ORIGINAL ARTICLE

Bile Acids and Biliary Carcinoma in Pancreaticobiliary Maljunction

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Abstract. Pancreaticobiliary maljunction is frequently associated with biliary carcinoma, whether or not there is choledochal dilatation. In this anomalous condition, pancreatic juice regurgitates and the mixture of bile and pancreatic juice stagnates in the biliary tree. In cystic choledochal dilatation, cancers arise, mainly in the dilated bile ducts, while in patients not having cystic dilatation, tumors arise in the gallbladder.

Gallbladder bile and/or bile duct bile from fifteen cases of pancreaticobiliary maljunction, including five cancer patients, was analysed biochemically and compared with control bile from 6 patients with a normal pancreaticobiliary junction. Bile levels of pancreatic enzymes were extremely high in the anomalous junction group. In the bile duct bile from patients with cystic choledochal dilatation with pancreaticobiliary maljunction, the concentrations of deoxycholic acid (DCA), lithocholic acid (LCA) and unconjugated bile acid fractions were increased regardless of the presence of cancer. Increases of these bile acid fractions, which are known to have a cancer-promoting effect, were also seen in gallbladder bile from the cancer patients without cystic dilatation. (Keio J Med 40 (3): 118–122, September 1991)

Key words: pancreato-biliary maljunction, pancreatic enzyme, bile acid fractions, carcinogenesis, cancer promoting factor

Introduction

Pancreaticobiliary maljunction is a congenital anomaly that is not so rare in Japan,¹ and is known to be commonly associated with congenital biliary dilatation since the report of Babbitt.² Irwin and Morison³ were the first to report a cancerous change of cystic choledochal dilatation and it has been widely recognized that cystic dilatation is often accompanied by cancer since the report of Kasai *et al.*⁴ More recently, pancreaticobiliary maljunction itself has been shown to be associated with a biliary carcinoma.^{5,6} When there is cystic bile duct dilatation, cancer arises mainly in the bile duct, while in patients not having dilated bile duct or having spindle- or cylindrical-shape choledochal dilatation cancer arises mainly in the gallbladder.

Regarding the causative factors of cancer in pancreaticobiliary maljunction, many controversies remain unresolved. The most evident physiological abnormality in the pancreaticobiliary maljunction is the reflux of pancreatic juice into the biliary tract and stagnation of

the mixture of bile and pancreatic juice. This study investigated the etiological factors of biliary carcinogenesis by biochemical analysis of the bile acids.

Materials and Methods

The subject of the present study were 15 patients with pancreaticobiliary maljunction that we treated from 1984 to 1989 (Table 1). Twelve cases had biliary dilatation (cystic type in 10, spindle type in 1 and cylindrical type in 1) and three had no biliary dilatation. There were 5 children aged from 2 to 12 years and ten adults. Five patients had cancer, which involved the bile duct in 2 cases and the gallbladder in 3 cases. Bile was collected at operation. As the control, gallbladder bile was collected from six adults (3 with peptic ulcer, 2 with motor vehicle trauma and one with hepatic hemangioma). All of the controls were shown to have a normal pancreaticobiliary junction by direct cholangiography at operation. Bile duct bile was collected from five choledocholithiasis patients through the T-tube postoperatively.

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Table 1 Case List of the Pancreaticobiliary Maljunction

Bile duct dilatation	Age	Dilatation type	Cancer	
			(-) bile duct	(+) gallbladder
(+) n = 12	child n = 5	cystic spindle cylindrical	5	
	adult n = 7	cystic spindle cylindrical	4 1	1 1
(-) n = 3	adult n = 3			3
Total n = 15			10	2 3 5

Four pancreatic enzymes were assayed according to the following methods; amylase by the C1-PNP-G7 enzyme method, elastase-I⁸ and trypsin⁹ by RIA, and lipase by the UV rate assay.¹⁰ Bile acid fractions were investigated by Okuyama's high performance liquid chromatography method using a Nihon-Bunkoh Ltd system with an immobilized enzyme column. Statistical analysis was performed using Student's unpaired t-test.

Results

Pancreatic enzymes in gallbladder bile

The activated amylase level in the gallbladder bile was significantly raised ($P < 0.05$) at $60,984 \pm 70,903$ IU/L/37°C ($M \pm S.D.$) in the maljunction patients ($n = 13$), when compared with the level of 45.2 ± 29.6 IU/L/37°C in the controls ($n = 6$). However, in the maljunction group, there was no statistical difference between the patients with ($8,122 \pm 14,796$, $n = 5$) and without ($136,525 \pm 72,805$, in 4 adults) cancer, or between the adults and children ($93,372 \pm 73,836$, $n = 4$) (Fig 1).

The trypsin level (Fig 2) in the maljunction group ($n = 13$) was $77,171 \pm 200,540$ ng/ml, while it was 698 ± 375 ng/ml in the control group (N.S.).

The lipase level (Fig 3) in the maljunction group ($n = 6$) was $4,829 \pm 7,180$ IU/L/37°C, compared with 120 ± 84 IU/L/37°C in the control ($n = 6$, N.S.).

Finally, the elastase-I level (Fig 4) in the maljunction group was $1,327,770 \pm 288,510$ ng/dl ($n = 9$), as compared with $1,980 \pm 976$ in the controls ($n = 6$, N.S.).

Bile acids fractions in gallbladder bile (Table 2)

The mean values for each bile acid fractions are listed in Table 2. In the maljunction group of children who had biliary dilatation without cancer ($n = 5$), the concentration of CA-related acids was slightly increased

Fig 1 Activated amylase level in the gallbladder bile.

Fig 2 Activated trypsin level in the gallbladder bile.

Fig 3 Activated lipase level in the gallbladder bile.

Fig 4 Activated elastase-I level in the gallbladder bile.

Table 2 Bile Acid Fractions in the Gallbladder Bile

(Mean %)																									
Case		bile acid				UDCA				CA				CDCA				DCA				LCA			
		GUDC	TUDC	free	Total	GC	TC	free	Total	GCDC	TCDC	free	Total	GDC	TDC	free	Total	GLC	TLC	free	Total				
BD child ($\bar{s}$ Ca)	n = 5	1.7	0.7		2.4	23.3	20.0	0.1	43.4	26.8	21.6	0.1	48.5	3.2	2.3		5.5	0.1	0.1		0.2				
BD adult ($\bar{s}$ Ca)	n = 5	1.4	0.9	2.2	4.5	13.2	16.7	4.7	34.6	34.0	19.6	3.8	57.4	2.6	0.9		3.5				0				
BD (cyst.) adult																									
Bile duct ca	n = 1	1.2	3.6	0.1	4.9	7.1	32.6	0.3	40.0	9.1	33.9	0.2	43.2	1.7	10.2		11.9				0				
BD (cylind) adult																									
Bile duct ca	n = 1	7.5			7.5	27.9	1.5		29.4	31.7	2.8		34.5	24.0	1.2		25.2	3.3			3.3				
BD (–) adult																									
Gallbladder ca	n = 2	3.0	0.9	0.8	4.7	34.2	6.9		41.1	41.0	7.0		48.0	2.7	1.0	1.0	4.7	1.1	0.4		1.5				
Control	n = 6																								
		3.9	0.3	0.1	4.3	23.8	9.3		33.1	36.1	12.7		48.8	10.4	2.3		12.7	0.8	0.2		1.0				
UDCA: urtho deoxycholic acid						DCA: deoxycholic acid								G. : glyco											
CA : cholic acid						LCA: lithocholic acid								T. : tauro											
CDCA: chenodeoxy cholic acid						free : unconjugated								BD: biliary dilatation											

compared with the control group (n = 6), whereas DCA- and LCA-related acids were decreased (N.S.). In the maljunction group of adults who had biliary dilatation without cancer (n = 5), the bile acid fraction pattern was similar to that in the children.

In the gallbladder bile obtained from an adult who had bile duct cancer and cystic dilatation, CA-related acids accounted for 40%, CDCA-related acids for 43%, DCA-related acids for 12%, and the UDCA-related acids for 5%, while LCA-related acids were not measurable.

This ratio was similar to those in the controls. In the gallbladder bile obtained from an adult with bile duct cancer and cylindrical type biliary dilatation, DCA-related acids accounted for 25%, with GDCA being 24%, and LCA-related acids were slightly increased to 3%. In the gallbladder bile of patients who had gallbladder cancer without bile duct dilatation (n = 2), LCA-related acids were slightly increased to 1.5%.

Table 3 Bile Acid Fractions in the Bile Duct Bile

(Mean %)																					
bile acid		UDCA				CA				CDCA				DCA				LCA			
		GUDC	TUDC	free	Total	GC	TC	free	Total	GCDC	TCDC	free	Total	GDC	TDC	free	Total	GLC	TLC	free	Total
Case																					
BD child ($\bar{s}$ Ca)	n = 3	4.5	1.1	0.1	5.7	41.6	11.5	0.2	53.3	18.2	13.8		32.0	5.3	3.4		8.7	0.1	0.1		0.2
BD adult ($\bar{s}$ Ca)	n = 5	1.2	0.7	2.6	4.5	12.9	19.9	2.8	35.6	33.8	22.1	0.6	56.5	2.6	0.7		3.3				0
BD (cyst.) adult																					
Bile duct ca	n = 1	1.4	3.5		4.9	5.3	33.4	1.5	40.2	6.9	37.7	0.5	45.1		9.7		9.7				0
BD (–) adult																					
Gallbladder ca	n = 3	1.2			1.2	42.9	5.6	0.5	49.0	44.1	4.5	0.4	49.0	0.8			0.8				0
Control	n = 5																				
		0.3			0.3	55.4	7.6		63.0	31.5	5.2		36.7				0				0

Bile acid fractions in bile duct bile (Table 3)

In the bile duct bile from anomalous junction children with choledochal dilatation ($n = 3$), DCA- and UDCA-related acids were increased, and LCA-related acids were detectable. In the bile duct bile from the adult maljunction group who had biliary dilatation without cancer ($n = 5$), CDCA-, DCA- and UDCA-related acids were increased.

In the bile duct bile from an adult who had the bile duct cancer associated with cystic dilatation, CA-related acids were decreased, while the CDCA- and UDCA-related acids were increased.

In the bile duct bile from three adults who had gallbladder cancer without choledochal dilatation, CA-related acids were slightly decreased, and CDCA- and, DCA-related acids were increased, compared with the control.

In the bile duct bile from all maljunction groups, unconjugated free bile acids were detected, although these were not detected in the controls.

Discussion

A total of 12,399 patients who had received biliary surgery and 569 cases of anomalous pancreaticobiliary junction were analyzed in 1985 by the Japanese Study Group on Pancreaticobiliary Maljunction.¹² In this series, incidence of biliary carcinoma excluding uncertain cases of maljunction was 21.7% (118/545), significantly higher than the rate of 11.6% (1392/11975) in patients with a normal junction. Among 245 cases of maljunction associated with the cystic choledochal dilatation, biliary cancer was found in 29 (11.8%), with the cancer arising in the dilated bile duct in 69.0% (20/29). In contrast, 44 out of 80 (55%) maljunction patients without dilatation developed gallbladder cancer, and 4 (5.0%) also had bile duct carcinoma. Regarding carcinogenic factors, Ohkawa *et al.* and Ogura¹⁴ have reported a role of activated pancreatic enzymes, especially trypsin,¹³ elastase,¹³ and phospholipase A2¹⁴ in this condition. When pancreatic juice regurgitates into the common bile duct, several enzymes can be activated by enterokinase or unknown mechanisms in the biliary system. In our current study, pancreatic enzyme levels were found to be extremely high in the gallbladder bile from maljunction cases when compared with the levels in the control with normal junction. This was proof of the regurgitation of pancreatic juice, but we could not find that any enzyme level was higher in the cancer group than in the patients without cancer. It thus seems difficult to explain biliary carcinogenesis in patients with an anomalous junction merely on basis of regurgitation of pancreatic juice or the activation of pancreatic enzymes from our data.

The close chemical relationship of bile acids and methylcholanthrene, the most active known carcino-

genic agent, has been noted since 1940.¹⁵ However, a few reports¹⁶ have dealt with the changes of bile acid fractions in the bile-pancreatic juice mixture in maljunction patients and only Kato¹⁷ measured such changes in relation to carcinogenesis.

Many clinical or experimental studies concerning the tumor enhancing effects of bile or bile acids have been performed in models of colonic,^{18–22} gastric²³ and hepatic carcinogenesis²⁴ as well as *in vitro*.^{25,26} Many investigators^{20,21,27} have pointed out a cancer-promoting effect of bile acids. Wilpart *et al.*²⁶ reported a co-mutagenic effect of secondary bile acids, while neither cholic nor chenodeoxycholic acid had the same activity. Cohen *et al.*²⁷ reported the effect of cholic acid, a primary bile acid, on N-methyl-N-nitrosourea-induced colon tumors in rats. Secondary bile acids are formed in the colon by the bacterial metabolism of primary bile acids. The secondary bile acids, deoxycholic acid and lithocholic acid, markedly damage the cellular membrane and they may also activate pancreatic enzymes like phospholipase A2 and lipase. Unconjugated bile acids are also toxic to the cell membrane.^{28,29}

In this study, it was found that DCA, LCA, and unconjugated bile acids were increased in the bile duct bile from cystic dilatation with pancreaticobiliary maljunction regardless of the presence or absence of malignancy. A slight increase of LCA was observed in the gallbladder bile from two gallbladder cancer patients without bile duct dilatation, and an increase of LCA and DCA was seen in gallbladder bile from a patient with cancer of the confluence of the common bile duct and the cystic duct associated with cylindrical choledochal dilatation. In such patients, stagnation of a mixture of pancreatic juice and bile would take place in the gallbladder.

In conclusion, we speculate that in patients with pancreaticobiliary maljunction the bile is mixed with regurgitated pancreatic juice and stagnates in the biliary system, and that concomitant changes in the bile acid fractions, *i.e.* an increase in DCA, LCA, and/or unconjugated bile acids, might be one of the etiological factors of biliary carcinoma.

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