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Abnormal Copper Accumulation in Non-cancerous and Cancerous Liver Tissues of LEC Rats Developing Hereditary Hepatitis and Spontaneous Hepatoma

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We studied the copper concentrations in the non-cancerous and cancerous liver tissues of LEC rats with hereditary hepatitis and spontaneous hepatoma by atomic absorption spectrophotometry. Copper concentration in the non-cancerous livers of 29-month-old male LEC rats was comparable to that in the livers of LEC rats aged 2, 3 and 8 months whose hepatic copper concentrations were more than 40 times those of normal LEA rats. Copper concentration in spontaneously developed hepatocellular carcinomas of the 29-month-old male LEC rats was lower than that in the surrounding non-cancerous liver tissues, but was still more than 39 times that of 8-month-old male LEA rats. These findings suggest that in LEC rats an abnormal copper metabolism may be maintained during the process of hepatic carcinogenesis.

Key words: LEC rats — Copper — Hereditary hepatitis — Hepatocellular carcinoma

LEC rats (Long-Evans rats with a cinnamon-like coat color), an inbred strain that has been separated from Long-Evans rats, spontaneously develop acute hepatitis about 4 months after birth. The clinical signs of the hepatitis resemble those of human fulminant hepatitis.[1,2] Genetic analysis has revealed that a single autosomal recessive gene is responsible for the hepatitis.[3] We have recently found that copper accumulates densely in the liver of LEC rats, while their serum levels of copper and ceruloplasmin are reduced[4]; this suggests that the hepatitis is closely associated with copper toxicity and that we may be dealing with a rat form of Wilson's disease in humans.

Since those LEC rats which have survived for more than one year frequently develop hepatocellular carcinoma (HCC),[5] they provide us with an opportunity to study the role of copper in carcinogenesis. In the present investigations we have studied the copper profiles in non-cancerous and cancerous liver tissues of LEC rats, and in this report we discuss their relationship to hepatic carcinogenesis.

LEC rats were established from the Long-Evans rats at the Center for Experimental Plants and Animals of Hokkaido University. Details of the origin and breeding history of LEC rats have been described previously.[1,2] This experiment was approved by the Animal Care and Use Committee of Hokkaido University School of Medicine.

We examined copper concentrations in the livers of 3 to 5 LEC rats aged 2 days and 1, 2, 3, and 8 months, both male and female, and 3 male LEC rats aged 29 months. Age- and sex-matched LEA rats (Long-Evans rats with an agouti coat color), which are a sibling line of LEC rats and develop neither hepatitis nor hepatoma, were used as controls. The animals were killed under ether anesthesia and the livers were quickly removed. All 3 male LEC rats aged 29 months had spontaneously developed multiple liver tumors. In this study we investigated in each rat the copper concentration in two of the tumors and their surrounding non-cancerous liver tissues. The tumor masses were excised from the liver, and a portion of each tumor was cut out for histologic examination. The samples were wet-ashed with 20 ml of 61% nitric acid, 10 ml of 60% perchloric acid and 3 ml of 97% sulfuric acid, and the copper concentrations were determined with an atomic absorption spectrophotometer (Model 180-30, Hitachi, Tokyo). The samples of liver tumors for histologic examination were fixed with 10% phosphate-buffered formalin. Paraffin sections were made and stained with hematoxylin and eosin.

The copper concentrations in the liver of LEC rats aged 2, 3 and 8 months were more than 40 times higher than those of age- and sex-matched LEA rats. Although copper had already accumulated densely in the liver of LEC rats of both sexes by the age of 2 days (male, 148.0 $\pm$6.8; female, 151.1 $\pm$ 10.4 μg/g wet weight), the concen-

Table I. Copper Concentration in the Liver of LEC and LEA Rats

Age	Sex	Copper concentration (μg/g wet weight)	
		LEC	LEA
2 days	Male	148.0±6.8[a]	49.5±1.0
	Female	151.1±10.4[a]	48.1±2.3
1 month	Male	75.7±7.5[a, d]	3.1±0.1
	Female	93.3±14.2[b, e]	3.0±0.1
2 months	Male	170.2±8.4[a, f]	3.2±0.2
	Female	188.7±7.8[a, f]	3.3±0.3
3 months	Male	253.6±72.0[c]	3.5±0.1
	Female	282.9±51.7[c]	2.9±0.1
8 months	Male	213.4±3.3[a]	3.4±0.1
	Female	152.7±27.4[c]	3.3±0.2
29 months	Male	210.1±34.7	nd

Data are means±SE with 3 to 5 rats.
a) $P<0.001$, *b*) $P<0.005$, *c*) $P<0.05$ vs. age- and sex-matched LEA rats; *d*) $P<0.001$, *e*) $P<0.05$ vs. sex-matched 2-day-old LEC rats; *f*) $P<0.001$ vs. sex-matched 1-month-old LEC rats by unpaired Student's *t* test. nd, not determined.

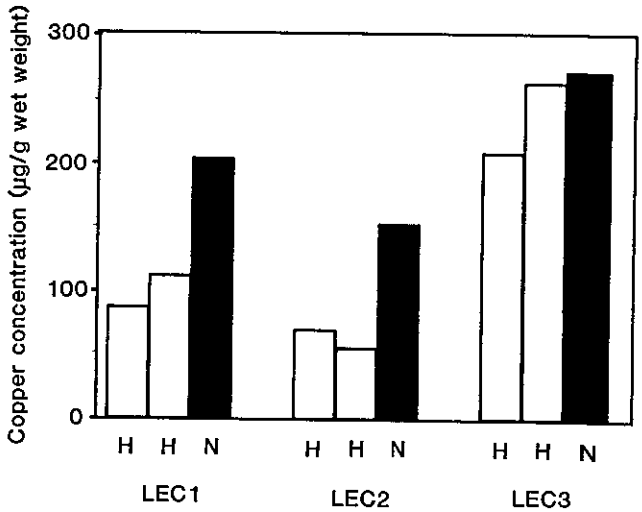

Fig. 1. Copper concentration in spontaneously developed hepatocellular carcinoma (H) and surrounding non-cancerous liver (N) of three 29-month-old male LEC rats, LEC1 to LEC3.

trations decreased by the age of 1 month (male, 75.7± 7.5; female, 93.3±14.2 μg/g wet weight), but rose again at the age of 2 months (male, 170.2±8.4; female, 188.7± 7.8 μg/g wet weight). The decrease of the copper level between the ages of 2 days and 1 month is comparable to that observed in age-matched LEA rats (Table I); this could be the consequence of a switch from a fetal to an adult mode of copper metabolism. Although there were no statistically significant age-related changes in copper concentrations in the liver of LEC rats after the age of 2 months, the concentration values of 3-month-old rats were about 1.5 times higher than those of 2-month-old rats (Table I). Since there is no cell necrosis in the liver of LEC rats before the onset of hepatitis, it seems likely that the hepatic copper in the 3-month-old rats may continue to accumulate until it reaches a toxic level at the age of 4 months, which then leads to the development of hepatitis.

Light microscopic examination confirmed that all the spontaneous liver tumors in LEC rats were HCCs. The copper concentrations in the HCCs of LEC1 and LEC2 were between 37% and 55% of those in the surrounding non-cancerous liver tissues, while the copper concentration in the HCC of LEC3 was much the same as that in non-cancerous liver tissues (Fig. 1). The mean value of copper concentrations in the HCCs (133.6±34.3 μg/g wet weight) was significantly lower than that in the non-cancerous liver tissues (210.1 ± 34.7 μg/g wet weight; $P<0.005$ by paired Student's *t* test), but was still more than 39 times higher than that of male LEA rats aged 8 months.

Our own histochemical studies have revealed that, in LEC rats, copper accumulates markedly in hepatocytes (unpublished results). Since copper also accumulated densely in the HCCs derived from LEC rats, it is likely that the abnormal copper metabolism in the hepatocyte was maintained during the process of carcinogenesis. We did not observe any necrosis in the tissues of the HCCs, either macroscopically or microscopically, which suggests that the copper in these HCCs may exist in a non-toxic form. The copper concentrations in the HCCs were lower than those in non-cancerous livers. We speculate that the proliferation of tumor cells may quantitatively dilute the copper in the tumor tissues, although we must still take into account any qualitative changes in the copper metabolism in the tumors.

The occurrence of HCC is rare in patients with Wilson's disease.[6, 7] It has been proposed that, as well as offering protection against chemical-induced hepatoma in copper-loaded rats,[8, 9] in Wilson's disease too, copper acts as a protective agent against carcinogenesis.[6, 7] Nevertheless, recent studies have indicated that copper can efficiently induce DNA damage *in vitro* in the presence of hydrogen peroxide and reducing agents.[10, 11] The high incidence of HCC and the abnormal copper accumulation in both non-cancerous and cancerous liver tissues in LEC rats suggest that abnormal copper metabolism may

be involved in the development of the HCCs. Takahashi *et al.* have recently reported that the liver of LEC rats aged 2 months is highly susceptible to the initiating effect of diethylnitrosamine and possibly to other hepatic carcinogens.[12] We therefore speculate that this phenomenon may be associated with the abnormal copper accumulation, although further study will be necessary to elucidate whether the accumulated copper itself initiates hepatic carcinogenesis.

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