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Copper and Inflammation

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Abstract

Bonta, Sorenson and others have shown that Cu(II) derivatives are effective anti-inflammatory agents. Some chemical and pharmacological properties of Cu(I) and metallic Cu are discussed. Thio complexes of Cu(I) were prepared and shown to be useful anti-inflammatory agents in rats. Hypotheses are stated concerning the possible therapeutic value of copper in its various oxidation states.

'Remember always that some ideas that seem dead and buried may at one time or another rise up to life again, more vital than ever before.' Louis Pasteur [1].

Introduction

The Ebers papyrus, a therapeutic compendium dating from the 18th dynasty of ancient Egypt (*ca.* 1550 B.C.), indicates the use of verdigris (basic copper acetate), blue vitriol (copper(II) sulphate) and pulverized metallic copper for treating granulomatous inflammation, especially in the eye. This papyrus is the oldest extant handbook of drugs; perhaps even the most ancient book, in the Western world [2–6].

Several recent reports provide an experimental basis for believing that copper, applied externally as the metal [7–9] or parenterally as *divalent* copper(II) salts and organic complexes [10–14], may generate anti-inflammatory activity in vivo or fortify the body's natural inflammalytic processes. Furthermore, low concentrations of copper(II) ions within the stomach can prevent drug-induced gastric injury [15]; this anti-ulcerant effect might be considered a rather specific example of a topical anti-inflammatory action.

This communication discusses some properties of monovalent copper(I) complexes, particularly those containing a Cu–S bond, which are the copper analogues of the aurothio complexes (containing monovalent gold), currently used as anti-arthritic drugs in the clinic [16–18].

Experimental

Preparation of (Cu, Ag)-thiocomplexes

Analytically pure Na_2 Cu-thiomalate and Na_2 Ag-thiomalate were prepared by reacting CuCl and AgCl with Na_2 thiomalate solutions under N_2 at 25°C. The Cu-thiomalate was collected as an air-sensitive yellow precipitate. The pale yellow silver compound was precipitated with ethanol.

Analytically pure Na_2 $[Cu_2(S_2O_3)_2] \cdot H_2O$ and $Ag_2S_2O_3 \cdot 2Na_2S_2O_3 \cdot 2H_2O$ were prepared similarly from the metal chlorides and $Na_2S_2O_3$ solutions. Copper(I) D-penicillamine was prepared from CuCl and collected as a pale yellow crystalline product that was air-sensitive.

For bioassays Cu(I) complexes were freshly dissolved in equimolar aqueous solutions of the appropriate complexing agent (thiomalate, thiosulphate, penicillamine) or in 5% (v/v) thiodiglycol.

Biological Assays

A. Acute anti-inflammatory activity was measured by reduction in rat paw edema initiated with carrageenan. Wistar-derived rats, of either sex, weighing 180–220 g were injected subcutaneously (neck) with aqueous solutions of test compound, adjusted to 300 milliosmolar with NaCl and pH > 5.0 with $NaHCO_3$. Forty minutes later, one rear paw was injected through the subplantar surface with 1 mg Na carrageenan in 0.1 ml saline; the other paw received

0.1 ml saline only. Paw thicknesses were determined with a micrometer screw gauge after 2, 4, 6 and 24 h.

B. Anti-arthritic activity was assayed in male Hooded rats in which chronic (adjuvant) polyarthritis had been induced 12 days previously by inoculating 0.5 mg delipidated heat-killed *M. tuberculosis* (human) dispersed in 50 μl squalane, into the base of the tail. Only animals showing significant inflammatory lesions in both rear paws on the 13th day were selected for drug testing. Copper derivatives were injected subcutaneously (neck and flanks) once daily for 4 days. Reference anti-inflammatory drugs were administered once daily p.o. after dispersion in 1% acacia. Changes in the following parameters between the 13th and 18th days were recorded for each rat: thickness of each rear paw and ankle, number of lesions on all four paws, tail and ears, maximum tail thickness (local inflammation), severity of front paw inflammation, body weight. These studies were repeated with two other strains of rat (Dark Agouti, a Wistar variant) after successful arthritis induction.

C. Local irritancy was determined by paw edema induced in 240–280 g rats, after injecting 0.1 ml isotonic neutral salt solutions (used in assays A and B above) into one paw and isotonic saline into the other paw. Paw thicknesses were determined after 1, 2 and 4 h. Irritancy of copper metal implants in adult rats (subdorsum) was studied by excising tissue around these implants 4 and 10 days later.

D. Antipyretic activity was measured in 120–150 g male Wistar rats, inoculated in one flank with 2 g/kg brewer's yeast suspended in saline. Rectal temperatures were measured 8 and 9 h later: animals showing consistent temperatures >38.9°C were selected for assay. (Animals not injected with yeast had average body temperatures of 36.9 $\pm$ 0.2°C, n = 30.) Reference antipyretic drugs (paracetamol, salicylamide 150 mg/kg) were given orally. Solutions/dispersions of Cu complexes, as used in assays A–C, were injected subcutaneously. Rectal temperatures were determined after 0.5, 1, 2, 3 and 5 h.

E. Toxicity assays. Body weight changes over 5 days, following a single parenteral injection (assay A) were recorded for all treated animals. Normal (i.e. non-inflamed) rats were also injected subcutaneously with single doses of selected Cu preparations, transferred to metabolism cages, permitted food and water *ad lib.* and urine collections made at 12 h intervals for 4 days to note any hematuria.

Results

Comparison of some Cu(I), Ag(I) and Au(I) thiocomplexes

Table 1 shows the *acute* anti-inflammatory activity of these thiocomplexes, together with some reference Cu(I) and Cu(II) compounds and

Table 1
Acute anti-inflammatory activity of some Cu(I) and other compounds in rats.

Series	Compound	Dose	ΔP.T. (mm) after	
			2 h	4 h
1	No drug (saline only)		2.45	3.55
2	Cuprothio compounds:			
	Cu(I)-D-Penicillamine (Pn)	100	0.65	1.90
	$Na_5Cu(I)_8Cu(II)_6$-D-$Pn_{12}Cl$	7	1.30	2.60
	$Na_2Cu(I)$-thiomalate	100	0.75	1.00
	$Na_2Cu_2(S_2O_3)_2 \cdot H_2O$	50	0.60	1.60
	Cu(I)-(thioacetamide)$_4$Cl	100	0.75	2.30
3	Other Cu compounds:			
	$Cu(I)(Cu_3CN)_4CO_4$	100	1.70	3.15
	Cu(I)Cl (with DMSO[a])	100	1.55	1.40
	Bis-glycinato-Cu(II)	100	0.95	1.70
	Cu(II) with 3.5 eqts. ascorbate	100	1.75	2.20
4	Other Group IB thiocomplexes:			
	Na_2Ag-thiomalate	100	2.30	4.05
	Na_2Au-thiomalate (Myochrysin)	100	2.75	3.90
	$Ag_2S_2O_3 \cdot 2Na_2S_2O_3 \cdot H_2O$	50	2.80	3.70
	$Na_3Au(S_2O_3)_2 \cdot 2H_2O$ (Sanochrysin)	200	1.90	2.40
5	Aspirin	1100	0.95	1.80
	Phenylbutazone	325	1.40	2.05

Data from groups of 3–5 animals. Cu(I) thio compounds showed variable stability in aq. media (oxidation to Cu(II)) and were dissolved in equimolar solutions of the thioligand (Na salt) to ensure maximal [Cu(I)]. Cu(I)-(thiourea)$_3$Cl was too toxic (>50 μmoles/kg) for testing being rapidly lethal.

All compounds given subcutaneously (s.c.), except those in Series 5 = given p.o. Dose (μmoles/kg) varied to give equivalent metal content.

Paw inflammation initiated with 1 mg Na carrageenan 45 min after giving test compound. ΔP.T. = increase paw thickness.

(Similar data obtained for paw inflammation initiated with 10 mg kaolin or 4 mg Na urate crystals).

[a] DMSO (=1 ml/kg s.c.) and thioligands alone had no effect.

Table 2
Therapeutic assay of some (Cu, Ag, Au)-thiocomplexes against late development of rat polyarthritis.

Compound	Av. increase in thickness (mm)		⊿Weight (g) days 13–17	Comments
	Rear paws	Tail		
None	1.35	0.45	−11	
Cu-Penicillamine (1 : 2)	−0.20	−0.70	−18	Cu(I) only
Cu-Penicillamine (1 : 1)	−0.05	−0.05	−14	Cu(I) with Cu(II)
Cu(I)-Thiosulphate	0.55	−0.25	+02	Beneficial
Ag(I)-Thiosulphate	0.20	−0.40	−12	? toxicity
Au(I)-Thiosulphate	×	×	×	100% mortality
Cu(I)-Thioacetamide	0.90	0.20	−06	Modest effect
Cu(I)-Thiomalate	0.60	−0.30	−08	Anemic feet
Ag(I)-Thiomalate	0.55	0.15	−09	? effective
Au(I)-Thiomalate	×	×	×	100% mortality
Cu(I)-Cl/thiodiglycol[a]	1,65	0.05	+02	Inactive
Cu(II) Cl_2/thiodiglycol[a]	0.95	−0.80	−08	Some effect
Cu(II)-bisglycine	0.05	−0.45	−13	Anemia
Phenylbutazone[b]	0.50	−0.55	−01	Reference drug

Hooded rats were inoculated with arthritogen 12 days previously. Compounds given s.c. = 100 μatoms metal/kg/day on days 13–16. Signs of inflammation recorded before 1st dose and 24 h after 4th dose.
Data from groups 4–6 animals. Controls injected with saline.

[a] 5% thiodiglycol-saline (1 ml/day) had no effect.
[b] Dose = 330 μmoles/kg/day.

Table 3
Some side-effects observed with (parenteral) copper in rats.

Presentation as	Obvious non-therapeutic effects
Cu(O): external	None (stain skin only)
internal	Local irritation: Encapsulation Pus reaction (with sterile implants) Haematoma
Cu(I)-Thiocomplexes[a]	Range of local irritancy (edema, etc): e.g. zero with Cu-penicillamine (1 : 1), modest with Cu-thiomalate Haematuria = transient, if any Range of toxicities (weight loss, mortality): e.g. modest with Cu(I) thiomalate/penicillamine, severe with Cu(I) acetonitrile/thiourea
Cu(II)-N,O complexes[a]	Range of local irritancy: e.g. zero with Cu-histidine, Cu-TRIEN, modest with Cu-salicylate, severe with $CuCl_2$, Cu-imidazole Haematuria after 24 h: zero with Cu-salicylate, severe with Cu-mimosine Range of toxicities: well tolerated, e.g. Cu-salicylate; weight loss, e.g. Cu-glycine; mortality, e.g. Cu-TRIEN Distress, particularly rapid diarrhoea: e.g. with Cu-histidine, Cu-oxidized penicillamine

All forms parenteral Cu may induce acute phase reactants in plasma (these are glycoproteins = markers of infection, inflammation, etc).

[a] After local doses of 1 μmole (irritancy) or 100 μmoles/kg for systemic effects.
TRIEN = triethylenetetramine.

known anti-inflammatory drugs. Silver and gold complexes were virtually inactive at the highest levels tested here (200 μmoles/kg).

Table 2 shows the anti-arthritic activity of some Cu(II) complexes in rats. We were unable to establish any useful effects of the two aurothio complexes in this assay at subtoxic doses (even with two other strains of rats in which poly-arthritis had been successfully induced). By contrast Cu(I)-thiocomplexes were effective in preventing the amplification of established inflammation as the adjuvant disease progresses beyond its first manifestations in all four paws, and compared favourably in this respect with proven anti-inflammatory drugs.

Further properties of some Cu compounds in rats

The copper(I) thiocomplexes caused only minimal lowering in the body temperatures of fevered rats, and could not therefore be considered to have antipyretic effects. In this regard, they are quite distinct from many wholly organic anti-inflammatory drugs, e.g. aspirin, indomethacin.

By contrast, Cu(II) and mixed valency complexes (Cu(I) with Cu(II)) were hypo-thermic even in normal animals.

Table 3 indicates the differing degrees of acute, rapidly-expressed, toxicity of some Cu(I) and Cu(II) complexes.

The cuprothiourea complex, $Cu(NH_2CSNH_2)_3 \cdot Cl$, was extremely toxic when administered subcutaneously in 5% dithiodiglycol-saline (100 μmoles/kg): the equivalent dose of thiourea (300 μmoles/kg) being well tolerated.

Discussion

Part of the acute-phase response in patients with rheumatoid arthritis and in rats with *severe* inflammation is a rapid rise in serum Cu levels [19–22] indicating extensive Cu mobilization in response to inflammagenic stressors. It is against this background that conventional anti-arthritic (and Cu-binding) drugs are used, e.g. salicylic acid, D-penicillamine. These may facilitate the transfer of part of the newly mobilized copper away from high M.W. complexes within the circulation, on to copper-ligands on or within hyper-reactive cells.

Hypothesis I. If copper-mobilization is part of a natural inflammalytic response, is it perhaps insufficient in chronic inflammation and therefore needs (some modest) reinforcement with exogenously applied copper?

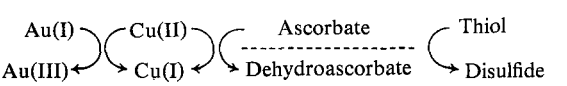
If this be true, then consequent therapeutic intervention should ensure minimal copper toxicity. Direct application of clearly subtoxic levels of Cu(II) complexes is nevertheless frequently accompanied by severe local irritation and/or delayed hemolysis, even hematuria [23]. By contrast, cuprothio derivatives, though locally irritant, seem to be better tolerated. Unfortunately Cu(I) is not stable in aqueous media, except when complexed with certain ligands such as those containing sulphur. When liganded with nitrogen or oxygen, it is rapidly oxidized (or undergoes dismutation) to yield Cu(II) – and metallic copper if no other oxidant is present.

Hypothesis II. Cu(I) compounds, if reasonably stable in a physiological milieu, may demonstrate a bioactivity more akin to that of monovalent gold (aurothio complexes) and distinct from that of the corresponding Cu(II) compounds.

This has been partly established by the differential toxicities of some Cu(I) and Cu(II) complexes, e.g. frequent hematuria and depression of even normal body temperature by some Cu(II) complexes.

Hypothesis III. Certain anti-arthritic, as opposed to anti-inflammatory, drugs may interact with available, but bio-inert, protein-bound Cu(II) to form bioreactive Cu(I).

This has been well established for penicillamine, which can strip albumin-bound Cu(II) [24] and form the very soluble mixed valency (anti-inflammatory) polymer with the composition, $[Cu(I)_8Cu(II)_6\text{-pen}_{12}Cl]^{5-}$ [25]. Inspection of the redox potential of Au(I), Au(III), Cu(I) and Cu(II) shows that it is thermodynamically feasible for aurothio anti-arthritic drugs to function as Cu(II)-reductants. One natural bioreductant is ascorbic acid which may be less effectively retained in vivo in patients with rheumatoid disease [26] and is certainly deficient in rats with severe local inflammation or established arthritis [27].



This is directly demonstrable by the quenching of the Cu(II) absorption spectrum (>600 nm) in neutral Cu-glycine solutions with either aurothiomalate, thiols or ascorbate.

Hypothesis IV. Metallic Cu is not bio-inert. This has been demonstrated by the solubility of metallic copper in human sweat [28] and endometrial fluids [29, 30]. This is relevant to the actions of copper intra-uterine devices (I.U.D.). Subdermal copper implants elicit edematous inflammation in rats that is almost certainly due to some local oxidative solubilization of the metal. Even metallic gold, which is more inert than copper, dissolves in solutions of α-amino acids in the presence of oxidants [31].

Physiological 'corrosion' of metallic copper can be represented as:

$$2\,Cu(O) + O_2 + 4HL = 2CuL_2 + 2\,H_2O$$

where HL is one of the many copper ligands (acids) found in sweat, endometrial secretions, or other biological fluids.

Hypothesis V. External metallic copper (including I.U.D.'s) may therefore be a source, or depot, of potentially bio-available soluble copper.

Both copper bracelets/rings and I.U.D.'s lose weight when worn [8, 32] – of the order 2–3 mg/day from the ring and *ca.* 50 μg/day from the I.U.D.

Hypothesis VI. The integument is not impermeable to soluble copper, derived from an external depot (e.g. copper metal).

The neutral copper(II)-bisglycine complex certainly can perfuse cat skin [33].

Hypothesis VII. The biodistribution, pharmacoactivity and toxicity of both copper(I) and copper(II) is largely determined by the nature of their ligand(s), that determine the physicochemical properties of the copper complex, e.g. charge, stability, solubility, etc.

In a physiological environment, all soluble copper is co-ordinated, i.e. ligand-associated, whether inert or bioactive. Exchange of ligands consequently alters bioactivity and distribution. For example, the uncharged Cu(II)-salicylate complex may penetrate biomembranes that exclude hydrated/charged metal complexes. If, once it has traversed the membrane barrier, such a permeating complex is immediately removed either physically (e.g. by the circulation) or chemically by irreversible transformation to a less liposoluble complex (e.g. containing Cu(I) after bioreduction), then a uni-directional copper flux might be established.

Even within a homogeneous phase, e.g. plasma, copper may be redistributed amongst various bioligands and exogenous drugs. Thus D-penicillamine effectively unbinds Cu(II) from its albumin carrier by displacing the equilibrium in favour of unbound Cu(II), that is removed by rapid reduction to Cu(I) and subsequent complexation with penicillamine [24].

Hypothesis VIII. Unlike Copper(II), Copper(I) mimics Zinc(II) chemically and might therefore behave biologically as either a zinc substitute (i.e. agonist) or a zinc-antagonist.

Hypozincemia may accompany the hypercupremia, associated with acute stress, infection, inflammation, etc. [34].

Final comments

These hypotheses suppose copper to be pharmacoactive, i.e. a possible suppressor of inflammation. A contrary view is to regard the copper bracelet as a slow-release, 'immunizing' or tolerizing, source of copper that desensitizes the host to the irritant/pathogenic effects of the natural hypercupremia associated with chronic inflammation (including perhaps pregnancy!). Until we understand the function of the endogenous (natural) copper in an inflamed animal, we cannot really expect to understand the role of Cu-mobilizing drugs or indeed exogenous (applied) copper. However, we can continue to test the hypotheses presented here and duly reconsider if a treatment as old as the recorded use of metallic copper is still of value today.

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