Biochemical mechanisms of increased AFP production by injured livers

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SUMMARY

The undifferentiated pattern of key glycolytic and gluconeogenic enzymes and the increased production of AFP in injured livers appeared to be caused by closely related but different mechanisms. The importance of the gene activation due to liver cell injury rather than the increased number of AFP producing cells following liver cell regeneration was discussed as a possible mechanism for the enhanced production of AFP in injured livers.

Our previous studies of key glycolytic and gluconeogenic enzymes in biopsied human livers from hepatitis and cirrhosis patients as well as in experimentally injured rat livers revealed increased activities of glucose 6-phosphate dehydrogenase (G6PDH), hexokinase (HK) and pyruvate kinase Type M_2 ($PK-M_2$) together with decreased activities of glucokinase (GK) and pyruvate kinase Type L (PK-L) in these injured livers[1~3]. A close similarity of the altered enzyme patterns to those of hepatocellular carcinoma and other undifferentiated liver cells implicitly predicts the appearance of α-fetoprotein (AFP) in serum of patients with non-malignant liver diseases, such as hepatitis and cirrhosis of the liver. In fact, the recent development of radioimmunoassay of AFP has demonstrated significantly increased levels of serum AFP in some of these patients as well as in rats treated with non-carcinogenic hepatotoxins[4~6].

In our recent studies on three interconvertible molecular forms of G6PDH with human livers, the appearance of abnormal G6PDH Band I in hepatitis and cirrhosis patients was found to be associated with increased levels of serum AFP[7].

In the present report, the levels of serum AFP and liver enzyme activities are compared under various clinical and experimental conditions in order to find out whether or not the undifferentiated alteration of the liver enzyme pattern is associated with the appearance of serum AFP in liver injuries.

MATERIALS AND METHODS

Activities of the enzymes listed in Table 1 were determined as described previously[1,3] on 20–30 mg portions of liver tissues obtained by percutaneous needle biopsy under peritoneoscopy from 91 patients with convalescent acute hepatitis, chronic hepatitis (active and inactive forms, with sublobular hepatic necrosis) and cirrhosis of the liver. Serum and liver AFP concentrations were estimated simultaneously by radioimmunoassay[4,5].

Experimental liver injuries were produced by a single oral administration of 0.5 ml of 20% CCl_4 in liquid paraffin/100 g body weight of overnight fasted, 35-day male Sprague-Dawley rats. Serum AFP concentrations of rats were determined by a double antibody technique[8] with an ^{131}I-labeled AFP purified from rat fetal serum[9]. The assay method for rat liver G6PDH was reported previously[2].

RESULTS AND DISCUSSION

Comparison of liver G6PDH activities with serum AFP levels in non-malignant liver diseases (see Materials and Methods) gave a positive correlation with a correlation coefficient of 0.44 (Fig. 1). The correlation coefficient between serum and liver supernatant AFP concentrations was 0.49. High levels of serum AFP were found in cases with increased G6PDH activities, whereas the cases with increased G6PDH activities were not necessarily associated with high AFP levels. Therefore, some additional factors other than those causing increased liver G6PDH activities seem to be present for the increased production of AFP.

Correlation coefficients among serum AFP levels and other liver enzyme activities are listed in Table 1. Serum AFP concentrations in these cases were less than 150 ng/ml. The correlation coefficients found between serum AFP levels and liver enzyme activities did not exceed 0.44, which was found for G6PDH *vs.* AFP, and were apparently less than those found among the enzymes, of which the alteration of activity by liver injury was shown to share a common mechanism[2]; e.g., a value of 0.77 was obtained between G6PDH and HK and 0.60 between G6PDH and PK–M_2. These results could be interpreted as indicating that the undifferentiated enzyme pattern and the increased production of AFP are caused by closely related but different mechanisms.

Comparisons of serum AFP levels and G6PDH and fructose 1,6-diphosphatase (FDPase) activities determined on hepatoma tissues obtained on operation or postmortem examination (within 6 hrs after death) from patients with primary hepatocellular carcinoma gave similar results. Even among

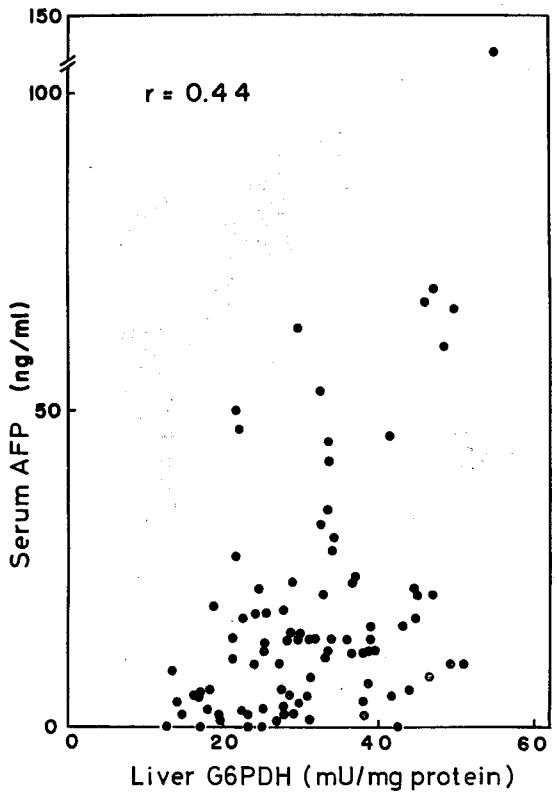

Fig. 1. Correlation between liver G6PDH activity and serum AFP concentration in non-malignant liver diseases.

the hepatoma tissues, the activities of G6PDH increased and FDPase decresaed independent of serum AFP levels (1.3×10–8.8×10^5 ng/ml), although the extent of enzyme deviation and AFP increase were generally much greater in hepatoma than in other non-malignant liver diseases. Accordingly, the possibility still remains that the mechanisms causing the undifferentiated enzyme pattern and AFP production as may be found in hepatoma cells could also exist in injured liver cells.

In experimental liver injuries of rats, the apparent difference existing between increased G6PDH and AFP formations were demonstrated by comparing the time courses of the increases in liver G6PDH and serum AFP levels following CCl_4 treatment (Fig. 2). The activity of liver G6PDH increased without a lag phase, while the concentration of serum AFP increased after a lag period of more than 24hrs. Furthermore, the maximum increase in G6PDH activity was found to be 4.7-fold and that of AFP concentration was 39-fold. These differences could be explained by

Table 1. *Correlations between liver enzyme activities and serum AFP concentration in non-malignant liver diseases*

	AFP	G6PDH	HK	GK	PK-M_2	PK-L	PFK	G6Pase	FDPase
AFP	—								
G6PDH	0.44* (91)	—							
HK	0.31* (88)	0.77* (86)	—						
GK	−0.30* (84)	−0.60* (83)	−0.56* (85)	—					
PK-M_2	0.21 (61)	0.60* (61)	0.35* (57)	−0.41* (54)	—				
PK-L	−0.37* (61)	−0.52* (61)	−0.53* (57)	0.61* (54)	−0.35* (61)	—			
PFK	−0.07 (34)	0.15 (34)	−0.05 (34)	0.17 (34)	0.27 (30)	0.18 (30)	—		
G6Pase	−0.21 (67)	0.05 (66)	0.03 (67)	0.22 (66)	0.06 (39)	0.34± (39)	−0.04 (32)	—	
FDPase	−0.11 (77)	−0.15 (76)	−0.17 (77)	0.15 (75)	−0.04 (48)	0.06 (48)	0.04 (33)	0.19 (65)	—

Correlation coefficients (r) are listed. ( ) No. of cases.
* $P<0.01$; ± $P<0.05$

assuming that the increased AFP production in the injured liver is closely associated with the liver cell regeneration[6] in contrast to the increase in G6PDH activity. The increased activities of liver G6PDH following CCl_4 treatment could be demonstrated without liver cell division and have been shown to represent *de novo* synthesis of G6PDH protein, which is sensitive to cyclohexamide but not to Actinomycin D, indicating that the increased synthesis of G6PDH by liver injury is caused by a disordered regulation at the level of translation and that this is not the consequence of liver cell regeneration[2,10].

In an attempt to find out whether the increased AFP levels in the liver injury are merely due to the increased numbers of potential AFP-producing cells as a result of liver cell regeneration or some gene activation taking place in some connection with liver cell division, the rates of liver DNA synthesis after CCl_4 treatment and partial hepatectomy were estimated in a comparative experiment[2]. The maximum incorporation of ^{14}C-orotate into DNA was 2-fold in CCl_4-injured liver and no more than 3-fold even in regenerating liver. This does not account for the 39-fold increase in AFP level in CCl_4-treated rats only by assuming that the increased number

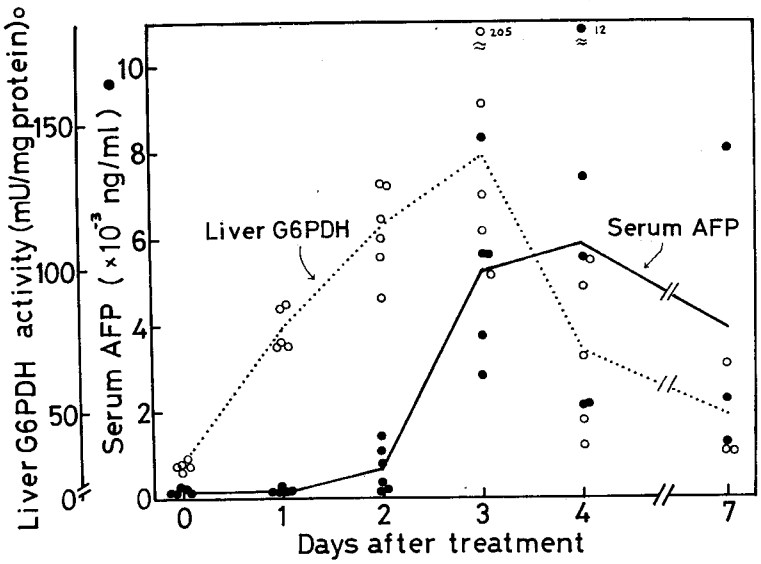

Fig. 2. Time courses of liver G6PDH activity and serum AFP concentration in rats following CCl_4 treatment.

of liver cell after regeneration is responsible for the increased AFP production. Furthermore, the synthesis of DNA started to increase after 24 hrs in CCl_4-treated rat liver, whereas a more than 2-fold increase in incorporation was found already at 24 hrs after partial hepatectomy.

In spite of such a large difference in the phase of DNA synthesis between CCl_4 treatment and partial hepatectomy, the AFP levels at 48 hrs after the treatments increased nearly to the same extent, suggesting that some additional mechanisms of gene activation leading to increased AFP production per cell appear to play a major role in increased production of AFP in CCl_4-injured liver. On the other hand, prior administrations of aminoacetonitril, which inhibits polyribosome breakdown, blocked the increase in AFP level as well as G6PDH activity after CCl_4 treatment. It is, therefore, most likely that the increased AFP synthesis in CCl_4-treated rat liver is due to a gene activation, or enhancement of the gene activity, not only at the level of transcription but also at the level of translation.

Whether the gene activation is caused by the liver injury *per se* or associated with liver cell division could not be assessed at this writing.

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