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Hypoglycemic Effect of Copper(II) Acetate Imidazole Complexes

ABDUL-SALAM ABDUL-GHANI,*[1] ABDUL-LATIF ABU-HIJLEH,[2] NABEEL NAHAS,[1] AND RIYAD AMIN[1]

[1]Department of Biology and Biochemistry; and [2]Department of Chemistry, Faculty of Science, Birzeit University, P.O. Box 14, Birzeit, West Bank, Palestine

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ABSTRACT

The effect of copper(II) complexes on glucose metabolism was studied in normal and streptozotocin-induced diabetic rats. The copper(II) complexes used were bis(acetato)tetrakis(imidazole) copper (II), $[Cu(OAc)_2(Im)_4]$, bis(acetato)bis(2-methylimidazole) copper(II), $[Cu(OAc)_2(2mIm)_2]$, bis(acetato)bis(1,2-dimethylimidazole) copper(II), $[Cu(OAc)_2(1,2dmIm)_2]$, and bis(acetato)bis(μ-acetato)tetrakis(*N*-methylimidazole) copper(II) hexaaquo, $[Cu_2(OAc)_4\text{-}(NmIm)_4] \cdot 6H_2O$. Intramuscular administration of various doses of $Cu(OAc)_2(Im)_4$ ranging from 10 to 100 mg/kg body mass to overnight fasted rats decreased blood glucose levels in a dose-dependent manner. Maximum hypoglycemic effect was observed 3 h after administration and lasted for at least 6 h. Treatment with 100 mg/kg body mass of $Cu(OAc)_2(Im)_4$ caused hypoglycemic shock, which was irreversible and even lethal. Blood insulin levels were reduced sharply during this hypoglycemic shock. Similar changes in blood glucose level were achieved using $Cu(OAc)_2(2mIm)_2$.

The same pattern of hypoglycemia, although less pronounced, was observed for $Cu_2(OAc)_4(NmIm)_4 \cdot 6H_2O$ and $Cu(OAc)_2(1,2dmIm)_2$. Binary copper(II) acetate complex, the ligand imidazole, and the inorganic form of copper, such as copper(II) chloride, had no significant effect on blood glucose level. These results indicate that the hypoglycemic activity of these complexes varies with the imidazole ligand and structure of the complex.

*Author to whom all correspondence and reprint requests should be addressed.

Intramuscular administration of $Cu(OAc)_2(Im)_4$ to diabetic rats caused a reduction in blood glucose levels and improved their tolerance for glucose.

Index Entries: Copper(II) complexes; streptozotocin-induced diabetes; hypoglycemia; glucose tolerance test.

INTRODUCTION

Over the last 20 years, copper complexes were found to possess anti-inflammatory *(1–3)*, antiulcer *(4)*, anticonvulsant *(5,6)*, and antidiabetic activities *(7)*.

The antidiabetic activity of copper complexes was first reported using two copper complexes, copper(II)$_2$ (3,5-diisopropyl-salicylate)$_4$ and copper(II)$_2$ (salicylate)$_4$ *(6,8)*. They improved glucose tolerance in streptozotocin-treated rats, whereas their parent ligands, salicylic acid and 3,5-diisopropylsalicylic acid, had no antidiabetic activity.

Inorganic copper has some antidiabetic properties *(7)*. It stimulates glucose incorporation into glycogen in diaphragm muscle *(9)* and epididymal adipose tissue of normal and streptozotocin-diabetic rats *(10)*. Cohen and Miller have reported that inorganic copper stimulates insulin release from the perfused rat pancreas *(11)*.

The present study was undertaken to investigate the hypoglycemic effect of four copper(II) acetate imidazole complexes:

1. $Cu(OAc)_2(Im)_4$;
2. $Cu_2(OAc)_4(NmIm)_4 \cdot 6H_2O$;
3. $Cu(OAc)_2(1,2dmIm)_2$; and
4. $Cu(OAc)_2(2mIm)_2$.

(OAc = acetate anion, Im = imidazole, NmIm = *N*-methylimidazol, 1,2dmIm = 1,2dimethylimidazol, and 2mIm = 2-methyl imidazole). The molecular structures of the complexes under investigation have been previously characterized *(12–14)* and are depicted in Fig. 1.

MATERIALS AND METHODS

Reagents and Materials

Copper(II) chloride, copper(II) acetate monohydrate, imidazole, and its methyl derivatives were purchased from Aldrich (Milwaukee, WI). Copper(II) acetate complexes with imidazole or methyl imidazoles were synthesized and characterized as previously described *(12–14)*. Dextrostix strips were purchased from Ames, (Miles, Paris), and streptozotocin (STZ) was obtained from Sigma (St. Louis, MO).

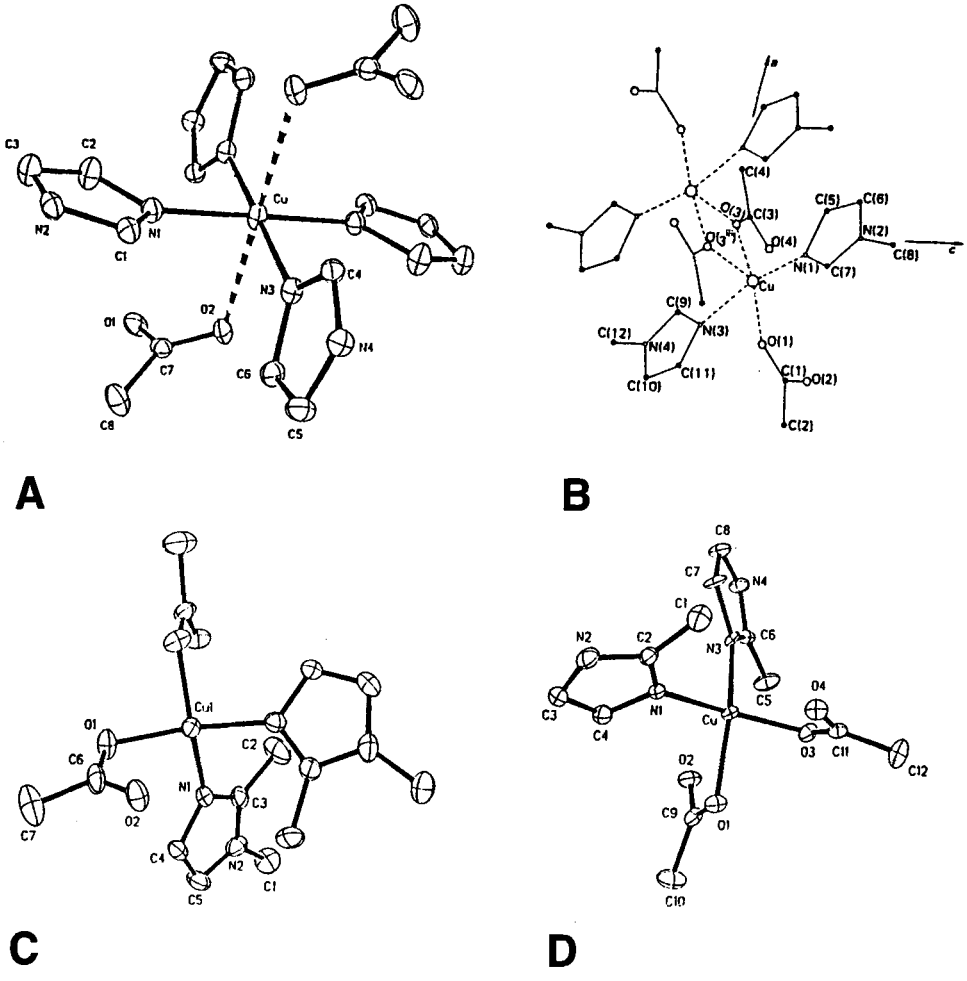

Fig. 1. The molecular structures of copper(II) complexes, $Cu(OAc)_2(Im)_4$ **(A)**, $Cu_2(OAc)_4(NmIm)_4 \cdot 6H_2O$**(B)**, $Cu(OAc)_2(1,2dmIm)_2$**(C)**, and $Cu(OAc)_2(2mIm)_2$ **(D)** (adapted from refs. (12–14)).

Animals and Administration of Drugs

Male 150–250 g Sprague Dawley rats were fasted for 18 h, but allowed water ad libitum before use in these experiments. Groups of 6–12 animals were injected intramuscularly (im) or intrapertoneally (ip) with 100 mg/kg body mass of the following compounds: $Cu(OAc)_2(Im)_4$, $Cu(OAc)_2(2mIm)_2$, $Cu(OAc)_2(1,2dmIm)_2$, and $Cu_2(OAc)_4(NmIm)_4 \cdot 6H_2O$, or vehicle containing imidazole, copper(II)$_2$ (acetate)$_4$ or copper(II) chloride. In other experiments, doses of 10, 20, 40, 50, 60, or 100 mg/kg

$Cu(OAc)_2(Im)_4$ were injected intramuscularly (im) to overnight fasted animals to study dose response.

Experimental diabetes was induced by subcutaneous (SC) injection of 35 mg streptozotocin/kg body mass dissolved in 100 m*M* sodium acetate buffer, pH 5.2. Diabetes was assessed by monitoring blood glucose level and by ip glucose tolerance test. Animals were considered as diabetic 3 wk after injection of STZ, when blood glucose levels were between 300 and 400 mg/dL. $Cu(OAc)_2(Im)_4$ 50 mg/kg was administered im to 18-h fasted diabetic animals 2 h before ip injection of 0.5 g glucose/kg body mass as a 25% solution with water. Blood samples were taken from the tail tip after 15, 30, 60, 90, and 120 min following glucose injection.

In other experiments, normal fasted rats were injected orally with different doses of $Cu(OAc)_2(Im)_4$ ranging from 50 to 1000 mg/kg, and in other groups, iv injection of smaller doses of 5–10 mg/kg of the same compounds were used.

The effect of $Cu(OAc)_2(Im)_4$ on blood insulin was determined after injecting 50 mg/kg im, while control animals were injected with an equal volume of saline. Three hours after injection, blood was collected from the abdominal artery, and insulin was determined using the Amersham (UK) Insulin Kit. Blood glucose was measured using a glucometer and Dextrostix strips *(15)*. Student's *t*-test was used for all statistical analyses.

RESULTS

Hypoglycemic Effect of $Cu(OAc)_2(Im)_4$

Table 1 and Fig. 2 show that intramuscular administration of various concentrations of $Cu(OAc)_2(Im)_4$ ranging from 10 to 100 mg/kg body mass to overnight fasted rats reduced blood glucose level in a dose-related fashion. The maximum hypoglycemic effect was observed 3–5 h after injection of $Cu(OAc)_2(Im)_4$. A 15% reduction was observed with 20 mg/kg, 34% with 40 mg/kg, 48% with 50 mg/kg, and 51% with 60 mg/kg 5 h after treatment. The hypoglycemic effects were not observable at 24 h after treatment. The dose of 10 mg/kg produced only a weak hypoglycemic effect, which was not significant ($P > 0.05$), and the high dose of 100 mg/kg produced irreversible hypoglycemic shock, which was lethal.

Oral administration of up to 1000 mg/kg produced no hypoglycemic activity, whereas iv administration of 10 mg/kg was lethal within 5–10 min without showing any signs of hypoglycemia.

Intramuscular injection of 50 mg $Cu(OAc)_2(Im)_4$/kg significantly reduced blood insulin concentration from a normal value of 40.10 ± 7.74 *(5)* μU/mL to 8.60 ± 3.22 (5) μU/mL ($P < 0.025$) (Table 2).

Table 1
Hypoglycemic Effect of Different Doses of $Cu(OAc)_2(Im)_4$

Dosage	Blood glucose mg%	Hypoglycemic effect	$P <$
0	90.24 ± 2.78 (6)	—	—
10 mg/kg	84.00 ± 2.71 (6)	7%	NS
20 mg/kg	76.47 ± 3.06 (7)	15%	0.05
40 mg/kg	59.78 ± 3.21 (7)	34%	0.0025
50 mg/kg	47.19 ± 2.83 (6)	48%	$P < 0.0005$
60 mg/kg	44.19 ± 2.46 (6)	51%	$P < 0.0005$
100 mg/kg	17.28 ± 2.55 (12)	81%	$P < 0.0005$

Blood glucose measured in the tail tip 300 min after treatment with different doses of $Cu(OAc)_2(Im)_4$. The data are normalized to the initial value of 100%, and all subsequent values expressed relative to the initial level. Hypoglycemic effect is the percentage reduction in blood glucose compared to the saline-treated animals. The number of animals indicated in parenthesis.

NS = nonsignificant.

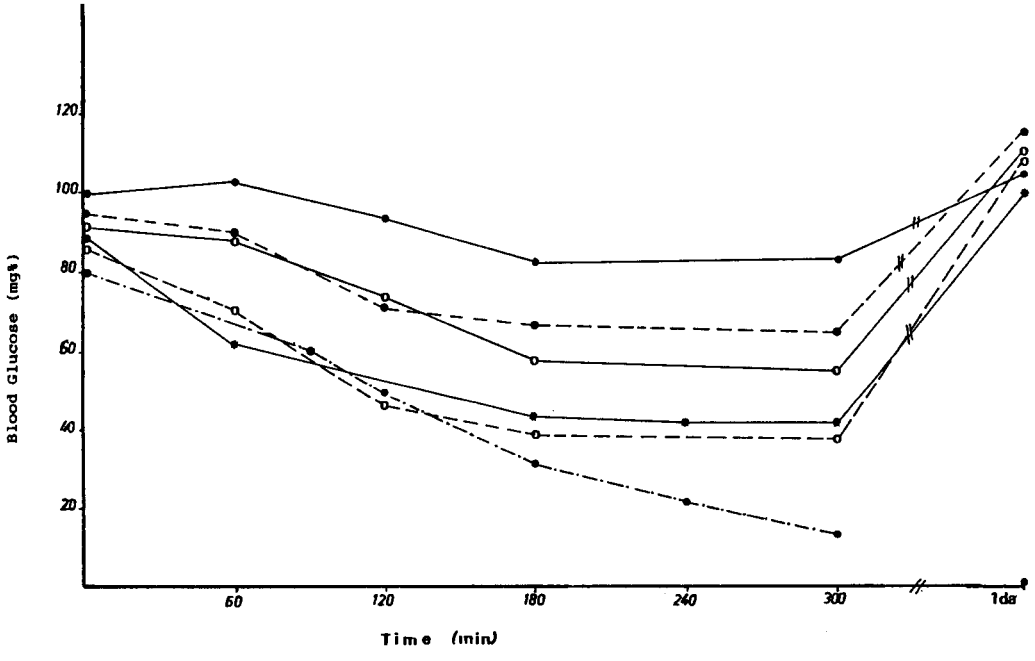

Fig. 2. Hypoglycemic effect of $Cu(OAc)_2(Im)_4$. Rats were injected im with various doses of $Cu(OAc)_2(Im)_4$. 10 mg/kg (●————●); 20 mg/kg (●– – –●); 40 mg/kg (○————○); 50 mg/kg (✳————✳); 60 mg/kg (○– – –○); or 100 mg/kg (●–·–·–·–●). Values are the mean for 6–12 rats/group. Error bars have been omitted for clarity.

Table 2
Changes in Blood Insulin Content
following im Injection of $Cu(OAc)_2(Im)_4$ 50 mg/kg

Blood insulin content (μU/mL)	
Control	$Cu(OAc)_2(Im)_4$, 50 mg/kg
30	10
62	2.5
58	5.5
16.5	3
34	22
40.10 ± 7.74 (5)[a]	8.60 ± 3.22 (5) ↓ 78% $P < 0.025$[b]

Normal values 25–80 μU/mL. The animals were killed 3 h after injection of CAI (50 mg/kg im). Blood was collected from the abdominal artery for the determination of insulin levels.

[a]The average ± SEM of the five animals tested.

[b]Percent reduction in insulin values.

Hypoglycemic Activity for Various Copper Complexes

The hypoglycemic effect of copper complexes varies with the complex structure (Table 3). $Cu(OAc)_2(Im)_4$, 100 mg/kg im, caused 77% reduction in blood glucose level 4 h after treatment compared to saline-treated animals ($P < 0.0005$). The same treatment with $Cu(OAc)_2(2mIm)_2$ caused a 76% decrease in blood glucose level, whereas $Cu_2(OAc)_4(NmIm)_4 \cdot 6H_2O$ and $Cu(OAc)_2(1,2dmIm)_2$ produced reductions of 43 and 37%, respectively. An inorganic form of copper, $CuCl_2$, was less active and reduced blood glucose only by 15%, which was nonsignificant. Binary copper(II) acetate complex and the ligand imidazole each administrated (100 mg/kg) caused a nonsignificant reduction of 15–16%. All changes exerted by these copper compounds persisted for 3–6 h after administration. Similar results were achieved with ip injection.

The Effect of $Cu(OAc)_2(Im)_4$ on Glucose Tolerance in Diabetic Animals

Intraperitoneal administration of 500 mg glucose/kg to fasted diabetic animals produced a significant rise in blood glucose, which reached a maximum level after 30 min ($P < 0.001$). Blood glucose levels remained higher than baseline values for about 2 h after glucose administration. The effect of $Cu(OAc)_2(Im)_4$ on glucose tolerance in diabetic animals is shown in Fig. 3.

Table 3
Hypoglycemic Effect of Different Copper(II) Compounds

	Blood glucose, mg%	Hypoglycemic effect
Saline	70.40 ± 4.35 (5)	—
Imidazole	60.52 ± 3.90 (5)	15%
Copper(II) chloride	60.07 ± 5.15 (5)	15%
Copper(II) (acetate)$_4$	59.03 ± 4.83 (5)	16%
Cu(OAc)$_2$(1,2dimIm)$_2$	44.50 ± 2.82 (6)[a]	37%
Cu$_2$(OAc)$_4$(NmIM)$_4$ · 6H$_2$O	40.05 ± 4.29 (6)[b]	43%
Cu(OAC)$_2$(2mIm)$_2$	16.62 ± 2.29 (6)[c]	76%
Cu(OAc)$_2$(Im)$_4$	16.28 ± 2.90 (12)[c]	77%

Blood glucose was measured in the tail tip 4 h after treatment with copper compounds (100 mg/kg im). Hypoglycemic effect is the percentage reduction in blood glucose compared to the saline-treated animals.

NS = nonsignificant.

[a]$P < 0.01$.

[b]$P < 0.02$.

[c]$P < 0.0005$.

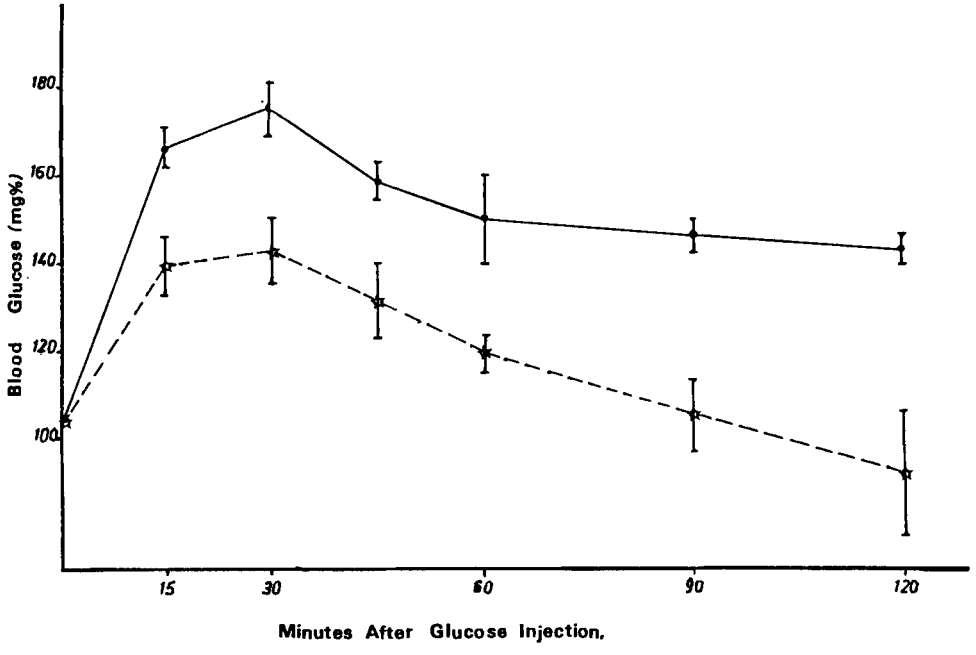

Fig. 3. Effect of Cu(OAc)$_2$(Im)$_4$ on the glucose tolerance test of diabetic rats, which were injected im either with saline (●——●) or Cu(OAc)$_2$(Im)$_4$ (☆- - - -☆) for 2 h before glucose tolerance test. Each point represents the mean of the glucose values of eight rats.

Pretreatment with $Cu(OAc)_2(Im)_4$, 50 mg/kg im, 120 min before injection of glucose reduced the maximum glucose value by 55%, as compared to saline-treated control animals ($P < 0.05$). Blood glucose levels were significantly decreased below initial values 2 h after ip injection of glucose.

DISCUSSION

$Cu(OAc)_2(Im)_4$ is clearly an effective hypoglycemic agent, when injected im or ip peritoneally into fasted rats. Doses between 20 and 60 mg/kg were most effective and nontoxic. This hypoglycemic effect is dose-dependent with a duration of action of about 6 h, whereas higher doses of 100 mg/kg were toxic and even lethal as a result of hypoglycemic shock, which induced seizures. Oral injection of copper(II) complexes was not effective in reducing blood glucose levels even with high doses. This could be owing to inactivation or modification of the copper compound by gastrointestinal secretions or inadequate absorption from the digestive tract. Intravenous injection of small doses caused a sharp increase in blood pressure, which was lethal after 1–3 min of injection. The decrease in blood glucose level is consistent with the 75% reduction in blood insulin content, since it is well known that reduction in blood glucose has a negative feedback on insulin release from the pancreas. This result indicates that the hypoglycemic effect of $Cu(OAc)_2(Im)_4$ is not owing to a direct effect on the pancreas.

The hypoglycemic activity of copper(II) complexes varies with complex structure with $Cu(OAc)_2(Im)_4$ being the most effective and $Cu(OAc)_2(1,2dmIm)_2$ the least effective, whereas inorganic form of copper and imidazole(s) have only a slight hypoglycemic activity, which was nonsignificant.

Copper(II) chloride has been shown to stimulate glucose incorporation into glycogen of diaphragm muscle *(9)* and epididymal adipose tissue of rats *(10)* in vitro, and to stimulate insulin release from the perfused pancreas of rats *(11)*. This may be a specific physiological role for copper since nickel *(16)*, cobalt *(17)*, manganese *(18)*, and zinc *(19)* are known to inhibit glucose-induced insulin release in vitro.

The fact that copper complexes were more active than inorganic copper suggests that the observed pharmacological activity is owing to the physiochemical properties of these complexes. It is presumed that copper complexes facilitate absorption, distribution, and utilization of copper, which is essential for activation of copper-dependent enzymes required to maintain normal physiological processes.

The exact mechanism underlying the effect of copper(II) acetate–imidazole complexes on blood glucose needs further clarification.

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