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Copper chelating anti-inflammatory agents; N^1-(2-aminoethyl)-N^2-(pyridin-2-ylmethyl)-ethane-1,2-diamine and *N*-(2-(2-aminoethylamino)ethyl)picolinamide: An *in vitro* and *in vivo* study

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Abstract

An *in vitro* and *in vivo* study of some copper chelating anti-inflammatory agents for alleviation of inflammation associated with rheumatoid arthritis (RA) has been conducted. Two copper chelating agents, N^1-(2-aminoethyl)-N^2-(pyridin-2-ylmethyl)ethane-1,2-diamine ([555-N]) and *N*-(2-(2-aminoethylamino)ethyl)picolinamide ([H(555)-N]) have been synthesized as their hydrochloride salt; their protonation constants and formation constants with Cu(II), Zn(II) and Ca(II) determined by glass electrode potentiometry at 298 K and an ionic strength of 0.15 M. Cu(II) formed stable complexes at physiological pH while the *in vivo* competitors, Zn(II) and Ca(II) formed weak complexes with both chelating agents. Both [555-N] and [H(555)-N] showed better selectivity for Cu(II) than for Zn(II) and Ca(II). Electronic spectra for species formed at physiological pH suggest a square planar geometry. Speciation calculations using a blood plasma model predicted that these copper chelating agents are able to mobilize Cu(II) *in vivo*, while bio-distribution studies of their ^{64}Cu(II)-labelled complexes at physiological pH showed tissue accumulation and retention indicating an encouraging biological half life.

Keywords: *In vitro*; *In vivo*; Potentiometry; Speciation; Bio-distribution

1. Introduction

The past decade has seen an upsurge of interest in metal ion therapeutics for both diagnosis and treatment of diseases [1,2]. Important in this regard is the ability of metal ions to bind proteins and peptides *in vivo*. Of particular interest, Cu(II) chelating agents have been observed to reduce inflammation associated with rheumatoid arthritis (RA) [3–5]. However for this to be possible, the Cu(II) would have to be bio-available, and compounds possessing the potential of facilitating transport of Cu(II) in human blood plasma to sites of inflammation have to be developed.

Although Cu(II) complexes under physiological conditions administered orally and intravenously have been reported [6] to increase the bio-availability of Cu(II) *in vivo*, it is difficult to move the coordinated metal ion through a series of body compartments without binding to proteins. One essential requirement for the Cu(II) complexes is the formation of electrically neutral species at physiological pH so as to enable easy perfusion into tissues with minimal renal loss. However design of chelating agents intended to achieve this requirement has been observed to be no guarantee [7] of the expected enhanced lipophilicity of these neutral species under physiological conditions. The ligands 3,6,9,12-tetraazatetradecanedioate (TTDA) and 3,6,9-triazaundecanedioate (DTDA) are strong chelators of Cu(II) *in vivo*, yet despite their complexes being formally neutral, are rapidly excreted into the kidneys. Indeed

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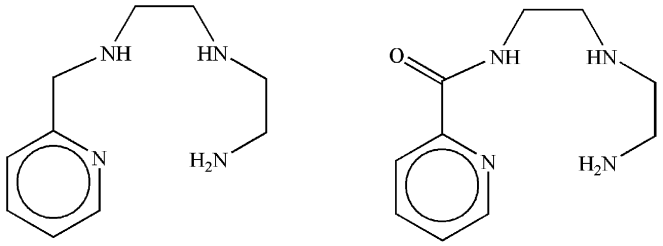

[555-N] [H(555)-N]

Fig. 1. Schematic structures of chelating agents studied.

these two ligands are good candidates for use in conditions of copper overload like Wilson's disease.

The foregoing conclusion therefore prompts specification of another requirement in the design of the copper chelating agents, for successful Cu(II)-transportation in human blood plasma for therapeutic purposes. Hydrophobicity is one of the most important physiochemical parameters governing transport, distribution and fate of chemicals in biological systems. A measure of hydrophobicity of a substance has been widely used in the estimation of bio-accumulation in animals and plants [8,9] as well as in prediction of toxicity and drug absorption [10]. While species neutrality is essential in the design of these chelating agents, in order to enhance their transport across membrane *in vivo*, it would be useful to enhance the hydrophobicity of the chelating agent so as to improve their tissue permeation and therefore minimize loss of the complexes formed from the body via renal filtration. With the total set of the design requirements in mind, the two copper chelating agents (Fig. 1) described in this paper have been designed to enhance their tissue permeation in the transdermal transportation of exogenous Cu(II) and also the mobilization of endogenous Cu(II).

In vitro studies involving the potentiometric determination of the equilibrium constants of [555-N] and [H(555)-N] with H^+, Cu(II), Zn(II) and Ca(II) were carried out. These constants, together with a computer model of blood plasma [11] were used to calculate the *in vivo* speciation of the systems. These results were verified using animal experiments.

complex containing a metal ion, M, ligand, L and proton, H, is formed according to the equilibrium given below (1), where p, q and r are the stoichiometric coefficients of the components in the complex was adopted in this study.

$$pM + qL + rH \rightleftharpoons M_pL_qH_r, \tag{1}$$

$$\beta_{pqr} = \frac{[M_pL_qH_r]}{[M]^p[L]^q[H]^r} \tag{2}$$

The species formed in the investigated systems can be characterized by this general equilibrium process (1) where charges are omitted. The formation constants (β_{pqr}) given by (2) for the generalized reaction (1) were evaluated from the pH-potentiometric titration data using the Equilibrium Simulation for Titration Analysis (ESTA) computer program [15].

Electronic spectra in aqueous solution were recorded in 1.00-cm quarts cells using a Varian UV-Cary 100 spectrophotometer equipped with a temperature-controlled cell holder.

Water/octanol partition coefficients were measured as a function of pH using the shake flask method [16–18]. The aqueous solutions in the vials, at different pH, were spiked with $^{64}CuCl_2$ solution of activity 7.50–9.00 mCi, before shaking with equal amount of water saturated octan-1-ol. An equal amount (1 ml) of each phase was counted in a Minaxi Auto gamma counter (5000 Series-Packard) using a window set at 340–540 keV [19–22].

Blood plasma modelling was carried out by incorporation of the determined formation constants into the blood plasma model consisting of data for 7 metal ions, 40 ligands, and more than 5000 complexes [7,11]. This enlarged database was efficiently and conveniently interrogated by the Evaluation of Constituent Concentrations in Large Equilibrium Systems (ECCLES) computer program [11] to yield results pertaining to the influence of the chelating agents on the equilibria in terms of the plasma mobilizing index (pmi) of each agent [23]. Pmi, defined as the ability to move metal from a protein bound form to a low-molecular weight form, can be represented by the following expression:

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

2. Experimental

Formation constants were measured at 298 K and ionic strength of 0.15 M (NaCl) using a procedure described previously [12]. The reagents used were all of analytical grade and the synthesis of the chelating agents has been described previously [13,14]. A convention where a

Bio-distribution studies were carried out on female balb/c mice with approval from the Research Animals Ethics Committee of the University of Cape Town (permission number 004/022). These studies were carried out using a procedure described previously [24]. In this study intravenous injection via the tail vein using copper-64 was used.