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BILE ACIDS OF PATIENTS WITH RENAL FAILURE (POSSIBILITY OF BILE ACID SECRETION IN THE DISTAL TUBULE)

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Bile acids of patients with renal failure and of healthy subjects were analyzed by capillary gas chromatography after group separation. The amount of bile acids in the total dialysate (150 L) of the patients was smaller than that in the 24-h urine of healthy subjects. Polar bile acid sulfates constituted 17.3% and 30.9% of the total bile acids in serum and urine of healthy subjects, respectively, 26.0% in predialysis serum of patients, and only 11.3% in dialysate of patients. The amount of bile acid sulfates in the hemodialysate converted during a 24-h period dialysis, was still smaller than that in 24-h urine of healthy subjects. We propose that the distal tubule secretes bile acid sulfates. © 1988 Academic Press, Inc.

In the healthy kidney, some of the bile acids in blood are excreted into urine as a minor component, while in patients on hemodialysis, bile acids in blood are identified in their hemodialysate (1-3). This suggests that in patients with renal failure, hemodialysis replaced the kidney's function to excrete bile acids as well as such compounds as electrolytes and metabolites. However, the mechanism of bile acid excretion in the healthy kidney is not clear; we only know that the renal excretion rate of sulfated bile acids is greater than that of nonsulfated bile acids (4-6) and bile acids except for the bile acid sulfates are reabsorbed in the proximal tubule (7).

Abbreviations:

αα, 7α,12α-dihydroxy-5β-cholan-24-oic acid; ββ, 7β,12β-dihydroxy-5β-cholan-24-oic acid; glyco-αα, glyco-7α,12α-dihydroxy-5β-cholan-24-oic acid; tauro-ββ, tauro-7β,12β-dihydroxy-5β-cholan-24-oic acid; glyco-αα 7α-sulfate, glyco- 7α,12α-dihydroxy-5β-cholan-24-oic acid 7α-sulfate; lithocholic acid (LC), 3α-hydroxy-5β-cholan-24-oic acid; $3\beta OH\Delta^5$, 3β-hydroxy-5-cholen-24-oic acid; deoxycholic acid(DOC), 3α,12α-dihydroxy-5β-cholan-24-oic acid; chenodeoxycholic acid(CDC), 3α,7α-dihydroxy-5β-cholan-24-oic acid; ursodeoxycholic acid(UDC), 3α,7β-dihydroxy-5β-cholan-24-oic acid; cholic acid(CA), 3α,7α,12α-trihydroxy-5β-cholan-24-oic acid; 7βCA, 3α,7β,12α-trihydroxy-5β-cholan-24-acid; norcholic acid(NCA), 3α,7α,12α-trihydroxy-5β-norcholan-23-oic acid.

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We examined the bile acids in patients with renal failure, to obtain further information on the mechanism of bile acid excretion in the kidney.

SUBJECTS, MATERIALS AND METHODS

Subjects

The subjects were 3 patients with renal failure who had had 5-hr hemodialysis periods two or three times weekly for over two years. None of the patients had any abnormalities in liver function as examined by a usual test.

Hemodialysis for the patients was carried out under the following conditions. The dialyzer used had a membrane through which substances of less than 3,000 in molecular weight could pass. During the hemodialysis, the blood flow in the dialyzer was set up to 100 to 200 ml/min, and dialysate flow, to 500 ml/min.

Specimens

To obtain serum for analysis, blood was taken before and after hemodialysis from the intra-shunt set up for hemodialysis treatment. The serum before dialysis was designated as predialysis serum, and the serum after completion of dialysis, as postdialysis serum. Two liters of dialysate were collected five times every hour from the start of dialysis, and the dialysate (10 L) combined for analysis corresponded to 1/15 of the total dialysate. Fasting serum and 24-h urine obtained from 3 healthy subjects were used as control.

Reagents

All chemicals were of analytical reagent grade, and organic solvents were distilled before use.

Four internal standards, 7β,12β-dihydroxy-5β-cholan-24-oic acid, glyco-7α, 12α-dihydroxy-5β-cholan-24-oic acid, tauro-7β,12β-dihydroxy-5β-cholan-24-oic acid, and glyco-7α,12α-dihydroxy-5β-cholan-24-oic acid 7α-sulfate were synthesized as reported previously(8, 9). Authentic 3α,7β,12α-trihydroxy-5β-cholan-24-oic acid was kindly supplied by Dr. K. Uchida (Shionogi Research Laboratory, Osaka, Japan), and norcholic acid by Prof. Dr. T. Hoshita (Hiroshima University, Hiroshima, Japan).

Extraction of bile acids

Prior to extraction of bile acids, 20 μg of ββ, 25 μg of glyco-αα, 25 μg of tauro-ββ and 25 μg of glyco-αα 7α-sulfate were mixed with 10 L of dialysate, 4 μg of ββ, 5 μg of glyco-αα , 5 μg of tauro-ββ and 6 μg of glyco-αα 7α-sulfate with 1.0-5.0 ml of serum, and 8 μg of ββ, 10 μg of glyco-αα, 10 μg of tauro-ββ and 12 μg of glyco-αα 7α-sulfate with 20 ml of urine.

Extraction of bile acids from each sample was performed as described previously (9). Dialysate adjusted to pH 10, was applied to an Amberlite XAD-2 column (30 X 1 cm I.D.), and then bile acids were eluted with 100 ml of ethanol containing 0.25% ammonium hydroxide. On the other hand, for extraction of bile acids from urine and serum, a 15 X 0.5 cm I.D. Amberlite XAD-2 column was used and bile acids were eluted with 50 ml of the same organic solution.

The bile acids extracted were fractionated to free, glycine-conjugated, taurine-conjugated, and sulfated bile acids by PHP gel column chromatography (9, 10). After either solvolysis followed by hydrolysis or only hydrolysis of each fraction, bile acids were extracted with ethyl ether from the acidified solution.

Capillary column gas chromatography

After methylation and dimethylethylsilylation (11), the bile acids were analyzed by gas chromatography (Model GC 4 CPF, Shimadzu, Japan) with a

capillary column (CBP-1 Hicap, 25 m, Shimadzu, Japan) which was maintained at 290°C.

Determination of bile acids

Lithocholic acid, 3β-hydroxy-5-cholen-24-oic acid, deoxycholic acid, chenodeoxycholic acid, ursodeoxycholic acid, cholic acid, 3α,7β,12α-trihydroxy-5β-cholan-24-oic acid and norcholic acid were identified by relative retention time of each bile acid to internal standard, αα or ββ (9).

The quantification of the above bile acids was carried out by applying the peak ratio of each bile acid to an internal standard on a calibration curve. The amounts of bile acids are represented as the weight of free form, and their sum was assumed to be the amount of total bile acids.

RESULTS AND DISCUSSION

Bile acids in blood have been reported to be transferred into the dialysate by hemodialysis and the high concentration of serum bile acid in patients with cholestasis or renal failure to be reduced by hemodialysis (2-3). In this study, 1,761 μg of bile acids was present in the total dialysate (Table 1). However, hemodialysis had little effect on the serum bile acid concentration in the present patients, who had a serum concentration of bile acid similar to that of the healthy subjects. Moreover, the amount of bile acids removed in the total dialysate was smaller than that in the 24-h urine of healthy subjects. These results suggest that 5-h hemodialysis did not replace the function of a healthy kidney to remove of bile acids in blood, but in the present patients, the bile acids transferred into dialysate were transferred into the systematic blood from the liver, because the serum bile acid concentration was maintained at the level in the healthy subjects.

Table 1. The amounts and composition of bile acids in several samples

	Amount of bile acids	Composition (%) of bile acids							
		LC	DOC	CDC	UDC	CA	7βCA	3βOHΔ^5	NCA
Healthy urine	5,233 μg/24 h	14.3	14.2	38.4	17.1	13.5	2.6		
Healthy serum	2.88 μg/ml	10.4	20.3	28.1	10.7	16.9	5.9	8.2	
Dialysate	1,761 μg/5 h	5.4	12.7	14.3	11.3	51.5	1.3		3.5
Predialysis serum	3.24 μg/ml	11.5	17.0	45.2	5.0	13.7	1.3	2.0	4.3
Postdialysis serum	3.11 μg/ml	30.9	15.2	27.3	8.1	5.9	1.5	2.9	8.1

Numbers represent the mean values obtained on 3 patients (lower 3 rows) and 3 healthy (upper 2 rows) subjects.

A characteristic finding was that cholic acid in dialysate constituted 51.5% of the total bile acids which was higher than that found in predialysis or postdialysis serum, healthy serum or healthy urine (Table 1). This was similar to the findings reported previously (3), and may be explained by the finding that although bile acids in blood do not pass through the dialysis membrane because they bind with protein (12-14), cholic acid has the lowest binding rate with protein (15, 16), and is also more water soluble than other bile acids (4, 5).

Table 2 shows the composition of chemical forms on bile acids in several samples. Polar bile acid sulfate constituted 17.3% of the total bile acids in serum of healthy subjects, while urinary bile acid sulfates constituted a higher proportion (30.9%). These findings are in agreement with the data reported by others (17-20). On the other hand, sulfated bile acids in dialysate, which may be regarded as urine, in patients with renal failure constituted only 11.3%, although polar bile acid sulfates in predialysis serum constituted a higher proportion than in healthy serum. The incomprehensible results may be explained by the effect of the number of hydroxyl groups in the bile acid molecule on the binding rate of bile acids with protein in blood, but not by their chemical form such as free, conjugated and sulfated bile

Table 2. The composition of chemical forms on bile acids in several samples

	Amount of bile acids	Composition (%) of chemical forms			
		F	G	T	S
Healthy serum	2.88 μg/ml	31.7	32.2	18.7	17.3
Healthy urine	5,233 μg/24 h	32.2	28.4	9.1	30.9 (1,617 μg)
Dialysate	1,761 μg/5 h	14.8	43.8	29.8	11.3 (199 μg)
Predialysis serum	3.24 μg/ml	11.3	19.3	43.4	26.0
Postdialysis serum	3.11 μg/ml	15.1	15.3	46.0	23.5

Numbers represent the mean values obtained on 3 patients (lower 3 rows) and 3 healthy subjects (upper 2 rows).
F, G, T, and S indicate free, glycine conjugated, taurine conjugated, and sulfated bile acid fractions, respectively.

acids (15, 16), and from the fact that free, glyco and tauro cholic acids are reabsorbed in the proximal tubule by an active transport mechanism in dogs (7). Moreover, bile acid sulfates are hardly reabsorbed in the intestine (6).

The amount (199 μg) of sulfated bile acids in 150 L of dialysate during 5-h hemodialysis was smaller than that in the 24-hr urine of healthy subjects. Now, let us suppose that in a unit time, the amount of bile acids filtrated in the glomerule is equal to the amount of bile acids transferred into dialysate, and also that the bile acid composition of filtrate in glomerule is equal to that of dialysate. Moreover, sulfated bile acids appear not to be reabsorbed in the renal tubule. Therefore, the amount of bile acid sulfates (1,617 μg) in the 24-h urine of healthy subjects is still larger than that (955 μg = 4.8 X 199 μg) in the whole dialysate, supposing that patients have received a 24-h hemodialysis. These findings can not be explained solely by the fact that bile acid sulfates are filtrated in the glomerule and then are hardly reabsorbed in the renal tubule (6, 7). These findings imply that urinary bile acid sulfates consist of both bile acids which are secreted in the renal tubule, perhaps in the distal tubule, as well as some pigments and urea, and bile acids which are not reabsorbed in the proximal tubule after the filtration in the glomerule. Therefore, we propose that the distal tubule secretes bile acid sulfates.

Whether sulfated bile acids which are present in blood are secreted in the distal tubule or wether the bile acids are sulfated during the process of bile acid secretion in the distal tubule in humans remains to be determined, although sulfotransferase activity has been detected in the cytosol obtained from the kidney and liver of rat, and the human liver, but not in that obtained from the human kidney (21-25).

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