Serum 7 α -hydroxy-4-cholesten-3-one concentrations in the evaluation of bile acid malabsorption in patients with diarrhoea: correlation to SeHCAT test

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Abstract

The synthesis of bile acids is regulated by a homeostatic mechanism in which bile acids returning to the liver from the intestine inhibit their own synthesis. Serum concentrations of the bile acid intermediate 7 α -hydroxy-4-cholesten-3-one reflect the rate of bile acid synthesis whereas bile acid malabsorption can be determined by the SeHCAT test. This study was done to evaluate the correlation between the two tests in humans. Twenty eight patients with chronic diarrhoea were included in the study. Fasting serum was collected for the determination of 7 α -hydroxy-4-cholesten-3-one, and on the same day the γ emitting bile acid analogue SeHCAT was given orally and its fractional catabolic rate assessed by repeated external counting over the upper abdomen during the next seven days. There was a highly significant positive correlation between the two tests ($R_s=0.80$, $p<0.001$). The results show a close relation between intestinal loss and hepatic synthesis of bile acids and imply that analysis of 7 α -hydroxy-4-cholesten-3-one in serum should now be evaluated as a possible convenient method for assessing bile acid malabsorption in patients with diarrhoea.

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SeHCAT, which is resistant to deconjugation by intestinal bacteria, otherwise behaves like taurocholic acid with regard to enterohepatic cycling, hepatic handling, active absorption from the terminal ileum, faecal excretion, and overall turnover in the enterohepatic circulation.²⁻⁶

The rate of synthesis of bile acids is determined by a homeostatic mechanism regulated by the amount of bile acids returning to the liver from the intestine (negative feed back). Thus patients with significant bile acid malabsorption can be expected to have an increased production of bile acids. Cholesterol 7 α -hydroxylase is considered to catalyse the rate limiting reaction in the biosynthesis of bile acids, converting cholesterol into 7 α -hydroxycholesterol.⁷ This sterol is then converted into bile acids via the intermediate 7 α -hydroxy-4-cholesten-3-one (Fig 1). Recent studies have shown that plasma concentrations of 7 α -hydroxy-4-cholesten-3-one reflect the cholesterol 7 α -hydroxylase activity and bile acid production in the human liver.^{8,9}

The aim of this study was to determine whether there is any correlation between the serum concentration of 7 α -hydroxy-4-cholesten-3-one and the result of the SeHCAT test. If so, analysis of serum 7 α -hydroxy-4-cholesten-3-one may eventually be used to identify bile acid malabsorption in humans.

Bile acids are necessary for the absorption of dietary fats and cholesterol from the intestine. They are produced in the liver from cholesterol, stored in the gall bladder, and are excreted into the intestine during meals.¹ More than 95% of the bile acids are then reabsorbed, the major portion by active uptake in the distal ileum. Malabsorption of bile acids can induce diarrhoea, a symptom common to many intestinal diseases. In recent years, the SeHCAT test has been used for the determination of bile acid malabsorption. Selenium-75 homocholic acid taurine (⁷⁵SeHCAT) is a synthetic analogue of the natural conjugated bile acid taurocholic acid with the γ emitting ⁷⁵Se in the side chain.^{2,3}

Methods

PATIENTS AND CONTROLS

The study comprised 28 patients with diarrhoea who were consecutively referred for the SeHCAT test (Table). All of the patients had undergone extensive investigations to evaluate their intestinal function. After a basal clinical and laboratory evaluation excluding heart, kidney, and liver disease, they were followed closely as outpatients and allowed to continue their normal diet. Any antidiarrhoeal drugs were stopped at least three days before the SeHCAT test. On the morning of the SeHCAT test the patients had been fasting overnight. Blood

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Figure 1: A simplified scheme for the conversion of cholesterol (I), into bile acids through 7 α -hydroxycholesterol (II), and 7 α -hydroxy-4-cholesten-3-one (III). The first reaction is catalysed by cholesterol 7 α -hydroxylase.

Data on patients with chronic diarrhoea studied with selenohomocholic acid taurine (SeHCAT) test and serum measurements of 7 α -hydroxy-4-cholesten-3-one

Patient	Age/sex (y)	Duration of symptoms (y)	SeHCAT FCR (pools/day)	7 α -hydroxy-4-cholesten-3-one (ng/ml) ⁸	Additional comments
1	32/F	–	0.035	7	
2	48/F	8	0.055	11	Cholecystectomy
3	46/F	2	0.062	8	
4	68/F	10	0.130	45	Radiation therapy
5	26/M	1	0.153	7	
6	44/F	4	0.159	<4	
7	68/M	50	0.177	7	Cholecystectomy
8	62/F	30	0.196	9	
9	39/F	2	0.204	11	Cholecystectomy
10	46/M	10	0.223	9	
11	53/F	5	0.273	35	Partial gastrectomy
12	30/F	10	0.287	11	Cholecystectomy
13	63/F	5	0.334	24	
14	58/M	32	0.335	32	Partial gastrectomy
15	43/F	12	0.337	51	Lactose intolerance
16	57/F	3	0.345	11	
17	53/F	10	0.374	16	
18	24/F	5	0.403	11	
19	69/F	9	0.421	63	Cholecystectomy, radiation therapy
20	58/F	20	0.454	101	Cholecystectomy, radiation therapy
21	54/F	6	0.466	97	
22	50/F	2	0.576	40	Cholecystectomy
23	76/M	30	0.654	44	Cholecystectomy
24	29/M	3	0.729	103	
25	63/F	6	0.746	111	Cholecystectomy, villous atrophy of ileum, lymphocytic colitis
26	35/M	3	0.782	45	Ileocaecal resection
27	68/F	3	1.140	57	Cholecystectomy, villous adenoma of ileum
28	38/M	6	1.964	170	Extensive ileal resection
Normal values			<0.335	<30	

*For SeHCAT controls (n=29), fractional catabolic rate (FCR)=mean (SEM) 0.225 (0.020) pools/day.

samples were taken, and after centrifugation serum was separated and stored at –20°C until analysed for 7 α -hydroxy-4-cholesten-3-one.

Twenty nine (19 male, 10 female) normal healthy subjects served as controls for the SeHCAT test and 20 (10 male, 10 female) were studied for comparison of serum 7 α -hydroxy-4-cholesten-3-one concentration. A basal clinical and laboratory evaluation was done in all subjects to exclude heart, liver, kidney, thyroid disease, and diabetes. Informed consent to participate in the study was obtained from each patient and control, and the protocol was approved by the ethics committee at Huddinge University Hospital.

SeHCAT TEST

A capsule of SeHCAT (10 μ Ci, Amersham International) was given orally with a drink of water and the initial count rate (100% value) was measured three hours after the intake of isotope (day 0), by an uncollimated gammacamera (Porta Camera, General Electric Nuclear Medical ApS, Horsholm, Denmark). The measurements were repeated on days 2, 4, and 7 after the administration of the capsule and the fractional catabolic rate (FCR) of the SeHCAT was calculated. Further details of the method have been given in a recent paper.¹⁰

ANALYSIS OF 7 α -HYDROXY-4-CHOLESTEN-3-ONE IN SERUM

7 α -Hydroxy-4-cholesten-3-one was analysed as described previously.⁸ Briefly, serum (1 ml) was diluted with two volumes of isotonic saline and extracted on a small column of octadecylsilane bonded silica at 64°C. After a wash with water

and 65% aqueous methanol, 7 α -hydroxy-4-cholesten-3-one was eluted with hexane:chloroform, 95:5 (v/v). Analysis was performed by high performance liquid chromatography (HPLC) on a column (250 $\times$ 4.5 mm) of LiChrospher (Hibar, Si 100, 5 μ m, Merck-Darmstadt) connected to a UV detector (254 nm) with hexane:isopropanol, 95:5 vol/vol as the mobile phase. ³H Labelled 25-hydroxyvitamin D₃ added before extraction and determined in the effluent from the HPLC column, served to correct for losses and differences in injection volumes.

STATISTICAL ANALYSIS

Correlations were evaluated by the calculation of Spearman's correlation coefficient (R_s).

Results

Patients included in the present study had diarrhoea that in some cases was of long duration. They had all been extensively studied to evaluate the cause of their diarrhoea and the possibility of bile acid malabsorption was studied by the SeHCAT test. The result of the SeHCAT test was then compared with the serum concentration of 7 α -hydroxy-4-cholesten-3-one. The table summarises the clinical data and results.

There was a wide range of SeHCAT fractional catabolic rates, with 15 patients displaying abnormal values (FCR $\geq$ 0.335 pools/d) indicating bile acid malabsorption. Twelve patients had both a pathological SeHCAT test and an increased serum concentration of 7 α -hydroxy-4-cholesten-3-one. Thirteen patients showed a normal SeHCAT test, and 11 of these had normal concentrations of 7 α -hydroxy-4-cholesten-3-one. Thus three patients (16, 17, 18) had borderline pathological SeHCAT tests but normal 7 α -hydroxy-4-cholesten-3-one concentrations, and two patients (4, 11) with normal SeHCAT tests displayed a slightly increased concentration of the bile acid intermediate. There was a highly significant positive correlation ($R_s=0.80$, $p<0.001$; fig 2) between the two tests.

Ten of the patients were cholecystectomised, four of whom had additional intestinal pathology. For the cholecystectomised patients without intestinal pathology, four (2, 7, 9, 12) had normal SeHCAT elimination and 7 α -hydroxy-4-cholesten-3-one values whereas two (22, 23) had bile acid malabsorption and a slight increase in bile acid synthesis as measured by the bile acid intermediate.

Twelve of the patients, including 10 with pathological SeHCAT results, were treated with cholestyramine. Eight responded well whereas four patients including the two with normal SeHCAT values did not respond. The other two patients who did not show a positive response had lymphocytic colitis (patient 25) and extensive ileal resection (patient 28).

Discussion

Bile acid malabsorption may be involved in the development of chronic diarrhoea in disorders of

Figure 2: Serum concentration of 7 α -hydroxy-4-cholesten-3-one v SeHCAT fractional catabolic rate in the patients ($R_s=0.80$, $p<0.001$). Closed circles denote cholecystectomised patients.

the terminal ileum, ileal resected patients, and in several clinical conditions including post-cholecystectomy, post vagotomy, and functional and idiopathic diarrhoea.¹¹⁻¹⁴ The dihydroxy bile acids, chenodeoxycholic acid and deoxycholic acid, increase intestinal motility and induce net water and electrolyte secretion.^{15,16}

In recent years, the SeHCAT test has been widely used to investigate bile acid malabsorption. This test is simple and avoids intubation or faecal collection as necessary in other tests. The elimination of the isotope from the body measured externally by the gammacamera reflects the fractional catabolic rate of the bile acids. Liver disease may, however, interfere with the SeHCAT results. Furthermore, the interpretation of the SeHCAT test may be influenced by the presence of bacterial overgrowth in the small intestine.

On the other hand, the serum concentration of 7 α -hydroxy-4-cholesten-3-one reflects the bile acid synthesis. In previous studies it was shown that the serum concentration increases in conditions where there is bile acid loss – for example, after ileal resection and cholestyramine treatment, and decreases where there is decreased bile acid synthesis – for example, in cirrhosis of the liver.⁸

In this study we found a good agreement between the two tests, particularly considering the fact that they measure two different variables – namely, bile acid losses and bile acid synthesis. The advantages in determining serum 7 α -hydroxy-4-cholesten-3-one concentration are that it is an even simpler method, that only a serum sample is needed, that no radioactivity needs to be used, and that it is less time consuming.

There were a few discrepancies in the results. It has previously been found that an enhanced bile acid loss may also be the consequence of diarrhoea and increased intestinal transit itself, and bile acid malabsorption is not often likely to be the primary cause of the diarrhoea.¹⁷⁻²⁰ It

could so happen that slight bile acid malabsorption may or may not affect bile acid synthesis. It is also possible that small intestinal overgrowth with deconjugating bacteria was present in some cases, which could counteract a concomitant effect of ileal dysfunction on bile acid synthesis. Such phenomena may have been present in three of our patients, in whom a slight bile acid malabsorption was found with the SeHCAT test, but in whom there was no increase in bile acid synthesis by the liver. On the other hand, the SeHCAT value corresponds to a seven day elimination of bile acids, whereas the concentration of 7 α -hydroxy-4-cholesten-3-one only reflects the synthesis at a certain time. As the concentration of 7 α -hydroxy-4-cholesten-3-one can vary during the day and acute increases have been found, a better correlation may have been obtained if a serum sample had been taken at more than one occasion from each patient. Such diurnal variations may possibly explain why two of the patients showed increased bile acid synthesis but normal SeHCAT values. As substantial interindividual differences in synthesis of bile acids exist, it may be expected that the SeHCAT test could be more sensitive in evaluation of ileal uptake, especially in the borderline case. At any rate, there were no discrepancies among the patients showing grossly abnormal values for either test.

Both the tests showed similar results in the cholecystectomised patients. Bile acid malabsorption and increased synthesis of bile acids were even detected in cholecystectomised patients without intestinal pathology. Contrary to what had been suggested by a previous report,¹² the SeHCAT test may thus be of value also in cholecystectomised patients.

In conclusion, there was a strong positive correlation between circulating concentrations of 7 α -hydroxy-4-cholesten-3-one and values obtained with the SeHCAT test in patients with diarrhoea. This suggests that the determination of the serum concentration of 7 α -hydroxy-4-cholesten-3-one may be useful for the assessment of bile acid malabsorption in such patients. Future prospective studies should now evaluate the clinical relevance of this method, which, through its analytical simplicity and limited patient efforts, should be open to wide use.

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