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Further Aspects on the Characterization of High and Very Low Density Lipoproteins in Patients with Liver Disease

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Abstract. Various liver disorders are often associated with decreased concentrations of serum α- and pre-β-lipoproteins. The decreased concentrations of high density lipoproteins (HDL) is primarily due to an impaired lipid binding capacity of apolipoprotein A (apo A), the major protein moiety of HDL. This results in an abnormally high protein/lipid ratio and in severe cases in a lack of neutral lipids in this density class. In contrast to normal α-lipoproteins this fraction does not stain for lipids but shows two distinct and nonidentical precipitin bands on immunoelectrophoresis and immunodiffusion. The isolated very low density lipoproteins (VLDL) from patients with liver disorders revealed a regular particle size and a protein/lipid ratio close to normal but developed β-mobility on electrophoresis. Analysis of the protein moieties of this fraction indicated a lack of apolipoprotein A. It is suggested that disturbed liver function leads to the synthesis of an altered apo A resulting (a) in α-lipoproteins with dissociated apo A peptides and (b) in very low density lipoproteins devoid of apo A. Both findings may be explained by the impaired capacity of this apolipoprotein A to bind neutral lipids.

Key words: Plasma lipoproteins, liver disease.

Abnormal serum lipid patterns are often associated with abnormal liver function. The example best known is the hypercholesterolemia and hyperphospholipidaemia accompanying cholestasis, a phenomenon recorded more than a century ago by Flint [1]. Since the advance several years ago of techniques for the isolation and characterization of serum lipoproteins, the protein-lipid particles, in which form all serum lipids circulate, efforts have been made to define more precisely the nature of the serum lipid disturbance in liver disease.

Recently, it has been well documented that the characteristic plasma lipid changes in patients with obstructive jaundice are due to the presence of a low density lipoprotein (LDL) of abnormal composition and properties [2—5], designated LP-X. It has previously also been demonstrated that various liver disorders are associated with decreased concentrations of the serum α-lipoprotein and pre-β-lipoprotein fractions, when lipoprotein electrophoresis was employed as criterion [6—8]. However, not much information is available on the characteristics of these two classes of lipoproteins in liver disease.

This communication is the initial report of our studies designated to evaluate the possible mechanism responsible for the alterations of the α- and pre-β-lipoproteins, which are often found in acute hepatitis, as well as in obstructive jaundice and chronic liver failure.

Methods

Patients

Blood samples were obtained from 21 hospitalized patients with liver disease including 11 patients with acute hepatitis, 7 patients with extrahepatic biliary obstruction and 3 patients with cirrhosis of the liver. The diagnosis was proved by clinical evidence, liver function tests and liver biopsy or laparatomy.

Plasma was prepared from fresh blood samples from the patients and the normal controls after they had fasted overnight. 0.005 M EDTA was used as an anticoagulant.

Isolation of Lipoprotein Fractions

Standard sequential preparative ultracentrifugation of the patient's plasma lipoproteins was performed [9] in a Spinco model L2 65 B ultracentrifuge using a Type Ti 50 rotor at the densities 1.006 g/ml, 1.063 g/ml and 1.21 g/ml. All lipoprotein fractions were washed at least twice by recentrifugation to eliminate traces of albumin. All isolated lipoprotein fractions were dialysed for 24 h against 0.9% NaCl containing 0.05% EDTA.

Lipoprotein Electrophoresis

Agarose-Agar electrophoresis was performed by a modification [10] of the method of Nobel [11]. Polyacrylamide electrophoresis was performed as described by Davis [12] in a Canalco Model 6 unit. The acrylamide monomer concentrations were 3.5% and 7.5%. A continous buffer system of Tris-glycine, pH 8.8, was used.

Lipid and Protein Analysis

Cholesterol was determined as described by Sperry and Webb [13]; phospholipids as described by Gerlach and Deuticke [14]; triglycerides as described by van Handel and Zilversmit [15]; and protein as described by Lowry, Rosenbrough, Farr and Randall [16].

Immunological Methods

The immunochemical properties of plasma lipoproteins were studied by double diffusion [17] and immuno-electrophoresis [18] in 1% Agar or 1% Agar-

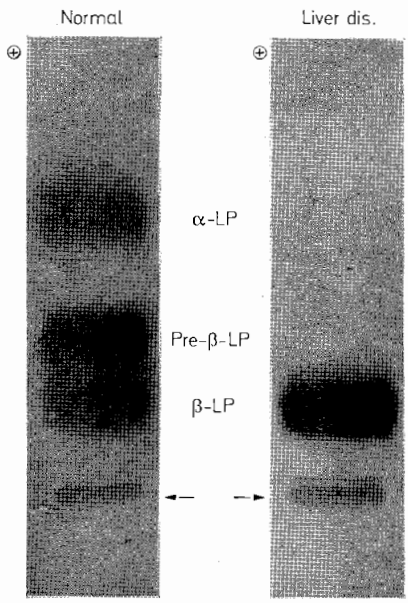

Fig. 1. Lipoprotein electrophoresis of fresh control plasma (normal) and of fresh plasma from a patient with liver disease (liver dis) in 1% Agarose gel. Oil O stain

ose gel employing barbital buffer, pH 8.6, ionic strength 0.05 The plates were developed and stained as previously described [4]. Radial immunodiffusion was performed as described by Mancini *et al.* [19] in 1.5% Agar gel. Rabbit anti-human sera α_1-LP, β-LP and albumin (Behring Werke AG, Marburg an der Lahn, Germany) were used after careful testing for specificity. The rabbit antiserum to apolipoprotein C was prepared in this laboratory as previously described [4, 5].

Delipidization of VLDL

Total delipidization of VLDL was performed by a modification [5] of the procedure of Scanu, Lewis and Bumps [20], using ethanol-diethyl ether.

Incubation of VLDL with α-lipoproteins

Isolated very low density lipoproteins from patients with liver disease were incubated in 0.9% NaCl at room temperature for 2 h with α-lipoproteins isolated from normal subjects or from patients with liver disease. 0.5 mg α-lipoprotein protein was added per mg VLDL protein.

Results

While a normal plasma lipoprotein pattern obtained by lipoprotein electrophoresis in agarose shows distinct bands for β-, pre-β- and α-lipoproteins, the plasma lipoprotein pattern of patients with severe liver dysfunction is often lacking the pre-β- and α-lipoprotein bands and has only one lipoprotein band in the β-position (Fig. 1).

However, this does not necessarily indicate a lack of the very low density lipoproteins normally migrating as pre-β-lipoproteins nor does it necessarily mean a lack of the high density lipoproteins normally migrating as α-lipoproteins and staining for lipids. We have therefore isolated the very low density lipoproteins from patients with liver disorders by ultracentrifugation and found a protein lipid composition close to normal and a normal mobility on polyacrylamide-electrophoresis (Fig. 2). This indicates a regular size of the particles. However, this fraction develops β-mobility on agarose electrophoresis (Fig. 2).

Using preparative ultracentrifugation and/or immunological techniques (Fig. 3) instead of lipid-electrophoresis we found no significant change in the concentration of apolipoprotein A, the major protein moiety of the α- or high density lipoproteins in patients with liver dysfunction. Applying immuno-electrophoresis (Fig. 4) the α-lipoproteins gave strong precipitin lines against normal anti α-lipoprotein serum, although this fraction was missing on lipid-electrophoresis. However, in contrast to normal α-lipoproteins reacting with one line, this fraction showed two distinct and nonidentical precipitin bands indicating a dissociation of apolipoprotein A into its two polypeptides [21—25]. This was also shown by double immuno-diffusion (Fig. 5). Whereas the normal α-lipoproteins reacted with one line stainable for lipids against anti-α-lipoprotein serum, the α-lipoproteins from patients with different liver disorders showed two precipitin arcs against the same anti-serum which did not stain for lipids, but for protein instead (Fig. 5). The protein-lipid-composition of the isolated HDL fraction revealed an increased protein/lipid ratio of more than 1.5 (see Table 1), compared to the normal ratio of 1.0 and in severe cases a lack of cholesterol and triglycerides, which together account for about 25% of normal α-lipoproteins. Since the plasma cholesterol and plasma triglyceride concentrations in these patients are usually higher than normal, our finding may suggest an impaired capacity of this apolipoprotein A to bind these lipid fractions and explains the lack of lipid stain which depends not only on the amounts but also on the type of lipid bound to the lipoprotein.

Analyses of the protein moieties of the very low density lipoproteins from patients with liver dysfunction also showed distinct differences compared to normal VLDL. While apolipoprotein B, the major protein moiety of the low density lipoproteins and the characteristic VLDL peptides (apo C) could be demonstrated, repeated immunological analyses of intact and delipidized very low density lipoproteins revealed a lack of apo A. Although present in small concentrations, apo A is a normal compound of the VLDL fraction (26—29) and is detectable by immunological means.

From our studies on the α- or high density lipoproteins in patients with liver dysfunction, we would like to suggest that the impaired capacity of apolipo-

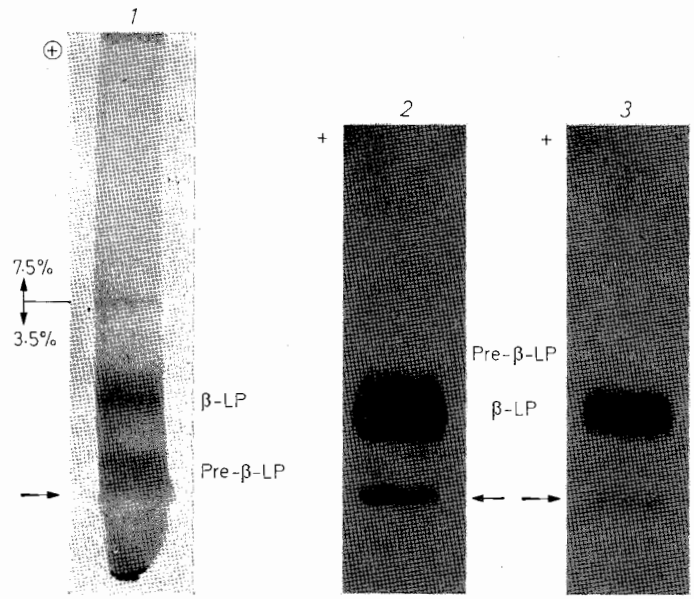

Fig. 2. Lipoprotein electrophoresis in polyacrylamide (1) and Agarose gel (2) of fresh whole plasma and of the VLDL fraction in Agarose gel (3) from a patient with liver disease

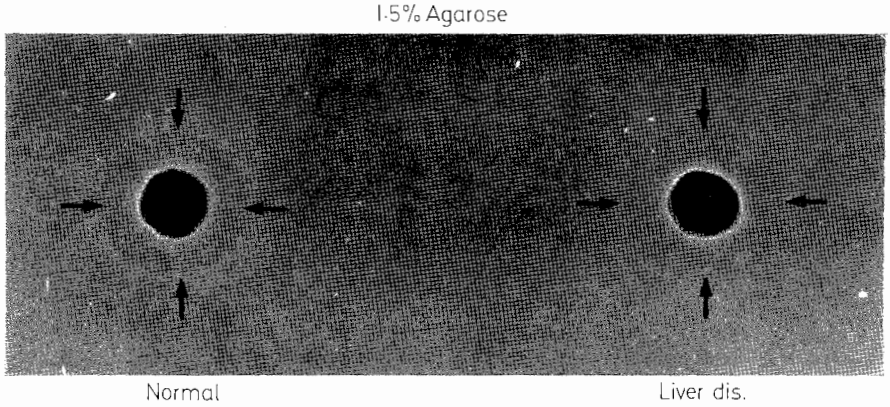

Fig. 3. Radial immunodiffusion pattern of fresh control plasma (normal) and of fresh plasma from a patient with liver disease (liver dis) in anti-α_1-lipoprotein serum-containing Agarose. → indicates the precipitin area

Table 1. Plasma lipid concentrations of the patients' whole plasma and protein-lipid-composition of the isolated VLDL and LDL fraction[a]

Fraction	Number of samples	Protein	Total cholesterol	Triglycerides	Phospholipids	Protein/Lipid
Whole plasma	21		mg/100 ml 351 ± 66	mg/100 ml 358 ± 64	mg/100 ml 405 ± 82	
HDL	9	% 50–71 (47.7)	% 2.0–11 (20.8)	% 0–3.1 (4.6)	% 29–47 (26.9)	mean 1.65 (0.9)
VLDL	8	% 3–17 (7.1)	% 7–28 (22.2)	% 45–81 (51.8)	% 7–19 (17.9)	mean 0.09 (0.08)

[a] The values in parentheses represent % composition of lipoprotein fractions in normal subjects [Bragdon et al., J. Lab. clin. Med. 48, 36 (1956)].

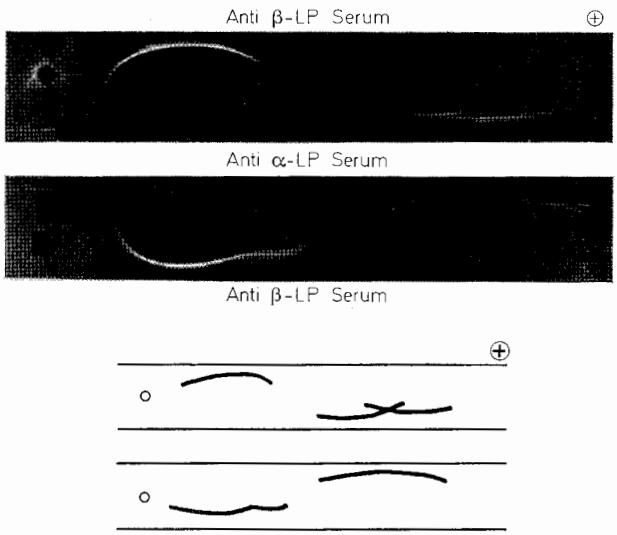

Fig. 4. Immuno-electrophoresis in 1% Agarose gel of fresh control plasma (lower row) and of fresh plasma from a patient with liver disease (upper row) against antibodies to normal α- and β-lipoproteins

hypothesis is supported by a series of *in vitro* incubation studies of isolated VLDL with isolated high density lipoproteins.

Isolated very low density lipoproteins from patients with liver disorders showed the initial β-mobility (Fig. 6) after incubation with high density lipoproteins, which were isolated from patients with liver disorders. The same VLDL fraction, however, developed normal pre-β-mobility after incubation with high density lipoproteins isolated from normal subjects.

Depending on a successful course of therapy the alterations of the plasma lipoprotein patterns in patients with severe liver dysfunction described in this paper normalized.

A follow-up of the lipoprotein patterns (Fig. 7) from a patient with complete biliary obstruction indicated normal pre-β-mobility of the very low density lipoproteins 12 days after the operation and a staining for lipids of the α-lipoproteins 16 days after the operation. The normalization of the lipoprotein pattern obtained by electrophoresis on agarose was paralleled

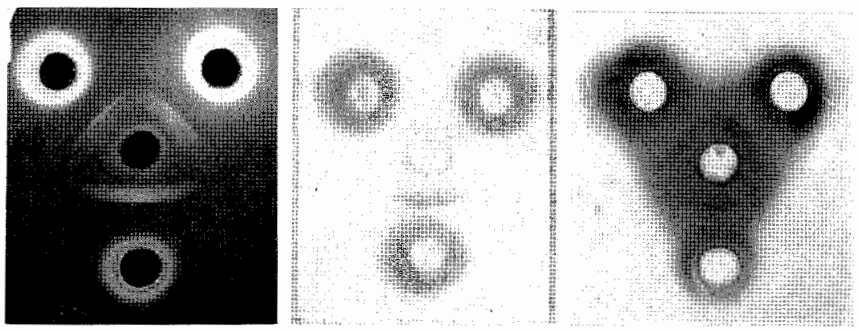

Fig. 5. Immuno-diffusion patterns in 1% Agar gel of fresh control plasma (lower wells) and of fresh plasma from two different patients with liver disease (upper wells) against antibodies to normal α-lipoproteins (central wells). (a) before staining, (b) after staining with Oil red O, (c) protein staining followed after lipid staining

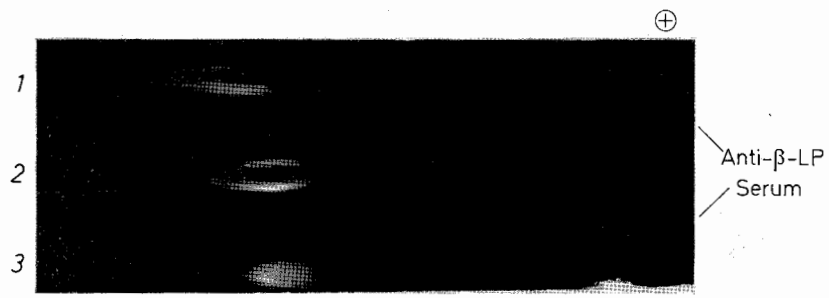

Fig. 6. Immuno-electrophoresis in 1% Agarose gel of lipoprotein fractions against antibodies to normal β-lipoproteins. (1) isolated VLDL from a patient with liver disease after incubation with α-lipoproteins isolated from the same patient. (2) the same isolated VLDL as in (1) after incubation with isolated normal α-lipoproteins. (3) normal VLDL

protein A to bind neutral lipids may also lead to a VLDL fraction devoid of apo A. It is well known that VLDL consists predominantly of triglycerides. This by a recombination of the two apolipoprotein A peptides. 16 days after the operation the two lines had completely consolidated and stained for lipids (Fig. 8).

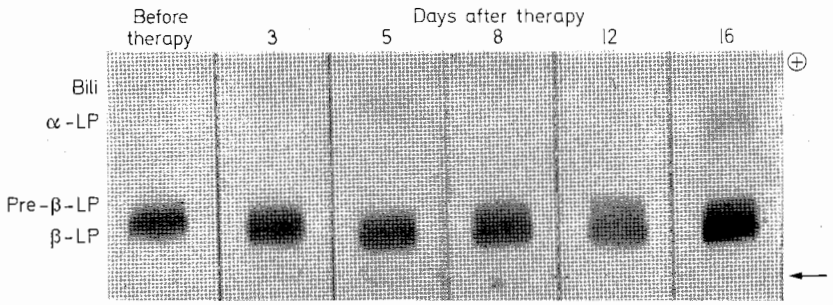

Fig. 7. Lipoprotein electrophoresis in 1% Agarose gel of fresh plasma from a patient with complete biliary obstruction under the course of therapy. Oil red O stain. Yellow bands in the albumin position before, 3 and 5 days after operation reflect the high plasma bilirubin concentration

Discussion

The absence of α- and/or pre-β-lipoproteins on lipoprotein electrophoresis is a phenomenon which can be found in patients with severe liver disfunction. This abnormal lipoprotein pattern may occur in patients with acute hepatitis, biliary obstruction as well as in patients with cirrhosis of the liver. It therefore has no differential diagnostic meaning, as earlier suggested [8].

The alterations of the very low and high density lipoproteins in patients with liver disease are independent of the occurrence of the abnormal lipoprotein (LP-X) characterizing cholestasis and seem to reflect the severity of liver damage rather than the cause of jaundice. The correlation between altered lipoprotein patterns and the severity of liver damage holds even for a larger number of patients currently investigated in our clinic. Results of this study will be presented separately [30].

The absence of α- and/or pre-β-lipoproteins on lipoprotein electrophoresis in patients with liver disease is not accompanied by the absence of very low density lipoproteins normally migrating as pre-β-lipoproteins nor by the absence of high density lipoproteins normally migrating as α-lipoproteins.

The isolated VLDL fraction shows a protein-lipid-composition close to normal and a regular particle size but is devoid of its apolipoprotein A compound. Most investigators at present agree that the apolipoprotein A content of the normal VLDL fraction is extremely low, although, detectable by chemical and immunological methods (26—29). It has been suggested that the pre-β-mobility of normal VLDL is derived from its apo A compound [28]. We would therefore like to propose that the altered electrophoretic mobility of the very low density lipoproteins in liver disease is due to the abnormal protein composition of this fraction. This hypothesis is supported by our incubation study of isolated VLDL with α-lipoproteins.

Analysis of the isolated α-lipoproteins revealed a dissociation of the two apolipoprotein A peptides (recently we were able to confirm this finding using specific antibodies to apo A-I and apo A-II, kindly provided by Dr. Kostner, Graz), an increased protein/lipid ratio and an impaired capacity to bind neutral lipids. This results in a diminished or negative stain reaction with Oil red O.

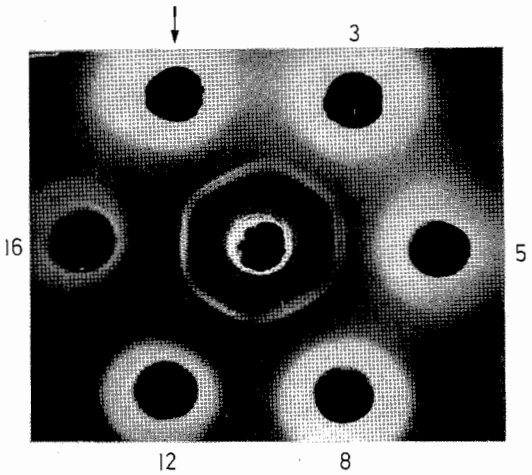

Fig. 8. Immuno-diffusion pattern in 1% Agar gel of the plasma from a patient with complete biliary obstruction before (↓) and the days after operation. Central well contains antibodies to normal α-lipoproteins. All plasma samples were stored at 4°C and contained 0.05% EDTA

With respect to the structural relations of the proteins to the lipids in plasma lipoproteins, the dissociation of apolipoprotein A into its two peptides seems to be of general and basic interest.

Our data suggest that disturbed liver function leads to the synthesis of an altered apo A resulting (a) in α-lipoproteins with dissociated apo A peptides and (b) in very low density lipoproteins devoid of apo A. Both findings may be explained by the impaired capacity of this apo A to bind neutral lipids. This study therefore indicates the important role of the apolipoproteins for maintaining the physico-chemical and chemical properties of the plasma lipoproteins and underlines the central function of the liver as the major source of plasma lipoproteins in this mechanism.

It therefore seems relevant to further investigate the tertiary structure and the carbohydrate moiety of

the apolipoprotein A peptides in patients with liver disease.

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