-methyleneimidazolyl-, 2-methylenepyridyl- and 2-aminoethyl-substituents is shown in Fig. 2b. 2-Aminobenzyl is understandably poor, but in view of the wide biological use of histidine as a chelating agent, it is surprising how poorly 4-methyleneimidazolyl fares. Under physiological conditions, it appears that the straight-chain polyamine is the best mobilizer of copper. Unfortunately, the situation is not as simple as shown because the site of substitution is also important. This is illustrated in Fig. 2c, using pyridine as the substituent. Substituting the central amino group with pyridine results in a decrease in mobilization, whereas substitution of the terminal groups increases the mobilization.

Since our ligands are all chelate ligands, the questions of denticity and ring size arise. Figure 2d shows the effect of changing the number of coordinating atoms. The large increase in mobilization in going from mono- to bidentate reflects not only the increased stability of the copper complex but also the increased difference in stability between Ca^{2+} and Cu^{2+}. Above a coordination number of 4 there is no significant increase in mobilizing ability.

The effect of ring size is illustrated in Fig. 2e using a quadradentate ligand as an example. $H_2N(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH_2$ forms three 6-membered rings upon coordination and, as expected, is far less effective at mobilizing copper than $NH_2(CH_2)_3NH(CH_2)_2NH(CH_2)_3NH_2$, which gives rise to one 5-membered and two 6-membered rings upon coordination. Triethylenetetramine, which forms three 5-membered rings upon coordination, is 1000-fold more efficient at mobilizing copper. The introduction of one 6-membered ring in the middle of the ligand results in a substantial increase in copper plasma mobilizing index, presumably as a result of decreased ring strain within the complex.

Since we wish to form a neutral Cu^{2+} complex, we have to introduce into the ligand two anionic groups. In Fig. 2f we compare the effect of phosphonate, phosphate, carboxylate and phenolate. There appears to be little to choose between the last three groups, since all are more efficient than phosphonate. Since each phosphate is dianionic, we should really consider the monoester of the phosphate in order to form a neutral complex. Interestingly, methyl substitution increases the efficiency 10-fold. This is most probably due to the inductive effect of the methyl group.

In conclusion, based on the results of our computer simulation studies, we have identified the thermodynamically desirable characteristics that a potential *in vivo* copper mobilizing agent should have, *viz.*: it should be a linear, diphenolate, dicarboxylate, or dialkyl phosphate substituted polyamine. While a dianionically substituted macrocycle would also have satisfied these thermodynamic conditions, they are unacceptable on kinetic grounds [15]. Of course, having designed a potential copper-based anti-inflammatory agent on theoretical grounds is no guarantee of its effectiveness *in vivo*, and to this end we have synthesized several such complexes and they are currently being tested against adjuvant arthritis in rats.

As a final justification of our approach, we quote Lord Kelvin: "If you can model it, ye ken it, If you cannae, ye dinnae!"

Acknowledgements

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