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—Original Article—

Copper-induced hypercholesterolemia of golden hamsters: Enhanced synthesis of cholesterol in the liver

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Summary: Effects of a subtoxic dose of copper on cholesterol metabolism were studied in male golden hamsters. Intraperitoneal injections of cupric acetate increased serum levels of cholesterol and phospholipids without liver damage. This lipidemia was associated with increased cholesterol of the liver. The participation of hemolysis was denied by peripheral red blood cell tests. Hepatic microsomal 3-hydroxy-3-methyl-glutaryl-CoA reductase was also elevated by copper administration. Biliary secretion of cholesterol increased but that of bile acids remained unchanged, suggesting no impaired degradation of cholesterol. We conclude that hepatic synthesis of cholesterol is enhanced by a subtoxic dose of copper, resulting in hypercholesterolemia. *Gastroenterol Jpn 1988;23:629–632*

Key words: *Cholesterol synthesis, Copper, Hypercholesterolemia*

Introduction

Copper is an important trace element component of some enzymes and proteins, while cholesterol is a major lipid membrane constituent. In spite of their quite different physiological properties, both copper[1,2] and cholesterol[3] largely share the same intrahepatic metabolic pathway, i. e. lysosomal accumulation and biliary excretion. Therefore, their interaction could be more complicated than is yet known[4]. For example, hypercholesterolemia and hypercupremia are characteristic of patients with obstructive jaundice. Because the estimated volume of cholesterol regurgitated in serum of bile-ligated animals is larger than that of cholesterol secreted in bile of non-bile ligated animals, enhanced cholesterol synthesis and secretion into the blood stream have been postulated[5]. Some bile constituent other than bile acids could be an activator of cholesterol synthesis. Using a subtoxic dose of copper, we produced hypercholesterolemia in non-bile duct ligated hamsters, which are sensitive to cholesterol load[6]. The underlying mechanism of altered cholesterol metabolism was investigated in these hamsters.

Materials and Methods

First Experiment

Inbred golden hamsters of GN strain, supplied by the Experimental Animal Center of Fujita Gakuen University, were fed a standard commercial diet: MF (Oriental Yeast Co. Ltd, Tokyo: crude fat content, 5.3%; total cholesterol, 0.15%) until the experiments began. Two doses of 0.5ml of 0.05% cupric acetate were intraperitoneally injected with an interval of 9 hours to the experimental animals, the same

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Table 1 Red blood cell examinations

(mean±SD)

	RBC ($\times 10^4$)	Hb (g/dl)	Ht (%)	Os-F (%NaCl)
control	770±13	16.6±0.4	42.0±1.7	0.55
20h	776±25	16.6±0.3	42.1±0.8	0.55

volume of saline to the control animals. Both experimental and control animals (n=6 for each group) had free access to food and water during the experiment. Twenty hours after the second injection, the animals were sacrificed under light nembutal anesthesia. Their heparinized bloods were prepared for examination of red blood cell counts (RBC), hemoglobin content (Hb), hematocrit (Ht) and osmotic fragility of red blood cells (Os-F). Blood chemistry included determination of triglyceride (TG), cholesterol, phospholipid (PL), alkaline phosphatase (AL-P), glutamic oxaloacetic transaminase (GOT), lactic acid dehydrogenase (LDH) and copper.

Lipids were extracted from the liver homogenates. TG and cholesterol were determined by enzymatic method[7,8],PL by the chemical method[9]. The microsomal fraction was obtained from liver homogenate, its 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase being measured by the method of Young.[10]

Second Experiment

For bile cannulation, another set of animals was prepared as for the first experiment (n=5 for each group). Twenty hours after the second injection, the animals were anesthetized by intramuscular injection of nembutal. Laparotomy was followed by ligation of the common bile duct, and cannulation of nylon tube into the gall bladder. Physiological saline was used to fill the peritoneal cavity and bile was collected for the first two hours. Bile flow was estimated by bile weight. Contents of bile acid, cholesterol and PL were determined as mentioned above.

Results

First Experiment

No changes were found in the red blood cell examinations (**Table 1**). The minimal dose of copper did not induce anemia in the hamsters. Hyperlipidemia, consisting of increased serum levels of cholesterol and PL was induced without liver disfunction except for elevated LDH activity (**Table 2**). Analysis of isozymes showed a relative increase of LDH_5. HMG-CoA reductase of hepatic microsomal fraction increased in response to copper load. The liver content of cholesterol increased after the copper load (**Table 3**), while that of other lipids and protein remained unchanged.

Second Experiment

There was no difference in bile flow between

Table 2 Blood chemistry

(mean±SD)

	TG (mg/dl)	cholesterol (mg/dl)	PL (mg/dl)	GOT (IU/l)	Al-P (IU/l)	LDH (IU/l)	Cu (μg/dl)
control	118±49	204±38	257±36	63±31	13.4±2.2	366±145	0.63±0.09
20h	135±52	356±48*	388±48*	55±12	9.3±1.6	2777±1424*	1.05±0.11*

*: different from control.

Table 3 Analysis of liver homogenate

(mean±SD)

	TG (μg/g liver)	cholesterol (μg/g liver)	PL (mg/g liver)	protein (mg/g liver)	HMG-CoA (pmol/min/mg)
control	942±503	299±167	14.7±4.8	24.6±0.9	1.58±0.7
20h	718± 49	717±239*	14.9±2.1	26.7±2.8	2.78±0.9*

*: different from control.

Table 4 Biliary lipids

(mean±SD)

	bile flow (g/h)		bile acid (mmol/l)		cholesterol(μg/ml)		PL (mg/ml)	
	1st 1h	2nd 1h	1st 1h	2nd 1h	1st 1h	2nd 1h	1st 1h	2nd 1h
control	0.36±0.07	0.40±0.11	5.9±1.5	4.6±1.2	113±60	79±35	1.12±0.28	0.60±0.19
20h	0.37±0.11	0.36±0.18	5.2±0.9	3.3±1.3	222±55*	153±69*	0.86±0.12	0.73±0.35

*: different from control.

the experimental animals and controls. Cholesterol, however, was secreted much more in the copper-loaded animals than the control animals (**Table 4**). Bile acid and PL contents of bile did not change. Gall stones were not observed in any animals studied.

Discussion

Lipoprotein X makes a major contribution to the increased plasma cholesterol of obstructive jaundice[5]. However, cholesterol of biliary origin in rat accounts for only a third of the excess found in plasma 24hrs after biliary obstruction. As an explanation for the hypercholesterolemia, Fredrickson et al.[11] demonstrated that cholesterol synthesis in rat liver strikingly increased following ligation of the common bile duct. This has been confirmed repeatedly, and so retention of some factor in bile has been postulated[5]. Here we report, for the first time, copper-induced hypercholesterolemia in non-bile duct ligated hamsters. The hypercholesterolemia was associated with increased hepatic cholesterol and increased HMG-CoA reductase of hepatic microsomes. Because this enzyme is a limiting enzyme of cholesterol synthesis, excess cholesterol in plasma, liver and bile can be largely explained by enhanced cholesterol synthesis in liver. Hypercholesterolemia, however, varies according to its inducer and the animal species used, so that the experimental condition and minor factors other than copper must also be considered.

Toxic doses of copper primarily affect cell membranes by generating peroxide. Among extrahepatic organs, red blood cells may be the most favorable target of ionized copper[12]. In the present experiments, hemolysis was negligible with the dose used. Both osmotic fragility of red blood cells and serum level of indirect bilirubin were normal. Liver function tests other than LDH were normal. Isozyme analysis of LDH indicated hepatic origin. Thus it is unlikely that hypercholesterolemia is induced by membrane-cholesterol release following hemolysis or impaired hepatic uptake of cholesterol.

Cholesterol and its main bile acid catabolites are secreted into bile. Neither associated hyperbilirubinemia nor elevated biliary enzymes in the serum were observed. Rather, biliary secretion of cholesterol increased significantly compared to control animals. The observed change of cholesterol distribution is clearly not a result of impaired biliary secretion of cholesterol or cholestasis.

The question arises as to whether depressed degradation of cholesterol is important for increased cholesterol in plasma and liver. The main degradation pathway of cholesterol is chemical conversion to bile acid through 7-alpha hydroxylation in liver and then biliary secretion[13]. Bile acids in bile did not change in the present study, in spite of increased cholesterol secretion. This means that cholesterol degradation occures as usual and is not inhibited in our experimental condition.

Zinc, in combination with copper, plays a role in cholesterol metabolism. For example, long-term cholesterol feeding of rats resulted in hypercholesterolemia associated with relative deficiency of copper to zinc, and supplementary copper protected against hypercholesterolemia[4]. In experimental cholestasis, hypercupremia was associated with reduced plasma

zinc level[14]. Neither hemolysis nor biliary obstruction, which are known to change serum zinc level, were involved in our acute experiment. Therefore, zinc seems less significant than copper in enhanced cholesterol synthesis. More important is the fact that acute copper load, in contrast with the chronic experiment[4], produced hypercholesterolemia in non-bile duct ligated animals.

Another aspect was that we used not rats but inbred golden hamsters. Hamsters are known to be sensitive to cholesterol load[6]. In fact, Wistar rats did not show any change in cholesterol contents of plasma and liver with the same dose of cupric acetate (data not presented). The reason why species differ in their reactions to copper is not clear, but one characteristic of inbred hamsters is the fact that their hepatocellular lipids are almost all of lysosomal origin, namely lipolysosomes[6]. These organelles were first reported in cholesteryl ester storage disease[15], then found widely in human livers[16]. In contrast, rats are resistant to cholesterol-induced lipolysosome proliferation[17]. In this sense, hamsters might have better cholesterol matabolism than rats.

We demonstrated that a subtoxic dose of copper induces hypercholesterolemia associated with increased hepatic cholesterol. Based on the elevation of hepatic HMG-CoA reductase, we postulate that hepatic synthesis of cholesterol is enhanced by copper, resulting in increased secretion into circulation and bile.

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