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Specific copper accumulation in liver of Formosan squirrel (Callosciurus erythraeus)

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Introduction

Copper is one of the essential elements and liver is the central organ of Cu homeostasis, regulating both storage and excretion. Wilson disease (WD) is well known as disorder of Cu homeostasis. WD patient and its animal models, LEC rat, accumulate Cu in their livers because of the decrease of Cu excretion to bile and defective supply of Cu to ceruloplasmin (Cp; Cu excretion root to bloodstream) due to hereditary mutation of ATP7B gene. Bedlington terrier accumulates excess hepatic copper because of mutation of COMMD1 gene, which is involved in Cu excretion to bile with cooperating with ATP7B.

We have discovered that Formosan squirrel (*Callosciurus erythraeus*), living in Japan and Taiwan, accumulated Cu in their liver at 420 μg/wet g on an average, and reported that Cu accumulation phenomenon in this animal wasn't due to environmental pollution.[1,2)] In this study, we focused on followed two points: The presence/absence of hepatotoxity caused by Cu accumulation and the distribution and the chemical form of Cu in the liver of Formosan squirrel with using HPLC-ICP MS.

Experimental

Thirty seven wild-living Formosan squirrels were trapped alive in Kamakura, Kanagawa, Japan under permission from the Kamakura City Hall. After blood and bile were collected, liver tissues were removed, and then samples were stored at -80 ℃ until chemical analysis. About 0.1 g of liver tissues was preserved in 10 % natural buffered formalin before washed for pathological test. The activities of ALT, AST, and Cp in the serum were determined with UV absorbance methods. About 2.0 g of liver samples were homogenized, then supernatant samples were prepared by ultracentrifuging at 105,000×g for 60 min at 4 ℃ . A portion of each fraction, serum and bile were wet-digested, then digested solution was diluted with Milli-Q water to 10mL. Concentrations of Cu, Zn and Cd were determined by the ICP-MS (HP-7500, Agilent, Japan). The distributions of Cu and other metals in the liver supernatants were determined on gel filtration HPLC column (Develosil 100Diol-5, 8.0×300 mm with a 8.0×35 mm guard column; Nomura Chemical, Tokyo) by eluted with 100 mM ammonium acetate, pH 6.5 (25 ℃) at the flow rate of 1.0 mL/min, with in-line detection with an ICP MS.

Results and discussion

Hepatic concentrations of Cu ranged from 6.3 to 1740 μg/wet g. From the result of HE stain, cellular infiltrations were shown in 14/27 liver of specimens in all. However, these were reversible degeneration and any gross anatomical changes, such as jaundice, hypertrophy and so on, were not shown. Moreover, cellular infiltrations didn't became severe according to hepatic Cu accumulation. Again, serum ALT and AST activity did not correlate with hepatic Cu concentration. These findings suggested that this species had any Cu detoxication mechanism.

Normally, excess Cu was detoxicated by metallothionein (MT), which is a family of low molecular weight, mainly cytoplasm-located, heavy metal-binding proteins. In the case of LEC rat and BT, most of accumulated Cu was bound to MT. Therefore, we focused on Cu bound to MT in this species. In the specimens, whose hepatic Cu concentration is higher than 100 μg/wet g, 60 % of Cu distributed in the insoluble fraction. From HPLC-ICP MS analysis, the Cu bound to MT increased according to hepatic Cu accumulation until about 500 μg/wet g of Cu were accumulated in the liver. Then amount of Cu bound to other protein, which is soluble and heavier than MT, increased.

From analysis of serum Cu distribution and Cp activities, serum Cu were bound to Cp despite low activity of Cp. Moreover, it was examined that Cu was excretion to bile in this species. These results suggested that Cu accumulation mechanism of Formosan squirrel was different from those of LEC rat and BT. Excess Cu in the liver of Formosan squirrel mainly bound to other than cytosolic MT.

References

1. Suzuki *et al.*, *Chemosphere*, **64**, 1296-1310 (2006).
2. Suzuki *et al.*, *Chemosphere*, **68**, 1270-1279 (2007).

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