Serum Leucine Aminopeptidase Isozyme and Histological Findings of Liver Biopsy

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When serum leucine aminopeptidase isozymes separated by agar-gel electrophoresis are observed after staining them on agar plate by histochemical techniques, only one band of activity is detected in the sera of healthy persons,[1] but examination of the sera of hepatobiliary disease patients show additional bands of LAP isozyme activity in locations different from that of the sera of healthy persons.[2] It has been reported that the appearance of such "pathological" LAP bands is indicative of hepatobiliary obstruction, and that the frequency is high when it is accompanied by hepatic disturbances.[3] In order to ascertain the types of hepatic disturbances which are related to the appearance of the "pathological" LAP isozymes, liver biopsies were done to make a comparison with the histochemical picture of the liver. The tissue specimen obtained by liver biopsy is only a small portion of the liver and even when there are diffuse hepatic disturbances, the hepatic parenchymal changes differ considerably by site so that is may not be possible to directly relate the appearance of pathological LAP isozymes to changes in the histological picture of the liver. Nevertheless the results which have been obtained explain to some degree the mechanism of the appearance of LAP isozyme α_2 and $\alpha_2\beta$.

MATERIAL AND METHOD

Liver biopsy was performed by the Vim-Silvermants method[4] on 41 cases of hepatic diseasses (inculuding 20 cases of hepatitis, 6 cases of liver cirrhosis, 4 cases of hepatic cancer, 1 cases of metastasis of gastric cancer to liver, 6 cases of cholelithiasis, 1 case of cholecystitis and 3 cases of malignant biliary obstruction.) The liver fragments obtained were fixed by 10 % formalin, and stained by hematoxylin eosine for examinanion of the histological picture.

The histological picture of the liver was classified as follows according to the method of Popper and Szanto,[5] and Shibata et al.[6] The relation of each to the appearance of pathological LAP isozyme was obtained.

(A) Liver cell damages:

1) Irregularity of the size and staining of nucleus, decreased basophilism of cytoplasm, formation of eosinophilic granules and increased sharpness of margins

2) Coagulative necrosis

(B) Inflammatory changes:
1) Leukocyte infiltration
2) Mobilization of Kupffer's cell
3) Localized necrosis of hepatic cords
4) Perilobular cellular infiltration
5) Cellular infiltration of Glisson's sheath
6) Proliferation of fibroblast

(C) Cicatrization
1) Formation of pseudobile canaliculi
2) Formation of bile canaliculi
3) Neovascularization
4) Modification of the lobular structure
5) Proliferation of fibrocytes and connective tissue

(D) Fatty degeneration

(E) Biliary stasis
1) Pigment granules in Kupffer's cells and liver cells
2) Bile thrombus

RESULTS AND DISCUSSION

The sera of patients with hepatobiliary diseases show 3 types of LAP zymograms including type Aα_1, type A$\alpha_1+\alpha_2$ and type A$\alpha_1+\alpha_2+\alpha_2\beta$. Isozymes α_2 and $\alpha_2\beta$ can not be seen in the sera of a healthy person. These had been tentatively called pathological LAP isozymes, but examination of the histological picture of the liver in cases demonstrating LAP isozymes α_2 and $\alpha_2\beta$ showed bile plugs and bile pigments in the liver cells or Kupffer's cells in all cases except two (Table 1). At the seme time they were accompanied by liver cell injuries.

From the standpoint of the histological picture of the liver of the 21 cases showing bile plugs or bile pigment in liver cells and Kupffer's cells, only 3 cases demonstrated neither isozyme α_2 nor $\alpha_2\beta$. Therefore, it seems that pathological LAP isozymes appear in cases of biliary stasis with liver cell injuries.

In general, changes of the liver cells show that even if there is comparatively severe injuries, pathological isozymes do not appear unless the injuries are accompanied by biliary obstruction. In a previous report on the results of liver function tests, it was mentioned that some degree of hepatic parenchymal disturbances is necessary for the appearance of pathological LAP isozymes. However, it was assumed[3] that when there is no hepatobiliary obstruction, there will be no appearance of such isozymes even though parenchymal disturbances are severe. The results we have obtained here are consistent with this assumption.

Review of the histological picture of the cases in which $\alpha_2\beta$ was detected

Table 1. LAP Isozyme and Histological Picture of the Biopsied Liver.

Cases	Clinical diagnosis	A* (1)	(2)	B (1)	(2)	(3)	(4)	(5)	(6)	C (1)	(2)	(3)	(4)	(5)	D	E (1)	(2)	Pathological LAP Isozyme
1		2+		+										2+		+	+	α_2
2		+					+									+	2+	α_2
3		+											+	+		2+		α_2
4		+											+	+			+	α_2
5		2+		+										2+				α_2
6		2+											+	3+		+		
7		+														+		
8		+			+		+								+			
9		2+												+				
10		+											+	+				
11	Hepatitis	+		+										2+				
12		+	+				+							+				
13		+		+					+					+	+			
14		+		+	+								+	+				
15		+	+											+				
16		+											+	+				
17		+		+														
18		+		+									+	2+				
19		+		+									+	2+				
20		+		2+			+						+	2+				
21		+									+			2+	+	+		α_2
22		+											+	2+		+		α_2
23		+		+														
24	Liver cirrhosis												+	2+				
25													+	+				
26													+	+				
27		+	+								+			+		+	+	α_2, $\alpha_2\beta$
28		+	+															α_2
29	Hepatic cancer	+	+														2+	α_2
30		2+											+	2+		+		
31	Metastatic hepatic cancer	+	+	+							+		+	+		+		α_2
32		+		+												+	3+	α_2, $\alpha_2\beta$
33		+												+		+	2+	α_2, $\alpha_2\beta$
34	Cholelithiasis	+		+						+				+		+	+	α_2
35		+		+										+		+	+	α_2
36		+											+	2+		+	+	α_2
37		2+	+													+		α_2
38	Cholecystitis	+																
39	Malignant	+											+	+			3+	α_2, $\alpha_2\beta$
40	biliary-	+		+									+	2+		+	3+	α_2
41	obstruction	2+	+													+		α_2

* A, B . . . . . See in text.

besides isozyme showed the bile plug to be more severe as compared with the group in which $\alpha_2\beta$ was not detected. This suggests that when biliary obstruction is severe, there is the appearance of isozyme $\alpha_2\beta$. In the previous report,[3] the appearance of isozyme $\alpha_2\beta$ was found more frequently in hepatobiliary obstruction with increased GPT to more than 50 units (Normal is less than 10

units.). Because of this, it was suspected that there might be relation to specific liver cell injuries. It was impossible to detect any certain relation with liver cell injuries from the histological patterns obtained by liver biopsy.

Although proliferation of fibrocytes and connective tissue was noted more or less in cases in which pathological isozymes appeared, it was impossible to directly correalate this with the appearance of pathological isozymes. In cases where there are only hepatic cell injuries without any biliary obstruction, pathological LAP isozymes are often not found, while patterns of biliary obstruction and hepatic cell injuries are noted in cases with pathological isozymes. In other words, biliary obstruction is first prerequisite and the presence of liver cell injuries are also needed. Possibly these two factors, namely, the presence of bile stasis and hepatic cell injuries contribute to the appearance of pathological isozymes in the sera.

CONCLUSION AND SUMMARY

Liver biopsy and examination of the serum LAP zymogram in 41 patients with hepatic diseases revealed that the appearance of pathological LAP isozyme α_2 and $\alpha_2\beta$ found in the sera of patients with hepatobiliary diseases is related with the simultaneous presence of biliary obstruction (bile plug and bile pigment granules in Kupffer's cells or hepatic cells) and hepatic cell injuries (irregularity of the size and staining of nucleus, disappearance of nucleus, decreased basophilism of cystoplasm, and formation of eosinophilic granules).

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