HISTOCHEMICAL STUDIES OF HEPATIC HEXOKINASE AND GLUCOSE 6-PHOSPHATASE IN CHRONIC LIVER DISEASES

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Two rate limiting enzymes of glycolysis and gluconeogenesis, *i.e.*, low-Km hexokinase (HK) and glucose 6-phosphatase (G6Pase), respectively, in liver tissues of patients with liver diseases, including acute hepatitis (convalescent stage), chronic hepatitis (active and inactive forms) and chronic hepatitis with sublobular hepatic necrosis and cirrhosis of the liver, were demonstrated histochemically. Generally, an intense staining of HK and a weak staining of G6Pase were observed in the degenerated hepatic cells in the lobule. In chronic hepatitis, active form, the HK activity was increased in the whole lobule with an emphasis in the central area, whereas the G6Pase activity was decreased in the central area. Chronic hepatitis with sublobular hepatic necrosis, which has been assumed to progress rapidly into the cirrhosis of the liver, was characterized by an uneven distribution of HK and G6Pase within the hepatic lobule; the activity of the former being increased and the latter decreased at the site of hepatic cell injuries. In cirrhosis of the liver, the HK activity was increased and G6Pase activity was retained well in the well defined nodule, whereas the latter was significantly decreased in the nodule of swollen and irregular hepatic cells with marked infiltration of round cells in the connective tissue.

Our previous studies of chronic hepatitis and its progression into cirrhosis of the liver by means of hepatic enzyme analysis have suggested that the activities of liver specific enzymes such as G6Pase decrease and the activities of liver non-specific enzymes such as HK increase in liver tissues of these patients (8, 9, 10). In an attempt to demonstrate histochemically these enzyme alterations in liver tissues, we have established methods to stain fructose 1,6-diphosphatase and HK (1, 2, 3). In the present studies, the localization and distribution of the altered HK and G6Pase activities in the hepatic tissues in acute hepatitis, chronic hepatitis and cirrhosis of the liver have been described. Characteristic staining patterns of chronic hepatitis with sublobular hepatic necrosis, which has been considered to progress easily into cirrhosis of the liver, have been also discussed (6).

MATERIALS AND METHODS

Liver tissues were obtained by needle biopsy under direct vision on peritoneo-

scopic examination from patients with viral hepatitis and cirrhosis of the liver. The samples used for the control study were a part of the liver tissue obtained by a wedge biopsy at the operation of cholecystectomy for cholelithiasis and a mucosal stomach cancer. The liver tissues obtained at the peritoneoscopic examination were all examined histologically for establishment of diagnosis. The number of patients studied were 4 cases for acute hepatitis, convalescent stage, 28 cases of chronic hepatitis including 5 cases of inactive form, 5 cases of active form and 19 cases of chronic hepatitis with sublobular hepatic necrosis of the liver and 14 cases of postnecrotic cirrhosis of the liver. Serum glutamic oxaloacetic transaminase and glutamic pyruvic transaminase activities, K_{ICG} and gamma globulin values, which were determined within one month prior to the operation, were used. HBs antigen was tested by the method of immunoelectrophoresis. Liver enzyme activities of HK and G6Pase were also obtained in some of these cases (12, 4).

Liver samples obtained at peritoneoscopic examination were processed and stained for HK as described in our previous paper (3). Control stainings for HK also were performed in each case (3). The staining of G6Pase was carried out as described by Wachstein and Meisel (13). Control staining were done as described by Kanamura (5). There was no staining when G6Pase was inactivated by incubating the section for 15 min at 37°C in 0.1 M acetate buffer, pH 5.0. An addition of 0.01 M Na molybdate, pH 7.0, a specific inhibitor for G6Pase, or an omission of substrate in the incubation mixture gave no deposition of lead sulfide as an activity of G6Pase (7).

RESULTS

A. Histochemical demonstration of HK and G6Pase activities in control cases (Table 1, Nos. 1 to 3)

HK (Fig. 1-a): The activity of HK was demonstrated as a dark blue deposit of formazan relatively homogeneously distributed throughout the lobule.

G6Pase (Fig. 1-b): The activity of G6Pase was demonstrated as a heavy deposit of lead sulfide and was distributed relatively homogeneous throughout the lobule of the liver, although there was a slight decrease of enzymatic activity in the central area.

B. Cases of chronic hepatitis, active form (Table 1, Nos. 4 to 8)

The distribution of HK in this group was almost uniform throughout the lobule with slight increase of intensity in the central area where the G6Pase activity was almost negatively stained (Fig. 2-a). The G6Pase activity in the peripheral area was retained at the normal level as in Fig. 2-b. The activity of the G6Pase was varied in the lobule as was described in Table 1. The HK activity was increased in the whole lobule, whereas the G6Pase activity was decreased in the central area.

In this area, parenchymal cells on hematoxylin and eosin staining revealed a pale stained cytoplasm with a infiltration of round cells, while the parenchymal cells in the peripheral area appeared intact, even though HK activities in these cells were elevated. However, in cases of chronic hepatitis, active form, which have distraction of the limiting plate, the activity of G6Pase was markedly decreased and the activity of HK increased in the parenchymal cells of the necrotic area.

C. Cases of chronic hepatitis, inactive form and acute hepatitis, convalescent stage (Table

FIG. 1. Enzyme histochemistry of human liver tissue, control patient.
a. HK, b. G6Pase, $\times 40$

I, Nos. 9 to 17)

The HK activity was increased in the whole lobule and the activity of G6Pase was decreased in the corresponding area, although the extents of the changes in these enzyme activities were generally less intense as compared to those in chronic hepatitis, active form. Similarly, histological changes in the parenchymal cells were minimum.

Correlations between the histochemical findings and liver enzyme activities (Table 1)

In accordance with the above histochemical observation, the activity of HK in the liver supernatants showed 2 to 3-fold elevation in cases of chronic hepatitis, active form. However, in cases of chronic hepatitis, inactive form and acute hepatitis, convalescent stage, the increase in HK activity was found only in some of these cases. Although, the activity of G6Pase in the central area was significantly decreased histochemically, the activities determined with homogenates revealed no marked decreases.

D. Cases of chronic hepatitis with sublobular hepatic necrosis (Table 2)

The staining patterns of HK and G6Pase activities were particularly interesting because of the uneven distribution of HK and G6Pase within the lobule; the area which showed decreased G6Pase activity gave an intense HK staining. These

FIG. 2. Histochemical demonstration of the HK and G6Pase of the human liver tissue, a case of chronic hepatitis, active form. (Table. 1, No. 5)
a. HK, b. G6Pase, $\times 40$ c. H.E. staining, $\times 100$

TABLE 1. Staining patterns of HK and G6Pase activities in the liver tissue and clinical and biochemical data in the cases of acute and chronic hepatitis and control.

	name	histo	GOT K.U.	GPT K.U.	K _{ICG}	γ -glob %	Liver HK mU/mg	Enzyme G6Pase protein	Enzyme HK	Histochem G6Pase
1	U.Y	C	17	20	—	16.0	2.4	45.9	C+, P+	C++, P##
2	M.K	C	19	32	—	17.5	3.4	65.6	C+, P+	C++, P##
3	H.T	C	27	23	—	13.2	2.5	—	C+, P+	C++, P##
4	Y.J	CHa	93	164	0.19	14.1	4.9	37.4	C++, P++	C±, P##
5	W.K	CHa	91	218	0.20	19.8	—	—	C##, P##	C±, P##
6	S.M	CHa	91	167	0.14	17.0	6.8	36.5	C##, P++	C±, P##
7	M.M	CHa	101	193	0.20	22.2	8.5	—	C++, P++	C±, P##
8	K.Y	CHa	63	91	—	14.0	—	—	C++, P++	C±, P##
9	S.C	CHi	91	129	0.15	25.0	7.9	42.3	C++, P++	C++, P##
10	M.H	CHi	46	68	0.19	17.0	2.1	47.5	C++, P+	C+, P##
11	O.C	CHi	55	153	0.12	10.0	7.2	—	C++, P++	C±, P##
12	K.S	CHi	113	218	0.22	15.4	—	—	C++, P++	C±, P##
13	M.K	CHi	49	50	0.18	17.0	2.6	41.8	C+, P+	C++, P##
14	O.H	AH	35	42	0.14	18.0	5.5	—	C++, P++	C++, P##
15	M.S	AH	24	32	0.18	14.8	2.1	50.8	C+, P+	C++, P##
16	K.Y	AH	19	16	0.19	21.5	—	—	C++, P++	C±, P##
17	T.S	AH	28	26	0.26	12.0	4.3	37.0	C++, P++	C++, P##

CHa, CHi and AH: chronic hepatitis active, inactive form and acute hepatitis, convalescent stage, respectively.

The number of plus signs represents the degree of relative intensities. The combination of plus and minus signs indicates low or little activity. C: central area of the lobule, P: peripheral area of the lobule.

enzyme alterations were found in the necrotic areas where swelling of the remaining parenchymal cells, or thread like hepatic cells with poor staining with eosin, rosette forming cells and evidence of hepatocellular degeneration were found (Fig. 3). The changes in enzyme activities were not limited to the area around the central hepatic vein but also found in the distracted limiting plate with infiltration of lymphocytes and plasma cells (Fig. 3, 4). In fact, the relation between peripheral part of the lobule and the central part of that was not necessarily clear because of the loss of the normal architecture of the lobule due to the necrosis of the parenchyma and subsequent fibrosis.

The staining patterns of HK and G6Pase in such a representative case are shown in Figs. 4 and 5. In these figures and also in Fig. 6, hepatic cells scattered in the necrotic area are shown to indicate the increased activities of HK and de-

Fig. 3. Histochemical demonstration of HK and G6Pase of the human liver tissue in the area of piecemeal necrosis.

a. HK, b. G6Pase, c. H. E. staining, $\times 100$

FIGS. 4 and 5. Histochemical demonstration of the HK and G6Pase of the human liver tissue, a case of chronic hepatitis with sublobular hepatic necrosis (Table 2, No. 19).
a. HK, b. G6Pase, $\times 40$

TABLE 2. Staining patterns of HK and G6Pase activities in the liver tissue and clinical and biochemical data in cases of chronic hepatitis with sublobular hepatic necrosis.

	name	HBsAg	HBsAb	GOT K.U.	GPT	K _{ICG}	γ -glob %	Liver HK mU/mg	Enzyme G6Pase protein	Enzyme HK	Histochem G6Pase
1	T.T	+	—	29	35	0.14	19.0	3.9	41.1	L++~+	L++~##
2	I.K	+	—	62	37	0.12	24.0	5.8	—	L++,S++	L##,S##
3	A.A	+	—	43	36	—	30.0	7.7	63.1	L##~++	L++~##
4	U.S	—	—	169	243	0.17	14.2	9.0	34.9	L##,S##	L++~##,S++
5	N.Y	—	—	110	153	0.16	29.0	7.2	56.5	L##~++	L++~##
6	N.K	+	—	60	60	0.13	23.5	8.9	60.0	L##~++	L++~##
7	N.S	—	—	39	44	0.20	15.9	—	—	L++~+	L++~##
8	M.J	+	—	370	410	0.17	26.0	11.7	63.7	L++~+	L+~++
9	K.Y	+	—	236	320	0.12	21.0	10.5	37.2	L##	L $\pm$ ~##
10	N.K	—	—	121	193	0.17	29.0	—	—	L##~++	L $\pm$ ~+
11	M.S	—	—	66	114	0.17	22.8	—	—	L++~+	L $\pm$ ~+
12	F.M	—	—	129	140	0.12	28.0	—	—	L##~++	L $\pm$ ~+
13	I.T	+	—	50	52	0.08	28.1	—	—	L++~+	L+~##
14	S.H			68	90	0.15	32.8	8.3	42.1	L##~+	L+~##
15	K.K	+	—	87	90	0.18	19.0	—	—	L##~++	L $\pm$ ~++
16	K.M	—	—	146	260	0.12	19.0	10.9	53.2	L##,S##	L $\pm$ ~+,S $\pm$
17	M.F	—	—	210	280	0.14	25.1	—	—	L##,S##	L $\pm$ ~++,S $\pm$
18	K.Y	+	—	230	580	0.14	25.1	—	—	L##~++,S+	L $\pm$ ~++,S $\pm$
19	T.S	+	—	210	320	0.18	20.0	12.5	43.7	L##,S++	L $\pm$ ~++,S $\pm$
19'	T.S	—	—	63	85	0.17	19.5	—	—	L++,S++	L $\pm$ ~++,S+

HBsAg, HBsAb: HBs antigen and antibody in the serum.

L: whole hepatic lobule, S: scattered hepatic cells in the necrotic area.

L+~++: difference of enzyme activity in the nodule.

creased activities of G6Pase in the corresponding area. The staining patterns of this group were divided into two groups according to the uneven distribution of G6Pase activity and that of HK in the lobule.

Among 19 cases with a diagnosis of chronic hepatitis with sublobular hepatic necrosis, there were 7 cases which showed that such a heterogeneous distribution of HK and G6Pase activity in the lobule was not so marked as compared with the latter 12 cases in Table 2. The borders between the hepatic parenchyma and the necrotic area were relatively well demarcated where the G6Pase activity was not reduced and the HK activity gave a moderate increase. In these 7 cases, the histological findings suggested the rather resolved or subsided stage. In other words, there were fibrosis with less infiltration of round cells in the necrotic area, and the parenchymal cells appeared intact. However, no significant differences in liver function tests were found between the 7 cases and the latter, although the increase in HK activity in the supernatants were more pronounced in the latter.

Fig. 9 shows the hospital course of the representative case of chronic hepatitis

TABLE 3. Staining patterns of HK and G6Pase activities in the liver tissue and clinical and biochemical data in cases of cirrhosis of the liver.

	name	histo	GOT K.U.	GPT	K _{ICG}	γ-glob %	Liver HK mU/mg	Enzyme G6Pase protein	Enzyme HK	Histochem G6Pase
1	O.Y	A	145	103	0.053	30.9	10.3	—	N++	N++
2	S.T	A'	41	58	—	26.0	6.0	68.2	N++	N++
3	N.K	A'	36	16	0.10	33.0	7.9	39.2	N++	N++
4	I.U	A	133	49	0.062	57.4	—	—	N##	N##
5	B.Y	A	37	28	0.13	23.0	2.3	76.4	N++~+	N++~##
6	N.S	A	33	51	0.016	15.1	4.2	44.4	N++~+	N++~##
7	N.M	A'	169	96	0.046	45.0	—	—	N++	N+~++
8	M.S	A'	76	67	0.15	19.3	5.8	—	N++	N++~##
9	S.I	A	92	76	0.072	29.4	—	—	N++	N++~##
10	Y.K	A'	158	200	0.17	29.8	5.0	—	N##	N+, ++, S±
11	K.Y	A	630	420	0.087	24.3	—	—	N##, S##	N+~++, S±
12	T.Y	A'	152	186	0.094	16.3	10.1	45.3	N##, S##	N±~+, S±~+
13	B.M	A'	40	41	0.14	22.9	10.8	32.0	N++, S++	N±~+, S±~+
14	O.Y	A	134	490	0.12	24.0	6.2	—	N++, S++	N++, S±~+

N: nodule, S: scattered hepatic cells in the necrotic area.

N+~++: difference of enzyme activity in the nodule.

with sublobular hepatic necrosis during which time he had the peritoneoscopic examination twice. The histochemical finding which was observed after the sharp elevation of serum GPT up to 1600 K. U. showed the characteristic uneven distribution of HK and G6Pase (Fig. 4). The finding of histochemistry done at the 2nd time was shown in Fig. 5. The heterogeneity of the staining pattern of HK and G6Pase was still observed but in a lesser degree as compared to the previous examination. Hepatic parenchymal cells which showed marked degeneration in the previous examination appeared improved in the 2nd examination.

E. Cases of the cirrhosis of the liver (Table 3)

Cases of the cirrhosis of the liver were grouped according to the staining intensities of HK and G6Pase as follows:

1. Nodules were stained uniformly without significant difference in intensity of HK and G6Pase in the nodules and among nodules (Nos. 1 to 4). The average activity of HK was increased and that of G6Pase was decreased in the whole nodule. Histologically, parenchymal cells were swollen and stained pale with hematoxylin and eosin and round cell infiltrations were significant in the necrotic area. Among liver function tests, a low K_{ICG} index was characteristic of this cirrhosis group.

2. The strongly increased activity of HK and the weak activity of G6Pase and

FIG. 6. Histochemical demonstration of HK and G6Pase of human liver tissue in the area of piecemeal necrosis.

a. HK, b. G6Pase, c. H.E. staining, ×100

FIGS. 7 and 8. Histochemical demonstration of HK and G6Pase of the human liver tissue, a case of cirrhosis of the liver. (Table 3, No. 6 and No. 11).

a. HK, b. G6Pase, $\times 40$

Fig. 9. Clinical course of the patient of a chronic hepatitis with sublobular hepatic necrosis. (Table 2, No. 19) +, -: HBs antigen in the serum reported positive, + or negative, -. ↓: peritoneoscopic examination. GPT activities: represented as Karmen unit.

vice versa in the corresponding area were observed within the same nodules (Nos. 5 to 9). The staining patterns of the representative case is given in Fig. 7. Most of these cases in this group appeared stable in clinical and laboratory data and histological findings. There was no piecemeal necrosis of inflammatory infiltration and the margins of the nodules were sharply defined, and the liver cells revealed no evidence of parenchymal liver cell damage.

3. In the 3rd group, the histochemical findings of HK activity in the nodules and in the hepatic cells in the necrotic area were increased, whereas, the G6Pase activity was significantly decreased in the corresponding cells and area (No. 10 to 14). In this group, the clinical data of the serum transaminase levels were in higher levels comparing with those of the other two groups. Fig. 8 showed one of these cases. The characteristic findings from the hematoxylin and eosin staining were swollen liver cells with pale stained cytoplasm and pyknotic nucleus throughout the nodule. And some liver cells were isolated within the advancing fibrous septa.

DISCUSSION

Our previous study with key glycolytic and gluconeogenic enzymes of hepatic tissues in hepatitis and cirrhosis of the liver has indicated the increase of the liver non-specific enzymes such as HK and the decreases of the liver specific enzymes such as G6Pase (8, 9, 10, 11). These enzyme alterations were found in the present study to be localized to the parenchymal hepatic cells which revealed several lines of histological evidence suggestive of the presence of hepatic cell injuries. In some cases with chronic hepatitis and cirrhosis of the liver, increased HK activities were found in apparently intact cells. This may indicate the presence of some sort of hepatic cell injury which could not be detected by light microscopy. In other words,

HK response appears to be a sensitive index to the injury, as was also demonstrated by principal factor analysis of hepatic enzyme patterns in these liver diseases (9).

Histochemical demonstration of G6Pase in liver tissues of hepatitis afforded us an interesting finding in a sense that marked decrease in G6Pase activity was observed particularly around the central hepatic vein in spite of no significant changes in G6Pase activity which was determined on homogenates. Although the possibility that a slight decrease in G6Pase activity is exaggerated during the process of histochemical staining could not be neglected completely, it is more likely that originally present zonal distribution with less G6Pase activity in the central area is readily intensified even with small decrease in the total G6Pase activity.

Based on these considerations, the histochemical demonstrations of HK and G6Pase are of particular use in elucidating the mechanism of progression of chronic hepatitis into cirrhosis of the liver. The uneven distribution of these enzymes in the hepatic lobule, which is characteristic of the chronic hepatitis with sublobular hepatic necrosis, may indicate the presence of unsettled injured cells, which may go into necrosis or regeneration leading to a formation of cirrhotic nodules. In cirrhosis of the liver, the presence of regenerated nodules with high HK and low G6Pase activities could be regarded as indicating an active state of cirrhosis. This is interesting in view of the fact that the incidence of the primary hepatoma is high in cirrhosis of the liver and that in the hepatoma tissue, generally, HK level is high and G6Pase level is low (9, 11).

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