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CHOLANOIC ACIDS AND CHOLESTEROL 7-α-HYDROXYLASE ACTIVITY IN HUMAN LEUCOCYTES[1]

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Evidence is presented for the identification of cholanoic acids and cholesterol 7α-hydroxylase activity in human leucocytes. Mononucleated cells contained most of the detectable cholanoic acids. Cholesterol 7α-hydroxylase activity was present only in the mononuclear cells. These data suggest that cholanoic acids in leucocytes may have originated by local biosynthesis. Because of their lipid solubilizing properties, the cholanoic acids might have a function in the phagocytic activity of leucocytes.

INTRODUCTION

The conversion of cholesterol into bile acids is catalyzed by microsomal enzymes and requires the transformation of cholesterol into 7α-hydroxycholesterol. Cholesterol 7α-hydroxylase, the enzyme that mediates this transformation, controls the overall rate of bile acid synthesis (1). Despite evidence that bile acids are present in tissues other than liver (2,3), it still is generally thought that this enzyme is localized only in liver microsomes (4,5). Herein, we report both the presence of cholanoic acids and cholesterol 7α-hydroxylase activity in human leucocytes and verification that 7α-hydroxycholesterol is the end product of the cholesterol 7α-hydroxylase assay.

MATERIALS AND METHODS

Venous blood samples from normal healthy subjects were obtained by venipuncture in vacutainer tubes containing heparin. Mononuclear cells were isolated by layering over Ficoll-Paque (Pharmacia Fine Chemicals) (6). Monocytes were collected by adherence to culture flasks and nonadhering lymphocytes were eluted. Granulocytes were isolated from the lower Ficoll-Paque band. Leucocyte microsomes were prepared by following the method of Young and Rodwell (7). Microsomal protein was determined by the Lowry procedure (8).

Cholanoic acids from lyophilized monocytes, lymphocytes, and granulocytes were extracted with choloroform:methanol (1:1). They were separated from other acidic lipids by thin-layer chromatography in a solvent system of Toluene:Ethanol:Methanol:Water:Ammonium hydroxide (50:20:14:3:1) (9). Reverse

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Table I. Cholanoic acids in human leucocytes[1] (range of 4 subjects).

Leucocyte	TC	TCDC	TDC	GC	GCDC	GDC
			μg per 10^6 cells			
Monocytes	5.35-5.65	9.46-13.5	ND[2]	14.15-20.29	8.4-13.83	ND
Lymphocytes	1.8-2.2	2.6-3.05	ND	4.15-4.21	3.04-3.26	0-2.09
Granulocytes	0.108-0.211	0.224-0.315	ND	0.219-0.348	0.314-0.421	ND

[1]TC = Taurocholic; TCDC = Taurochenodeoxycholic; TDC = Taurodeoxycholic; GC = glycocholic; GCDC = Glycochenodeoxycholic; GDC = glycodeoxycholic acids.

[2]Not detectable.

phase High Pressure Liquid Chromatography (HPLC), coupled with an ultraviolet detector set at 193 nm, was used for identification and quantitation of conjugated bile acids (10). Samples prepared in the elution solvent mixture (2 propanol: 8.8 mM potassium phosphate buffer, pH 2.5, 160:340), were analyzed on a 4 mm x 30 cm μ Bondapak/C-18 column (Waters Associates) by eluting at 1 ml/min. Quantitation was done by comparing the sample peak area with corresponding standards.

Cholesterol 7α-hydroxylase assays were done by using the Carlson et al. method (11) with the following modifications. A 0.3-ml sample of microsomal suspension in homogenizing buffer (7) was incubated at 0°C for 10 minutes with 0.3 ml of potassium phosphate buffer (pH 7.4) containing 35 mM cysteamine, 0.15 ml of 4.5 mM $MgCl_2$, and 0.15 ml of cholesterol suspension (0.1 μCi of 4[^{14}C] cholesterol, 54 μCi/mmol, solubilized with the aid of 0.36 mg of Tween 80). This allowed equilibration of exogenous and microsomal cholesterol. A substrate cofactor solution (0.15 ml) containing 0.75 mM NADP, 3 mM glucose 6 phosphate, and 0.15 units of glucose 6 phosphate dehydrogenase was then added, and the mixture was incubated for 40 minutes, at 37°C with shaking. Blanks containing boiled microsomes were run also. The reaction was stopped with 2 ml of ethanol. The assay conditions were such that, during incubation, 7α-hydroxycholesterol formed from cholesterol was not converted into intermediates further along the pathway or to secondary metabolites.

Confirmation of 7α-hydroxycholesterol as the end product of this assay was accomplished by using electron-impact mass spectrometry. A Finnigan GC/MS/DS model 2709 was used to obtain the spectra by using the following conditions: 25 eV ionizing voltage, ion source temperature 260°C, and 8.0 Kv acceleration voltage. Samples were introduced into the source chamber with a solid sampling probe. Commercially obtained 7α-hydroxycholesterol (Steraloids Inc.) was used as a reference standard.

Table II. Cholesterol 7α-hydroxylase activity in human leucocytes (mean ± SD of 4 subjects). Percentage conversion of cholesterol to 7α-hydroxycholesterol per 40 min per mg microsomal protein.

Leucocyte	Activity
Monocytes	0.760 ± 0.252
Lymphocytes	0.301 ± 0.112
Granulocytes	Not Detectable

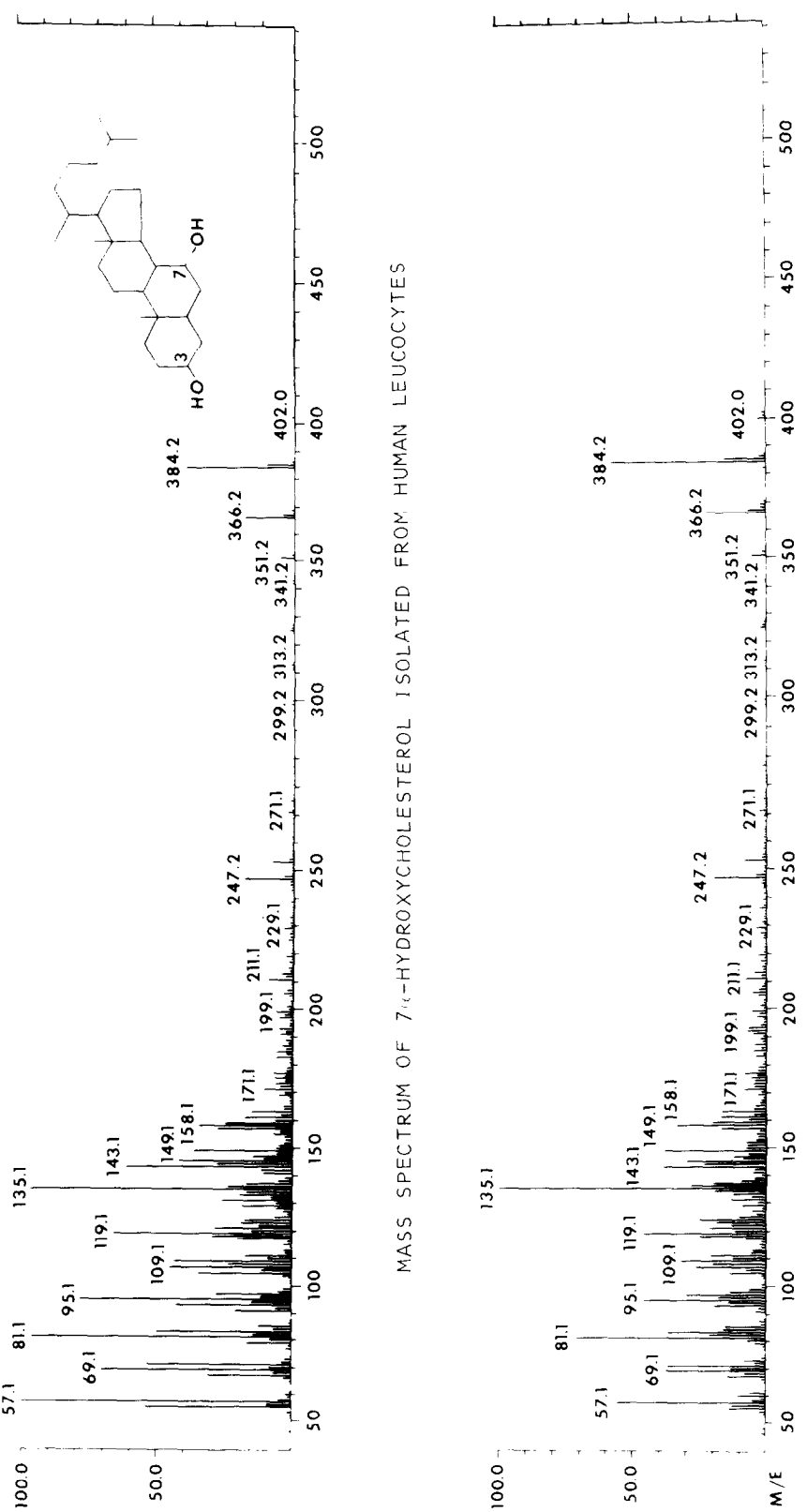

Figure I: Electron impact mass spectra of 7α-hydroxycholesterol from human leucocytes compared with an authentic standard.

Table III. Fragment ions and relative intensities of 7α-hydroxycholesterol isolated from human leucocytes vs. standard.

	m/e	human leucocyte	standard
M^+	402	2	1
$[M-n\ .\ 18]^+$	384	58	41
$[M-2\ .\ 18]^+$	366	23	20
$[M-2\ .\ 15+15]^+$	351	5	2
	313	0.5	0.5
	299	0.5	0.5
	271	3	3
$[M-149]^+$	253	12	10
	247	15	18
	229	4	5
	211	10	11
	199	7	8
	171	9	12
	158	35	45
	149	39	43
	143	39	61
	135	100	100
	119	47	66
	109	33	44
	95	46	78
	81	71	96
	69	37	70
	57	59	100

RESULTS AND DISCUSSION

The quantitation by HPLC of the cholanoic acids found is shown in Table 1. All cell types contained cholanoic acids. The granulocytes had very little as compared with concentrations in monocytes and lymphocytes (the mononuclear leucocytes). The cholesterol 7α-hydroxylase activity in different leucocytes (Table 2) is expressed as the percentage of [^{14}C] cholesterol added to incubation medium that is converted to [^{14}C] 7α-hydroxycholesterol, because the amount of endogenous cholesterol participating in the reaction was not determined. Most of the activity is in mononuclear leucocytes, monocytes being 2.5 times as active as lymphocytes. This finding is consistent with the observation made by Fogelman et al. (12) that monocytes are 5 times more active than lymphocytes in cholesterol biosynthesis. We could not detect any activity in granulocytes, and this was not unexpected because inability of granulocytes to synthesize cholesterol has been demonstrated (13).

The mass spectrum (Figure 1) of 7α-hydroxycholesterol exhibited a molecular ion at m/e 402. The fragmentation observed had predominant M-18 (80%) and M-2x 18 (33%) peaks corresponding to the loss of water molecules.

The peak at 253 suggested side-chain scission between C-17 and C-20. The spectrum of the sample isolated from the leucocytes was virtually identical with that of a reference standard (Table 3).

Unidentified fragments may be the result of thermal degradation in the sample probe and to possible interference from dissolved silica from the TLC plates.

Cholanoic acid presence and synthesis in leucocytes has not been reported before. The probability of formation of the cholanoic acids in the mononuclear leucocytes by local biosynthesis is suggested by the presence of cholesterol 7α-hydroxylase, considered to be the rate-limiting enzyme in the biliary acid biosynthesis. Hydroxylated sterols do not accumulate in cells, so further reactions to form the bile acids identified are likely. The function and importance of these compounds in phagocytosis and immune responses will be an important sequel to these findings.

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