ウィルソン病の動物モデルである LECラット肝細胞ライソゾームでの銅と鉄の蓄積
Compound overloading of copper and iron in hepatocellular lysosomes of LEC rats as a Wilson disease animal model

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Summary

Wilson disease is associated with primary copper toxicosis and LEC rats may be an animal model of Wilson disease with an ATP7B mutation and hypoceruloplasminemia. Recent studies indicate that Wilson disease shows various compound overloading of copper and iron. We tested a hypothesis that the animal model of Wilson disease is also affected by compound overload of copper and iron.

Copper and iron were identified in the hepatocellular dense bodies of LEC rats. Most dense bodies were positive for acid phosphatase, therefore excess amounts of copper and iron were stored in hepatocellular lysosomes.

LEC rats are a good animal model for studying compound overload of copper and iron in Wilson disease.

Keywords: LEC rat, Wilson disease, copper, iron, lysosome

INTRODUCTION

The liver is a central organ for homeostasis of trace elements of iron and copper. Excess amounts of iron and copper are otherwise hepatotoxic and are stored in hepatocellular lysosomes as hemosiderin and cuprothionein, respectively[(1)].

Wilson disease (WD) is associated with primary copper toxicosis. The hepatic copper transporter ATP7B is defective and biliary excretion of copper was completely blocked in this disease[(2)(3)]. Excess copper is first stored in the liver, and then in extrahepatic organs including the central nervous system. In the liver, copper is stored in hepatocellular lysosomes as cuprothioneins. Recent studies indicate that lysosomes are also loaded with iron, and may be replaced by iron during long-term copper cheletion because of more severe hypoceruloplasminema[(4)]. Both iron and copper are transition elements and primary sources in radial generation. As a result, multi-organ damage may be inevitable when excess amounts of iron and copper are accumulated in the liver and other organs.

Long-Evans cinnamon (LEC) rats are an animal model of Wilson disease with an ATP7B mutation and hypoceruloplasminemia[(5)]. There is evidence to suggest that transition metals of both iron and copper may be involved in fulminant hepatitis of LEC rats[(6)(7)]. Because these transition elements are finally stored in hepatocellular lysosomes, ultrastructural element analysis on LEC rats, an animal model of WD, may provide information on the pathogenesis of progressive liver damage in patients with WD.

MATERIALS and METHODS

To identify lysosomes under electron microscopy, portions of LEC rat liver were fixed in 4% paraformaldehyde at 4℃, and 0.1 M cacodylate buffer, pH 7.2, for 60 minutes. After rinsing in 0.1 M cacodylate

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.buffer at 4℃, pH 7.2, with 0.25 M sucrose, specimens were sliced in agar gel. Sections were incubated in 0.05 M acetate buffer, pH 5.0, with lead nitrate, sucrose, and 3% β-glycerophosphate, pH 5.0-5.2, at 37℃ for 10 minutes[8]. After a short rinsing period in distilled water, liver pieces were finally embedded in epoxy resin, TAAB812. Ultra-thin sections were mounted on gold grids. Unstained sections were examined under a TEM (JEOL)[1]. Sections were also analyzed by another microscope equipped with an EDS (JEM-2800).

RESULTS

Under electron microscopy, hepatocellular organelles including the nucleus, mitochondria, and dense bodies were visualized in ultrathin sections. After the acid phosphatase reaction, hepatocellular dense bodies became positive for lead precipitation (Figure 1). As a result, co-deposits of intrinsic elements of copper and iron-, and extrinsic lead were identified in some hepatocyte dense bodies (Figure 2). There was no significant lead precipitation outside of the dense bodies.

DISCUSSION

WD is associated with primary copper toxicosis, but recent studies indicate the co-existence of copper and iron in patients with WD[4]. Similar observations with WD patients were obtained in the current study using the LEC rat animal model of WD. Hepatocyte dense bodies rich in iron and copper were positive for acid phosphatase, suggesting that copper and iron are simultaneously accumulated in hepatocyte lysosomes-, as cuprothionein and hemosiderin, respectively.

The etiology of iron overload in patients with WD may be complex[9], but the main reason for iron deposits in LEC rats may be due to hypoceruloplasminemia. It is likely that long-term copper chelation for WD patients induce further iron accumulation in the liver via more severe hypoceruloplasminemia. A response to the secondary iron overload, if needed, is not discontinuation of copper chelation, but phlebotomy.

Compound overloading of copper and iron were confirmed in hepatocyte lysosomes in LEC rats, which could be a good model for studying the pathogenesis of progressive liver disease in WD[1][7].

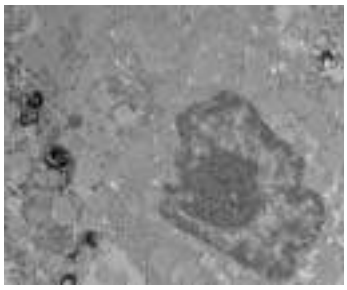

Figure 1. An electron micrograph of a liver specimen with the acid phosphatase reaction. Dense bodies are scattered throughout the cytoplasm of hepatocytes. As shown in the next figure, these dense bodies are positive for lead, indicating lysosomal origin. Furthermore, some of these dense bodies are positive for copper and iron. Therefore, copper and iron are stored in hepatocellular lysosomes of LEC rats.

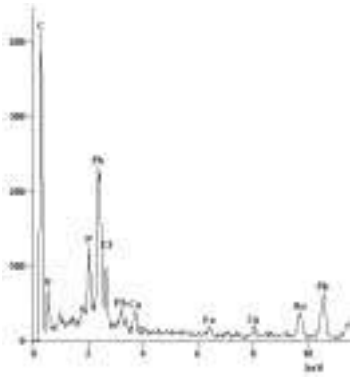

Figure 2. An X-ray spectrum of a dense body in a liver specimen exposed to an electron beam.
Element analysis showed peaks of carbon (C), oxygen (O), phosphorus (P), chlorine (Cl), calcium (Ca), iron (Fe), copper (Cu), lead (Pb), and gold (Au). Lead precipitation is a reaction product of acid phosphatase, and copper and iron are intrinsic elements deposited in the dense bodies of lysosomal origin.

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