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Effect of Bis(acetato)tetrakis(imidazole) Copper(II) in Delaying the Onset and Reducing the Mortality Rate of Strychnine- and Thiosemicarbazide-Induced Convulsions

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ABSTRACT

The anticonvulsant activity of bis(acetato)tetrakis(imidazole) copper(II), $Cu(OAc)_2(Im)_4$, was studied in normal mice using chemical convulsions induced by strychnine, thiosemicarbazide, picrotoxin, and pentelenetetrazol. Intraperitoneal administration of $Cu(OAc)_2(Im)_4$, 50 mg/kg body mass, has delayed the onset of strychnine (3 mg/kg)-induced convulsion by 204% ($p \leq 0.005$) and thiosemicarbazide (20 mg/kg)-induced convulsant by 61% ($p \leq 0.005$). The changes in the onset of picrotoxin- (6 mg/kg) and pentelenetetrazol (50 mg/kg)-induced convulsions were not significant. The same dosage of the copper compound was effective in delaying the lethal time and reducing the mortality rate of treated animals. The anticonvulsant activity of $Cu(OAc)_2(Im)_4$ complex against strychnine was not related to its constituents because the inorganic form of copper such as copper chloride, copper acetate, and the parent imidazole has no

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anticonvulsant activity. Other copper(II) complexes like copper(II)aspirinate and bis(acetato)bis(2-methyl imidazole) copper(II) were less effective.

Index Entries: Copper(II) acetate–imidazole; strychnine; anticonvulsants; thiosemicarbazide; picrotoxin; pentylenetetrazol; onset of convulsion.

INTRODUCTION

Copper is recognized as an essential metalloelement, which is required for normal metabolic processes. Many of the key enzymes such as hemocyanin cytochrome oxidase, superoxide dismutase, tyrosinase, dopamine-β-hydroxylase, and amine oxidases are copper-dependent enzymes *(1–3).* The pharmacological activities of copper complexes are consistent with the putative role of Cu-Zn superoxide dismutase and other copper oxidase metalloenzymes in the prevention of tissue damage *(4).*

Copper complexes have been shown to have many pharmacological activities such as anti-inflammatory *(5–8),* antiulcer *(9),* antitumor *(2,10,11),* antidiabetic *(2,12,13),* radio-protectant *(14–16),* as well as anticonvulsant activity *(10,12,17,18).* In many cases, the copper complex of some antiepileptic drugs has been shown to be more effective than their parent ligand *(19,20).* The hypothesis that seizures results from an inadequate supply of copper and loss of copper-dependent enzyme activity is consistent with the high-affinity transport of copper through the brain–blood barrier *(21)* and the fact that postmortem samples of brain tissue from epileptic patients have a very low copper concentration *(22).* Seizures as well as cerebral degeneration occur in copper-deficient animals *(23,24),* and chelating agents that produce tremors in animals also reduce brain copper levels *(25).*

The present study was undertaken to investigate the protective effect of some copper complexes on chemical convulsions induced by strychnine, thiosemicarbazide, picrotoxin, and pentylenetetrazol.

MATERIALS AND METHODS

Reagents and Materials

Strychnine, picrotoxin, thiosemicarbazide, and pentylenetetrazol were purchased from Sigma Chemicals Co. (St. Louis MO). Copper(II) chloride and imidazole were purchased from Aldrich (Milwaukee, WI). Copper(II) acetate complexes were synthesized and characterized as previously described *(26,27).*

Animals and Administration of Drugs

White female mice weighing approx 30 g were used throughout the experiments. Chemical convulsions were induced by intraperitoneal injection of strychnine (3 mg/kg), thiosemicarbazide (20 mg/kg), picro-

Table 1
Effect of $Cu(OAc)_2(Im)_4$ (50 mg/kg) on the Onset of Convulsions Induced by Strychnine, Thiosemicarbazide, Picrotoxin, and Pentylenetetrazol

Convulsants	Dose mg/kg	Control	Treated	Change %	P
Strychnine	3	4.65 ± 0.56 (8)	14.13 ± 1.92 (12)	204	≤ 0.005
Thiosemicarbazide	20	52.83 ± 0.98 (6)	85.04 ± 4.24 (23)	65	≤ 0.0005
Picrotoxin	6	11.57 ± 0.98 (5)	14.99 ± 1.24 (11)	30	NS
Pentylenetetrazol	50	3.36 ± 0.44 (6)	4.62 ± 1.08 (9)	38	NS

Note: Mice were injected intraperitoneally with strychnine (3 mg/kg), thiosemicarbazide (20 mg/kg), picrotoxin (6 mg/kg), or pentylenetetrazol (50 mg/kg) and the onset of convulsions was recorded. Treated animals were injected with $Cu(OAc)_2(Im)_4$ (50 mg/kg [ip]) for 30 min before injection of the convulsants. Control animals were injected intraperitoneally for the same period with the same volume of saline. Anticonvulsant activity is the percentage change at the onset of convulsion between treated and control animals. Values are mean ± SEM for the number of animals indicated in parentheses. NS = nonsignificant.

toxin (6 mg/kg), and pentylenetetrazol (50 mg/kg) and the animals were kept under constant observation for measuring the behavioral changes, onset of seizure, lethal time, and the mortality incidence recorded after a 24-h period. Treated animals were injected intraperitoneally (ip) for 30 min with 50 mg/kg body mass of the following compounds: $Cu(OAc)_2(Im)_4$, Cu(II)asparinate, or a vehicle containing imidazole, copper(II)$_2$(acetate)$_4$, or copper(II)chloride. Control animals were pretreated for the same period with saline (0.9% [w/v] NaCl).

Values were expressed as mean values ± standard error of the mean for the number of experiments indicated in parentheses, and the Student's *t*-test was used for all statistical analyses.

RESULTS AND DISCUSSION

Chemical Convulsions

Table 1 shows that intraperitoneal injection of mice with strychnine (3 mg/kg) produced convulsions after a latent period of 4.65 ± 0.56 (8) min, thiosemicarbazide (20 mg/kg) produced convulsions after 52.83 ± 0.98 (6) min, picrotoxin (6 mg/kg) produced clonic convulsions after 11.57 ± 0.98

Table 2
Effect of Bis(acetato)tetrakis(imidazole) Copper(II) on the Onset of Convulsions, Death, and Mortality Rate of Mice Treated with Strychnine and Thiosemicarbazide

Pre-treatment	Treatment	Onset of Convulsion (min.)	Death (min.)	Mortality Rate
Saline	Strychnine (3 mg/kg)	4.65 ± 0.56 (8)	6.37 ± 0.54 (8)	100%
$Cu(OAc)_2(Im)_4$ (50 mg/kg)	Strychnine (3 mg/kg)	14.13 ± 1.92 (12) 204% (P≤0.005)	17.13 ± 2.25 (12) 169% (P≤0.005)	80%
Saline	Thiosemicarbazide (20 mg/kg)	52.83 ± 0.98 (6)	67.00 ± 6.92 (6)	100%
$Cu(OAc)_2(Im)_4$ (50 mg/kg)	Thiosemicarbazide (20 mg/kg)	85.4 ± 4.24 (23) 61% (P≤0.0005)	143.73 ± 10.73 (15) 115% (P≤0.005)	65%

Note: Mice were injected intraperitoneally with strychnine (3 mg/kg) or thiosemicarbazide (20 mg/kg); the onset of convulsions, lethal time, and mortality rate was recorded. Other animals from the same batch were pretreated for 30 min with $Cu(OAc)_2(Im)_4$ (50 mg/kg [ip]) before injection of the convulsants. Values are mean ± SEM. for the number of animals indicated in parentheses.

(5) min, and pentylenetetrazol (metrazol) (50 mg/kg) produced clonic tonic convulsions after 3.36 ± 0.44 (6) min.

In this experiments, we used minimal doses to induce chemical seizures, and the convulsants are well known for their effect on GABA and glutamate neurotransmission. Strychnine blocks inhibition mediated by glycine *(28),* thiosemicarbazide inhibits GAD activity by an action on its coenzyme pyridoxal phosphate *(29),* and picrotoxin acts by blocking the inhibitory synaptic action of GABA *(30).* Pentylenetetrazol (metrazol) is well known to be a general excitant in the central nervous system *(31).* From our results, it is clear that strychnine is the most potent convulsant, because an intraperitoneal injection of a small dosage (3 mg/kg) was enough to induce convulsions after 4.65 ± 0.56 (8) min and death after 6.37 ± 0.54 (8), with a mortality rate of 100% (*see* Table 2)

Anticonvulsant Activity of Copper(II)acetate–Imidazole

The synthesis and spectroscopic and X-ray structural characterization of the copper(II)acetate–imidazole complex, $Cu(OAc)_2(Im)_4$, was previously reported by one of our colleagues *(27).* It was prepared by the reaction of excess imidazole with $Cu_2(OAc)_4$. Its spectral parameters are

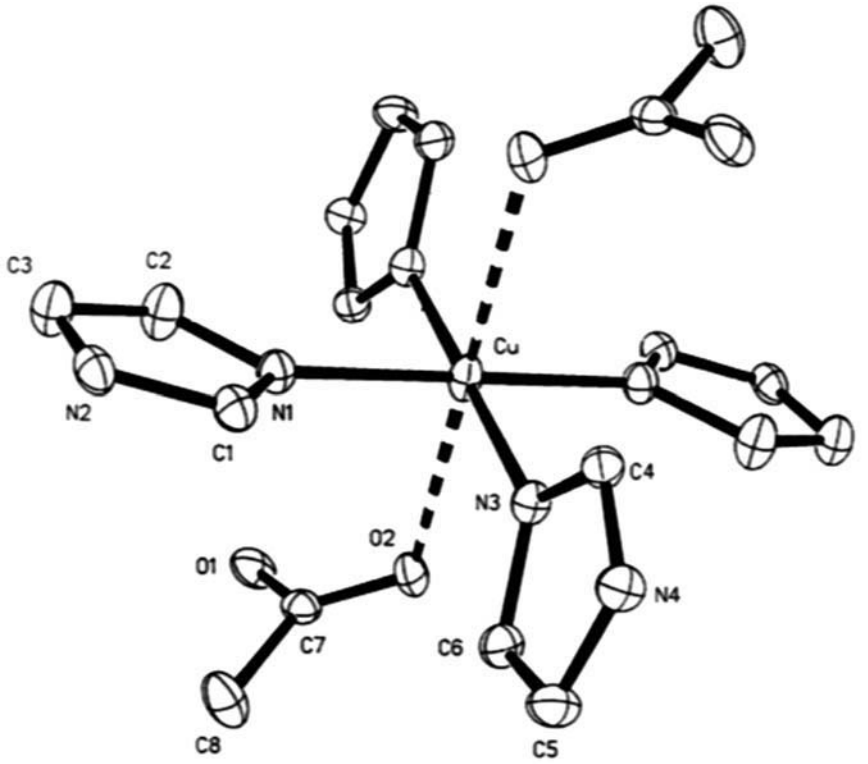

Fig. 1. Oak Ridge Thermal Ellipsoid Plot (ORTEP) drawing of $Cu(C_3H_4N_2)_4(O_2CCH_3)_2$ showing 50% probability of thermal ellipsoids.

characteristic of a tetragonally elongated monomeric copper(II) complex having a $CuN_4 \ldots O_2$ chromophore that was verified by single-crystal X-ray structural analysis *(27)*. The structure of the complex is shown in Fig. 1. The Cu ion resides in a distorted octahedral environment that consists of four coordinated imidazole nitrogen atoms in a plane with two weak interacting carboxylate oxygen atoms in a plane disposition along a vector that is nearly perpendicular to the plane of the imidazole nitrogen atoms (*see* Fig. 1) *(27)*. The complex is blue violet, very stable at room temperature and at atmospheric air, and is soluble in most organic solvents (alcohols, dimethylsulfoxide [DMSO], acetonitrile, etc.).

Copper(II)acetate–imidazole, $Cu(OAc)_2(Im)_4$ (50 mg/kg), injected intraperitoneally for 30 min before treatment with the convulsants, delayed the onset of strychnine convulsion by 204% ($p < 0.005$) and the onset of thiosemicarbazide by 65% ($p < 0.0005$). The same copper complex has increased the onset of picrotoxin- and metrazol-induced convulsions by 30% and 38%, respectively, but the effect was not significant (*see* Tables 1 and 2).

Pretreatment with the copper complex $Cu(OAc)_2(Im)_4$ delayed the onset of strychnine convulsion by 204% and the time to death by 169% and

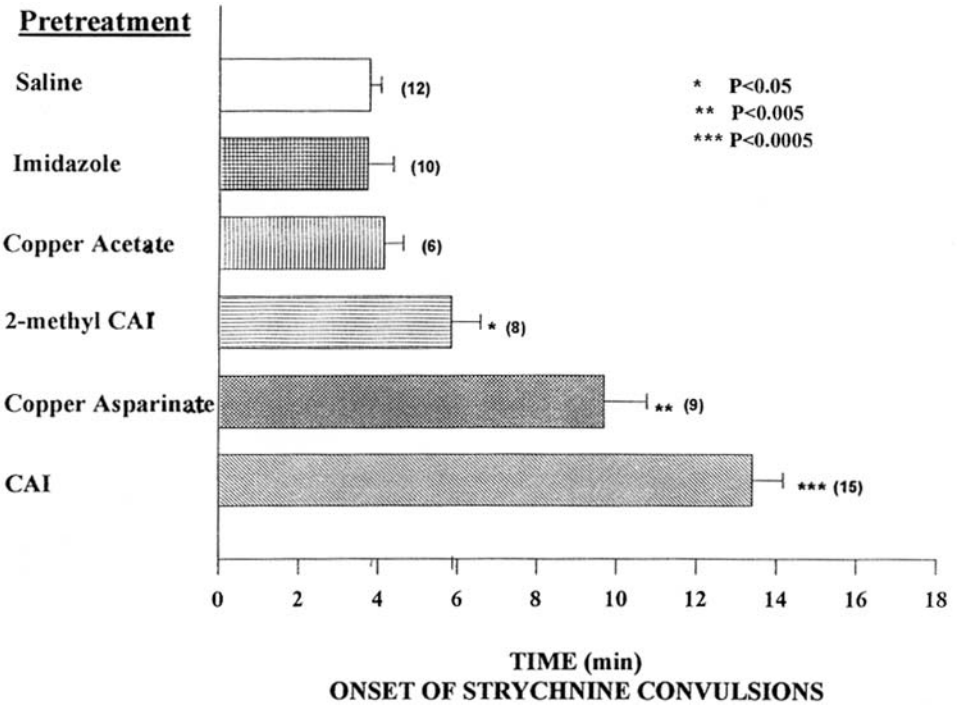

Fig. 2. The anticonvulsant activity of copper compounds was tested by measuring the onset of strychnine convulsion following pretreatment with different copper complexes and inorganic compounds: copper acetate imidazole (CAI), copper asparinate, copper acetate 2-methyl imidazole (2-methyl CAI), copper acetate, imidazole, and saline. All of these compounds were injected (ip) 30 min before injection of strychnine (3 mg/kg).

reduced the mortality rate by 20%. With thiosemicarbazide, the copper complex delayed the onset of convulsion by 65% and the time to death by 115% and reduced the mortality rate by 35%. The same copper complex was found previously to have an antidiabetic activity *(13),* which indicates the general protective mode of copper complexes in repairing of tissue injury that occur in many of these diseases *(2,18).*

In previous reports, all of the metal complexes of 3,5-diisopropylsalicylate (DIPS), including copper, iron, and zinc, were found to have anticonvulsant activities, by preventing metrazol and maximal electroshock-induced seizures *(15,32).* The anticonvulsant activity of $Cu(II)_2(3,5\text{-}DIPS)_4$ was related to the decrease in nitric oxide synthesis in animal models of epilepsy.

Anticonvulsant Activities of Other Copper Complexes and Ligands

Figure 2 shows that copper acetate imidazole, $Cu(OAc)_2(Im)_4$ was the most effective copper complex when tested against strychnine induced

seizures. It has delayed the onset of strychnine convulsions by 256% (p <0.0005). Copper asparinate has delayed the onset of convulsions by 159% ($p < 0.005$) and copper acetate-2-methylimidazole by 57% ($p < 0.05$). Pretreatment with saline, imidazole or copper acetate have no effect on the onset of strychnine induced seizures.

The dimethyl (DMF) and diethylether ternary complexes of $Cu(II)_2(3,5\text{-}DIPS)_4$ were found to have anticonvulsant activity in the maximal electroshock model of grand mal epilepsy, and $Cu(II)_2(3,5\text{-}DIPS)_4(DMF)_2$ was also effective against the metrazol model of seizure *(33).* Binuclear and mononuclear 1,10-phenanthroline and salicylate ternary copper(II) complexes were effective in preventing MES-induced seizures and ineffective in preventing metrazol-induced seizures *(20).* $Cu(II)_2(aspirinate)_4(DMF)_2$ was an effective anticonvulsant in the MES model of seizure, but ineffective against metrazol-induced seizures *(34).*

In vitro, it was reported that copper can modulate glutamate, GABA, and glycine receptors, spontaneous glutamate-mediated excitatory synaptosomal activity was completely blocked by copper *(35),* and copper could have a presynaptic effect on transmitter release via inhibition of voltage-gated calcium channels.

It has been suggested that copper(II) complexes of anti-inflammatory and anticonvulsant drugs are often more active, less toxic, and desirable drugs than the parent ligand themselves *(20,36,37).* It seems that copper complexes increase the bioavailability of copper and the activation of copper-dependent enzymes, which are essential for normal physiological processes and prevention of tissue damage. Copper complexes have superoxide dismutase mimetic activity that prevents tissue damage by dismutation of free active radicals such as superoxide and hydroxyl radicals *(12).*

In conclusion, the results reported here add further evidence to the copper complexes, which can play a part in inhibition of chemically induced seizures in addition to there antidiabetic and anti-inflammatory activity. Copper(II)–imidazol complexes can provide protection against seizures induced by strychnine and thiosemicarbazide without a having significant effect on picrotoxin- and pentylenetetrazol-induced convulsions. Further experiments are needed to elucidate the mechanism of action of the copper(II)acetate–imidazole complexes.

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