ON THE POLYPEPTIDE COMPOSITION OF AN ABNORMAL HIGH DENSITY LIPOPROTEIN (LP-E) OCCURRING IN LCAT*-DEFICIENT PLASMA

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1. Introduction

The enzyme lecithin-cholesterol-acyl transferase (EC 2.3.1.43) is responsible for the transfer of a fatty acid from the β-position of lecithin to cholesterol, and is probably the main source of esterified cholesterol in human plasma [1]. Primary definciency of the LCAT-enzyme is a rare hereditary disorder [2–5] accompanied by drastic changes in the composition and structure of serum lipoproteins.

Qualitative and quantitative disturbances of the lipoprotein patterns in patients with familial LCAT-deficiency include the presence of the abnormal cholestatic lipoprotein X in the LDL fraction, VLDL particles with β-mobility. Low concentrations of apoproteins A and B, and two abnormal high density lipoproteins [5–11].

Similar changes in the properties of plasma lipoproteins may occur in liver disease [5], which is known to be frequently accompanied by reduced LCAT-activity [12–14].

In the present study we have isolated a morphologically identical abnormal HDL (LP-E) from the plasma of patients with familial LCAT-deficiency and from those with secondary LCAT-deficiency due to liver disease. The lipoprotein isolated from both groups has a characteristic polypeptide composition. It can be detected by immunoelectrophoresis using a specific antiserum against apoprotein E, a normal polypeptide component from VLDL [15].

2. Material and methods

Lipoprotein fractions were isolated from the plasma of ten normolipaemic blood donors, from four patients with obstructive liver disease, and from two patients with familial LCAT-deficiency (G.M. and P.M.) [5]. All four patients with liver disease were LP-X positive and had low activities of plasma lecithin-cholesterol-acyl transferase (table 1). Sequential ultracentrifugation was performed in a Beckman L2-65 ultracentrifuge in rotors of type 40.2 or 60 Ti according to the method of Havel et al. [16] with minor modifications. In all initial centrifugation steps plasma or fractions were layered under an equal volume of the appropriate density solution. Fractions VLDL and HDL_2 were recentrifuged at least once at solvent densities 1.006 g/ml and 1.125 g/ml respectively. Lipoprotein fractions were extensively dialysed against 0.9% NaCl, 0.05% EDTA, pH 7.0 at +4°C.

The isolation of apoprotein E and preparation of

* Abbreviations: LCAT = Lecithin-cholesterol-acyl transferase; VLDL = Very low density lipoproteins; LDL = Low density lipoproteins; HDL = High density lipoproteins PAGE = Polycrylamide gel electrophoresis.

Table 1
LCAT-activity, plasma cholesterol and occurrence of abnormal lipoproteins in the patients with familial LCAT-deficiency and the patients with obstructive jaundice studied

Patient	LCAT-activity mU/ml	Total cholesterol mg/100 ml	Cholesterol unesterified %	LP-X	LP-E
G.M.[a]	0.065	130	83	+	+
P.M.[a]	0.13	64	67	+	+
K.K.[b]	0.33	500	54	+	+
H.T.[b]	0.02	320	66	+	+
H.H.[b]	0.16	430	98	+	+
A.M.[b]	0.38	167	62	+	+
Controls	0.76 ± 0.13	186 ± 18	37 ± 2.3	–	–

[a] Patients with familial LCAT-deficiency.
[b] Patients with obstructive jaundice.

the antisera is described elsewhere [15]. Immunoelectrophoresis was performed in a micromodification according to Scheidegger [17]. Polyacrylamide gel electrophoresis in the presence of SDS was carried out as described by Weber and Osborn [18]. The samples were treated with 1% SDS and 5% 2-mercaptoethanol at 90°C for 3 min prior to electrophoresis. Cholesterol was determined according to Watson [19]. LCAT-activity was assayed by a slight modification of the method of Kattermann and Wolfrum [20]. LP-X was determined according to Seidel et al. [21].

Electron microscopy of lipoprotein fractions was performed by negative staining with potassium phosphotungstate in a Philips EM-300 at 80 KV using initial magnifications of 40 000 and 120 000.

2. Results and discussion

All HDL_2-fractions isolated from the plasma of four individuals with liver disease and concomitant secondary LCAT-deficiency were found anomalous in size and shape, as was reported previously for an $alpha_1$-lipoprotein subfraction from patients with familial

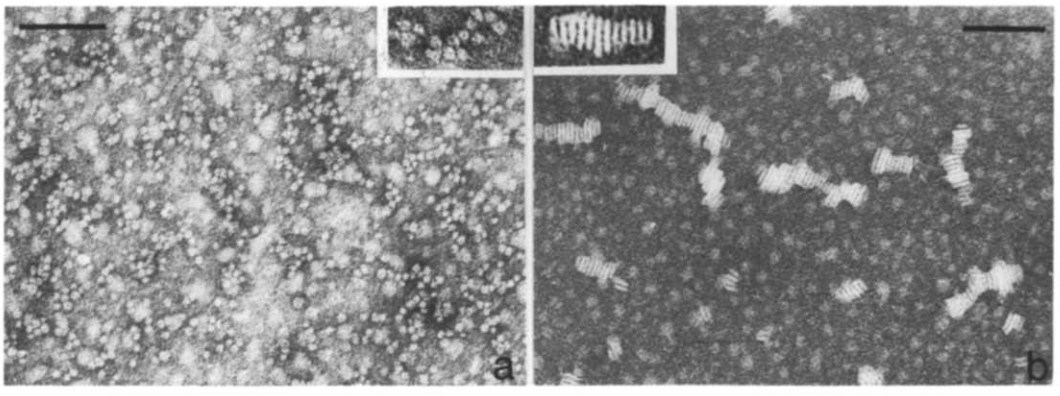

Fig. 1. Electron micrographs of $alpha_1$-lipoproteins of the HDL_2-fractions from a normal blood donor (A) and from a patient with LCAT-deficiency (K.K.) due to liver disease (B). Magnification × 112 000. Bar indicates 1000 A. Insert × 200 000.

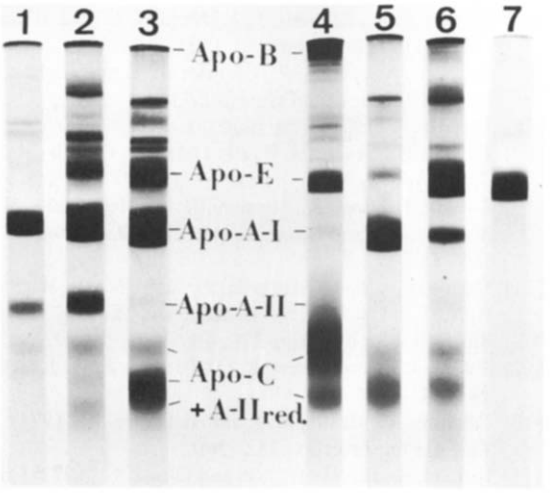

Fig. 2. SDS-PAGE of lipoprotein fractions. 10% monomer concentration. Samples on gels 1 and 2 are run without prior reduction by β-mercaptoethanol to demonstrate the presence of Apo-A-II. (1) HDL_2 from a normal blood donor; (2) and (3) HDL_2 from a patient with familial LCAT-deficiency; (4) VLDL; (5) HDL_2 from a normal blood donor; (6) HDL_2 from a patient with liver disease; (7) Apo E isolated from VLDL.

LCAT-deficiency [5,7,9]. No morphological differences could be detected by electron microscopy between abnormal HDL from the patients with liver disease and those with familial LCAT-deficiency.

The particles were disc-like in appearence with mean diameters of about 180Å and a thickness of 30Å. They tended to aggregate into long coin-like stacks (fig. 1).

All HDL_2-fractions were homogenous upon electron microscopy. They did not contain any normally migrating or small molecular weight $alpha_1$-lipoproteins [5,6] in disc-electrophoresis.

Gel electrophoresis of the abnormal HDL in the presence of SDS revealed an unusual polypeptide composition (fig. 2). In addition to Apo-A-I, Apo-A-II and the Apo-C polypeptides detected in normal HDL_2 by this technique, the abnormal HDL_2 contained a major component with an apparent molecular weight of about 40 000 [15]. This polypeptide was the main constituent of all four abnormal fractions from patients with liver disease, as judged from the gel electrophoretic pattern (fig. 2).

The HDL_2-subfraction from ten normal controls contained a faint band in the corresponding position in some cases, which by scanning accounted for less than 1% of the total protein mass.

However, a polypeptide component with the same apparent molecular weight in SDS-PAGE was consistently found as a third major component in normal VLDL, in addition to the well-documented Apo B and Apo C proteins in this fraction (fig. 2). We have isolated this polypeptide [15] and refer to it as apoprotein E, in accordance with the nomencalture proposed by Alaupovic [22].

An antiserum against Apo E stimulated in rabbits showed one precipitation arc in immunoelectrophoresis with some normal human sera in a position corresponding to the pre-β-lipoproteins (VLDL). In all six pathological sera studied an additional precipitation line was formed in the position between normal pre-β- and $alpha_1$-lipoproteins (fig. 3). The antigenic material corresponding to this line was found exclusively in the abnormal HDL_2 fraction from the patients with LCAT-deficiency.

Apparently Apo E from VLDL and the 40 000 dalton polypeptide from abnormal HDL are identical both in size and immunologically. Moreover they show identical bands in analytical isoelectric focussing [23].

In preliminary studies none of the twenty sera from healthy blood donors gave a reaction corresponding to the abnormal HDL, wheras among 20 sera from patients with different forms of liver disease eleven positive reactors were found.

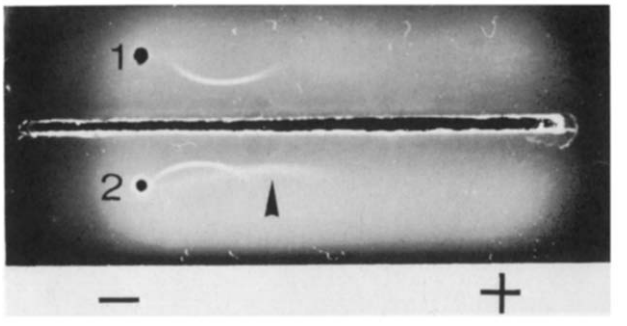

Fig. 3. Immunoelectrophoresis of sera from a normal blood donor (1) and a patient with LCAT-deficiency (2). Trough contains anti-Apo-E, mixed with anti-LP-B to mark the position of LP-E (arrow).

Thus this abnormal HDL-component can be detected by immunoelectrophoresis using a specific antiserum against apoprotein E, a normal polypeptide component from VLDL. We propose the name LP-E for this abnormal component.

The findings presented here suggest that Apo E is involved in the LCAT-reaction. It is accumulated in the HDL_2 when LCAT is deficient. It may be speculated that Apo E normally exchanges between HDL_2 and VLDL. Such a mechanism could explain recent findings by Marcel and Vezina [24] on the activation of LCAT by addition of triglyceride-rich lipoproteins to plasma.

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