AN ABNORMAL HIGH DENSITY LIPOPROTEIN IN CHOLESTATIC PLASMA ISOLATED BY ZONAL ULTRACENTRIFUGATION

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1. Introduction

Alterations of plasma lipoprotein distribution and composition are common features in human cholestasis. The most well-known change occurs in the low density lipoprotein class, with the appearance of an abnormal component called lipoprotein X (LP-X). This lipoprotein has been extensively studied in recent years [1–4]. High density lipoproteins, however, also undergo changes during biliary obstruction [2,5,6]. It is known that plasma levels of HDL decrease during the course of cholestasis, but the different HDL-species have not been investigated as thoroughly as LP-X. This may be due to difficulties in obtaining pure preparations using the common differential flotation technique [7] applied to lipoproteins with density ranges which might differ from those of the normal plasma. Density gradient centrifugation in swing-out rotors can solve this problem, but these rotors can only hold small samples. By zonal ultracentrifugation in density gradients, however, samples large enough for studies of lipid and protein composition can be prepared [8].

The present communication describes the isolation by zonal centrifugation of an abnormal HDL occurring in small amounts during prolonged cholestasis. The abnormal HDL_1, which has a density range between those of normal LDL and HDL_2, has been investigated with respect to its immunochemical properties and lipid and apoprotein composition.

2. Materials and methods

Venous blood samples were collected in the fasting state (14 hr) from five patients with pronounced obstructive jaundice with a duration of at least two weeks. Three of the patients had pancreatic cancer, and the other two had stones in their common bile ducts. Lipoprotein isolation was started within 4 hr after blood collection.

A Ti-14 zonal rotor was used in a Beckman model L2-65B ultracentrifuge for the isolation of the abnormal HDL. The loading and unloading procedures have been described elsewhere [3]. A NaBr-gradient was prepared as follows: 0–50 ml, d = 1.00 g/cm^3; 50–115 ml, d = 1.00–1.17 g/cm^3; 115–420 ml, d = 1.17–1.20 g/cm^3; 420–480 ml, d = 1.20–1.27 g/cm^3; 480–570 ml, d = 1.27–1.29 g/cm^3; 570–610 ml, d = 1.29–1.39 g/cm^3. The density varied linearily with rotor volume for all steps. All solutions were 0.01 M in Tris–HCl, pH 7.4, and contained 1 mM EDTA. The density of the sample (15–50 ml) was adjusted to 1.395 g/cm^3 by adding solid NaBr. At larger sample volumes the total volume of the gradient was reduced to allow at least 20 ml NaBr solution, d = 1.40 g/cm^3, to be used as cushion. Centrifugation was carried out at 44 000 rev/min for 20 hr at a temperature of +15°C.

Electroimmuno assay of lipoproteins was performed as described by Laurell [9] with antisera reacting with apoproteins A, B and C, respectively [6]. Crossed

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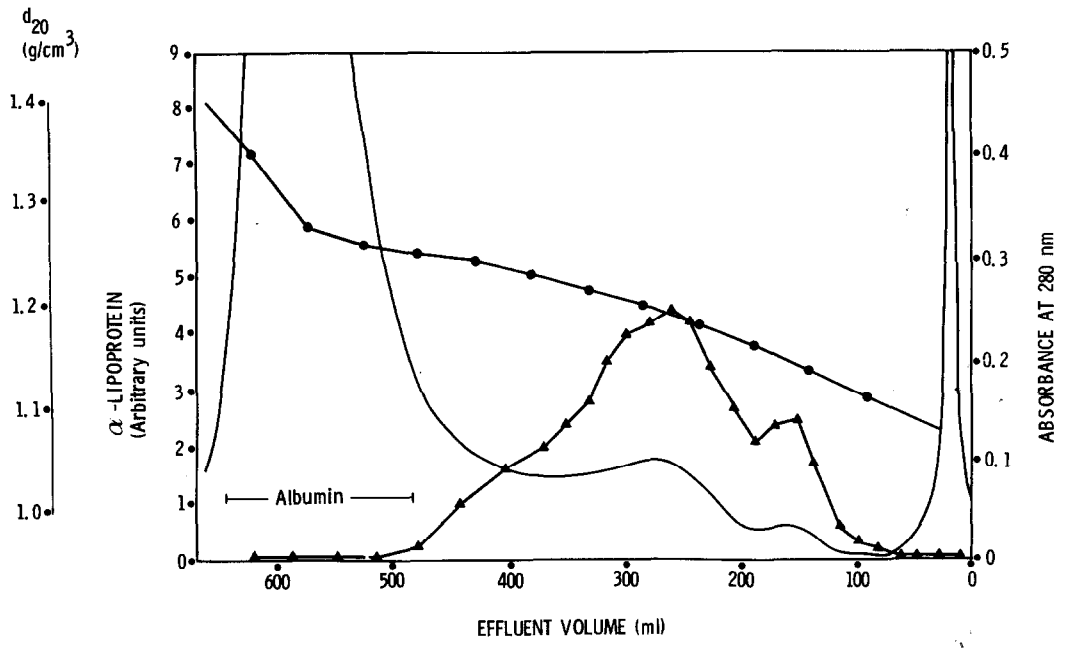

Fig. 1. Zonal ultracentrifugation of 15 ml plasma from a healthy male. (———) Absorbance at 280 nm continuously recorded. (●–●–●) Density. (▲–▲–▲) Electroimmuno assay of α-lipoprotein. Albumin could be detected immunochemically only within the indicated area.

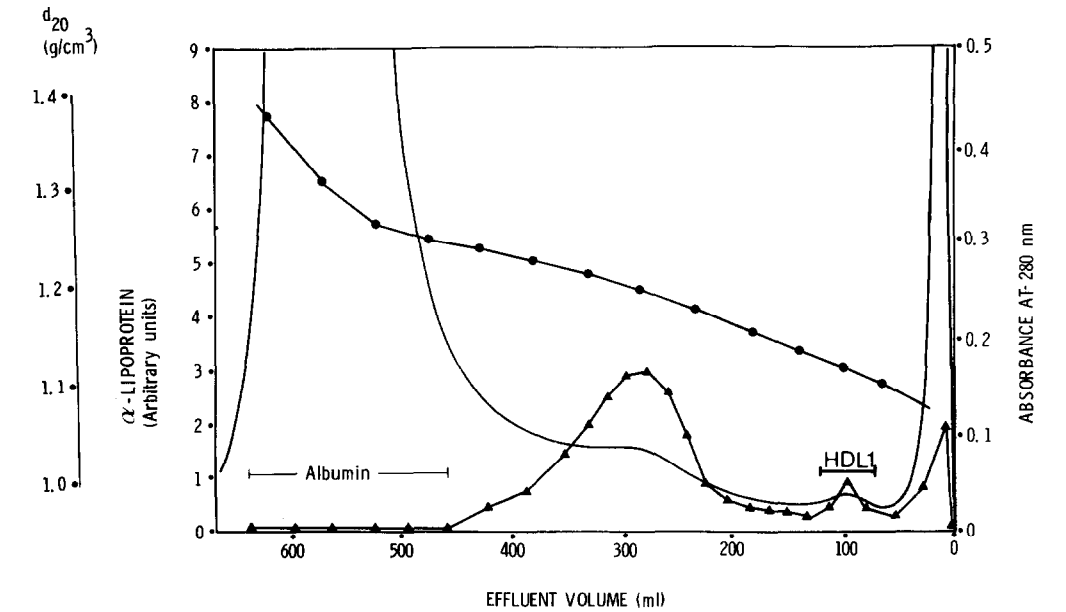

Fig. 2. Zonal ultracentrifugation of 15 ml plasma from a patient after three weeks of obstructive jaundice. (———) Absorbance at 280 nm continuously recorded. (●–●–●) Density. (▲–▲–▲) Electroimmuno assay of α-lipoprotein. Albumin could be detected immunochemically only within the indicated area.

immunoelectrophoresis was carried out according to Ganrot [10]. The immunoprecipitates were first stained with Sudan Black and after inspection stained with Coomassie Brilliant Blue.

Electrophoresis of the apoproteins was performed according to Davies [11] in 7.5% polyacrylamide gels containing 8 M urea, after delipidization with tetramethylurea [12]. The proteins were stained with Coomassie Brilliant Blue R250 in 10% TCA and densitometric recordings were made with a Beckman ACTA CIII spectrophotometer equipped with a type-2 gel scanner.

SDS-gel electrophoresis was carried out as described by Weber and Osborn [13] after heating the samples at 90°C for 3 min.

Unesterified cholesterol and cholesterol esters were determined according to Abell et al. [14] after separation on thin-layer chromatography. Phospholipids and triglycerides were determined as described by Belfrage et al. [15] and Laurell and Tibbling [16] respectively.

3. Results

Under the conditions described for the zonal ultracentrifugation it is possible to isolate HDL_2 and HDL_3 from normal plasma, partially separated from each other, from VLDL-LDL, and from the rest of the plasma proteins, as indicated by the albumin distribution (fig.1).

The HDL distribution in plasma from a patient after three weeks of extrahepatic biliary obstruction is shown in fig.2. HDL_2 had virtually disappeared, the amount of HDL_3 was reduced, and the density distribution of HDL_3 was displaced towards higher density. A new HDL component which appeared at a lower density (1.110 g/cm^3) than that of normal HDL_2 (1.140 g/cm^3) was present in a small amount (40 mg/100 ml plasma). Because of the relatively low density we designate this lipoprotein HDL_1.

The HDL_1, isolated from cholestatic plasma, contained 19% (17–21%) unesterified cholesterol, <2% cholesterol esters, <2% triglycerides, and 55% (49–59%) phospholipids. These figures are mean values for five patients, with ranges given within brackets. The lipids constituted 76% (72–79%) of the lipoprotein.

The abnormal HDL_1 migrated as a rather broad zone with β_1-mobility in agarose electrophoresis. No other components except trace amounts of albumin was found after staining with Coomassie Blue. Crossed immunoelectrophoresis against anti-apo A and anti-apo C revealed only one component with the same localization as the abnormal HDL_1 in agarose electrophoresis. No immunochemical reaction was obtained with anti-apo B. In contrast to normal HDL, the abnormal HDL_1 gave a distinct reaction against anti-apo C in crossed immunoelectrophoresis with a broad, somewhat asymmetrical precipitate.

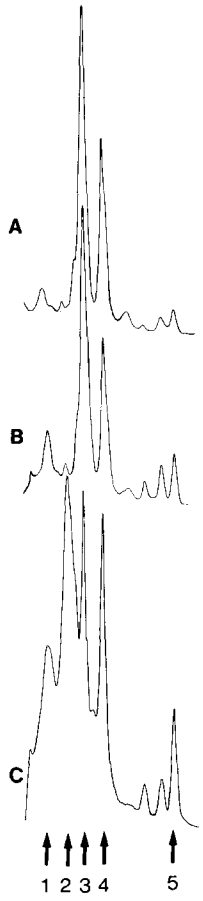

Fig. 3. Urea polyacrylamide gel electrophoresis of TMU-delipidated lipoproteins. A: Normal HDL_3. B: Normal HDL_2. C: Cholestatic HDL_1. The densitometric tracings indicate absorbance at 565 nm. The following apoproteins are indicated by arrows. 1: C I. 2: 'Arginine-rich'? 3: A I. 4: A II. 5: C III-2. The significant peaks between 4 and 5 correspond to A-III, C-II and C III-1.

Immunochemical results indicated that the protein moiety contained about 5% albumin immunochemically unmasked and more loosely associated than in LP-X, as judged from crossed immunoelectrophoresis in anti-albumin containing gel, where the complex dissociated and the albumin migrated with the same mobility as free albumin. The complex was, however, strong enough to withstand recentrifugation.

Peptide analysis by polyacrylamide gel electrophoresis in urea revealed an apoprotein composition that differed significantly from normal HDL_2 and HDL_3 (fig.3). The C-apoproteins were more prominent in HDL_1 with a remarkably high proportion of CIII-2. The ratio between AI and AII was reversed compared to normal HDL. A major band appeared between AI and CI with a mol. wt of 38 000, estimated from SDS-gel electrophoresis. This component was also present in a LP-X and in a VLDL preparation from pooled human serum (fig.4).

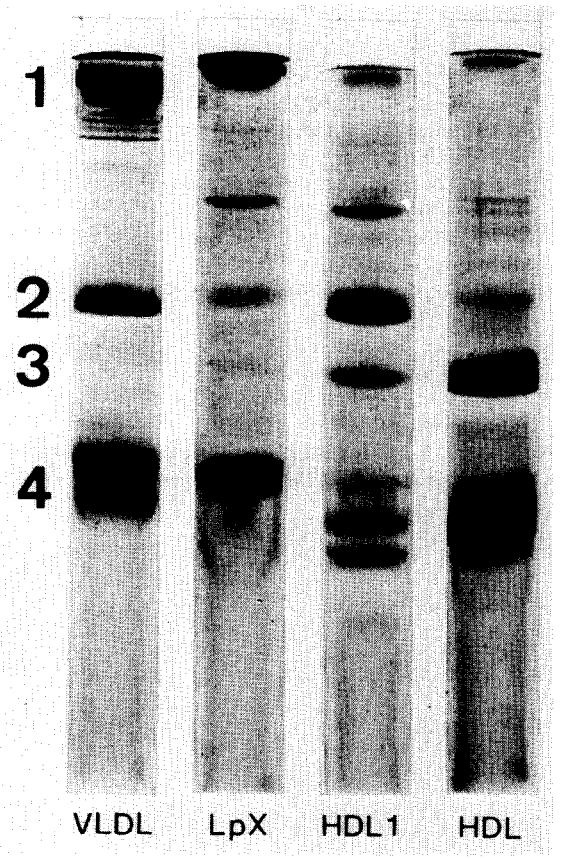

Fig. 4. SDS-gel electrophoresis of lipoproteins isolated by zonal ultracentrifugation. VLDL and HDL_{2+3} were obtained by polyanion precipitation of pooled plasma. LP-X and HDL_1 were obtained from a patient with obsrtructive jaundice after three weeks. The figures indicate: 1: apo-B, 2: 'Arginine-rich' peptide?, 3: A-I, and 4: apo-C + apo-A II.

4. Discussion

The abnormal HDL_1 occurring in small amounts at prolonged cholestasis differs markedly from the HDL components in normal plasma. The high lipid content is reflected in the low density at which HDL_1 is recovered after zonal centrifugation under the conditions outlined above. Also, the apoprotein composition of HDL_1 has some features common to LP-X, with a rather high content of C-apoproteins and with the presence of albumin. The albumin is, however, present in smaller amounts and is immunochemically unmasked.

The major apoprotein in the abnormal HDL_1, which is also present in LP-X and in VLDL from normal plasma, is probably identical to the recently described 'arginine-rich peptide' [12,17] judged from its positions in urea and SDS polyacrylamide gel electrophoresis. Utermann et al. [18] recently described an abnormal HDL (LP-E), occurring in patients with LCAT-deficiency and also present in plasma from patients with obstructive liver disease. The main apoprotien (apo-E) in this lipoprotein may well be identical to the earlier described arginine-rich peptide. Considering the high content of unesterified cholesterol in lipoprotein X as well as in the abnormal HDL_1 in cholestasis, it is interesting to note that the arginine-rich peptide has been suggested to be involved in the transport of cholesterol and cholesterol esters [17].

Our current studies on the lipoprotein alterations during cholestasis include VLDL and the whole HDL class. It also remains to be established whether the described HDL_1 is unique to cholestasis or if the same or a similar lipoprotein exists in the normal lipoprotein metabolism, possibly as a short-lived intermediate.

Acknowledgements

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