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Hypolipidemic Activity of Tetrakis-μ-(trimethylamine-boranecarboxylato)-*bis*(trimethylamine-carboxyborane)-dicopper(II) in Rodents and Its Effect on Lipid Metabolism

I. H. HALL *[x], W. L. WILLIAMS, Jr.*, COLLEEN J. GILBERT*, A. T. Mc PHAIL [‡], and B. F. SPIELVOGEL [‡]

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Abstract □ A binuclear copper(II) complex was shown to have potent hypolipidemic activity in rats and mice at low doses, *i.e.*, 2.5-10 mg/kg/d. The agent moderately lowered liver ATP-dependent citrate lyase, acetyl CoA synthetase, and phosphatidate phosphohydrolase activities *in vivo*. The appetite of the animal was reduced by drug treatment, and orally administered cholesterol absorption from the intestine was markedly lowered. Higher lipid levels were found in the chyme and the feces, indicating accelerated excretion of lipids by the drug, probably *via* the biliary route. Organs, *e.g.*, liver and small intestine, as well as serum lipoprotein levels, demonstrated lower lipid content after drug administration. Thus, this chemical class of agents may have potential as a hypolipidemic agent in humans.

Keyphrases □ Binuclear copper(II) complex—antihyperlipidemic activity in rodents, effect on lipid metabolism □ Antihyperlipidemic agents—binuclear copper(II) complex, activity in rodents, effect on lipid metabolism □ Cholesterol—antihyperlipidemic effect of binuclear copper(II) complex

Recently, a series of amine-cyanoboranes and amine-carboxyboranes were observed to be hypolipidemic in mice between 5 and 20 mg/kg ip (1). From *in vitro* studies of these series, the ability to lower serum cholesterol appeared to correlate positively with the inhibition of the liver regulatory enzyme β-hydroxy-β-methyl glutaryl CoA (HMG CoA reductase) reductase activity, and the reduction of serum triglyceride was correlated with inhibition of fatty acid synthetase activity (1). Subsequently, a binuclear copper(II) complex derived from trimethylamine-carboxyborane, *viz.*, tetrakis-μ-(trimethylamine-boranecarboxylato)-*bis*(trimethylamine-carboxyborane)dicopper(II) was synthesized, and its effects on lipid metabolism is reported.

EXPERIMENTAL SECTION

Preparation of Tetrakis-μ-(trimethylamine-boranecarboxylato)-*bis*(trimethylamine-carboxyborane)dicopper(II)—Cupric chloride was purchased commercially[1]. Trimethylamine-carboxyborane was prepared as described previously (2). Trimethylamine-carboxyborane (1.8703 g, 15.9 mmol) was dissolved in 1 M NaOH (16 mL) and water (20 mL). Dropwise addition of 23 mL of a solution of $CuCl_2 \cdot 2H_2O$ (1.36 g, 8 mmol) in water (40 mL) produced a dark green solution which was allowed to stand overnight. Subsequent filtration through a fine-fritted funnel removed a greenish-brown sludge and left a dark-green filtrate, which was allowed to evaporate in the atmosphere. After 6 d, the solution evaporated, leaving many small green crystals in a clear liquor. These crystals were filtered and washed with chloroform (40°C); no $(CH_3)_3N \cdot BH_2COOH$ crystals were evident. The green crystals were then splashed with a minimal amount of cold water to ensure removal of any trace of sodium chloride and dried *in vacuo*. The yield was 0.49 g (23%), mp 165°C (dec.); IR: ν_{BH}, 2350, $\nu_{C=O}$ 1665 cm^{-1}.

Anal.—Calc. for $C_{24}H_{68}B_6Cu_2N_6O_{12}$: C, 34.95; H, 8.31; N, 10.19. Found: C, 35.00; H, 8.49; N, 10.15. The structure was determined by single-crystal X-ray analysis (3).

Antihyperlipidemic Screens in Normal Rodents—Compounds to be tested were suspended in 1% aqueous carboxymethylcellulose and administered to male CF_1 mice (~25 g) intraperitoneally for 16 d or male Holtzman rats (~350 g) orally by an intubation needle for 14 d. On days 9 and 14 or 16, blood was obtained by tail vein bleeding, and the serum was separated by centrifugation for 3 min. The serum cholesterol levels were determined by a modification of the Liebermann-Burchard reaction (4). Serum triglyceride levels were determined with a commercial kit[2] for a different group of animals bled on day 14 or 16.

Testing in Induced Hyperlipidemic Mice—Male CF_1 mice (~25 g) were

[1] Allied Chemicals, Morristown, N.J.
[2] Fisher, Hycel Triglyceride Test Kit.

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Table I—Effects of the Binuclear Copper(II) Complex on Serum Cholesterol and Triglyceride Levels of Male CF_1 Mice and Sprague–Dawley Rats[a]

Compound	Mice			Rats		
	Serum Cholesterol		Serum Triglycerides	Serum Cholesterol		Serum Triglycerides
	Day 9	Day 16	Day 16	Day 9	Day 16	Day 16
Control (1% carboxymethylcellulose)	100 ± 7[b]	100 ± 6[c]	100 ± 7[d]	100 ± 6[e]	100 ± 6[f]	100 ± 8[g]
Binuclear copper(II) complex						
2.5 mg/kg	61 ± 5[h]	62 ± 6[h]	50 ± 6[h]	—	—	—
5.0 mg/kg	67 ± 6[h]	63 ± 5[h]	69 ± 6[h]	—	—	—
10.0 mg/kg	71 ± 6	63 ± 5[h]	47 ± 5[h]	63 ± 5[h]	65 ± 5[h]	36 ± 4[h]
20.0 mg/kg	52 ± 5[h]	53 ± 4[h]	—	—	—	—

[a] Expressed as percent of control (mean ± *SD*); $n = 6$. Compound was administered intraperitoneally to mice and orally to rats. [b] 118 mg%. [c] 122 mg%. [d] 137 mg%. [e] 73 mg%. [f] 78 mg%. [g] 110 mg%. [h] $p \leq 0.001$.

Table II—Effects of Binuclear Copper(II) Complex on *In Vitro* CF_1 Mouse Liver Enzyme Activities[a]

Enzyme Parameter	Liver Enzyme Activity, % of Control			
	Control	50 μm	100 μm	200 μm
Mitochondrial citrate exchange	100 ± 7[b]	105 ± 6	104 ± 6	103 ± 7
ATP-dependent citrate lyase	100 ± 6[c]	110 ± 6	98 ± 5	105 ± 7
Acetyl CoA synthetase	100 ± 6[d]	104 ± 5	87 ± 4[e]	84 ± 5[f]
HMG CoA reductase	100 ± 9[g]	102 ± 8	101 ± 9	105 ± 8
Cholesterol side-chain oxidation	100 ± 5[h]	—	152 ± 7[f]	—
Acetyl CoA carboxylase	100 ± 6[i]	96 ± 6	93 ± 5	92 ± 5
Fatty acid synthetase	100 ± 8[j]	86 ± 7	96 ± 8	87 ± 7
sn-Glycerol-3-phosphate acyl transferase	100 ● 7[k]	108 ± 6	92 ± 8	89 ± 6
Phosphatidate phosphohydrolase	100 ± 7[l]	77 ± 5[f]	63 ± 6[f]	35 ± 3[f]

[a] Expressed as mean ± *SD*; $n = 6$. [b] 30.8% exchange of mitochondrial citrate. [c] 30.5 mg of citrate hydrolyzed/g of wet tissue/20 min. [d] 28.5 mg of acetyl CoA formed/g of wet tissue/20 min. [e] $p \leq 0.005$. [f] $p \leq 0.001$. [g] 384,900 dpm of cholesterol formed/g of wet tissue/60 min. [h] 6080 dpm of CO_2 formed/g of wet tissue/18 h. [i] 32,010 dpm/g of wet tissue/30 min. [j] 37,656 dpm/g of wet tissue/20 min. [k] 537,800 dpm/g of wet tissue/20 min. [l] 16.7 μg Pi/g of wet tissue/15 min.

placed on a commercial diet[3] which contained butterfat (400 g), cellulose[4] (60 g), cholesterol (53 g), choline dihydrogen citrate (4 g), salt-mixture oil[5] (40 g), sodium cholate (20 g), sucrose (223 g), vitamin-free casein (200 g), and total vitamin supplement for 10 d. After the cholesterol and triglyceride levels were assayed and observed to be elevated, the mice were administered test drugs at 10 mg/kg/d ip for an additional 12-d period. Serum cholesterol and triglyceride levels were measured after 12 d of drug administration.

Toxicity Studies—Animals weights were obtained periodically during the experiments and expressed as a percentage of the weight of the animal on day 0. After dosing for 14 d with test drugs, selected organs were excised, trimmed of fat, and weighed. The organ weights were expressed as a percentage of the total body weight of the animal. Food consumption was assessed daily[6].

The acute toxicity (LD_{50} value) (5) was determined in male CF_1 mice by administering test drugs intraperitoneally from 5 to 50 mg/kg as a single dose. The number of deaths recorded over 7 d in the group was determined for each dosage.

Enzymatic Studies—*In vitro* enzymatic studies were determined with 10% homogenates of male CF_1 mouse liver with 50–200 μM of test drugs. *In vivo* enzymatic studies were determined with 10% homogenates of liver from male CF_1 mice obtained after administering the agents for 16 d at a dose ranging from 2.5 to 20 mg/kg/d ip. The liver homogenates for both *in vitro* and *in vivo* studies were prepared in 0.25 M sucrose and 0.001 M EDTA.

Acetyl CoA synthetase (6) and ATP-dependent citrate lyase (7) activities were determined spectrophotometrically at 540 nm as the hydroxyamate of acetyl CoA formed after 30 min at 37°C. Mitochondrial citrate exchange was determined by the procedure of Robinson *et al.* (8, 9) with sodium [^{14}C]bicarbonate (41 mCi/mmol) incorporated into mitochondrial [^{14}C]citrate after isolating rat mitochondria (9000×*g* for 10 min) from the homogenates. The exchanges of the [^{14}C]citrate were determined after incubating the mitochondrial fraction, which was loaded with labeled citrate and test drugs, for 10 min. The radioactivity was then measured in the mitochondrial and supernatant fractions in scintillation fluid[7] and expressed as a percentage. Cholesterol side-chain oxidation was determined by the method of Kritchevsky and Tepper (10) using [26-^{14}C]cholesterol (50 mCi/mmol) and mitochondria isolated from rat liver homogenates. After 18 h of incubation at 37°C with test drugs, the generated $^{14}CO_2$ was trapped in the center well in [2-[2-(*p*-1,1,3,3,-tetramethylbutylcresoxy)ethoxy]ethyl]dimethylbenzylammonium hydroxide[8] and counted[7]. HMG CoA reductase activity was measured with [1-^{14}C]acetate (56 mCi/mmol) and a postmitochondrial supernatant (9000×*g* for 20 min) incubated for 60 min at 37°C (11). The digitonide derivative of cholesterol was isolated and counted (12). Acetyl CoA carboxylase activity was measured by the method of Greenspan and Lowenstein (13). Initially, the enzyme had to be polymerized for 30 min at 37°C, and then the assay mixture containing sodium [^{14}C]bicarbonate (41.0 mCi/mmol) was added and incubated for 30 min at 37°C with test drugs. Fatty acid synthetase activity was determined by the method of Brady *et al.* (14) with [2-^{14}C]malonyl CoA (37.5 mCi/mmol), which was incorporated into newly synthesized fatty acids that were extracted with ether and counted[7]. Acyl transferase activity was determined with L-[2-^{3}H]glycerol-3-phosphate (7.1 Ci/mmol) and the microsomal fraction of the liver homogenates (15). The reaction was terminated after 10 min, and the lipids were extracted with chloroform–methanol (1:2) containing 1% concentrated HCl and counted. Phosphatidate phosphohydrolase activity was measured as the inorganic phosphate released after 30 min from phosphatidic acid by the method of Mavis *et al.* (16). The inorganic phosphate released after development with ascorbic acid and ammonium molybdate was determined at 820 nm.

Liver, Small Intestine, and Fecal Lipid Extraction—In male CF_1 mice that were administered test drugs for 16 d, the liver, small intestine, and fecal materials (24-h collection) were removed, and a 10% homogenate in 0.25 M sucrose–0.001 M EDTA was prepared. An aliquot (2 mL) of the homogenate was extracted by the methods of Floch *et al.* (17) and Bligh and Dyer (18), and the number of milligrams of lipid was determined. The lipid was taken up in dichloromethane, and the cholesterol level (3), triglyceride levels[9], neutral lipid content (19), and phospholipid content (20) were determined.

[^{3}H]Cholesterol Distribution in Rats—Male Holtzman rats (350 g) were administered the test agent for 14 d orally. On day 13, 10 μCi of [^{3}H]cholesterol (40.7 mCi/mmol) was administered intraperitoneally in mice and orally in rats, and feces were collected for the next 24 h. Twenty-four hours after cholesterol administration, the major organs were excised, and samples of blood, chyme, and urine were obtained. Homogenates (10%) of the tissues were prepared, combusted[10], and counted[7]. Some tissue samples were plated on filter paper[11], dried, digested for 24 h in base[8] at 40°C, and counted[7]. Results are expressed as dpm/mg of wet tissue and dpm/total organ.

Cholesterol Absorption Study—Male Holtzman rats (~400 g) were administered the test drug orally for 14 d at 10 mg/kg/d. On day 13, 10 μCi of [1,2-^{3}H]cholesterol (40.7 Ci/mmol) was administered to the rats orally. After 24 h, the blood was collected, and the serum was separated by centrifugation (21). Both the serum and the precipitate were counted[7].

Bile Cannulation Study—Male Holtzman rats (~400 g) were treated with

[3] Basal Atherogenic Test Diet; U.S. Biochemical Corp.
[4] Celufil.
[5] Wesson.
[6] Wayne Blox Rodent Chow.
[7] Fisher Scintiverse in a Packard Scintillation Counter.
[8] Hyamine hydroxide; New England Nuclear Corp.
[9] Bio-Dynamics/bmc Triglyceride Kit.
[10] Packard Tissue Oxidizer.
[11] Whatman No. 1.

Table III—*In Vivo* Effects of the Binuclear Copper(II) Complex on CF_1 Mouse Liver Enzyme Activities After 16 d Dosing at 10 mg/kg/d i.p.[a]

Compound	ATP-Dependent Citrate Lyase	Acetyl CoA Synthetase	HMG CoA Reductase
Control (1% carboxylmethylcellulose)	100 ± 7	100 ± 8	100 ± 8
Binuclear Copper(II) Complex			
2.5 mg/kg	70 ± 6[b]	62 ± 6[b]	112 ± 8
5.0 mg/kg	79 ± 7[b]	77 ± 7[b]	108 ± 9
10.0 mg/kg	72 ± 7[b]	73 ± 7[b]	102 ± 8

	Acetyl CoA Carboxylase	Fatty Acid Synthetase	*sn*-Glycerol-3-phosphate Acyl Transferase	Phosphatidate Phosphohydrolase
Control (1% carboxymethylcellulose)	100 ± 6	100 ± 7	100 ± 6	100 ± 7
Binuclear Copper(II) Complex				
2.5 mg/kg	103 ± 6	102 ± 7	95 ± 6	55 ± 5[b]
5.0 mg/kg	95 ± 5	90 ± 6	95 ± 7	53 ± 5[b]
10.0 mg/kg	120 ± 7	94 ± 5	119 ± 7	18 ± 3[b]

[a] Expressed as percent of control (mean ± *SD*); $n = 6$. [b] $p \leq 0.001$.

test drugs at 10 mg/kg/d orally for 14 d. The rats were anesthetized with chlorpromazine[12] (25 mg/kg) followed 30 min later by pentobarbital[13] (22 mg/kg ip). The duodenum was isolated from the small intestine, and ligatures were placed around the pyloric sphincter and distally to a site approximately one-third of the way down the duodenum. Sterile isotonic saline was injected into the sectioned-off duodenal segment. The saline expanded the duodenum and the common bile duct. Once the bile duct was identified, a loose ligature was placed around it, an incision was made, and plastic tubing[14] was introduced into the duct. Once past the ligature, the tubing was tied in place, and the ligatures around the duodenum were removed. Once bile was freely moving down the cannulated tube [1,2-^{3}H]cholesterol (40.7 Ci/mmol) was injected intravenously into the rats. The bile was collected over the next 6 h and measured (in milliliters). Aliquots were counted[7] and analyzed for cholesterol content (4).

Plasma Lioprotein Fractions—Male Holtzman rats (~400 g) were administered test drugs at 20 mg/kg/d for 14 d. On day 14, blood was collected from the abdominal aorta. Serum was separated from whole blood by centrifugation at 3500 rpm. Aliquots (3 mL) were separated by density gradient ultracentrifugation by the methods of Hatch and Lees (22) and Havel *et al.* (23) into the chylomicrons, very low-density lipoproteins, high-density lipoproteins, and low-density lipoproteins. Each of the fractions was analyzed for cholesterol (4), triglycerides[9], neutral lipids (19), phospholipids (20), and protein levels (24).

RESULTS

Data are expressed in the tables as percent of control ± *SD*. The probable significant level (p) between each test group and the control group was determined by the Student's t test.

The binuclear copper(II) complex proved to be a potent hypolipidemic agent after intraperitoneal administration in mice and oral administration in rats (Table I). The reduction of serum cholesterol in normal mice was dose dependent, with 20 mg/kg/d resulting in the maximal effect of 47%. Serum triglyceride levels were reduced by >50% at 2.5 and 10 mg/kg/d. In rats administered 10 mg/kg/d, a similar reduction in serum cholesterol levels was observed as in mice, *i.e.*, 37%. However, in rats administered 10 mg/kg/d, serum triglyceride levels were reduced 64%, which was greater than the 53% reduction in mice at this dose. In hyperlipidemic-induced mice, serum cholesterol levels were elevated 183% (354 mg%) above normal values (125 mg%), which were reduced 59% to 148 mg% by drug administration over 12 d. Hyperlipidemic mice serum triglyceride levels, which were elevated 168% (367 mg/dL) above control values (137 mg/dL), were lowered 67% by drug administration to 122 mg/dL.

Examination of the *in vitro* liver enzyme studies (Table II) demonstrated that enzymes involved in the generation of the key intermediate, acetyl CoA which is required for cholesterol and fatty acid synthesis as well as cholesterol synthesis, were not affected by the presence of the drug. The degradation of cholesterol by oxidation of the side chain was accelerated significantly (52%) in the presence of drug. Fatty acid synthesis enzyme activities and *sn*-glycerol-3-phosphate acyl transferase (one of the regulatory enzymes of triglyceride synthesis) activities were not affected by the agents; however, the other regulatory enzyme of triglyceride synthesis, phosphatidate phosphohydrolase activity, was suppressed with increasing concentration of the drug.

Studies *in vivo* after dosing for 16 d in mice (Table III) showed that there was a moderate suppression of ATP-dependent citrate lyase and acetyl CoA synthetase activity at 2.5 mg/kg/d. Phosphatidate phosphohydrolase activity was reduced in a dose-dependent manner, with 10 mg/kg/d causing 82% reduction.

Lipid concentrations in the rat liver and small intestine were reduced significantly by drug treatment (Table IV). The cholesterol, triglyceride, and neutral lipid levels were reduced in both organs, and phospholipids were reduced in the small intestine. The lipid content in the livers of mice treated with the drug at 2.5–10 mg/kg/d for 16 d demonstrated similar changes (Table V); however, more dramatic effects were observed in the ability of the drug to lower triglyceride levels more than cholesterol levels. Higher lipid levels were observed in fecal samples of rats after administration of drugs for 2 weeks, particularly in the triglyceride, neutral lipid, and phospholipid levels (Table IV).

After separation of the rat serum lipoprotein fractions (Table IV), it became evident that the cholesterol, neutral lipids, and triglyceride content of each fraction was reduced. The phospholipid and protein content of the fractions were not markedly affected by drug treatment.

In rats after 14 d of treatment, the organ weights (Table VI) had not significantly altered, although in general, the treated animals had lower organ weights. In the control rats over the 2-week period, there was a 9.8% increase in body weight, and for the treated rats, there was only a 0.2% increase. The drug therapy did interfere with appetite, reducing (26%) the grams of food per day per rat from 30.35 to 22.39.

The absorption of [^{3}H]cholesterol after oral administration (Table VI) was reduced 85% over a 24-h period in rats treated for 14 d. [^{3}H]cholesterol distribution in major organs after drug therapy generally showed reductions in dpm/organ compared with the control, *e.g.*, [^{3}H]cholesterol was reduced 42% in the liver, 18% in the heart, 46% in the brain, and 91% in the kidney. There were higher contents of cholesterol in the stomach (198%), chyme (113%), large intestine (27%), small intestine (81%), and urine (336%). The LD_{50} in CF_1 mice by the intraperitoneal administration route was 39.2 mg/kg.

DISCUSSION

Tetrakis-μ-(trimethylamine-boranecarboxylato)-*bis*(trimethylamine-carboxyborane)dicopper(II) was found to be a potent hypolipidemic agent in mice and rats. However, its mode of action seemed to be somewhat different from previously reported amine-cyanoborane and amine-carboxyborane analogues in that HMG CoA reductase and fatty acid synthetase activities were essentially unaltered both in the *in vivo* and *in vitro* studies (1). The binuclear copper(II) complex was active in the dosage range previously reported for borane derivatives as hypolipidemic agents (1); nevertheless, it was more potent than commercially available clofibrate, which at doses of 150–200 mg/kg lowers serum cholesterol 6–15% and serum triglyceride levels 25% (25). Not only is the binuclear copper(II) complex active orally and intraperitoneally, it is effective in hyperlipidemic mice, lowering serum cholesterol to near normal levels and serum triglycerides below normal levels after administration for 12 d. The mode of action of this agent appeared to be by interference with cholesterol absorption from the GI tract and accelerated excretion of cholesterol *via* the fecal route. Triglyceride levels appear to be reduced due to suspension of activity of the regulatory enzyme of *de novo* triglyceride synthesis. Lamb *et al.* (15) have shown a positive correlation between lowering of serum triglyceride levels and the inhibition of hepatic and intestinal phosphatidate phosphohydrolase activity with 1-methyl-4-piperidyl-bis(*p*-chlorophenoxy)acetate and 1,3-bis(*p*-methylphenoxy)-2-propane. Similar findings

[12] Thorazine, chlorpromazine hydrochloride; Smith, Kline and French Laboratories.
[13] Nembutal, sodium pentobarbital; Abbott Laboratories.
[14] PE-10 Intramedic polyethylene tubing.

Table IV—Effects of the Binuclear Copper(II) Complex on Rat Liver, Small Intestine, and Serum Lipoprotein Fraction Lipid Content after 14-d Dosing [a]

	Lipid Extracted, mg	Cholesterol	Triglycerides	Neutral Lipids	Phospholipids	Protein
Liver						
Control	100 ± 7	100 ± 7[b]	100 ± 6[c]	100 ± 6[d]	100 ± 8[e]	100 ± 6[f]
Treated	51 ± 5[g]	65 ± 6[g]	76 ± 6[g]	76 ± 7[g]	93 ± 9	99 ± 7
Small intestine						
Control	100 ± 6	100 ± 7[h]	100 ± 5[i]	100 ± 5[j]	100 ± 7[k]	100 ± 7[l]
Treated	43 ± 5[g]	47 ± 6[g]	40 ± 4[g]	17 ± 3[g]	46 ± 5[g]	111 ± 8
Feces						
Control	100 ± 7	100 ± 6[m]	100 ± 6[n]	100 ± 8[o]	100 ± 7[p]	100 ± 5[q]
Treated	123 ± 5[g]	110 ± 6	133 ± 7[g]	131 ± 7[g]	183 ± 8[g]	68 ± 6[g]
Lipoprotein fraction						
Chylomicrons						
Control	—	100 ± 7[r]	100 ± 7[s]	100 ± 8[t]	100 ± 7[u]	100 ± 6[v]
Treated	—	88 ± 6	58 ± 5[g]	67 ± 7[g]	96 ± 6	99 ± 7
Very Low-Density						
Control	—	100 ± 6[w]	100 ± 6[x]	100 ± 7[y]	100 ± 8[z]	100 ± 7[aa]
Treated	—	32 ± 3[g]	43 ± 4[g]	62 ± 5[g]	110 ± 7	96 ± 7
Low-Density						
Control	—	100 ± 6[bb]	100 ± 7[cc]	100 ± 6[dd]	110 ± 8[ee]	100 ± 8[ff]
Treated	—	68 ± 6[g]	70 ± 6[g]	78 ± 7[g]	105 ± 6	101 ± 6
High-Density						
Control	—	100 ± 7[gg]	100 ± 6[hh]	100 ± 7[ii]	100 ± 6[jj]	100 ± 6[kk]
Treated	—	64 ± 5[g]	31 ± 5[g]	72 ± 8[g]	86 ± 7	108 ± 8

[a] At 10 mg/kg/d orally; expressed as percent of control (mean ± *SD*), $n = 6$. [b] 24.03 mg of cholesterol/g of tissue. [c] 44.11 mg of neutral lipid/g of tissue. [d] 6.37 mg of triglyceride/g of tissue. [e] 7.19 mg. [f] 4.5 g of protein/g of wet tissue. [g] $p \leq 0.001$. [h] 7.82 mg/g. [i] 6.98 mg/g. [j] 1.12 mg/g. [k] 2.06 mg/g. [l] 42 mg/g. [m] 28.47 mg/g. [n] 33.94 mg/g. [o] 1.86 mg/g. [p] 1.39 kg/g. [q] 6.99 mg/g. [r] 337 mg/mL. [s] 67 mg/mL. [t] 420 mg/mL. [u] 149 mg/mL. [v] 184 μg/mL. [w] 190 mg/mL. [x] 98 mg/mL. [y] 22 mg/mL. [z] 26 mg/mL. [aa] 50 mg/mL. [bb] 210 mg/mL. [cc] 10 mg/mL. [dd] 45 mg/mL. [ee] 41 mg/mL. [ff] 122 μg/mL. [gg] 544 mg/mL. [hh] 620 mg/mL. [ii] 27 mg/mL. [jj] 153 mg/mL. [kk] 657 μg/mL.

Table V—*In Vivo* Effects of the Binuclear Copper(II) Complex on CF_1 Mouse Liver Lipid Content after 16 d of Dosing [a]

Compound	Lipid, mg	Cholesterol	Triglycerides	Neutral Lipids	Phospholipids	Protein
Control (1% carboxymethylcellulose)	100 ± 6	100 ± 7[b]	100 ± 5[c]	100 ± 6[d]	100 ± 7[e]	100 ± 5[f]
Binuclear Copper(II) Complex						
2.5 mg/kg	82 ± 5[g]	71 ± 6[g]	40 ± 3[g]	79 ± 5[g]	67 ± 6[g]	105 ± 6
5.0 mg/kg	85 ± 6[h]	74 ± 7[g]	38 ± 4[g]	86 ± 5[h]	92 ± 7	92 ± 5
10.0 mg/kg	95 ± 6	77 ● 7[g]	36 ± 5[g]	87 ± 4[h]	152 ± 8[g]	101 ± 8

[a] Expressed as percent of control (mean ± *SD*); $n = 6$. [b] 12.24 mg of cholesterol/g of tissue. [c] 4.77 mg of triglyceride/g of tissue. [d] 28.35 mg of neutral lipid/g of tissue. [e] 4.39 mg of phospholipid/g of tissue. [f] 4.5 mg of protein/g of tissue. [g] $p \leq 0.001$. [h] $p \leq 0.010$.

have also been made with phthalamide (26), saccharin (27), and 1,8-naphthalamide (28), cyclic imide derivatives, with mouse liver. These compounds, as well as clofibrate, also suppressed *sn*-glycerol-3-phosphate acyl transferase activity, whereas the binuclear copper complex(II) did not. Clofibrate accelerates cholesterol excretion *via* the biliary route (29); however, its effects on liver metabolism include inhibition of HMG CoA reductase. Thus, the copper complex appears to be different in its mode of action than standard therapeutic agents on the market today, *e.g.*, clofibrate and the resin cholestyramine.

The serum lipoprotein fractions after 14 d of dosing at 10 mg/kg/d demonstrated consistent reductions in cholesterol, triglyceride, and neutral lipid content. Supposedly, the chylomicron and very low-density fractions contain the highest triglyceride levels, which the binuclear complex(II) reduced significantly. The low- and high-density lipoprotein fractions contain the majority of the cholesterol and its esters under normal conditions. The cholesterol content of the low-density fraction is important in controlling the amount of cholesterol being deposited in atherogenic plaques. Supposedly, the high-density fraction returns the cholesterol from peripheral tissue to the liver. The binuclear copper(II) complex reduces cholesterol approximately equally in both the low- and high-density lipoprotein fractions. Thus, the ratio of cholesterol content of low-density to high-density lipoprotein fractions probably was not altered by drug therapy; however, the absolute content of each fraction was reduced significantly.

Table VI—Effects of the Binuclear Copper(II) Complex on Rat Organ Weights and Orally Administered [^{3}H]Cholesterol after Dosing for 14 d [a]

Organ[a]	Organ Weight, g		dpm/Total Organ	
	Control	Treated	Control	Treated
Brain	1.866	1.666	29,115	15,783
Lung	1.766	1.933	15,350	17,106
Heart	1.333	1.021	24,582	20,060
Liver	12.000	10.600	47,429	27,695
Kidney	2.900	2.167	5,875	565
Spleen	0.566	0.501	19,388	13,241
Adrenal	0.036	0.031	—	—
Stomach	2.066	1.766	11,375	33,901
Small intestine	8.333	6.433	15,291	16,514
Large intestine	4.100	4.233	47,109	59,665
Chyme	5.866	5.9333	102,772	218,742
Feces	5.702	8.633	320,454	294,644

[a] At 10 mg/kg/d; $n = 6$.

Lipids removed from the serum were not deposited in the organs as demonstrated by reduced lipid content in the liver and small intestine, [^{3}H]cholesterol content in the major organs, and the weights of the organs. Adrenal weights were not altered by drug administration, indicating that there was no compensatory hyperplasia of the adrenal cortex due to stimulation of steroidogenesis because of low serum levels. The appetites of the animals were reduced by the drug; however, the magnitude of the effects of binuclear copper(II) complex on lipid metabolism and blood levels is unknown. Thus, tetrakis-μ-(trimethylamine-boranecarboxylato)-*bis*(trimethylamine-carboxyborane)dicopper(II) appears to be a potent hypolipidemic agent at low doses, lowering both cholesterol and triglyceride levels. Further chemical derivatives of this type and mechanisitic studies need to be investigated in the future.

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NOTES

Choline Magnesium Trisalicylate: Comparative Pharmacokinetic Study of Once-Daily and Twice-Daily Dosages

MONTE J. LEVITT ˣ and JULES KANN

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Abstract □ This randomized crossover study compared the pharmacokinetics of choline magnesium trisalicylate tablets administered once daily (3000 mg of salicylate) or twice daily (1500 mg of salicylate) for six d. Serum salicylate levels were measured by HPLC. Mean "trough" concentrations fell within the therapeutic range (5–30 mg/dL) with either regimen and were relatively constant, indicating that the steady state had been reached. The 24-h area under the salicylate curve ($AUC_{0-24\ h}$) after the final 3000-mg salicylate dose averaged about twice the mean 12-h AUC after the last 1500-mg dose, indicating that the two dosing regimens were equally bioavailable. Clinical observations and results of laboratory safety studies indicate that both dosage schedules of the drug are well tolerated. The present findings support the once-daily therapeutic use of choline magnesium trisalicylate.

Keyphrases □ Choline magnesium trisalicylate—pharmacokinetic comparison of once-daily and twice-daily dosages, humans □ Bioavailability—choline magnesium trisalicylate, once-daily and twice-daily dosage schedules, humans □ Pharmacokinetics—choline magnesium trisalicylate bioavailability after once-daily and twice-daily dosages, humans

Complexity of dosage schedule may cause nonadherence to self-administered drug regimens (1, 2), particularly among ambulatory, fully active patients. Conversely, simplicity of treatment, as reflected in a single daily drug dose, is desirable from the standpoint of compliance (2, 3). In terms of this criterion, conventional salicylates, though widely regarded as first-line antiarthritic drugs, are not highly conducive to patient cooperation. These substances, notably aspirin, must often be taken at frequent intervals during the day, primarily to minimize GI irritation; daily quantities high enough to yield sustained therapeutic blood levels may be expected to cause prohibitive side effects when given as only one or two doses. Paradoxically, however, the inadequate compliance such a complex drug regimen might entail could undermine maintenance of therapeutic blood salicylate levels (4).

Published data indicate the feasibility of reducing the frequency of salicylate doses in arthritis. A recently developed nonacetylated drug of this class, choline magnesium trisalicylate (I) has been successfully used on a twice-daily basis in patients with rheumatoid arthritis or osteoarthritis (5–8). It was of interest to determine whether pharmacokinetic data would support even less frequent administration of this drug. We therefore conducted the present crossover bioavailability study in healthy volunteers to compare once-daily and twice-daily treatment schedules of I.

EXPERIMENTAL SECTION

Protocol—Twenty-four healthy males, 19–34 years old (mean, 28 years), whose weights were within 10% of ideal (58.6–96.1 kg; mean, 74.7 kg), took part in the study, which was conducted in accordance with a protocol approved by an institutional review board. The subjects had given written informed consent and were judged to be in good health on the basis of physical findings and the results of blood chemistry determinations, hematological work-up, routine urinalysis, and tests for fecal occult blood. None had a history of sensitivity to salicylates or of serious GI, hepatic, renal, or hematological

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