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# Hereditary Low Level of Plasma Ceruloplasmin in LEC Rats Associated with Spontaneous Development of Hepatitis and Liver Cancer

Takao Ono, Syuiti Abe and Michihiro C. Yoshida[1]

*Chromosome Research Unit, Faculty of Science, Hokkaido University, Kita 10 Jo, Nishi 8 Chome, Kita-ku, Sapporo 060*

**Both young (5 weeks old) and old (61–100 weeks old) hereditary hepatitis LEC rats showed a markedly low level of plasma ceruloplasmin (Cp) ferroxidase activity as compared with that of age-matched LEA and BN strain rats. This trait was genetically examined by the use of (BN × LEC) $F_1$ hybrid and ($F_1$ × LEC) backcross rats. The $F_1$ hybrids never developed hepatitis and showed a similar level of Cp to that found in the parental BN rats. Among the backcross rats with about 1:1 segregation rate for hepatitis, affected rats had a remarkably decreased level of Cp, as found in LEC rats, whereas unaffected rats exhibited a similar level of Cp to that of BN, $F_1$ and LEA rats. These results indicate that the low level of Cp is heritable in a single autosomal recessive mode in LEC rats. The observed tight link between the low Cp level and the hepatitis in LEC rats suggests that defective copper metabolism may be associated with the occurrence of hepatitis in LEC rats, since Cp is a copper-binding protein primarily involved in copper transport from the liver.**

Key words: LEC rats — Hereditary hepatitis — Ceruloplasmin

We have previously reported a hereditary hepatitis LEC rat strain that develops acute hepatitis with severe jaundice about 4 months after birth.[1,2] Approximately 30–40% of the rats died of submassive necrosis in the liver within a week after the onset of jaundice.[2,3] The remainder, after recovery from jaundice, developed chronic hepatitis and finally hepatocellular carcinoma.[4] Genetic analysis revealed that a single autosomal recessive gene, *hts*, is responsible for the hepatitis in LEC rats,[5] but the mechanism of the disease remains obscure. The clinical features of hepatic lesions in LEC rats were similar to those found in human fulminant hepatitis.[3] Thus, the LEC rats provide a valuable animal model for studying hepatitis and liver cancer.

Recently, abnormal accumulation of copper in the liver and kidney and marked decrease in the levels of plasma or serum ceruloplasmin (Cp) diamine oxidase activity were observed in LEC rats (N. Takeichi, personal communication). Cp is a copper-binding protein primarily involved in the transport of copper from the liver.[6] Cp is synthesized in the liver, and binds six to eight atoms of copper per molecule.[7,8] Therefore, it is probable that the LEC rats may have impaired copper metabolism. In the present study, we examined the level of Cp in LEC and other strain rats and in their hybrids, to ascertain whether the low level of Cp is heritable in LEC rats, in association with the occurrence of hepatitis.

Three inbred strains of rats, LEC, LEA and BN, maintained at the Center for Experimental Plants and Animals of Hokkaido University, were used. Both LEC and LEA strain rats were isolated from a closed colony of Long-Evans rats, but LEA rats do not develop hepatitis.[2] Five-month-old male LEC rats that had recovered from jaundice were mated with age-matched female BN rats to produce $F_1$ hybrids. Backcross rats were obtained from matings with female $F_1$ hybrids and parental male LEC rats. Cp in blood plasma collected from these animals at different ages was measured as ferroxidase activity[7,9] by using a Determiner Cp kit (Kyowa Medics, Tokyo), according to the manufacturer's instructions. A parallel histological study on paraffin sections of liver tissues with hematoxylin-eosin and dimethylaminobenzylidine rhodanine staining was made for confirmation of hepatic lesions and copper accumulation.

The levels of plasma Cp ferroxidase activity in LEC, LEA and BN strain rats, (BN × LEC) $F_1$ and ($F_1$ × LEC) backcross hybrids are summarized in Table I. The level of Cp was markedly lower in LEC rats than in BN or LEA rats. Although the Cp ferroxidase activity tended to increase with age, no statistically significant difference was observed between young and older rats except for BN strain. The levels of Cp in older BN and LEA rats were similar, whereas the level of Cp in older LEC rats with hepatic lesions remained low, nearly one-fifth of that in the two other strains (23 mU/ml versus 5 mU/ml). These results indicate that Cp ferroxidase activity is low in both young and older LEC rats, irrespective of the occurrence of hepatic lesions.

[1] To whom reprint requests should be addressed.

Table I. Level of Plasma Ceruloplasmin in Three Strain, $F_1$ and Backcross Rats

| Animal | No. and sex of subjects ♀ | ♂ | Age (weeks) | Ceruloplasmin ferroxidase activity (mU/ml) mean±SE |
|---|---|---|---|---|
| LEC-young | 4 | 3 | 5 | 2.58±0.66[b] |
| LEC-old[a] | 1 | 5 | 61–100 | 5.04±1.59[b] |
| BN-young | 3 | 4 | 7 | 15.75±1.19 |
| BN-old | 4 | — | 56 | 23.33±0.78[c] |
| LEA-young | 3 | 3 | 7 | 19.15±1.20 |
| LEA-old | 1 | 2 | 33 | 23.23±2.57 |
| $F_1$ (BN×LEC) | 5 | 3 | 54–63 | 20.79±1.34 |
| BC ($F_1$×LEC) | | | | |
| with hepatitis | 9 | 8 | 40–53 | 6.24±0.61[b] |
| without hepatitis | 11 | 5 | 40–53 | 22.60±1.06 |

*a*) All rats developed hepatic lesions including chronic hepatitis or hepatoma and cholangiofibrosis.
*b*) Statistically significant as compared with each of young or old BN and LEA, and $F_1$ rats ($P<0.005$ in all cases, *t*-test).
*c*) Statistically significant as compared with young BN rats ($P<0.005$).

As shown in Table I, the $F_1$ hybrids at 53–63 weeks of age showed a similar level of Cp to that of aged BN and LEA rats and never developed any hepatic lesions. The Cp data were obtained from eight of 19 $F_1$ hybrids derived from four litters of two different mating pairs. On the other hand, 19 of 35 backcross rats from three litters of two different mating pairs developed fulminant hepatitis at 17 to 22 weeks after birth, and two died of the disease. The 17 other affected rats survived for 40–53 weeks and developed chronic hepatitis as found in parental LEC rats[4]; this was confirmed by histological examination. But none of them had hepatocellular carcinoma at the time of examination. The remaining 16 unaffected backcross rats never developed hepatic lesions. The rate of segregation of hepatitis in the backcross rats appeared to be approximately 50%, indicating a single autosomal recessive mode of inheritance as previously described.[5] The backcross rats without hepatitis showed a similar level of Cp to that of old BN, LEA and $F_1$ hybrid rats, whereas the backcross rats with hepatitis exhibited a significantly decreased Cp level as compared with each group of young or old BN, LEA or $F_1$ hybrid rats ($P<0.005$ in all cases, see Table I). These results indicate that the low level of Cp is heritable and tightly linked with the occurrence of hepatitis.

The histological examination with rhodanine staining in the liver of young and older LEC and of backcross rats with hepatitis revealed a frequent localization of copper in Kupffer cells but not in hepatocytes (Fig. 1). On the other hand, LEA, BN, $F_1$ and backcross rats without hepatitis showed no sign of copper accumulation in the liver. Thus, the observed abnormal copper accumulation in the liver seems to be associated with the hereditary hepatitis and the low level of Cp ferroxidase activity.

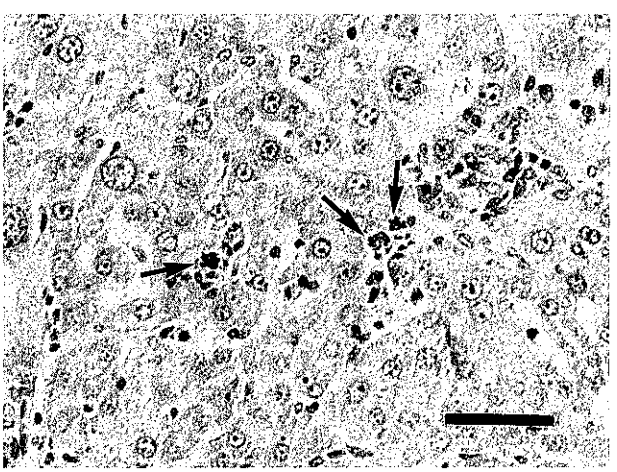

Fig. 1. Rhodanine-stained liver section from a male backcross rat that shows chronic hepatitis with enlarged hepatocytes having large nuclei. Accumulated copper is observed as coarse granules (arrows) in Kupffer cells but not in hepatocytes. The bar indicates 50 $\mu$m.

The level of Cp is low in newborns of all mammalian species so far examined, but increases with age,[10-12] as seen in LEA and BN rats examined here. It has been

reported that Cp gene is transcribed to a normal adult level of mRNA in newborn Wistar strain rats, suggesting a post-translational regulation in the case of neonatal low Cp production.[11] If this is the case, the observed low Cp levels in LEC rats may be accounted for by a genetically altered translational process rather than a defective Cp gene. Another possibility, however, can not be excluded, i.e., that an unknown intermediate(s), which is normally involved in the incorporation of copper into apo-Cp, may be genetically defective in LEC rats, particularly since a possible metabolic link has recently been suggested among apo-Cp, Cu (I)-thionein and activated leucocytes in porcine blood plasma.[13] These possibilities should be examined by further studies.

The present study clearly demonstrated that the low level of Cp ferroxidase activity is heritable in a single autosomal recessive mode, in tight association with the occurrence of hepatitis in LEC rats. Although it is unknown whether the decreased Cp level is the primary defect in the hereditary hepatitis of LEC rats, their abnormal copper metabolism may play a role in the development of hepatitis. The present findings also suggest that the low Cp level may become a useful marker for detecting individuals with hereditary hepatitis among $F_2$ and backcross hybrids between LEC and other strains of rats.

In addition, markedly low levels of Cp are found in patients with Wilson's disease, one of the human inherited disorders of the liver and the nervous system, in which damage is caused by chronic copper accumulation in the target organs.[14–18] Although the primary defect of Wilson's disease is still unknown, these pathogenetic features are very similar to those of the LEC rats, thus providing a useful model for Wilson's disease.

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