Copper Complexes: a Physiological Approach to the Treatment of 'Inflammatory Diseases'

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Plasma concentrations of low molecular weight copper-containing components are known to increase in response to arthritis, epilepsy, and cancer. Each of these diseases are recognized as having inflammatory components. Evidence is provided to show that administration of low molecular weight copper complexes produce antiinflammatory effects in animal models of inflammation, anticonvulsant effects in animal models of seizure, and anticancer effects in animal models of cancer. These data are reviewed in support of the hypothesis that the elevation of plasma copper-containing components represents a physiologic response which may lead to remission. Promotion of this physiologic response would appear to be a valid approach to the treatment of arthritis, epilepsy, cancer, and other diseases with inflammatory components.

Introduction

Arthritis

Since the original publication by Heilmeyer and Stuwe [1] there have been many confirmations that plasma or serum copper-containg components are elevated in arthritic diseases [2]. Compared to normal healthy individuals, patients with rheumatoid arthritis have higher mean serum or plasma copper concentrations which are directly related to disease severity or activity as measured by increased body and local temperatures, immobility, duration of disease, pain, edema, and erythrocyte sedimentation rate, as well as diminished strength and decreased hemoglobin values. Small sex-related differences in normal individuals are obscured by marked increases found for both male and female patients. Copper concentrations increase in association with the onset and persistence of active disease but return to normal with remission.

It is now known from animal studies that the rise in serum copper is accompanied by a fall in liver copper concentrations [3–5]. Since serum copper-containing components are synthesized in the liver and appear in serum after the onset of disease, it seems reasonable to suggest that this is a physiologic response to arthritis, which facilitates remission. Alternatively, a lack of this response was suggested to result in chronic or persistent disease [2]. This view is consistent with the observations that blood serum or plasma levels of these same copper-containing components increase in animals in response to inflammations [2, 5–7] and that these inflammations are more severe in animals with established copper deficiency [8–12]. All of the above support the role of the increased copper-containing components in blood as 'putative modulators' of inflammation [13].

The low or normal serum concentrations found for arthritic patients with chronic disease deserves some comment. These values may represent a failure of this aspect of the physiologic response as a result of depleted liver copper stores. Depletion may be due to increased turnover resulting in copper excretion, which is known to be elevated in arthritis [14, 15], or failure to replete these stores because of either a loss of appetite or an inadequate diet. Over thirteen studies document that human diets supply less than an adequate daily intake of copper [16–19]. Failure of serum copper to increase maximally as a physiologic response to disease could lead to chronic disease.

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Epilepsy

Epileptic patients also have elevated blood copper concentrations [20–22]. The importance of copper-dependent enzymes for normal brain development and function [23–25] is reflected by the fact that the brain contains more copper than any other non-storage tissue in the human body [26].

A symptom of copper deficiency in humans and animals is tremors or seizures, which subside with copper supplementation [25, 27–32]. Seizures following treatment with tremor-inducing drugs are accompanied by a concomitant reduction in brain copper levels [33–36]. Also, brain norepinephrine and epinephrine concentrations are reduced in association with seizures [24, 33, 37, 42]. This latter observation is particularly relevant since a copper-dependent enzyme, dopamine-β-hydroxylase, is required for the synthesis of norepinephrine and epinephrine.

The hypothesis that seizures result from the loss of copper from some copper-dependent site which may be replaced with the physiological release of liver copper stores is consistent with the observation that inorganic copper injected into the carotid blood supply readily crosses the blood-brain barrier within 15 seconds and the amount crossing the barrier is increased by coadministering amino acids [43]. Additional support for this hypothesis comes from the observations that *post-mortem* samples of brain tissue from epileptic patients have markedly decreased copper concentrations [27] and that etiologies of epilepsy in children [44] and adults [45] are associated with inflammatory conditions in the central nervous system (CNS).

Impaired copper metabolism in the CNS may lead to the seizure state and prolonged reduction of some copper-dependent process may be characterized as chronic epileptic disease.

Cancer

Copper metabolism has been studied in a variety of neoplastic diseases [46]. It is now known that patients with acute leukemia have elevated serum or plasma copper concentrations [47]. The elevation in serum copper correlated with an increase in number of bone marrow blast cells. A decline in symptoms or remission of disease following therapy correlated with a decrease in serum copper concentration [46–49], enabling accurate prognoses based upon serum copper determinations.

Chronic leukemia is associated with a near-normal plasma copper concentration and a markedly decreased hematocrit, which may be a symptom of 'copper deficiency'. Since remissions do not occur in cases of chronic lymphocytic and myeloid leukemias as well as myelomas, serum copper levels do not return to normal [46].

Children and adults with active Hodgkin's disease have elevated plasma copper concentrations. Copper levels return to normal with remission and increase with relapse, enabling accurate prognosis [50–53]. Since the return to normal copper levels with remission is a constant feature of this disease, it has been suggested that a normal serum copper level be included among the criteria for complete remission [54]. Some patients in remission have been reported to have an elevated serum copper level [55]. However, it is uncertain what stage of remission these patients had achieved or whether they were about to have a relapse. If a patient had active disease and was entering into a remission phase, it may require some time before the serum copper level returns to normal. The increase in serum copper was correlated with an increase in ceruloplasmin concentration [56, 57] which was attributed to a lack of catabolism by the liver as opposed to the alternative interpretation of increased ceruloplasmin synthesis.

A good correlation was also found between increased serum copper concentration and disease activity in non-Hodgkin's lymphomas [46]. Patients who responded to therapy had a return to normal serum copper levels, but nonresponders had a persistently elevated serum copper level [58]. Relapse was associated with an elevated copper concentration prior to the onset of symptomatic relapse.

Elevation of serum copper levels has also been reported for patients with various carcinomas. The degree of elevation in women with cervical carcinoma increased as the stage of disease progressed, and those patients who responded favorably to treatment had a nearly normal serum copper level [59]. Patients with bladder carcinoma also have elevated serum copper levels which correlate with the stages of this disease [60]. An elevation in serum copper has also been reported in patients with mammary [46, 61], bronchial [62], gastric [63, 64], colonic [64], rectal [64], and liver [64] carcinomas as well as osteosarcoma [65]. The degree of elevation in serum copper in osteosarcoma has been correlated with the extent and activity of this disease. The highest copper levels were associated with metastatic disease and the poorest prognosis [66]. Increases in serum copper with liver tumors can be partially accounted for by a failure in liver-mediated copper excretion.

Elevated serum copper levels were also found in dogs with radiation-induced and 'spontaneous' osteosarcoma [67]. No clinical signs of metastasis were observed following tumor removal, by limb amputation, and there was a return to normal or near-normal serum copper levels. Dogs with nonmalignant, nonosteosarcoma lesions were found to have normal serum copper levels.

In each of the above neoplastic diseases, the elevation of serum copper was found to correlate with disease activity, and a return to normal levels

with remission. These observations are consistent with the view that the increase in serum copper is a physiological response which may facilitate remission.

The increase in serum or plasma copper concentration found in all cancers studied has special significance since it has recently been recognized that many, if not all, tumor cells have decreased copper-dependent superoxide dismutase (SOD) enzymatic activity [68]. The elevation of blood copper following release from the liver stores may induce SOD or other enzymatic activity in cancer cells and play a role in the facilitation of remission and the subsequent return to normal copper levels.

Alterations in blood copper concentration associated with arthritic diseases, epilepsies, and cancers can be generally summarized as shown in Fig. 1.

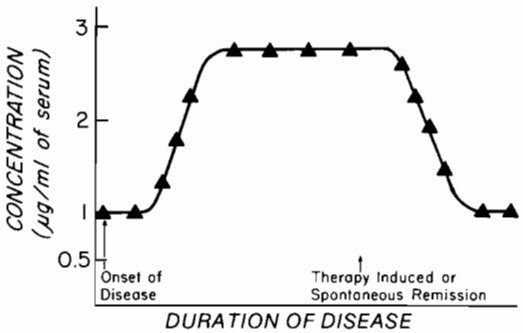

Fig. 1. Alteration of serum copper content in the general acute phase response to various diseases.

With the onset of disease, leukocyte endogenous mediator, also known as leucocyte activating factor or endogenous pyrogen, produced by chemotactically attracted polymorphonuclear leukocytes initiates events leading to increased synthesis of ceruloplasmin in the liver and its release into the blood along with copper–albumin and amino acid copper complexes [5]. This release is most pronounced in active disease. This physiologic response appears to be maintained throughout the course of the disease, until the etiologic lesion is overcome which is perceived as 'a spontaneous or therapy-induced remission'. With the onset of remission the concentration of blood copper-containing components returns to normal.

The elevation in blood copper compounds may represent mobilization of stored copper to supply affected tissues in need of copper for the synthesis of copper-dependent enzymes. Enzymes known to be copper dependent [23–25] are listed in Table I.

The prevention of superoxide-induced inflammation associated with the accumulation of superoxide [70] and the replacement of connective tissue components, collagen and elastin, required for repair of damaged tissues [71] would seem to make superoxide dismutase and lysyl oxidase extremely important enzyme requirements for tissue maintenance.

TABLE I. Recognized Copper-Dependent Enzymes and Their Biochemical Function.

Cu-dependent enzyme	Function
Cytochrome c oxidase	Cellular respiration
Superoxide Dismutase	Dismutation of superoxide anion radical
Tyrosinase	Conversion of tyrosine to Dopa
Dopamine-β-hydroxylase	Conversion of dopamine to norepinephrine
Lysyl oxidase	Conversion of procollagen to tropocollagen and of proelastin to elastin in the connective tissues
Soluble pyridoxal-dependent monoamine oxidase (and perhaps the insoluble membrane bound flavin-dependent monoamine oxidases [69]	Oxidation of catecholamines to aldehydes

Reestablishing normal catecholamine metabolism would seem to be essential for normal neuronal function. In addition, it has been pointed out that other copper-dependent processes are required for modulation of prostaglandin syntheses [25], lysosomal membrane stability [25], and the activity of histamine [25], which are also important in the inflammatory response.

In the not too distant past it was thought that the elevation in blood copper levels associated with arthritic diseases was pathologic. This notion still persists in the interpretation of the significance of elevated blood copper compounds found in patients with epileptic or neoplastic diseases. However, observations that copper complexes have antiinflammatory activity, anticonvulsant activity, and anticancer activity suggest that these elevations in blood copper compounds are physiologic and play a role in bringing about remissions of these disease states.

Results and Discussion

Antiinflammatory Activities of Copper Complexes

To date, over 70 copper complexes have been studied as antiinflammatory agents. The results of these studies have been reviewed recently [72] and confirm as well as extend the original observations that copper complexes of inactive ligands and active antiinflammatory drugs are more active than the parent ligand or inorganic copper. The following is a brief presentation of some of the data from the original report suggesting that copper complexes were the active metabolites of the antiarthritic drugs [73].

As shown in Table II, $Cu(II)_2(acetate)_4$ was found to be active in the initial test (carrageenan paw

TABLE II. Antiinflammatory Activities of Some Copper Complexes.

Compound	Carrageenan Paw Edema	Cotton Wad Granuloma	Adjuvant Arthritis	Copper (%)
$Cu(II)_2(acetate)_4$	A at 8	I at 100	I at 30	31.8
anthranilic acid	I at 200	NT	I at 30	
3,5-dips acid	I at 200	NT	I at 30	
$Cu(II)(anthranilate)_2$	A at 8	A at 25	A at 1.2	18.9
$Cu(II)(3,5\text{-dips})_2$	A at 8	A at 5	A at 1.2	12.5
Aspirin	A at 64	A at 200 i.g.	A at 6	
$Cu(II)_2(aspirinate)_4$	A at 8	A at 10	A at 1.2	15.0
D-Penicillamine	I at 200	I at 100	I at 30	
$Cu(I)D\text{-pen}(H_2O)$ 1.5	A at 8	A at 10	NT	26.7
$Cu(II)(D\text{-pen disulfide})(H_2O)_2$	A at 8	A at 25	A at 30	15.4

A = lowest active dose tested; I = inactive; NT = not tested. All compounds were given by subcutaneous injection unless indicated as intragastric (i.g.) and expressed as milligrams per kilogram of body weight.

TABLE III. Copper-Salicylate Therapy of Acute Rheumatic Fever, Rheumatoid Arthritis, Cervical Spine–Shoulder and Lumbar–Spine Syndromes, and Sciatica [69].

Disease	Total number of patients	Symptom free	Clinical results		
			Improved	Slightly improved	Unchanged
Acute rheumatic fever[a]	78	78(100%)			
Rheumatoid arthritis[a]	620	403(65%)	143(23%)		74(12%)
Cervical spine–shoulder & lumbar–spine syndromes[a]	162	95(57%)	52(32%)		18(11%)
Sciatica without lumbar involvement[b]	120	76(63%)	38(32%)	6(5%)	
with lumbar involvement[b]	160	95(59%)	39(24%)	10(6%)	16(11%)
Total	1140	744(65%)	272(24%)	16(1%)	108(10%)

[a] Symptom free: The absence of articular inflammation, disappearance of nonarticular inflammation, return of articular mobility-deformation only as a result of irreversible changes, normal ESR, no radiological evidence of progression. Free from pain and fever. Improved: ESR was still elevated, articular swelling—though only slight—still present, disturbances in articular mobility with only little sign of activity still evident, no increase in deformities, no radiological evidence of progression. Arthralgia only occasionally. No fever. Unchanged: General condition unchanged, painful, no change in inflammatory signs, radiological evidence of progression, elevated ESR, restricted mobility, deformation and fever of varying degrees, but not significantly decreased for evaluation as improved. [b] Symptom free: Disappearance of (subjective) symptoms, Lasague's sign negative, normal reflexes with equal quality on both sides, no disturbances in sensitivity, no tenderness on pressure and mobility restored. Improved: Not completely relieved from (subjective) symptoms or persistence of one or several symptoms listed in the symptom free classification, yet no longer any impairment of mobility. Slightly improved: Impairment of walking ability still demonstrable, though only moderate, with persistence of one or several symptoms. Unchanged: No response to treatment at all.

edema) for antiinflammatory activity but inactive in the two follow-up antiinflammatory screens (cotton wad granuloma and adjuvant arthritis).

Ligands such as anthranilic acid and 3,5-diisopropylsalicylic acid (3,5-dips) which were anticipated to be inactive were found to be inactive. However, their copper complexes were found to be potent antiinflammatory agents in all three models of inflammation. These observations supported the notion that complexed copper was a more active antiinflammatory form of copper and led to the suggestion that copper complexes of active antiinflammatory agents might be more active than the parent antiinflammatory drugs. Representative data obtained by comparing aspirin and D-penicillamine with their copper complexes are also presented in Table II. These data showed that these complexes were more effective than the parent drugs and supported the hypothesis that the active metabolites of the antiarthritic drugs are their copper complexes. Since the amount of copper in these complexes does not appear to correlate with the activity of these complexes, it is suggested that the pharmacologic activity is due to the physico-chemical properties of the complexes.

These results explain the earlier observations by Fenz, Forestier, and Hangarter that copper complexes were effective in treating arthritic and other degenerative diseases in Man [74]. The results obtained by Hangarter with his copper-salicylate preparation (Permalon) are presented in Table III. These results are particularly noteworthy since most of these patients had previously been unsuccessfully treated with drugs commonly used to treat these diseases.

Copper complexes were also found to be unique antiinflammatory agents when it was demonstrated that they were potent antiulcer agents, as shown in Table IV.

TABLE IV. Antiulcer Activity of Some Copper Complexes.

Compound	Shay Antiulcer Activity[a]
$Cu(II)_2(acetate)_4(H_2O)_2$	225
$Cu(II)(anthranilate)_2$	4.5
$Cu(II)(3,5\text{-dips})_2$	2.3
$Cu(II)_2(aspirinate)_4$	11.3
$Cu(II)_n(niflumate)_{2n}$	4.5
$Cu(II)(D\text{-pen disulfide})\cdot 3H_2O$	4.5
$Cu(II)_n(fenamole)_n(acetate)_{2n}$	4.5
$Cu(II)_n(fenamole)_{2n}(HCl)_{2n}$	4.5
$Cu(II)(salicylate)_2\cdot 4H_2O$	4.5
$Cu(II)_2[1\text{-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetate}]_4(CH_3COCH_3)_2$	4.5
$Cu(II)_n(4\text{-n-butyl-1,2-diphenyl-3,5-pyrazolidinedione})_{2n}$	4.5

[a] All doses were given intragastrically and are expressed as the lowest active dose in milligrams per kilogram of body weight.

$Copper(II)_2(acetate)_4$ had only very weak antiulcer activity and the antiarthritic drugs, from which the copper complexes were synthesized, are known to be potent ulcer causing agents. Nevertheless, their copper complexes have been found to be effective antiulcer agents in six different models of ulcer [72]. Most remarkable is the observations by West and his colleagues that copper complexes of the antiarthritic drugs are able to treat or prevent ulcers due to the parent drug [75–77].

Anticonvulsant Activity of Copper Complexes

Being aware of the fact that tremors and seizures are a constant feature of copper deficiency in Man and animals and that copper metabolism is abberant in epileptics, we submitted copper complexes to the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) when it was announced that this agency of the National Institutes of Health had established an Antiepileptic Drug Development Program to screen compounds for anticonvulsant activity. The two recognized models of seizure used to screen these compounds are the maximal electroshock (MES)- and Metrazol(M)-induced seizures. Routine testing is done following intraperitoneal (IP) injection and challenge with electroshock or Metrazol (pentylenetetrazol) 30 minutes and 4 hours after injection of the test compound at doses of 30, 100, 300, and 600 mg/kg. Compounds found to be effective at 30 minutes are viewed as rapid in onset (due to rapid distribution to the CNS) and those found to be only active at 4 hours are viewed as slower in onset. We have also requested subcutaneous (SC) administration and these data are provided as the testing work load permits. Subcutaneous administration has been helpful in determining whether or not hypnotic activity can be distinguished from anticonvulsant activity based upon the decreased rate of absorption associated with this route of administration as opposed to the more rapid rate of absorption associated with IP administration.

At the outset three copper salicylate complexes were submitted for evaluation. One of the three, $Cu(II)(3,5\text{-dips})_2$, was found to have a rapid onset and prolonged duration of activity in protecting mice against both MES-induced and M-induced seizures, as shown in Table V.

Although the data for the copper complexes presented in Table VI are incomplete and preliminary, there are some interesting results. The copper complex of Dilantin was found to have a rapid onset and prolonged duration in inhibiting only MES seizures. Dilantin is also known to only inhibit MES seizures. Phase II data for $Cu(II)(Dilantin)_2$ indicate that it has a time of peak effect of 4 hrs, which is longer than the time of peak effect for Dilantin (1 hr).

The other two complexes $Cu(II)(salicylate)_2$ and $Cu(II)(3,5\text{-ditertiarybutylsalicylate})_2$, were effective in protecting against M-induced seizures but inactive against MES-induced seizures at the times and doses routinely studied. When $Cu(II)(salicylate)_2$ was studied at a higher dose it was effective in both models of seizure [78]. A group of amino acid copper complexes were also found to protect against M-induced seizures but had no activity in protecting against MES-induced seizures. Both $Cu(II)Cl_2$ and $Cu(II)_2(acetate)_4$ were found to be ineffective in protecting against both MES- and M-induced seizures following SC administration [78]. Since the ligands were not known to have anticonvulsant activity and $Cu(II)Cl_2$ or $Cu(II)_2(acetate)_2$ did not have activity, it was suggested that the anticonvulsant activity was due to the complexed form of copper. The lack of a correlation between the amount of copper in these complexes and their anticonvulsant activity suggested that the activity was in part due to their physico-chemical properties. This then led to the hypothesis that copper complexes of the antiepileptic drugs might be the active metabolites of these drugs.

In preliminary studies, the copper complex of amobarbital was found to be a more potent anti-

TABLE V. Anticonvulsant Activities of Some Copper Complexes [78–79].

Complexes	%Cu	MES	Metrazol
Salicylate			
Cu(II)(salicylate)$_2$	19	I[a]	A[b] at 30 at 30 min; A at 300 at 4 hr
Cu(II)(4-tertiarybutylsalicylate)$_2$·1/2H$_2$O	14	I	A at 300 at 30 min; A at 300 at 4 hr
Cu(II)(3,5-dips)$_2$	13	A at 300 at 4 hr; A at 100 at 6 hr; A at 300 at 8 hr	A at 30 at 30 min; A at 100 at 4 hr; A at 100 at 6 hrs; A at 100 at 8 hr
Amino Acid			
Cu(II)(L-threoninate) (L-serinate)	22	I(i.p.)	A at 30 at 30 min and 4 hr
Cu(II)(L-threoninate) (L-alaninate)	24	I(i.p.)	A at 30 at 30 min and 4 hr
Cu(II)(L-valinate)$_2$H$_2$O	20	A at 600 at 30 min (i.p.)	A at 30 at 30 min and 4 hr
Cu(II)(L-threoninate)$_2$H$_2$O	20	I(i.p.)	A at 30 at 30 min and 4 hr
Cu(II)(L-alaninate)$_2$	27	I(i.p.)	A at 30 at 30 min and 4 hr
Cu(II)(L-phenylalaninate)$_2$	16	I(i.p.)	A at 30 at 30 min
Cu(II)(L-cystinate)$_2$H$_2$O	20	I(i.p.)	A at 100 at 30 min
Cu(II)(L-tryptophan)$_2$	14	I(i.p.)	A at 100 at 30 min and 4 hr (i.p.)

[a] Inactive (I) at doses studied. [b] Activity (A) at doses and times indicated. All doses (mg/kg) were given subcutaneously or, when indicated, intraperitoneally (i.p.).

convulsant than sodium amobarbital [79]. We then began to synthesize copper complexes of other classes of antiepileptic drugs and submitted them to NINCDS for Phase I studies, designed to detect anticonvulsant activity, and, if active in Phase I, Phase II follow-up studies to quantitate the anticonvulsant activity at the time of maximum effect.

The data provided in Table VI for the inhibition of MES- and M-induced seizures are the lowest effective doses (mg/kg). If a compound is found to be effective and nontoxic in Phase I evaluations, which are done to detect anticonvulsant activity, it is further examined in Phase II studies to determine time of peak effect and ED_{50}. Since Phase II evaluations are done only after Phase I, we have Phase II data for a smaller number of compounds. Unfortunately, there are no Phase I data for the antiepileptic drugs since there is no need to attempt to detect anticonvulsant activity for these established anticonvulsant agents. We do have Phase II-ED_{50} data for some of these compounds and they have been included in Table VI. Table VI also contains Phase II time of peak effect data for the parent drugs.

In Phase I studies, which are to be repeated, Cu(II)$_2$(Valproate)$_4$ appeared to be ineffective against MES seizures but had some inhibitory activity against M seizures. This compound had a rapid onset and short duration of activity following IP administration and, consistently, a prolonged onset of activity at a higher dose following SC administration. The parent compound was also weakly effective against MES seizures and more effective against M seizures.

With few exceptions the Phenobarbital complexes were also found to have rapid onset and prolonged durations of activity in both models of seizure. Although the data do not allow a rigorous comparison of these compounds, it is interesting that the pyridine and imidazole complexes were somewhat less effective than the aquo complexes. All three solvates had prolonged onsets of action following SC administration. The aquo Phenobarbital complexes were most effective regardless of the route of

TABLE VI. Phase I and Some Phase II Anticonvulsant Data for Copper Complexes of Antiepileptic Drugs.

Compound	Route[b]	Challenge Time	Seizure Model[a]	
			MES	Metrazol
Cu(II)(Dilantin)$_2$(H$_2$O)$_3$	IP	30 min	30	100
		4 hr	30	100
Cu(II)(Dilantin)$_2$(H$_2$O)$_3$	IP[c]	4 hr[d]	13	NT[e]
Dilantin	IP[c]	1 hr	7	NT

(Continued on facing page)

TABLE VI. *(Continued)*

$Cu(II)_2(Valproate)_4$	IP	30 min	I	100
		4 hr	I	I
$Cu(II)_2(Valproate)_4$	SC	30 min	I	I
		4 hr	I	600
Valproic Acid	IP[c]	15 min	272	149
$Cu(II)(Phenobarbital)_2(H_2O)_{5.5}$	IP	30 min	30	5
		4 hr	30	5
$Cu(II)(Phenobarbital)_2(H_2O)_{5.5}$	SC	30 min	30	30
		4 hr	30	30
$Cu(II)(Phenobarbital)_2(H_2O)_3$	IP	30 min	30	5
		4 hr	30	5
$Cu(II)(Phenobarbital)_2(H_2O)_3$	SC	30 min	30	30
		4 hr	30	30
$Cu(II)_n(Phenobarbital)_n(H_2O)_{2n}(H_2O)_{3n}$	SC	30 min	100	30
		4 hr	30	30
Phenobarbital	IP[c]	1 hr	22	13
$Cu(II)(Phenobarbital)_2(Pyridine)_2$	IP	30 min	100	30
		4 hr	100	30
$Cu(II)(Phenobarbital)_2(Pyridine)_2$	SC	30 min	I	I
		4 hr	30	30
$Cu(II)_n(Phenobarbital)_n(pyridine)_{2n}(H_2O)_{3n}$	SC	30 min	100	30
		4 hr	30	30
$Cu(II)_n(Phenobarbital)_n(pyridine)_{2n}(H_2O)_{3n}$	SC	30 min	30	30
		4 hr	100	30
$Cu(II)(Phenobarbital)_2(imidazole)_2$	IP	30 min	100	300
		4 hr	100	30
$Cu(II)(Phenobarbital)_2(imidazole)_2$	SC	30 min	I	100
		4 hr	30	100
$Cu(II)(Amobarbital)_2(H_2O)_{2.5}$	IP	30 min	300	30
		4 hr	I	I
$Cu(II)(Amobarbital)_2(H_2O)_2$	IP	30 min	100	100
		4 hr	I	I
$Cu(II)(Amobarbital)_2(H_2O)_{2.5}$	IP[c]	30 min	87	57
$Cu(II)(Amobarbital)_2(pyridine)_2$	IP	30 min	300	100
		4 hr	300	30
$Cu(II)(Amobarbital)_2(pyridine)_2$	SC	30 min	I	600
		4 hr	100	600
$Cu(II)(Amobarbital)_2(imidazole)_2$	IP	30 min	600	300
		4 hr	I	I
$Cu(II)(Amobarbital)_2(imidazole)_2$	SC	30 min	I	I
		4 hr	I	600
$Cu(II)(Lorazepam)_2(Cl)_2H_2O$	IP	30 min	20	1
		4 hr	30	1
$Cu(II)(Lorazepam)_2(Cl)_2H_2O$	SC	30 min	20	1
		4 hr	100	1
Lorazepam	IP[c]	1 hr	24	0.02

[a]The numerical values are the lowest active doses in milligrams per kilogram of body weight, I = inactive, MES = maximal electroshock. [b]IP = intraperitoneal, SC = subcutaneous. [c]Phase II data. [d]Time of peak activity in Phase II studies and ED 50 values for inhibition of seizures. [e]Not Tested.

administration and recent data show that the tri- and penta-aquo complexes are effective in preventing the Metrazol-induced seizure at a dose much lower than the lowest dose routinely used as the lowest dose in Phase I studies, 30 mg/kg. Activity at 5 mg/kg would appear to indicate greater activity than Phenobarbital which has an ED_{50} of 13 mg/kg.

Copper(II)(amobarbital)$_2$ complexes also appear to have rapid onsets and short durations of activity following IP administration, which appears to be reversed with SC administration. The NINCDS has no Phase I or Phase II data available for amobarbital.

The copper complex of Lorazepam appears to have a rapid onset of action and prolonged duration following IP and SC administration. This complex appears to be quite active. However, this complex involves complexation at the 4-nitrogen and its stability may not be very great. As a result, more data is also required to distinguish this complex from its parent compound.

Since all of these ligands are known to be active anticonvulsants, simultaneous comparisons of the ligands and their copper complexes are ultimately required to determine whether or not these complexes are more active than the ligands. Data from Phase I and II studies will be used to select pairs of compounds for simultaneous evaluations.

Antineoplastic Activity of Copper Complexes

Dietary copper and small molecular weight copper complexes have been known to have antineoplastic activity for some time [25]. However, these antineoplastic effects were not well understood until Oberley and Buettner [68] pointed out that neoplastic cells were deficient in superoxide dismutase activity. Both copper-dependent and manganese-dependent superoxidases have lower activity in neoplastic cells [80]. Oberley *et al.* then demonstrated that the copper-dependent superoxide dismutase (Cu-ZnSOD) was effective in decreasing growth of solid tumors produced by injecting sarcoma 180 cells into the muscle of the hind leg of mice [81]. Duration of survival was also increased in these tumor-bearing mice. It was then found that small molecular weight copper salicylate complexes, which were known to dismutate superoxide, were also effective in decreasing the rate of Ehrlich-cell tumor growth in this same model [81]. These complexes and the tumor model in which they have been reported to be effective are listed in Table VII.

Consistent with these observations, Kimoto *et al.* have reported that Cu(II)(glycylglycylhistidine) has antineoplastic activity against Ehrlich tumor cells in *in vitro* and *in vivo* model systems when co-administered with ascorbic acid [80]. Forty percent of the mice given both ascorbic acid plus Cu(II)(glycylglycylhistidine) survived to day 60. All of the control mice and Cu(II)(glycylglycylhistidine)-treated mice had died by day 30. It was most interesting that the 60-day survivors rejected tumor growth even after intraperitoneal inoculation with additional Ehrlich cells and survived 'without an accumulation of ascites.'

The antiinflammatory, anticonvulsant, and antineoplastic activities of copper complexes are consistent with the hypothesis that the increase in blood copper-containing components associated with arthritic diseases, epilepsy, and cancers is a physiological response. Remission of these disease states may occur when this and all other physiological responses that participate in overcoming the biochemical lesions responsible for the particular disease state are appropriate. The use of copper complexes in therapy may be viewed as a physiological approach to the treatment of these diseases. Designation of the particular complex or list of complexes which may be most effective in overcoming a particular disease state awaits the description of the specie or species of copper that is or are most important in the physiological response to that disease.

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TABLE VII. Antineoplastic Copper Salicylates.

Compound	Neoplastic Cell Model	Reference
Cu(II)$_2$(acetylsalicylate)$_4$(DMSO)$_4$	solid Ehrlich tumor	[81]
Cu(II)(acetylsalicylate)$_2$(pyridine)$_2$	solid Ehrlich tumor	[81]
Cu(II)$_2$(acetylsalicylate)$_4$	ascites neuroblastoma tumor	[82]
	solid Ehrlich tumor	[83–84]
Cu(II)(salicylate)$_2$	solid Ehrlich tumor	[84]
Cu(II)(3,5-diisopropylsalicylate)$_2$	solid Ehrlich tumor	[81–84]
Cu(II)(3,5-ditertiarybutylsalicylate)$_2$	solid Ehrlich tumor	[84]

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