Clinical Values of Monitor of Individual Bile Acids in Serum to Primary Liver Cancer by Absolute Ethanol Injection Therapy

Xinhua Ju[1], Zhenlong Xia[1], Yukui Zhang[2]

[1] Department of Hepatobiliary Surgery, The Second Clinical College, China Medical University, Shenyang 110004,China;
[2] National Chromatographic R.&A. Center, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116011, China

Abstract **Objective** To study directive significance of serum levels of individual bile acids determination to intrahepatic tumor by ethanol injection therapy. **Methods** Using precolumn derivatization of high performance liquid chromatography, measured the serum levels of 9 kinds bile acids in 5 patients with primary hepatic cancer (PHC), who were treated before and after ethanol injection therapy or both hepatic artery chemotherapy embolism and ethanol injection therapy during operation, 5 patients with liver cirrhosis (LC) and 5 healthy persons, respectively. **Results** Serum levels of total bile acids and glycocholic acids were significantly higher in patients with PHC than healthy persons ($P<0.05$) and lower than in patients with LC ($P<0.01$). Ratio of serum levels of cholic acids to chenodeoxycholic acids increased temporarily after treatment; ratio of serum glycine-conjugated bile acids to total bile acids decreased. Serum total bile acids increased, and hidn't recover until the 7th day after treatment. **Conclusions** The results suggested that serum levels of individual bile acids as an effective indicator of liver function may be valubale to monitor PHC therapy.

Key Words primary liver cancer; serum bile acids; alsolute ethanol injection; therapy

Because primary liver cancer (PLC) mostly belongs to middle or later stages and its resected rate is lower, absolute ethanol injection to tumor (EIT) or hepatic artery chemotherapy embolism (HACE) is preferred method in unresected therapy. About 80~90% patients accompanied by liver cirrhosis,so the damage of liver function becomes an important factor what influences treatment effects. Production and metabolism of bile acids closely relate to liver function. This study applys to precolumn dirivatization method of high performance liquid chromatography (HPLC)[1] to detect serum levels of individual bile acids. The advantage of this method is to be not limited by enzyme activity and it has higher sensitivity to compare with enzymatic hydrolysis of current method with HPLC [2-4]. Up to now it has not been reported in inland of China. Through serum levels of individual bile acids are detected after intrahepatic tumor by EIT, our aim is to study directive significance in detection of serum levels of individual bile acids.

MARERIAL AND METHODS

Patients and therapy

In PLC group 5 patients were all male and the ages ranged beteen 33~64 year-old.Those diagnosis accorded with diagnosis criteria of liver cancer which were adoped by the national anticancer association. 2 patients were treated by EIT under guiding B mode of ultrasound. The dose of Absolute ethanol was 10~28ml. At the same time, 3 patients were treated by EIT or both EIT and HACE during operation. Chemotherapy drugs were 5-fluorouracil 250mg, mitomycin 16mg, epirubicin hydrochloride 20mg plus pieces of jelly sponge and lipiodol 10ml. Serum samples were collected before and 1, 3,7 day after treatment respectively. 5 patients in liver cirrhosis(LC) group were diagnosed by pathology. 5 persons in healthy control (HC) group were without hepatobiliary diseases and malignant tumor. 5ml with peripheral veins blood of empted stomach were extracted, seperated by serum and stored at -30°C untill determination.

Experimental reagents

Cholic acid (CA), deoxycholic acid (DCA), chenodeoxycholic acid (CDCA), ursodeoxycholic acid (UDCA), lithocholic acid (LCA), glycocholic acid (GCA), glycodeoxycholic acid (GDCA), glycochenodeoxycholic acid (GCDCA), glycolithocholic acid (GLCA) and 4-bromomethyl-7-methoxycoumarin (BMC) were purchased

from Sigma company in U.S.A.

Apparatus and test method

Apparatus of HPLC was Daojun LC-9A and RF-530 fluorescence detector made by japan. Dirivatization method of HPLC was discribed literature[5].

RESULTS

Detecting serum level of total bile acid (TBA) with PLC

Serum levels of total bile acids in patients with PLC were significantly higher than that in healthy persons ($P< 0.05$) and lower than in patients with LC ($P<0.01$). Serum levels of total bile acids in patients with LC were significantly higher than that in healthy persons($P<0.01$) (table 1). Serum levels of GCBA in PLC were significantly higher than that in healthy persons($P<0.05$), But it was significantly lower than in patients with LC($P<0.01$).

Comparison of serum levels of bile acids before and after EIT for PLC

Serum TBA increased at 3rd day, and didn't recover until the 7th day after treatment. Ratio of serum levels of cholic acids to chenodeoxycholic acids increased temporarily 1st day, and dropped close to the level before treatment at 3rd day after treatment. Ratio of serum glycine-conjugated bile acids to total bile acids decreased, and didn't recover until the 7th day after treatment ($P>0.05$) (table 2).

Comparison of serum levels of bile acids before and after EIT for PLC

Serum alanina aminotransferase(ALT) increased significantly 1st day after treatment ($P<0.05$), and recovered to previous level at 7 th day. Bilirubin increased at 1 st day, and it was high levels at 7 th day still. Liver function of total protein, albumin and alkaline phosphatase didn't change much ($P>0.05$) (table 3).

Table 1 Comparison of serum total bile acids of PLC, LC and healthy control ($\bar{X}\pm Sx$)

Group	TBA (μmol/L)
Healthy control	5.056 ± 1.852
Liver cirrhosis	89.783 ±41.973 [1)]
Primary liver cancer	17.516 ± 9.572 [2),3)]

note : 1) compare with healthy control, $P<0.01$;
2) compare with LC, $P<0.01$;
3) compare with healthy persons, $P<0.05$

Table 2 Serum levels of bile acids before and after absolute ethanol injection for PLC(mol/L)

items	before treatment	after treatment		
		1st day	3rd day	7th day
T B A	17.516	16.225	26.096	25.855
CA/CDCA	0.513	0.934	0.558	0.519
GCBA/TBA	0.610	0.462	0.468	0.405

Table 3 liver function before and after absolute ethanel injection for PLC

items	before treatment	after treatment		
		1st day	3rd day	7th day
TP(g/L)	67.8	6.37	65.9	67.0
A(g/L)	33.8	35.0	36.7	34.3
ALT(u/L)	32.2	152.6 [1)]	68.2	33.2
AKP(u/L)	77.8	72.6	62.2	81.2
bilirubin(μmol/L)	22.8	53.0	43.9	36.7

note : 1) compared with pretreatment, $P<0.05$

DISCUSSION

The bile acids were synthetic production of liver cells that utilized cholesterol as material. Organism reabsobed bile acids from enterohepatic circulation into liver where the free-bile acids were transformed into combination-bile acids. Glyco-combination bile acids were dominant component in combination bile acids. When the liver was diseased and metabolism of bile acids were taken place hindrance. xiao reported[6] serum bile acids in PLC increaced. Our experiment showed that serum levels of TBA and GCA in patients with PLC were significantly higher than that in healthy persons, but lower than in patients with LC. Serum bile acids in patients with PLC increased because cancer cells of liver destroyed bile duct, release of bile acids increased. But serum bile acids in patients with LC increased mainly related to hepatocyte degeneration, necrosis, hyperplasia of fibrous tissue, regeneration of hepatocyte nodules resulting in liver function damaged and liver cirrhosis leading to portal hypertension which created interhepatic and extrohepatic vein shunt.

At present detection of routine liver function often can not been reflected degree of liver diseases exactly. those detection result sometimes showed discordance with pathologic lesion of liver[7]. Many data revealed individual bile acids were an effective indicator for liver function[8]. In our study, ALT increased temporarily after treatment, serum bilirubin increased was a little longer than ALT in lasting time, but 3 remainder items of liver function didn't change significantly. However, the results of serum bile acids revealed that the ratio of serum levels of CA to CDCA increased temporarily, serum TBA increased and the ratio of GCBA to TBA decreased. The two latter items of liver function recovered more slowly than routine detection liver function, which showed sensitivity of detecting of individual bile acids was superior to routine items of liver function. After EIT and HACE tumor and paratumor tissues turned out coadulation, necrosis and liver function decline which cause bile acid reflux and entering blood, TBA levels in extrermity blood increased. Hepaatocyte damage lead to GCBA increased and ratio of GCBA to TBA decreased. In addition, the ability of hepatocyte regeneration was very strong at initial stages of liver damage and synthesis bile acid increased.As liver damage was severe or its function was incomplete, bile acids can not been decorated by 12α−carboxyl of microsome, it was easy to synthere CDCA, so the ratio of CA to CDCA decreased. Azer et al reported when donor liver was dysfunction, ratio of CA to CDCA diclined 24 hours more early than other biochemical dectetion [9]. Changes of serum bile acids were considered as reliable indicator of reserved function of liver[10]. On the basis of trending of moniterig serum levels of individual bile acids, it is certainly valuable for correctly evaluated damage degree of liver function and its prognosis.

REFERENCES

1. Gatti R, Roda A, Cerre C, et al. HPLC-fluorescence determination of individual free and conjugated bile acids in human serum. Biomed Chromatogr, 1997, 11(1):11-15.
2. Zhao RH, Li BY, Chen N, et al. High performance liquid chromatographic determination of individual bile acids in serum for automatic diagnosis of various liver and biliary diseases. Biomed Chromatogr, 1993, 7(3):139-142.
3. Chisaki K, Nakajima T, Iwasawa K, et al. Enhancement of endothelial nitric oxide production by chenodeoxycholic acids in patients with hepatobiliary diseases. Jpn Heart J, 2001, 42(3): 339-353.
4. Okuyama S. Analysis of free, glycine-and taurine-conjugated individual bile acids using high performance liquid chromatography and immobilized 3α-hydroxysteroid dehydrogenase in column form. The Japanese Journal of Clinical Pathology, 1981, 29(5): 446-458.
5. Wang GF, Stacey NS, Earl J. Determination of individual bile acids in serum by high performance liquid chromatography. Biomed Chromatogr, 1990, 4(4):136-140.
6. Xiao B, Yan AL, Lai ZS. Detection serum glycocholi acids of liver diseases and non-hepatobiliary diseases. Academic Journal of The First Military Medical College, 1995, 15(1): 43-44.
7. Azer SA, Murray M, Farrell GC, et al. Selectivity and sensitivity of changes in serum bile acids during induction of cirrhosis in rats. Hapatology, 1993, 18(5):1224-1231.
8. Azer SA, Klaassen CD, Stacey NH. Biochemical assay of serum bile acids: methods and applications. Br J Biomed Sci, 1997, 54(2):118-132.
9. Azer SA, Caughan GM, Stacey NH, et al. Daily determination of individual serum bile acids allows early detection of hepatic allograft dysfunction. Hepatology, 1994, 20(6):1458-1464.
10. Maniwa Y, Tanimura H, Ishimoto K, et al. The significance of measurement of bile acid fractions in the serum and bile after hepatectomy. Japanese Journal of Clinical Surgeon Association, 1991, 52(11): 2588-2593.