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Ascitic Bile Acids in Patients with Liver Cirrhosis

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ABSTRACT

Ascitic bile acids of 8 patients with liver cirrhosis were analyzed in order to elucidate the clinical significance.

The results were as follows.

1) Ascitic bile acid concentrations in intractable ascites were high, although low in tractable ascites.

2) Among the 8 patients, 4 had serum bile acid analysis performed and a significant correlation of bile acid concentration was found between ascites and serum ($P<0.01$).

3) Ascitic bile acid concentration was positively correlative with ascitic protein concentration ($P<0.05$).

4) In one patient whose group separation on Piperidinohydroxypropyl Sephadex LH-20 was determined, free bile acids were found to predominate the major part of the ascitic bile acids.

These results suggest that ascitic bile acids reflect serum bile acids, and that bile acids with protein are filtered into ascitic fluid. Further patient studies will be necessary to shed light on the relationship between intractability of ascites and high ascitic bile acid concentrations.

Although bile acids have been detected in bile, serum, feces, urine, and other compartments of body fluids, no report had been made about ascitic bile acids until we described their existence in intractably ascitic fluid of a cirrhotic patient[4]. In our previous study, however, we could not sufficiently discuss their clinical implications since ascitic bile acids were analyzed in only one case. So, in the present study, ascitic bile acids of various stages of liver cirrhosis were investigated to determine whether ascitic bile acids which have escaped from the enterohepatic circulation have any clinical significance.

MATERIALS AND METHODS

Chemicals: Sep-Pak C_{18} bonded cartridge was purchased from Waters Associates, Inc. (Milford, Ma) and Sephadex LH-20 from Pharmacia Fine Chemicals (Uppsala, Sweden). 7α, 12α-dihydroxy-5ß-cholan-24-oic acid was synthesized as previously reported[3]. Piperidinohydroxypropyl Sephadex LH-20(PHP-LH-20) was prepared according to the method described by Goto et al[2]. All other chemicals were reagent grade.

Patients (Table 1): To analyze the ascitic bile acids, ascitic fluids of 8 consecutive patients who were hospitalized for liver cirrhosis in the 2nd Department of Surgery, Hiroshima University Hospital in the past 3 years were obtained by paracentesis or laparotomy. Among the 8 patients, 6 patients had much ascitic fluid. Cases 1,2,3,4 and 5 had intractable ascites. Cases

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Table 1. Clinical data of 8 patients with cirrhotic ascites

Case	Age(yr.)	Portal hypertension	Ascitic volume	Ascitic protein (g/dl)	Prognosis
1 TI	65	—	Remarkable	0.7	Dead due to hepatic coma
2 ST	61	—	Remarkable	3.0	Dead due to hepatic coma
3 TI	60	+	Remarkable	3.6	Dead due to hepatic coma
4 KM	57	+	Remarkable	3.3	Dead due to hepatic coma
5 TK	67	+	Remarkable	2.4	Alive
6 MK	52	+	Moderate	1.0	Dead due to cerebral apoplexy
7 KM	34	—	Remarkable	0.5	Alive
8 TM	52	+	Slight	1.3	Alive

1,2,3 and 4 died of hepatic coma. The amount of ascitic fluid in Case 7 was decreased by conservative therapy. In Case 6, about 1 week before death due to apoplexy a moderate volume of fluid was clinically found and obtained at autopsy. As for Case 8 with esophageal varices a small quantity of ascitic fluid was found at laparotomy, and never observed postoperatively.

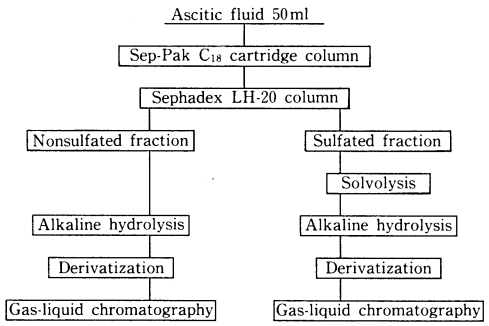

Fig. 1. Outline of procedure for analysis of ascitic bile acids

Methods

1) Bile acid analysis; Analytical procedures of ascitic bile acids on gas-liquid chromatography are summarized in Fig. 1. A 50 ml sample of ascitic fluid obtained from each patient was mixed with 300 ml of ethanol for deproteinization. After being left for a day, ethanolic solution was filtered and evaporated to dryness. The following procedures were as described previously[6], except for using Sep-Pak C_{18}[9] cartridge instead of Amberlite XAD-2 for bile acid extraction. In brief, the residue was dissolved in water and percolated through the Sep-Pak C_{18} cartridge and eluted with methanol after washing the cartridge. The resulting sodium salts were divided into two fractions of differing polarity by column-chromatography on Sephadex LH-20, separating nonsulfated from sulfated fraction. The nonsulfated fraction was eluted with a mixture of chloroform/methanol (1:1, vol/vol), containing 0.01M sodium chloride. The sulfated fraction was eluted with methanol. Then a 100 μg of 7α, 12α-dihydroxy-5ß-cholan-24-oic acid was added to each fraction as an internal standard. After the sulfated fraction was solvolyzed, the bile acids of both fractions were deconjugated by alkaline hydrolysis (at 120°C for 3 hr) followed by extraction. Then the bile acids were analyzed by gas-liquid chromatography as their methyl ester trimethylsilyl ether derivatives. All gas-liquid chromatographic analyses were carried out on model Shimadzu GC-7A with 2% Poly I-110 and 3% OV-17 columns.

To investigate the conjugation of the ascitic bile acids, ascitic bile acids in Case 7 were also examined through PHP-LH-20 column. Because the ascitic fluid of Case 8 was of such small volume, ascitic total bile acids were determined only by the enzymatic method[7].

2) Collection of ascites and other examinations; Ascitic fluid of each patient was obtained in fasting state. In order to follow the change of ascitic bile acids, ascitic fluid was collected 3 times every one month in Case 4. In Cases 2, 5, 6, and 8, peripheral blood was drawn simultaneously with the collection of ascitic fluid and serum total bile acids were examined by the enzymatic method. Routine liver function tests were carried out on a sampling of ascitic fluid, and ascitic protein levels were also determined in all cases.

Table 2. Serum biochemical data in liver function tests on a sampling of the ascites

Case	Age(yr.)	Total protein	Albumin	Total bilirubin	Direct bilirubin	GOT	GPT	TTT	ZTT
1 TI	65	6.3	2.2	1.7	0.8	64	47	12	36
2 ST	61	6.1	1.6	6.3	5.1	1241	296	10	41
3 TI	60	7.6	3.1	23.2	15.5	111	87	4	12
4 KM	57	7.5	3.9	3.5	2.3	153	51	10	26
5 TK	67	6.7	3.1	2.2	1.7	131	65	16	16
6 MK	52	5.8	2.2	5.7	—	138	42	4	10
7 KM	34	5.5	1.8	1.3	0.8	108	37	10	14
8 TM	52	7.0	3.7	0.7	0.4	47	26	7	8
		(6.3-8.2)	(3.6-5.1)	(1.0↓)	(0.4↓)	(5-35)	(30↓)	(4↓)	(4-12)
		g/dl	g/dl	mg/dl	mg/dl	U.	U.	U.	U.

Numbers in parentheses indicate normal range.

Table 3. Levels of the ascitic bile acids in 8 patients with liver cirrhosis (mg/liter)

Case	Fractions	CA	CDC	DC	UDC	Total	%sulfated bile acids
1 TI	NS	16.94	7.52	—	—	24.26	0
	S	—	—	—	—		
2 ST	NS	7.46	16.62	—	—	24.08	0
	S	—	—	—	—		
3 TI	NS	7.31	9.72	0.07	—	19.51	12.4
	S	0.12	1.56	—	0.73		
4 KM	NS	9.91	16.46	1.76	7.49	41.17	13.5
	S	0.85	3.19	0.54	0.97		
5 TK	NS	7.56	13.07	—	0.57	21.20	0
	S	—	—	—	—		
6 MK	NS	2.70	1.44	0.54	0.15	7.15	35.1
	S	0.41	1.33	0.49	0.28		
7 KM	NS	0.76	0.20	—	—	1.28	25.0
	S	0.12	0.20	—	—		
8 TM*						0.65	—
Mean ± SD						17.44±13.75	12.3±13.8

*Case 8 was analyzed according to enzymatic method.
NS; nonsulfated bile acid, S; sulfated bile acid, CA; cholic acid
CDC; chenodeoxycholic acid, DC; deoxycholic acid, UDC; ursodeoxycholic acid

Table 4. Conjugation and composition of ascitic bile acids in Case 7 with liver cirrhosis (%)

	Free bile acid	Glycine bile acid	Taurine bile acid	Sulfated bile acid	Total
Cholic acid	40.6	13.3	5.5	9.4	68.8
Chenodeoxycholic acid	15.6	—	—	15.6	31.2
Total	56.2	13.3	5.5	25.0	100.0

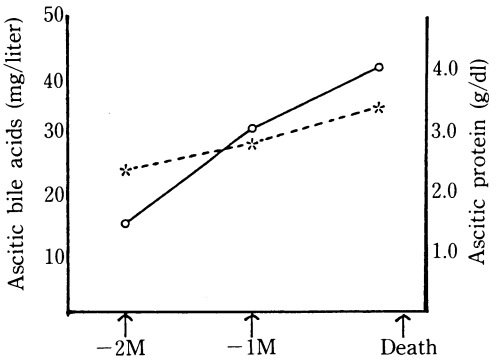

Fig. 2. Changes of ascitic bile acid and ascitic protein concentration.
○—○; ascitic bile acids, *...*; ascitic protein.

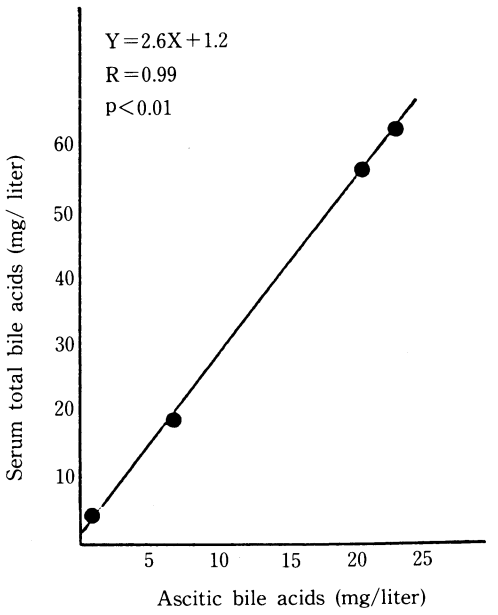

Fig. 3. Relationship between ascitic bile acid and serum total bile acid concentration in 4 patients (Case 2, 5, 6, and 8) with liver cirrhosis.

Table 5. Peripheral serum total bile acid levels according to enzymatic method (mg/liter)

Case 2	62.8
Case 5	56.7
Case 6	18.5
Case 8	3.6

RESULTS

The levels of ascitic protein in each patient are shown in Table 1, and the serum biochemical

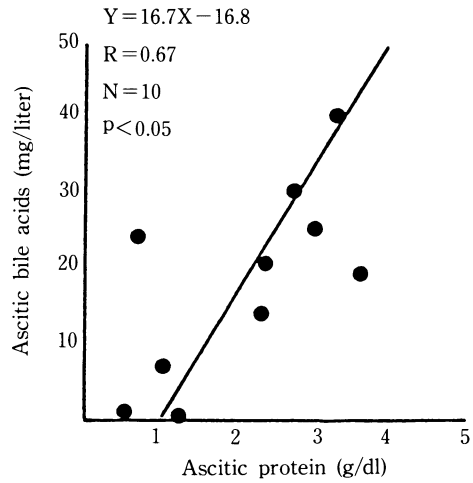

Fig. 4. Relationship between ascitic bile acid and ascitic protein concentration of 10 samples in 8 patients with liver cirrhosis (Case 4 contains 3 samples).

data of liver function tests in Table 2. The values of ascitic bile acids widely ranged from 0.65 ml/liter to 41.17 mg/liter as given in Table 3. The mean concentration of ascitic bile acids was 17.44 ± 13.75 (mean ± SD) mg/liter. In Cases 1, 2, and 5, sulfated bile acids were not detected. There was no definite tendency about bile acid composition. The levels of both ascitic bile acids and ascitic protein increased over time in Case 4 (Fig. 2). The percentage of conjugation and composition of ascitic bile acids in Case 7 is shown in Table 4. Free bile acids composed the major part of ascitic bile acids in Case 7. Serum bile acid levels analyzed according to the enzymatic method in Cases 2, 5, 6, and 8 (Table 5) showed a positive correlation to the ascitic bile acid levels as shown in Fig. 3 ($P<0.01$). In 10 ascitic samples which contained 2 samples aspirated on other days in Case 4 (Fig. 2), a positive correlation was found between ascitic bile acid and ascitic protein levels ($P<0.05$, Fig.4).

DISCUSSION

As shown in Fig. 3, bile acids in ascitic fluids were statistically correlated with those in serum. Therefore, ascitic bile acids may originate from serum bile acids. Although ascitic fluid is generally considered to be an ultrafiltrate of serum in cirrhosis, it has not been discussed whether bile acids of serum are filtered into abdominal

fluid. In the present study, it has been confirmed that ascitic bile acids are also a filtrate of serum in cirrhosis, and that they reflect serum bile acids to some extent.

Among the 8 patients, 5 patients (Cases 1 to 5) with intractable ascites which could not be improved by any conservative treatments had high ascitic bile acid concentrations, more than 20 mg/liter (Table 3). On the other hand, ascitic bile acid value in Cases 6, 7, and 8 with ascitic fluids which were relatively tractable for treatment were low, less than 8 mg/liter (Table 3). Furthermore, in Case 4 with intractable ascites, ascitic bile acid concentration increased in proportion as the clinical status deteriorated (Fig. 2). These results seem to suggest that there is some relationship between intractability of ascites and high ascitic bile acid concentrations. That is to say, there is one possibility that ascitic bile acids play some role on the formation of intractable ascites. There is another possibility that high ascitic bile acid value in intractable ascites is a result of severe liver cirrhosis, because it is frequently associated with intractable ascites. Although ascitic bile acid concentration is conventionally considered to be a result of, rather than a cause of, intractable ascites, the former possibility should not be ignored. Heaton pointed out[5] that one of the roles of the enterohepatic circulation was to keep bile acids, which possess detergent properties, away from other parts of the organism in which they might be harmful. In other words, while a detergent is precious in the right place, it is also noxious in the wrong place. Future animal experiments need to be done to ascertain whether ascitic bile acids which have escaped from the enterohepatic circulation are noxious and play some role in the formation of intractable ascites. In any case, it is evident that a knowledge of standardized levels of ascitic bile acids would give some information as to the state of a cirrhotic patient.

It is well known that bile acid in serum is bound to serum protein[8]. To our surprise, ascitic bile acids were found to be significantly correlated with ascitic protein (Fig. 4). This result in our study suggests that serum bile acid bound to protein is filtered into ascitic fluid. The similar results were described by Burch et al[1]. In their paper, zinc which was bound to serum protein was reported to be positively correlated with protein in ascitic fluids of cirrhotic patients. These findings suggest that substances bound to serum protein, such as bile acid or zinc, are filtered from the serum into ascitic fluid with carrier proteins.

In Case 7, the percentage of free bile acids is greater than that of conjugated bile acids in ascitic fluid (Table 4). It is unknown whether there is a difference in the filtering rate between free and conjugated bile acid into ascitic fluid. Free bile acid has less molecular weight than the conjugated one, so that free bile acid is considered to be filtered through the vascular wall more easily than the conjugated one. However, we would like to defer such a hasty conclusion until the conjugations of bile acids in serum, especially in the portal vein, have been made more clear in a larger number of cirrhotic cases.

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