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Bile Salt Sulphation in Man

Liver bile salt sulphotransferase activity in patients with primary biliary cirrhosis

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ABSTRACT

Bile salt sulphation in primary biliary cirrhosis was studied by measurements of the liver bile salt sulphotransferase levels in 16 patients. Although the enzyme activity varied among the patients it did not correlate with the severity of cholestasis. Furthermore, the mean bile salt sulphotransferase magnitude in patients with primary biliary cirrhosis did not differ significantly from corresponding enzyme activity in patients with non-cholestatic, alcohol induced liver disease. The present data indicates that chronic cholestasis, as evidenced in patients with primary biliary cirrhosis, does not lead to increased levels of liver bile salt sulphotransferase. It is suggested that mechanisms other than enzymic induction are responsible for the increased bile salt sulphate synthesis as observed in primary biliary cirrhosis.

INTRODUCTION

Sulphation, identified by Palmer (14) as one pathway of the human bile salt metabolism, occurs to only a minor extent in the healthy man (1, 12, 21, 26). Patients with cholestasis eliminate large amounts of bile salt sulphates in the urine (1, 12, 21, 23, 26). Although the pathophysiological significance of bile salt sulphation has not been determined it is regarded to be important for the elimination of potentially toxic bile salts (22). However, few data concerning the enzymatic mechanisms for bile salt sulphate synthesis in clinical conditions with cholestasis are available. The enzyme, bile salt sulphotransferase, which transfers sulphate groups form 3'-phosphoadenosine-5'-phosphosulphate (PAPS) to bile salts, has been identified in the human liver, (5, 8), small intestine (9) and adrenals (11). In the present investigation bile salt sulphation in primary biliary cirrhosis (PBC), a liver disease characterised by a chronic intrahepatic cholestasis (16), has been studied by measurements of the bile salt sulphotransferase levels in percutaneous liver biopsy specimens.

MATERIAL AND METHODS

Patients

The study was carried out on patients admitted for clinical investigation of liver disease to the Department of Internal Medicine, University Hospital, Uppsala, Sweden. Sixteen patients with primary biliary cirrhosis were examined (Table I). Drug treatment at the time of the study included prednisolone (pat. no. 2, 5 and 10), spironolactone (pat. no. 1 and 2), cholestyramine (pat. no. 4, 7, 11 and 14), cimetidine + levothyroxin (pat. no. 14). The criteria for the diagnosis were: a) blood chemistry analyses of liver function indicative of cholestasis; b) liver histology diagnostic or highly suggestive of PBC. c) normal extrahepatic bile ducts on cholangiography. d) positive serum mitocondrial antibody.

Routine percutaneous liver biopsies were performed ad modum Menghini (13). Liver histology was analysed and classified in four stages by one pathologist. (Stage 1 = florid duct lesion, stage 2 = ductular proliferation, lymphoid aggregates with preserved lobular architecture, stage 3 = fibrosis with septum formation and disturbed lobular architecture, stage 4 = cirrhosis (16).

Liver function was also evaluated by the intravenous galactose tolerance test (24).

Eight patients with alcoholic liver disease were also included in the study (Table II). The patients had no drug therapy. The diagnosis was based on a history of alcohol abuse together with abnormal liver function tests. The clinical investigation did not reveal any alternative etiology to the liver disease. In these patients blood chemistry analyses of liver function were not indicative of cholestasis.

Chemicals

Chemicals used were of analytical grade and obtained from Kebo Grave, Stockholm, Sweden, unless otherwise specified. The sodium salt of glycolithocholate (Calbiochem) was stored dessicated at +4°C. For preparation of buffers and other solutions deionized water was used.

Carrier-free (^{35}S)-labelled sodium sulphate was purchased from the Radiochemical Centre Ltd, Amersham, England.

Radioactive 3'-phosphoadenosine-5'-phosphosulphate ((^{35}S)PAPS) was biosynthesised and purified as earlier described (10). Radioactive samples were counted in 5 ml Lumagel® in a Packard Tricarb 3000 liquid scintillation counter.

Total serum bile salts were determined by a ready made enzymatic kit (Sterognost-3 α Flu, Nygaard & Co, Oslo, Norway).

Tissue preparation

The percutaneous liver biopsy specimens were divided into two parts, one for histological examination and the other for analysis of sulphotransferase activity. The latter specimen was immedeatly transported to the laboratory in ice cold 0.15M KCl containing 0.02 M Tris/HCl (pH 7.5), 0.001 M, EDTA and 0.001 M 2-mercaptoethanol. Unless otherwise specified all further procedures were carried out at +4°C. The tissue was homogenised in 0.5 ml of the aforementioned buffer and after addition of another 0.5 ml of the buffer the homogenate was centrifuged for 60 min. at 105000 x g in a Beckman L2-65B ultracentrifuge. The clear supernatant was pipetted off and stored at -70°C in 0.2-0.3 ml portions until used.

Sulphotransferase assay

A modification (10) of the sulphotransferase assay described previously (8) was used. The incubation mixture contained: a) 2.5 nmol glycolithocholate added as an ethanolic solution and evaporated to dryness in vacuo; b) 7.5 nmol (^{35}S)PAPS, specific activity 50 cpm/pmol, in 100 µl 0.15 M KCl containing 0.02 M Tris/HCl (pH 7.5), 0.004 M EDTA and 0.001 M 2-mercaptoethanol; c) 50 µl of liver cytosol containing 10-40 µg of protein.

Incubations were carried out in duplicate for 30 min. at 37°C followed by butanol extraction and quantification of radioactive sulphate esters by liquid scintillation (8). One enzyme unit was defined as the amount of enzyme necessary to catalyze the formation of one pmol glycolithocholate sulphate per 3 minute. Specific enzyme activity is expressed as units/mg cytosol protein.

The protein content of liver cytosol was determined by the method of Lowry (7).

Statistics

Students t-test was used for testing the statistical significance between two means. Means ($\bar{x}$) and standard deviation (S.D.) were calculated by conventional methods (2).

RESULTS

Liver bile salt sulphotransferase activity towards glycolithocholate in patients with PBC, Table I, varied between 42 and 193 units/mg cytosol protein. No obvious difference with respect to the sulphotransferase activity was found between drug treated patients and patients without drugs. The lowest enzyme activities belonged to the two men included in the PBC group. No correlation was found between bile salt sulphotransferase activity and serum concentration of bilirubin, total bile salt and alkaline phosphatases or to the intravenous

Table 1. Primary biliary cirrhosis. Patient data.

Patient No.	Age years	Sex	Liver histology stage I-IV	S-bilirubin µmol/l (4-21)*	S-alk. phosph. µkat/l (0.8-4.8)	S-bile salt µmol/l (2.7-7.3)	AMA titre (neg)	I.v. galaktose tolerance test T ½ min (< 17 min)	Liver bile salt sulphotransferase units/mg cytosol protein
1	74	♀	II	7	11.5	2.0	1/400	21	94
2	52	♀	III	19	14.0	22.0	1/400	13	117
3	43	♀	III	9	16.7	7.0	1/400	18	100
4	48	♀	III	82	42.0	17.0	1/100	18	126
5	64	♂	III	30	6.0	17.0	1/400	19	42
6	47	♂	III	27	17.5	22.0	1/100	15	76
7	57	♀	IV	78	11.7	39.3	1/400	23	188
8	66	♀	IV	174	9.3	54.0	1/100	44	79
9	56	♀	IV	14	11.6	4.6	1/400	23	146
10	62	♀	IV	20	10.4	31.6	1/400	-	121
11	56	♀	IV	19	29.0	17.0	1/400	13	133
12	39	♀	IV	29	25.0	12.0	1/25	17	125
13	42	♀	IV	151	35.0	59.0	1/100	11	72
14	36	♀	IV	343	19.2	270.0	1/400	16	122
15	59	♀	IV	12	37.0	25.0	1/400	28	124
16	59	♀	IV	34	12.8	10.0	1/400	21	193
Mean±SD	54±11			66±89	19.3±11.0	38±64.0		20±8	116±40

* Normal range of serum values is given in paranthesis.

galactose tolerance test. Moreover, no correlation was found between the bile salt sulfotransferase level and the known duration of the disease.

The bile salt sulphotransferase activity in patients with alcoholic liver disease, Table II, varied between 70 and 136 units/mg cytosol protein. Also in this group the lowest values were found in the male patients.

When the mean liver bile salt sulphotransferase activity in patients with PBC (116 ± 40 units/mg cytosol protein) and patients with alcoholic liver disease (106 ± 26 units/mg cytosol protein) were compared no significant difference was found.

DISCUSSION

In the present work liver bile salt sulphotransferase activity was measured in primary biliary cirrhosis. The patients had varying degree of cholestasis as indicated by the serum concentrations of bilirubin and bile salts. The enzyme levels differed by 500 % when the highest and lowest values were compared. However, the variations in bile salt sulphotransferase activity were not correlated to the degree of cholestasis. Furthermore no correlation was found between the bile salt sulphotransferase activity and liver function as evidenced by the intravenous galactose tolerance test. We have not measured the amounts of bile salt sulphates excreted in the urine from these patients. However, other investigations have shown that patients with PBC constantly have an increased urinary elimination of bile salts which mainly appear in the sulphated form (15). Moreover, the magnitude of the bile salt sulphate elimination in the urine seem to correlate well with the serum bile salt concentration (15, 26). Thus, the present data suggest that patients with PBC, a clinical condition characterized mainly by the symptoms of a chronic cholestasis, do not increase their sulphation capacity by induction of liver bile salt sulphotransferase when the cholestasis progresses. This assumption is further supported by the observation that the mean bile salt sulphotransferase activities in PBC and in alcoholic liver disease with no clinical signs of cholestasis, respectively, do not differentiate significantly. These results also agree with the reported absence of bile salt sulphotransferase induction in the livers from bile duct ligated hamsters (4). The individual data of the male patients in the PBC group and in the alcohol liver disease group with respect to bile salt sulphotransferase activity had a tendency to be lower than the corresponding enzyme activity in females. We have not observed a sex difference with respect to human bile salt sulphotransferase activity in the liver in previous studies (9). However, steroid sulphotransferase activity, including bile salt sulphotransferase, in the rat and hamster seem to be significantly influenced by sex hormones (3, 17, 18, 19, 20). Female rat and hamster livers

Table 2. Alcoholic liver disease. Patient data.

Patient No.	Age years	Sex	Liver histology	S-bilirubin µmol/l (4-21)	S-alk. phosph. µkat/l (0.8-4.8)	S-bile salt µmol/l (2.7-7.3)	I.v. galaktose tolerance test T ½ min (< 17 min)	Liver bile salt sulphotransferase units/mg cytosol protein
1	54	♂	normal	8	4.8	1.5	15	70
2	41	♂	steatosis	6	5.3	4.5	12	78
3	38	♀	steatosis	20	2.1	6.5	14	127
4	45	♂	cirrhosis	11	2.0	3.5	23	106
5	73	♂	inflammation, siderosis	4	3.3	4.5	31	129
6	63	♂	normal	9	2.8	2.5	20	80
7	22	♀	normal	7	2.2	2.5	11	118
8	44	♀	steatosis	19	3.5	10.0	16	136
Mean±SD	48 ± 16			11 ± 6	3.3±1.2	4.4 ± 2.7	18 ± 7	106 ± 26

contain two to six times the activity in males (17, 20). Even if such a sex difference might be present in man and is taken into consideration because of the uneven sex distribution between the PBC and alcoholic liver group it would rather support the observed absence of bile salt sulphotransferase induction in primary biliary cirrhosis.

The increased biosynthesis of bile salt sulphates as observed in PBC (15, 26) may instead be caused by a better substrate availability for bile salt sulphotransferase when the bile salt concentration increases in the cholestatic liver as suggested by Barnes (4). In addition extrahepatic sulphation, e.g. in the small instestine or adrenals (9) may contribute to the enhanced sulphation of bile salts in primary biliary cirrhosis.

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