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Increase of UDP-glucuronosyltransferase activities toward xenobiotics during the development of hereditary hepatitis in LEC rats

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Abstract—UDP-glucuronosyltransferase activities were induced spontaneously during the development of hepatitis in LEC (Long Evans Cinnamon-like coat color) rats. Transition of hepatic microsomal UDP-glucuronosyltransferase activities was observed during the development of the LEC rat, which displayed spontaneous fulminant hepatitis with severe jaundice at about 12–16 weeks after birth. UDP-glucuronosyltransferase activities toward various substrates in 8-week-old LEC and LEA (Long Evans Agouti coat color; control) rats were similar. After 8 weeks of age, the transferase activities of LEA rats towards all substrates tested, except for bilirubin, decreased slightly during the next 24 weeks. In LEC rats, the transferase activities towards serotonin and several phenolic xenobiotics, such as 4-nitrophenol, 1-naphthol and 4-methylumbelliferone, but not 4-hydroxybiphenyl, increased about 2-fold at 16 weeks of age. During the 24 weeks following the first 8 weeks of age, the high level activities towards the xenobiotics continued, with the exception of bilirubin transferase activity which decreased gradually. These results suggest that a form of UDP-glucuronosyltransferase, which catalyzes the glucuronidations of serotonin and these xenobiotics except for 4-hydroxybiphenyl, is induced during the development of hepatitis in the LEC rat.

Key words: UDP-glucuronosyltransferase; LEC rat; hepatitis

LEC* rats have been reported to develop acute hepatitis with severe jaundice about 16 weeks after birth [1], and 30–40% of the rats die of submissive necrosis in the liver within a week after the onset of jaundice [2]. Accumulation of copper in the liver and a decrease in the levels of ceruloplasmin ferroxidase activity were suggested to be associated with the occurrence of hepatitis in LEC rats [3]. Furthermore, the surviving rats spontaneously developed liver cancer [2]. Sugiyama *et al.* [4, 5] reported that the activity of drug-metabolizing enzymes and the induction of UDP-glucuronosyltransferase activity before the onset of liver cancer in LEC rats were similar to those observed in hyperplastic nodules induced by chemical carcinogens. In this paper, we report the selective increase of UDP-glucuronosyltransferase activities toward xenobiotics during the development of hereditary hepatitis in LEC rats.

Materials and Methods

LEC and LEA male rats were bred and supplied by the Center for Experimental Plants and Animals of Hokkaido University. The rats were maintained under conventional conditions with temperature and light controls. Cholic acid was purchased from Wako Pure Chemical Industries, Osaka, Japan, and was purified further and converted to sodium salt as described previously [6].

Preparations and microsomes. Animals were killed by exsanguination under ether anesthesia, and the livers were removed. After being perfused with 0.15 M KCl solution, the livers were frozen immediately in liquid nitrogen and stored at −80° until used for the following experiments. The livers were minced and homogenated with 4 vol. of 0.15 M KCl solution. The homogenate was centrifuged for 15 min at 9000 *g*. The supernatant fraction was further centrifuged at 105,000 *g* for 60 min to obtain microsomes.

Analytical procedure. UDP-glucuronosyltransferase activities towards various substrates were assayed at the following concentrations of aglycone by the methods described in the respective references: 0.5 mM 1-naphthol [7]; 0.5 mM 4-nitrophenol [8]; 0.5 mM 4-hydroxybiphenyl [9]; 1.0 mM serotonin (5-hydroxytryptamine) [10]; and 0.1 mM bilirubin [11] in microsomes fully activated by sodium cholate [12]. Protein was determined by the method of Lowry *et al.* [13], using bovine serum albumin as the standard.

Results and Discussion

UDP-glucuronosyltransferase activities towards various substrates in liver microsomes of LEC and LEA (control) rats at 8, 16, 24 and 32 weeks of age are shown in Fig. 1. During a period of 24 weeks, the transferase activity of LEA rats toward bilirubin did not alter, but that of LEC rats decreased gradually (Fig. 1A). In LEA rats, all transferase activities, with the exception of bilirubin transferase, declined slightly (Fig. 1). The transferase activity toward 4-hydroxybiphenyl in LEA and LEC rats decreased gradually (Fig. 1B). UDP-glucuronosyltransferase activities towards serotonin and several phenolic xenobiotics, such as 4-nitrophenol, 4-methylumbelliferone and 1-naphthol, in LEC rat liver microsomes increased about 2-fold over the control after 16 weeks of age (Fig. 1, C–F). A form of UDP-glucuronosyltransferase that could glucuronidate serotonin and these phenolic xenobiotics has been purified and named "GT-1" [6] (corresponds to phenol GT). We have obtained preliminary immunoblotting data that a single band (54 kDa) corresponding to GT-1 was increased in liver microsomes of LEC rats.

It was reported that drastic histological changes and an increase in GOT and GPT in serum occurred at about 13–14 weeks of age in LEC rats, called the "early acute hepatitis" stage [14]. In the present study, the activity of GOT in the serum of LEC rats also increased about 10-fold at 16 weeks of age, and then decreased (data not shown) as previously described [14]. UDP-glucuronosyltransferase activities towards xenobiotics were found to increase in LEC rats after this stage. The increase in the activities toward these substrates was suggested to be an increase in GT-1, which glucuronidated these xenobiotics [6] as previously reported in liver nodules of 2-acetylaminofluorene-treated rats [15, 16]. We have shown that

* Abbreviations: LEC, Long Evans Cinnamon-like coat color; LEA, Long Evans Agouti coat color; GOT, glutamic-oxaloacetic transaminase; and GPT, glutamic-pyruvic transaminase.

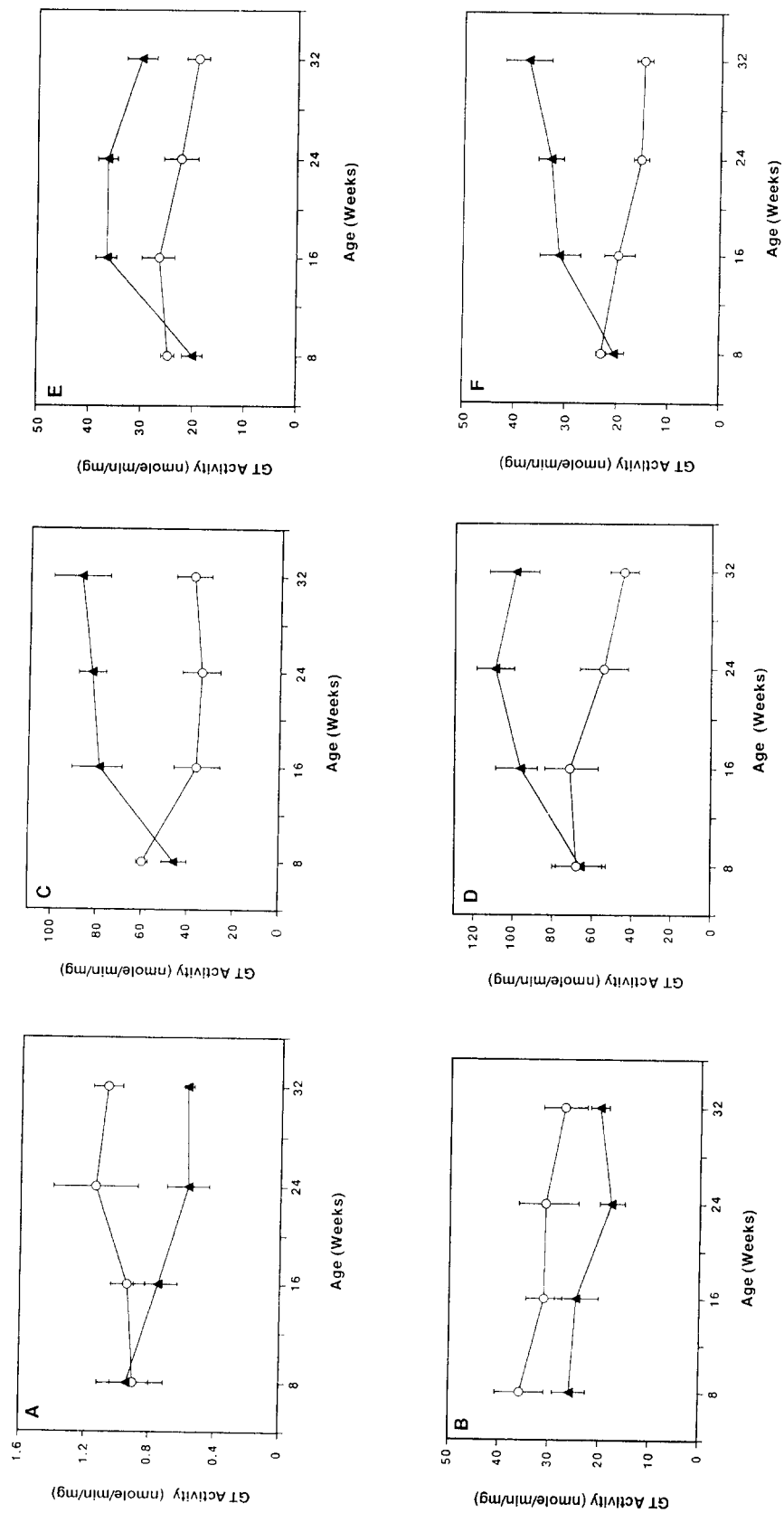

Fig. 1. Transition of hepatic microsomal UDP-glucuronosyltransferase activity during the development of the LEC rat. UDP-glucuronosyltransferase activities towards bilirubin (A), 4-hydroxybiphenyl (B), 4-nitrophenol (C), 4-methylumbelliferone (D), 1-naphthol (E), and serotonin (F) in LEA (○) and LEC (▲) rats were determined after the microsomes were fully activated by the addition of sodium cholate (final concentration; 0.02%) as described in Materials and Methods. Values are the means ± SEM of three rats.

GT-1 protein was increased by treatment of rats with 3-methylcholanthrene, and that the increase accounted for the increase in the level of transcription and translation of mRNA encoding GT-1 [17]. The increase of GT-1 activity in the liver of LEC rats may also result from an increase in the transcription and translation of DNA encoding GT-1, such as that of placental glutathione *S*-transferase in hereditary hepatitis of LEC rats [18]. Alterations in drug-metabolizing enzymes, such as a decrease of cytochrome P450 content [4, 5] and UDP-glucuronosyltransferase activity towards bilirubin (Fig. 1A) and an increase of GT-1 activity (Fig. 1, C–F) and glutathione *S*-transferase activity [18], appeared during the development of hepatitis, before the onset of liver nodules in LEC rats. The alteration was quite similar to that observed in the liver nodules of 2-acetylaminofluorene-treated rats [15, 16]. Recently, Ritter *et al.* [19] isolated a human gene complex, *UGT1*, which encodes at least six different GT mRNAs containing bilirubin GT and phenol GT (corresponds to our GT-1 in the rat) with each having independent regulation. It is very interesting that the regulation of the gene complex of UDP-glucuronosyltransferase and of other enzyme genes changes during the development of hepatitis. Alteration of these drug-metabolizing enzymes during the shift from liver hepatitis to liver nodules and cancer suggests genetic programming in LEC rats, and may be considered a signal for expression of the nodules and precarcinogenesis.

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