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The plasma level of 7α-hydroxy-4-cholesten-3-one reflects the activity of hepatic cholesterol 7α-hydroxylase in man

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Circulating levels of 7α-hydroxy-4-cholesten-3-one have been compared with activities of the rate-limiting enzyme in bile acid synthesis, microsomal cholesterol 7α-hydroxylase, measured in liver biopsies obtained from patients undergoing surgery for gallstone disease. Some patients were treated with cholestyramine or bile acids prior to operation in order to alter the feed-back inhibition of the enzyme. The levels of the sterol were similar in untreated patients and in patients treated with ursodeoxycholic acid (median concentration 17 and 13 ng/ml, respectively), and so were the activities of the enzyme (median activity 7.0 and 5.5 pmol/min/mg protein, respectively). The sterol levels and enzyme activities were significantly increased in patients treated with cholestyramine (91 ng/ml and 45 pmol/min/mg protein) and decreased in patients treated with chenodeoxycholic acid (< 2.0 ng/ml and 0.7 pmol/min/mg protein). There was a strong positive correlation ($r = 0.90$, $P < 0.00001$) between levels of 7α-hydroxy-4-cholesten-3-one in plasma and the activities of cholesterol 7α-hydroxylase in the whole patient group. The results show that analysis of 7α-hydroxy-4-cholesten-3-one in plasma is a sensitive and convenient method to determine relative rates of bile acid production in man.

Sterol metabolism; Bile acid biosynthesis; Cholestyramine treatment; Ursodeoxycholic acid treatment; Chenodeoxycholic acid treatment; Human plasma

1. INTRODUCTION

Several liver and intestinal diseases are associated with a changed production of bile acids. Since bile acids are major excretion products of cholesterol [1], such changes will affect the turn-over rate of cholesterol in the body. Cholesterol 7α-hydroxylase is considered to catalyze the rate-limiting reaction in the biosynthesis of bile acids [1], converting cholesterol into 7α-hydroxycholesterol. This sterol is then converted into bile acids *via* 7α-hydroxy-4-cholesten-3-one (Fig. 1). Relative rates of bile acid production may therefore be evaluated in vitro by determination of the activity of cholesterol 7α-hydroxylase in liver biopsies [2,3]. Since sampling is a major obstacle using this assay there is a need for a simple method to determine rates of bile acid synthesis in man. Plasma levels of 7α-hydroxycholesterol have previously been found to correlate positively to the activities of cholesterol 7α-hydroxylase but only in patients with increased enzyme activity [4]. Recently it was reported that plasma levels of 7α-hydroxy-4-cholesten-3-one seemed to reflect both an increased and a decreased production of bile acids [5]. This conclusion was based on a study on patients with predictable changes of bile acid synthesis. We have now examined this apparent relationship by comparing plasma levels of 7α-hydroxy-4-cholesten-3-one and 7α-hydroxycholesterol with activities of cholesterol 7α-hydroxylase in patients treated with bile acids or cholestyramine and the results are reported.

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2. MATERIALS AND METHODS

2.1. *Patients and samples*

Patients participating in this study had gallstones and were subjected to a cholecystectomy at the Huddinge University Hospital. None of the patients had signs of diabetes mellitus, hyperlipoproteinemia, or diseases affecting the liver, thyroid and kidney functions. Informed consent was obtained from each patient and the study was approved by the local Ethical Committee. The patients were divided into the following groups: (A) two men and six women (25–65 years old, median age 36 years) were untreated prior to surgery and served as controls; (B) one man and five women (37–63 years old, median age 54 years) received ursodeoxycholic acid, 15 mg/kg body weight per day, for 3–4 weeks prior to surgery; (C) one man and nine women (23–69 years old, median age 49 years) received chenodeoxycholic acid, 15 mg/kg body weight per day, for 3–4 weeks before the operation; (D) one man and seven women (25–67 years old, median age 56 years) were treated with cholestyramine (Questran, Bristol) in a dose of 8 g twice daily 2–3 weeks pre-operatively. The patients were hospitalized in the surgical ward 1–2 days before the operation. They were given the regular hospital diet. Blood samples were collected after a 12-h fast just prior to operation. Following centrifugation, plasma or serum was separated and immediately stored at −20°C until analyzed. The cholecystectomy was performed between 8 a.m. and 9 a.m. After opening of the abdomen, a 2–3 g liver piece was cut out from the left liver lobe. A small specimen was sent for histological examination. The rest of the biopsy was immediately placed in ice-cold homogenizing medium and transported to the laboratory.

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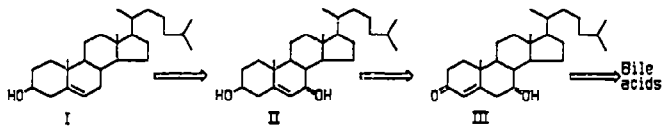

Fig. 1. A simplified scheme for the conversion of cholesterol (I) into bile acids *via* 7α-hydroxycholesterol (II) and 7α-hydroxy-4-cholesten-3-one (III). The first reaction is catalyzed by cholesterol 7α-hydroxylase.

2.2. *Analytical procedures*

The liver homogenate (10% w/v) was prepared in 50 mM Tris-HCl, pH 7.4, containing 50 mM NaCl, 0.3 M sucrose, 10 mM EDTA and 10 mM DTT. The microsomal fraction was prepared as described previously [3]. The microsomal content of protein was determined by the method of Lowry et al. [6]. The activity of cholesterol 7α-hydroxylase in the microsomal fraction was assayed by isotope dilution-mass spectrometry [3]. Essentially the same analytical method was used for analysis of 7α-hydroxycholesterol in plasma/serum [3,4]. Levels of 7α-hydroxy-4-cholesten-3-one in plasma or serum were determined by high-performance liquid chromatography with UV detection as described previously [5].

2.3. *Statistical analysis*

Statistical evaluation of data included calculation of Spearman's rank correlation coefficient and Kolmogorov-Smirnov's two sample test. The median and interquartil range were used as measures of central tendency and variation, respectively [7].

3. RESULTS

The activity of cholesterol 7α-hydroxylase in liver tissue was compared with the level of 7α-hydroxy-4-cholesten-3-one in plasma obtained from patients undergoing surgery for gallstone disease. Some patients were treated with cholestyramine or bile acids prior to operation in order to alter bile acid production by affecting the feed-back inhibition on cholesterol 7α-hydroxylase. The results are summarized in Table I.

As expected from previous studies, treatment with cholestyramine resulted in an increased and chenodeoxycholic acid in a decreased activity of cholesterol 7α-hydroxylase [4]. Treatment with ursodeoxycholic acid had no apparent effect on the enzyme activity. Changes in the levels of 7α-hydroxy-4-cholesten-3-one paralleled those of the enzyme activity. A significant elevation of levels was seen in plasma from patients treated with cholestyramine ($P<0.001$). The levels were subnormal ($P<0.001$) and often below the detection level in plasma from patients treated with chenodeoxycholic acid, whereas patients treated with ursodeoxycholic acid had normal levels. There was no overlap between levels of the controls and those of patients treated with cholestyramine or chenodeoxycholic acid. The correlation between levels of 7α-hydroxy-4-cholesten-3-one and corresponding activities of cholesterol 7α-hydroxylase for the entire patient group is illustrated in Fig. 2 and was highly significant ($r_s = 0.90$, $P<0.00001$). However, with the exception of values for patients treated with ursodeoxycholic acid, there was no correlation between sterol levels and enzyme activities within each group. Whether this was due to the limited number of patients within the groups or to other factors is not known.

For reasons of comparison, plasma levels of 7α-hydroxycholesterol were also determined. As expected, the levels of 7α-hydroxycholesterol were elevated in patients treated with cholestyramine [4]. However, in contrast to those of 7α-hydroxy-4-cholesten-3-one, levels were not decreased in patients treated with chenodeoxycholic acid (Table I).

4. DISCUSSION

Determination of rates of bile acid synthesis is important as a diagnostical aid in liver and gastrointestinal diseases and during dietary, hormonal and pharmacological manipulations of plasma cholesterol levels. However, available methods such as isotope-dilution techniques, analysis of fecal bile acid excretion or assay of the rate-limiting enzyme, cholesterol 7α-hydroxylase

Table I

Concentrations of 7α-hydroxy-4-cholesten-3-one in plasma and activities of hepatic microsomal cholesterol 7α-hydroxylase in untreated patients and patients treated with ursodeoxycholic acid, chenodeoxycholic acid, or cholestyramine. The corresponding concentrations of 7α-hydroxycholesterol are shown for comparison.

Patients[a]	n[b]	Activities of cholesterol 7α-hydroxylase[c] (pmol/min/mg protein)	Concentrations in plasma[c] (ng/ml)	
			7α-hydroxy-4--cholesten-3-one	7α-hydroxy-cholesterol
Untreated	8	7.0:5.5-11.1	17:10-26	17:11-20
Treated with:				
Ursodeoxycholic acid	6	5.5:4.2-9.8	13:10-24	12:8.0-17
Chenodeoxycholic acid	10	0.7:<0.1-2.1[d]	<2.0:<1.6-2.2[d]	7.0:2.0-25
Cholestyramine	8	45:32-50[d]	91:62-140[d]	85:52-127[d]

[a] Details on patients and doses are given in section 2.
[b] Number of patients.
[c] Values are expressed as median: lower quartil-upper quartil.
[d] Median significantly different from that of the untreated group ($P<0.001$).

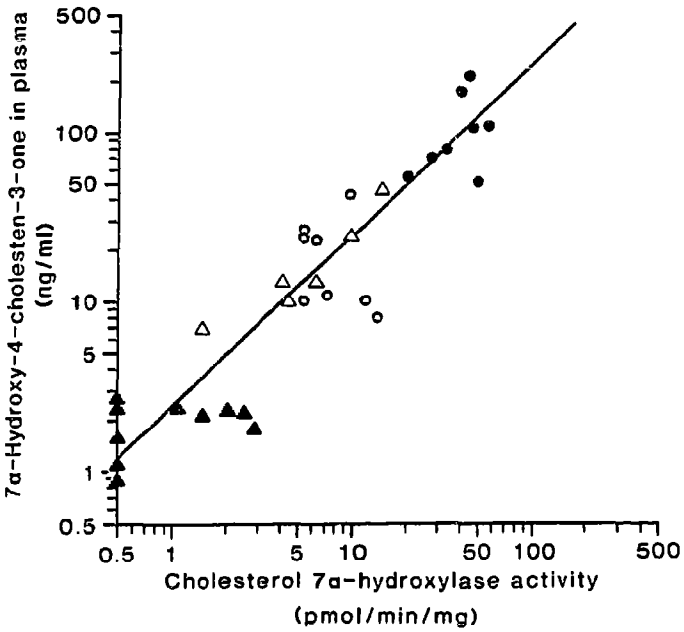

Fig. 2. The relationship between concentrations of 7α-hydroxy-4-cholesten-3-one in plasma and activities of cholesterol 7α-hydroxylase in liver tissue from untreated patients (○) and patients treated with ursodeoxycholic acid (△), chenodeoxycholic acid (▲) and cholestyramine (●).

in liver biopsies [2,3] are complicated and time-consuming.

This study shows that there is a strong positive correlation between the hepatic enzyme activity and the plasma level of 7α-hydroxy-4-cholesten-3-one. Analysis of this sterol may therefore be a convenient method for evaluating the relative rate of bile acid synthesis in humans. Its precursor 7α-hydroxycholesterol has previously been proposed to serve this purpose [4], but this compound only reflects normal and increased enzyme activities.

Also from a methodological point of view, measurement of 7α-hydroxy-4-cholesten-3-one has merits as compared to that of 7α-hydroxycholesterol. The former compound can be accurately determined with a simple HPLC method [5] whereas analysis of 7α-hydroxycholesterol requires more sophisticated methods [4]. Furthermore 7α-hydroxycholesterol can be formed by auto-oxidation of cholesterol and may be artifactually produced in the handling of plasma samples. This is not the case with 7α-hydroxy-4-cholesten-3-one [8]. Thus, it may be concluded that 7α-hydroxy-4-cholesten-3-one is a better marker for the activity of cholesterol 7α-hydroxylase than 7α-hydroxycholesterol.

Theoretically the level of 7α-hydroxy-4-cholesten-3-one in plasma should be dependent upon several factors other than cholesterol 7α-hydroxylase activity, e.g. substrate availability, activity of the hepatic 3β-hydroxysteroid-Δ^5-dehydrogenase, rate of diffusion/secretion of the sterol into blood and rate of metabolism/elimination. These factors may vary between patients and could possibly contribute to the poor correlation between sterol levels and enzyme activities observed within the patient groups. In spite of this, levels of 7α-hydroxy-4-cholesten-3-one in plasma are normally within a relatively narrow range (6–30 ng/ml) [5], suggesting that such factors are relatively constant.

It should be emphasized that circulating levels of 7α-hydroxy-4-cholesten-3-one do not necessarily reflect the total production of bile acids in man under all conditions. Thus a portion of bile acids can be formed *via* pathways by-passing cholesterol 7α-hydroxylase as the rate-limiting step [9]. The relative importance of such pathways is not known at present.

In conclusion, the strong correlation between circulating levels of 7α-hydroxy-4-cholesten-3-one and activities of cholesterol 7α-hydroxylase in human liver shows that analysis of 7α-hydroxy-4-cholesten-3-one can be used in studies on bile acid synthesis in man. Since plasma samples can be collected with short time intervals, the method is particularly attractive to use when rapid changes in the activity of the cholesterol 7α-hydroxylase are to be studied in vivo.

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