ABNORMAL LOW DENSITY PLASMA LIPOPROTEINS OCCURRING IN DOGS WITH OBSTRUCTIVE JAUNDICE

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1. Introduction

Obstruction of the bile ducts in man is almost invariably accompanied by the appearance of an abnormal plasma lipoprotein in the LDL* class called lipoprotein X [1,2]. This lipoprotein has a unique lipid composition characterized by a high content of phospholipids and a cholesterol ester/cholesterol ratio considerably below 1.0. Furthermore, the protein content is low and the protein part consists mainly of C-proteins and albumin, whereas no B-protein is found [1,3]. The occurrence of an analogous component with a lipid composition similar to that of human LP-X has recently been observed in dog's plasma after experimentally induced biliary obstruction [4,5].

As part of a current study of the abnormal lipoprotein metabolism in liver-biliary disease, we have studied the plasma lipoprotein distribution in dogs by zonal ultracentrifugation after induction of obstructive jaundice. The present report describes the occurrence of an abnormal cholestatic LDL component with a cholesterol ester/cholesterol ratio that does not differ from that of normal canine LDL.

2. Materials and methods

2.1. *Animal experiments*

Eight mongrel dogs weighing 16 to 22 kg were fed on commercial dog food during the whole experimental period. The common bile duct was sectioned in six dogs under anaesthesia with sodium thiomebumal (20–30 mg/kg intravenously). Two other dogs were sham-operated. All blood samples were drawn after 12 h fasting with EDTA as anticoagulant.

2.2. *Zonal ultracentrifugation*

Zonal ultracentrifugation was performed with a Ti-14 zonal rotor in a Beckman L2-65B ultracentrifuge. A 600 ml NaBr gradient (1.00–1.15 g/cm^3, linear to rotor volume) containing 0.01 M Tris-HCl and 0.001 M EDTA pH 7.4 was employed for the separation of VLDL–LDL. The density of the plasma samples was adjusted to 1.16 g/cm^3 with solid NaBr before introduction to the rotor. Centrifugation was carried out at +15°C for 3 h at 48 000 rev/min (max 172 000 *g*). In some experiments a modified gradient (1.08–1.19 g/cm^3) and a shorter running time (1.5 h) was used. The rotor was center-unloaded and the u.v.-absorbance at 280 nm was continuously monitored.

2.3. *Chromatography on hydroxyapatite*

Chromatography on hydroxyapatite was performed with a commercial product obtained from Bio Rad, Calif. (Bio Gel HPT). The absorbance at 280 nm of the effluent was continuously monitored.

2.4. *Analytical methods*

Electroimmunoassay was performed as described by Laurell [6], with the use of an antiserum to human β-lipoprotein showing a distinct cross-reaction against dog LDL. Agar gel electrophoresis was performed as described by Seidel [7]. SDS-polyacrylamide gel electrophoresis was performed in a discontinuous system [8].

* *Abbreviations:* VLDL, very low density lipoprotein; LP-X, lipoprotein X; LDL, low density lipoprotein; B-protein, major apoprotein of human LDL; C-proteins, the major apoproteins of human VLDL; SDS, sodium dodecylsulphate.

Unesterified cholesterol and cholesterol esters were determined according to the method of Abell [9] after separation by thin layer chromatography. Triglycerides were determined as described by Belfrage et al. [10] and phospholipids according to the method of Laurell and Tibbling [11].

3. Results and discussion

All animals, except the two sham-operated ones, showed unequivocal signs of obstructive jaundice after this period judged from the increase of plasma bilirubin and alkaline phosphatases and from histological examination of liver tissue. The zonal ultracentrifugal patterns of VLDL–LDL in the dogs' plasma before and 7 days after the operation are shown in fig.1. In the normal dogs LDL class was partially resolved into two subclasses, tentatively designated LDL_1 and LDL_2. Both of these reacted distinctly with antiserum to normal human LDL (anti-β-lipoprotein).

The plasma from all animals with bile obstruction showed a remarkable increase in LDL which also contained an additional LDL_1 fraction without immunoreactivity against anti-β-lipoprotein. This component (provisionally called cholestatic lipoprotein, CLP) was obtained partially resolved from LDL_1 at a density around 1.060 g/cm^3, a localization similar to that of human LP-X [12], shown for comparison in fig.1. This pattern remained essentially unchanged from 7 to 14 days after the operation. The ultracentrifugal patterns from the sham-operated animals did not differ from the preoperative patterns during this period. Like human LP-X, the CLP fraction migrated towards the cathode in agar gel electrophoresis.

In order to study the part of LDL not containing B-protein (CLP), 75 ml of plasma were separated by zonal ultracentrifugation in the modified gradient (see Materials and methods), which gave a somewhat better resolution in the LDL_1 region.

The CLP fraction was further fractionated by hydroxyapatite chromatography. As seen in fig.2 most of the lipoprotein material was eluted with low concentrations of phosphate buffer (peaks I and II). This material migrated cathodically in agar gel electrophoresis [7]. The small amounts of material appearing

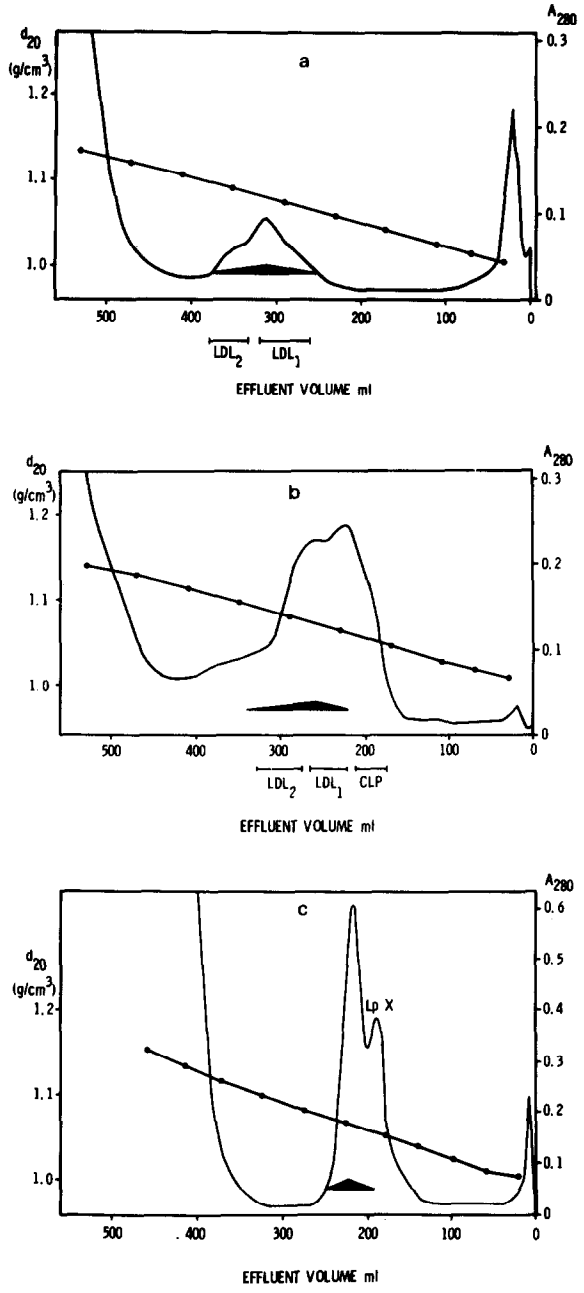

Fig.1. Zonal ultracentrifugal LDL patterns obtained with (a) 28 ml normal canine plasma; (b) 28 ml canine plasma 7 days after ligation of the common bile duct; (c) 15 ml human cholestatic plasma. Run time 3 h at 48 000 rev/min. (—) A_{280}; (●—●) the density gradient after centrifugation determined at 20°C; (▲) the distribution of material reacting with anti-human-β-lipoprotein in electroimmuno assay. Fractions were pooled as indicated by the bars.

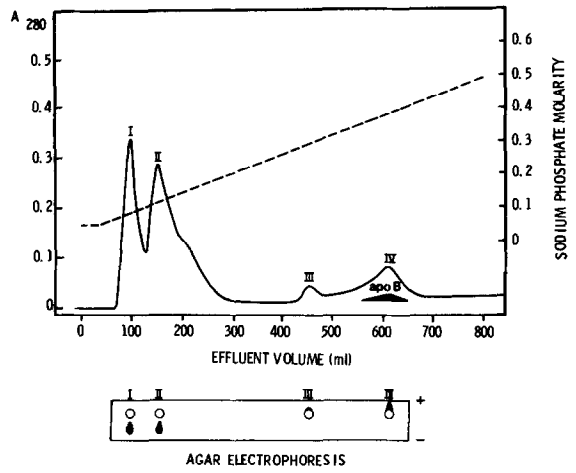

Fig.2. Hydroxyapatite chromatography of the CLP fraction obtained by zonal ultracentrifugation (fig.1(b)). Column dimensions: 2.6 × 30 cm. Elution with a linear gradient ranging from 0.05 M to 0.6 M sodium phosphate buffer, pH 7.0 (—) A_{280}; (▲) distribution of immunoreactivity against anti-human β-lipoprotein; (——) phosphate concentration. The lower part of the figure shows the results of agar electrophoresis according to Seidel et al. [7].

late in the diagram (peak IV) showed a distinct precipiting reaction with human anti-β-lipoprotein, and eluted in the same place as the main part of *normal* canine and human LDL. The small peak III has not as yet been characterized with regard to its protein and lipid composition. The lipid composition of separated fractions is presented in table 1. CLP is characterized by its high content of phospholipids and low content of triglycerides, and this was also characteristic for the cholestatic LDL_1 in contrast to normal canine LDL_1, indicating the presence of abnormal components also in this fraction. Surprisingly enough CLP had a cholesterol ester/cholesterol ratio of about 4, a value even exceeding that of the normal LDL_1. This ratio is quite different from that found for human LP-X and the finding also contrasts with previous results on the canine LP-X analogue [4,5]. When cholestatic canine plasma was subjected to the fractionation procedure used for the preparation of LP-X by other authors [3,5], the lipid composition of the isolated material did not differ from that given for the abnormal component isolated by zonal ultracentrifugation and hydroxy apatite chromatography (table 1). The lipoprotein material migrating cathodically in agar gel electrophoresis [7] was also recovered, and again the lipid composition was the same as previously found for CLP.

The presence of an apoprotein analogous to human B-protein in canine LDL_1 and LDL_2 before and after induction of cholestasis was established by the distinct immunoprecipitates obtained on electroimmuno assay against an antiserum to human B-protein (anti-β-lipoprotein). No distinct immuno-precipitates were observed when CLP was tested in the same way.

The SDS-gel electrophoretic patterns (fig.3) of normal canine LDL_1 and LDL_2 were very similar to each other and contained one distinct component with a mol. wt. around 28 000 and several faint bands

Table 1
Lipid and protein composition (wt %) of normal canine LDL_1 and of lipoproteins isolated after 14 days of obstruction

	Protein	Triglycerides	Unesterified cholesterol	Cholesterol esters	Phospholipids
Normal LDL_1	24.5	18.0	6.5	21.5	29.5
Cholestatic LDL_1	13.5	7.0	9.0	30.5	40.0
Abnormal lipoprotein (CLP) after zonal ultracentrifugation	9.0	2.0	10.0	39.5	39.5
Abnormal lipoprotein after hydroxyapatite chromatography					
fraction I	9.0	<2	9.0	35.5	44.5
fraction II	9.0	<2	8.5	36.5	44.0

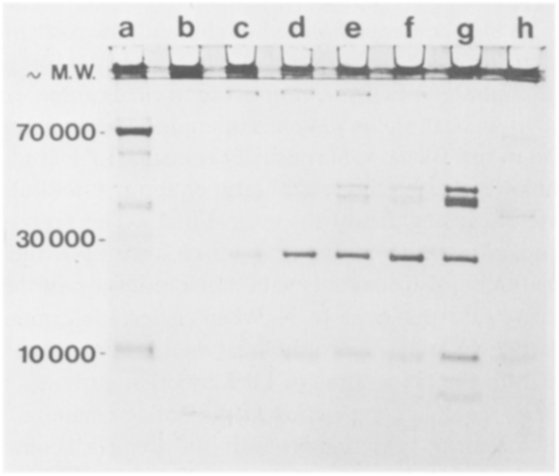

Fig.3. SDS-gel electrophoresis of LDL-fractions isolated by zonal ultracentrifugation. All samples contained 30 μg of protein. (a) mixture of human VLDL and human plasma albumin; (b) human LDL; (c) normal canine LDL; (d) normal canine LDL_2; (e) cholestatic canine LDL_1; (f) cholestatic canine LDL_2; (g) abnormal lipoprotein (CLP); (h) human LP-X.

with mol. wts ranging between 7 000 and 70 000, which is in contrast with the composition of human LDL but consistent with earlier observations in dogs [13]. The 28 000 dalton apoprotein of canine LDL apparently corresponds to the human apoA-I as judged from molecular size and from its immunological cross reaction in gel immunodiffusion with a specific antiserum against human apoA-I. The LDL_1 and LDL_2 from cholestatic dogs were still very similar to each other, but showed a relative increase of several components also present in small amounts in normal canine LDL. This increase was even more noticeable in CLP, especially in the 40 000 dalton region. Hydroxyapatite chromatography of CLP obtained by zonal ultracentrifugation did not change the apoprotein pattern seen in fig.3g.

In conclusion, the isolated abnormal canine lipoprotein fraction resembles the human lipoprotein X in the following respects: both are lipoproteins in the LDL class occurring in cholestasis with cathodic migration in agar gel electrophoresis, with high contents of phospholipids, and with low contents of triglycerides, and apparent lack of B-protein. The canine CLP, however, differs strikingly from human LP-X considering the cholesterol ester/cholesterol ratio and apoprotein composition. Although the cholestatic canine lipoprotein bears much in common with human LP-X, the dissimilarities observed by us, imply that obstructive jaundice does not affect the metabolism of cholesterol in dog in the same way as in man.

Acknowledgements

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