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Increased plasma α (1 → 3)-L-fucosyltransferase activities in patients with hepatocellular carcinoma

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Alpha(1 → 3)-L-fucosyltransferase (α1,3FT) activity was determined in plasma of patients with chronic liver diseases, namely, chronic hepatitis (CH), liver cirrhosis (LC) and hepatocellular carcinoma (HCC). The plasma α1,3FT activity was significantly higher ($p < 0.01$) in chronic liver diseases than that in normal controls. The enzyme activity in plasma of patients with HCC was also significantly higher than that in LC ($p < 0.05$) or that in CH ($p < 0.01$). However, no significant difference was observed in the enzyme activity between LC and CH. Plasma α1,3FT activity in patients with HCC was not significantly changed before and after transcatheter arterial embolization. In addition, the enzyme activity in the homogenate of the cirrhotic liver tissue was higher than that in the preparation of the hepatoma tissue in the same patient. These results suggest that the increased plasma α1,3FT activity in patients with HCC reflects mainly the enzyme activity of cirrhotic liver tissue, not that of hepatoma tissue. The significance of the elevated levels of plasma α1,3FT and its decreased hepatoma tissue activity in patients with HCC, compared with that in LC, remains to be clarified.

Keywords: α(1 → 3)-L-fucosyltransferase, liver cirrhosis, hepatocellular carcinoma

Introduction

Structural changes in the carbohydrate moiety of glycoprotein have previously been studied in relation to neoplastic transformation and changes have been reported for many glycoproteins, for example, alpha-fetoprotein [1], γ-glutamyltransferase [2], transferrin [3], and cholinesterase [4]. However, in some cases, such as cholinesterase [5], α-1-acid glycoprotein [6] and C1-inhibitor and hemopexin [7], the glycoproteins have already undergone aberrant glycosylation in liver cirrhosis (LC), a disease which is thought to be a precancerous state. Altered glycosylation is thought to be due to the changes in the activities of specific glycosyltransferases. *N*-Acetylglucosaminyltransferase III [8] is reported to increase in serum and tissue in patients with LC and hepatocellular carcinoma (HCC). The activity of galactosyltransferase [9], galactosyltransferase isoenzyme II [10] and α(1 → 3)-L-fucosyltransferase (α1,3FT) [11, 12] is reported to increase in various cancers. Yazawa *et al.* [13] reported that serum α1,3FT activity was elevated in HCC as well as in other cancers. It is well known that most cases of HCC develop from LC in Japan [14]. However, it is not clear whether α1,3FT activity is elevated in the plasma of patients with chronic hepatitis (CH) or LC as well as in HCC. In the present study, we investigated the α1,3FT activity in the plasma of patients with chronic liver diseases. We also measured α1,3FT activity in hepatoma tissue and its adjacent cirrhotic liver.

Materials and methods

Plasma samples

Plasma samples were obtained from 21 cases of HCC with LC, 19 cases of LC, 28 cases of CH and 58 normal controls (NC) and these specimens were stored at −80 °C until used. Plasma samples of four patients with HCC

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were obtained before and after treatment with transcatheter arterial embolization (TAE). The diagnosis of chronic liver disease was made on the basis of liver function tests, ultrasonography, computed tomography, celiac angiography and histological examination of liver specimens obtained at liver biopsy. All patients with CH, LC or HCC were positive for HBs antigen or HCV antibody. Patients with alcoholic and autoimmune liver diseases were excluded from the present study.

Tissue samples

Three hepatoma samples were obtained at operation or at autopsy and stored at −80 °C until used. Two specimens of normal liver tissue were also obtained at autopsy. Tissue was also removed from the adjacent cirrhotic liver. Enzyme activities were measured in the respective homogenates of hepatoma, its surrounding cirrhotic liver tissue and normal liver. Each specimen was sonicated in four volumes of phosphate-buffered saline followed by the homogenization with a Polytron Ultrax homogenizer. The homogenate was filtered through gauze and the supernatant was used for the measurement of the enzyme activity.

Assay of α(1 → 3)-L-fucosyltransferase activity

α1,3FT activity was determined as described previously [15]. The principle of the assay was a sandwich-type immuno-radiometric procedure. H type 2 trisaccharide (Fucα1 → 2Galβ1 → 4GlcNAcβ) covalently attached to bovine serum albumin (BSA) was used as an acceptor and incubated with plasma samples in the presence of guanosine diphosphate-fucose. The resulting product, Y tetrasaccharide (Fucα1 → 2Galβ1 → 4[Fucα1 → 3]GlcNAcβ-BSA), was detected by a sequential use of anti-BSA antibody-coated bead and ^{125}I-labelled anti-Y antibody. One unit was equivalent to the enzyme activity that could synthesize 75 fmol of Y hapten per hour per assay. The working range for this assay was 20–320 U ml^{-1}.

Protein determination

Protein was determined by the method of Lowry *et al.* [16].

Determination of alpha-fetoprotein (AFP), a protein induced by vitamin K deficiency or the antagonist-II (PIVKA-II), and other laboratory parameters

The serum AFP and PIVKA-II concentrations were determined with a commercially available radioimmunoassay kit, Alpha-fetoprotein RIA Bead, (normal value; < 25 ng ml^{-1}) (Dainabot, Tokyo), and an enzyme immunoassay kit, Eitest Mono-P II, (normal value; < 0.1 U ml^{-1}) (Eisai Co. Ltd, Tokyo), respectively. Other parameters were measured in the central laboratory of our hospital.

Statistical analysis

Comparisons between multiple groups were performed by ANOVA with Scheffe's test. The Wilcoxon test was used for the comparison of clinical data between cirrhotic and hepatoma patients. A level of $p < 0.05$ was accepted as statistically significant.

Results

Plasma α1,3FT activity in patients with chronic liver diseases

The plasma α1,3FT activity (mean ± SD, U ml^{-1}) in patients with HCC, LC, CH and NC was 143.4 ± 55.2, 96.4 ± 57.0, 55.8 ± 32.7 and 25.2 ± 8.5, respectively. The plasma α1,3FT activity was significantly ($p < 0.01$) higher in chronic liver disease than that in NC. The enzyme activity in HCC was also significantly higher than that of LC ($p < 0.05$) or CH ($p < 0.01$). However, no significant difference was observed in enzyme activity between LC and CH. When the mean enzyme activity + 3SD of the NC group was used as a cut-off value, 90.5% (19/21) of the HCC group, 63.2% (12/19) of the LC group and 28.6% (8/28) of the CH group had enzyme activity above this value (Fig. 1).

Tissue α1,3FT activity

As shown in Table 1, α1,3FT activity both in the homogenates of hepatoma and cirrhotic liver was higher than that in normal liver. On the other hand, the enzyme activity in the cirrhotic liver was elevated compared with that in hepatoma tissue in the same patient.

Correlation between α1,3FT activity and AFP or PIVKA-II

There was no significant correlation between α1,3FT activity and the level of AFP or PIVKA-II in plasma or sera from hepatoma patients (data not shown).

Ages and biochemical data for cirrhotic and hepatoma patients

As all our hepatoma patients had LC, we determined whether or not there were significant differences in age or in various biochemical parameters between LC and HCC. The mean age of patients with LC was not significantly different from that in patients with HCC. All laboratory measurements, except for γ-glutamyltranspeptidase (γGTP) were similar in both groups. γGTP activity in patients with HCC was significantly higher ($p = 0.02$) than that in patients with LC (Table 2). However, there was no correlation between γGTP and α1,3FT activity (data not shown).

Effect of TAE on α1,3FT activity

The effect of TAE on plasma α1,3FT activity was examined in four patients with HCC. As shown in Fig. 2,

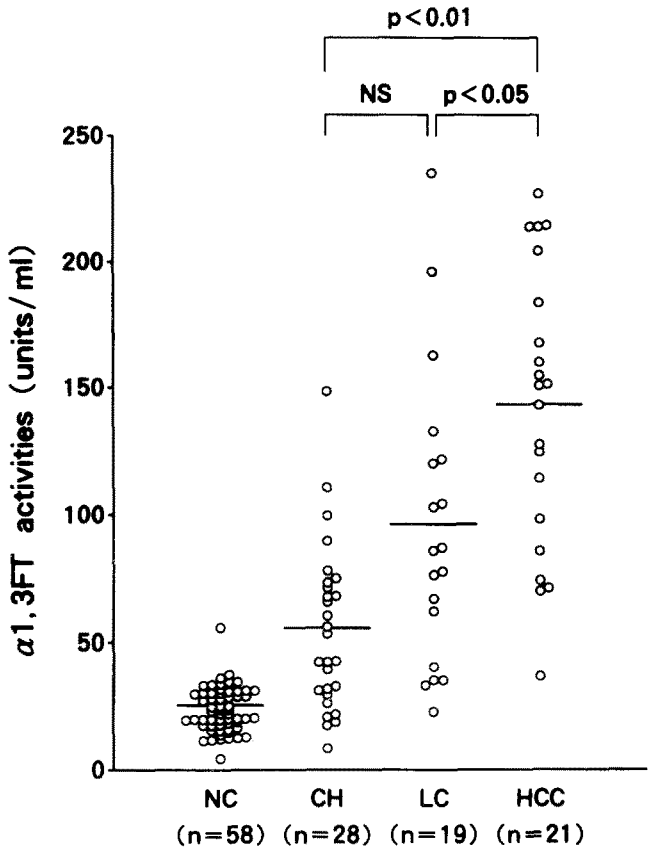

Figure 1. α1,3FT activity in plasma of patients with chronic hepatitis, liver cirrhosis, hepatocellular carcinoma and normal controls. α1,3FT, α(1 → 3)-L-fucosyltransferase; NC, normal controls; CH, chronic hepatitis; LC, liver cirrhosis; HCC, hepatocellular carcinoma.

Table 1. α(1 → 3)-L-Fucosyltransferase activities in the homogenates of hepatoma tissues and normal livers.

	Cancerous tissue (U per mg protein)	*Adjacent non-cancerous tissue (U per mg protein)*
Case 1	10.6	52.4
Case 2	2.7	22.2
Case 3	35.7	61.4
Normal liver 1		2.0
Normal liver 2		5.4

Table 2. Ages and biochemical data for cirrhotic and hepatoma patients.

	Liver cirrhosis (n = 19)	*Hepatocellular carcinoma (n = 21)*	
Age (year)	60.0 ± 12.9	66.6 ± 6.7	NS
Alb. (g dl^{-1})	3.5 ± 0.7	3.2 ± 0.5	NS
γ-Glob. (g dl^{-1})	2.0 ± 0.5	2.3 ± 0.5	NS
GOT (KU)	76.6 ± 28.7	96.8 ± 64.5	NS
GPT (KU)	63.8 ± 30.0	62.6 ± 30.0	NS
γGPT (U l^{-1})	56.2 ± 51.6	101.4 ± 100.0	$p = 0.02$
T. Bil. (mg dl^{-1})	1.0 ± 0.5	1.5 ± 1.3	NS
Alp (BLU)	3.7 ± 1.6	4.5 ± 2.7	NS

The values indicate the mean ± SD. Normal ranges: Alb. (albumin), 3.5–4.8; γ-glob. (-globulin), 0.6–1.4; GOT (glutamic oxaloacetic transaminase), 8–40; GPT (glutamic pyruvic transaminase), 5–35; γGTP (γ-glutamyl transpeptidase), 0–90; T. Bil. (total bilirubin), 0.2–1.2; Alp (alkaline phosphatase), 0.8–2.9. NS, not significant.

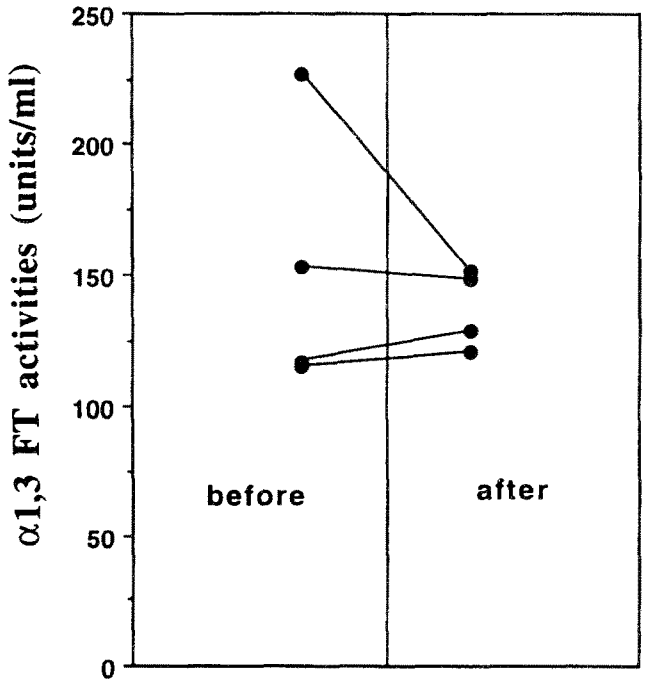

Figure 2. Effect of TAE on α1,3FT activity in four patients with HCC. TAE, transcatheter arterial embolization; α1,3FT, as in Fig. 1.

the level of plasma α1,3FT activity decreased significantly after TAE in only one case. On the other hand, two of the patients, whose plasma α1,3FT activity did not decrease after TAE treatment, had normal values for serum AFP and PIVKA-II. However, the effectiveness of TAE treatment was confirmed by computed tomography. In the third patient with no decrease in plasma α1,3FT activity serum AFP decreased from 912 to 406 ng ml^{-1}; the PIVKA-II level also decreased in this case from 0.44 U ml^{-1} to 0.12. Serum AFP and PIVKA-II of the patient with decreased plasma α1,3 activity decreased from 300 to 117 ng ml^{-1} and from 7.56 to 1.63 U ml^{-1}, respectively.

Discussion

α1,3FT activity has been measured using the serum of patients with various diseases and reported to increase in malignant diseases [11–13, 15]. The results presented

here indicated that plasma α1,3FT activity was significantly ($p < 0.01$) elevated in chronic liver diseases compared to that in NC. Among chronic liver diseases, the enzyme activity in HCC was significantly higher than that in LC or CH. However, no significant decrease in enzyme activity was observed after treatment of 3/4 of the hepatoma patients with TAE (Fig. 2). This may suggest that most of the enzyme was not produced in cancerous liver tissue.

Hutchinson *et al.* [17] reported that the activities of α-2/α-3 and α-6 L-fucosyltransferases were all significantly greater in plasma from patients with HCC than in plasma from cirrhotic patients or normal subjects ($p < 0.025$). In contrast, they also reported that activities of α-2/α-3 fucosyltransferases were significantly lower ($p < 0.025$) in homogenates prepared from tumorous liver tissue than in those prepared from nontumorous tissue from hepatocellular carcinoma and cirrhotic patients. For the α-6 enzyme in homogenates this situation was reversed. Their conclusion was that plasma fucosyltransferases are specifically elevated in HCC, but different mechanisms appear to underlie the changes seen for α-2/α-3 and α-6-L-fucosyltransferases. While they did not measure α-2 and α-3 enzyme activities separately, we measured α1,3FT activity in homogenates of both hepatoma tissue and the cirrhotic liver surrounding HCC. The results indicated that α1,3FT activity was higher in cirrhotic liver than in the cancerous tissue of the same patient. The explanation for the increased plasma and the decreased hepatoma α1,3FT activity in HCC needs to be clarified.

The hepatoma patients in this study also had cirrhosis. Therefore, we compared other biochemical measurements in the LC and HCC groups. Among the variables examined, γGTP activity was significantly higher in HCC than that in LC. It is well known that γGTP activity increases markedly with the appearance of preneoplastic foci and hyperplastic nodules during chemical carcinogenesis, and that the increased enzyme activity is retained in hepatoma [18]. It is generally accepted that γGTP activity is very low in normal adult liver [18]. Sawabu *et al.* [19] reported that γGTP activity of human serum was markedly elevated in HCC and that intensive staining for γGTP activity was observed in most HCC cells. These results strongly suggest that increased activity of serum γGTP reflects the enzyme activity in preneoplastic and HCC tissues. In addition, sugar chains of γGTP purified from human HCC were reported to differ from those from the normal liver [18, 20]. Our present data demonstrates that α1,3FT activity increases in cirrhotic liver tissue, compared to that in hepatoma tissue in the same patient. Therefore, the biological significance of elevated α1,3FT activity in hepatocarcinogenesis seems to differ from that of γGTP.

Recently, we reported [21] that *Aleuria aurantia* lectin (AAL)-reactive, but not *Lens culinaris* agglutin (LCA)-reactive, cholinesterase (ChE) was significantly increased in the serum of patients with LC and HCC. AAL interacts with various fucosyl residues, including Fucα1 $\rightarrow$ 2Galβ1 $\rightarrow$ 4GlcNAc, Galβ1 $\rightarrow$4(Fucα1 $\rightarrow$ 3)GlcNAc, Galβ1 $\rightarrow$ 3(Fucα1 $\rightarrow$ 4)GlcNAc, and Fucα1 $\rightarrow$ 6GlcNAc residues [22]. Meanwhile, LCA specifically interacts with 2 $\rightarrow$ 6Manα1 $\rightarrow$ 6($\rightarrow$ 2Manα1 $\rightarrow$ 3)Manβ1 $\rightarrow$ 4GlcNAcβ1 $\rightarrow$ 4(Fucα1 $\rightarrow$ 6)GlcNAc $\rightarrow$ Asn [23]. From the specific binding properties of AAL and LCA to oligosaccharides and structural analysis of sugar chains of serum ChE [21], $AAL^{+}LCA^{-}$ChE may have oligosaccharides containing a Lewis X antigenic determinant, namely Galβ1 $\rightarrow$ 4(Fucα1 $\rightarrow$ 3)GlcNAc residue. This may mean that α1-3 fucosyl residue is highly expressed in the sugar chains of serum ChE in patients with LC and HCC. The present results along with our previous data [21] suggest that increased plasma α 1,3FT activity in patients with HCC mainly reflects the enzyme level in cirrhotic liver tissue and not that in hepatoma tissue. Further investigations are needed to clarify the biological and clinical significance of the elevation of α1,3FT in chronic liver diseases, especially in HCC.

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