QUANTITATIVE DETERMINATIONS OF HDL₂ AND HDL₅ IN PATIENTS WITH LIVER DISEASE

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Summary

To elucidate the role of the liver in the metabolism of HDL subfractions, the levels of HDL₂ and HDL₅ were determined in the sera obtained from patients with liver disease. The determinations were carried out either by zonal ultracentrifugation or by gradient gel electrophoresis combined with HDL cholesterol measurement. Mean HDL₅ cholesterol level in patients with liver cirrhosis was about one third of the normal controls whereas no significant changes were observed in HDL₂ cholesterol concentration. HDL₅ cholesterol levels in patients with chronic hepatitis were about a half of the controls. The levels of HDL₅ cholesterol correlated significantly to the levels of serum albumin and to choline esterase activities. The results suggest either that HDL₅ is synthesized in the liver or that there is a metabolic defect in the conversion of HDL₂ to HDL₅ in liver disease.

Key Words: HDL₂, HDL₅, gradient gel electrophoresis, chronic hepatitis, cirrhosis.

Introduction

Human plasma high density lipoprotein (HDL) is a class of lipoproteins with subfractions of HDL₂ (d. 1.063–1.125 g/ml) and HDL₅ (d. 1.125–1.21 g/dl). Chemically they are characterized to contain two principal apoproteins called A-I and A-II accompanying with several minor components¹. Structural aspects of HDL have extensively been studied but the function and metabolism of HDL have been elucidated in parts². There is a high prevalence of coronary heart disease in populations whose HDL is low³,⁴), however, it is not clear how HDL₂ and/or HDL₅ are implicated to prevent atherosclerosis. Metabolic conversion between HDL₂ and HDL₅ has been proposed by several investigators⁵–⁷) demonstrating relationships between these two subfractions.

In previous papers, we showed the decrease in HDL levels in patients with liver disease⁸–¹⁰). In this study, we have investigated changes in HDL subfractions in patients with liver disease to elucidate the role of the liver in the metabolism of HDL subfractions.

Methods

Isolation of HDL₂ and HDL₅ by zonal ultracentrifugation

HDL₂ and HDL₅ were isolated by zonal ultracentrifugation essentially similar to the method described by Patsch et al.¹¹). 17 ml of
freshly prepared serum was adjusted to a density of d.1.40 g/ml by addition of 7 gm of NaBr and was diluted with 10 ml of NaBr solution with a density of 1.40 g/ml. The density gradient was produced in a Hitachi RZ-48Tii rotor (capacity: 660 ml, Hitachi Koki, Ibaragi, Japan) in the density range of 1.00-1.40 g/ml using distilled water and NaBr solution with a density of 1.40 g/ml. The nonlinear density gradient was prepared by a programmed pump (Hitachi DGP) which was driven according to the program shown by Patsch et al. 11). After filling the rotor with the gradient, the serum-NaBr mixture was loaded to the rotor from the rotor's periphery while the rotor was running at 3,500 rpm. The rotor was then accelerated and spun at 41,000 rpm at 15°C for 24 hours. After the run, the contents were collected in 10 ml fractions while monitoring the absorbance at 280 nm. In addition, the absorbances and the refractive indices of all 10 ml fractions were measured with a Hitachi Spectrophotometer model 100-40 at 280 nm and with an Abbe refractometer (Shimazu Bausch & Lomb 3L, Shimazu Seisakusho, Kyoto, Japan) at 20°C, respectively. The density of individual fractions were determined by finding a corresponding figure in a standard density-refraction curve which had been constructed measuring the refractive index of the NaBr solutions of various concentration at 20°C. The densities of the standard NaBr solutions were determined by a pycnometer. The effect of protein on the refractometry was minimal in the conditions utilized. No measurable differences of refractive index were observed between protein free solution and the HDL solution with absorbance of 1.13 at 280 nm.

HDL fractions were concentrated by means of ultrafiltration in a Diaflo cell, model 52, using a UM-2 membrane (Amicon Corp., Lexington, Mass., USA) and dialyzed against 0.15 M NaCl containing 0.05° (w/v) EDTA for three days. Concentrated fractions were subsequently analyzed for their content of lipids and apoproteins.

Electrophoretic separations of HDL2 and HDL3 and their quantitation

HDL subfraction analyses were performed in 50 patients with liver disease (33 males and 17 females) and in 18 normal controls (9 males and 9 females). HDL2 and HDL3 were separated from prestained whole serum by a gradient polyacrylamide gel electrophoresis. The detailed method has been described elsewhere10). Briefly, 0.2 ml of freshly prepared serum was mixed with 0.1 ml of 1% (w/v) Sudan Black B in ethylene glycol and 0.05 ml of 40% sucrose solution. After keeping the mixture in a dark room at 4°C for 16 hours, dyes in excess were removed by low speed centrifugation.

Gradient gel electrophoresis was carried out on a Pharmacia electrophoresis apparatus GE-4 with PAA 4/30 gradient gels (Pharmacia Fine Chemicals, Uppsala, Sweden). 0.02 ml of prestained serum samples were applied to the gel and ran at 120 V for 16 hours at 10°C in a Tris/borate buffer (0.09 M Tris HCl, 0.08 M borate, and 0.003 M EDTA at pH 8.35). The electrophoresis apparatus was covered with a fitted box to prevent color fading due to exposure to the light. At the end of electrophoresis, gel were scanned at 590 nm in a densitometer (Type: KEMIC, Atago Kogaku, Tokyo, Japan).

For confirmation of lipoprotein bands, HDL subfractions isolated by zonal ultracentrigugation were applied on a gradient gel with molecular weight markers (HMW Kit E, Pharmacia Fine Chemicals, Uppsala, Sweden) and electrophoresis was carried out in the same condition stated above. The gel was stained with 0.2 g/dl Coomassie blue G-250 in solvent which contained 10% acetic acid and 50% methanol. The migration distances were measured from