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Identification of Nascent High Density Lipoproteins Containing Arginine-Rich Protein in Human Plasma

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Summary: A high density lipoprotein fraction accumulates in the plasma of patients with alcoholic hepatitis when a severe lecithin:cholesterol acyltransferase (EC 2.3.1.43) deficiency is present. The major apoprotein present in this fraction is arginine-rich protein, the fraction is a preferred substrate for lecithin:cholesterol acyltransferase, and by electron microscopy appears as stacked bilayer discs. It is proposed that the lipoprotein represents the accumulation of nascent high density lipoprotein and is the principal pathway through which arginine-rich protein is secreted by the liver in man. The results also suggest that apoprotein AI is acquired by normal high density lipoprotein during the course of lipoprotein metabolism.

INTRODUCTION

Circulating plasma lipoproteins appear to be derived from the metabolism of nascent lipoproteins of hepatic or intestinal origin. Hamilton et al (1) showed that nascent HDL accumulating in rat liver perfusates in the presence of LCAT inhibitor contained decreased levels of cholesteryl esters and protein and was enriched in unesterified cholesterol and phospholipid. Electron microscopy of negatively stained preparations of nascent HDL revealed disc-shaped lipid bilayer particles with a mean edge thickness of 46 $\pm$ 5 Å and diameter of 190 $\pm$ 25 Å, frequently forming rouleaux. The major apoprotein present in rat nascent HDL was arginine-rich protein with relatively small amounts of apo AI, the major constituent of circulating plasma HDL and a required activator of LCAT (2). It was demonstrated that rat nascent HDL rather than plasma HDL was the preferred substrate for purified human LCAT. Marsh (3) added labeled amino acid

The following abbreviations will be used in this paper: HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; apo, apoprotein; LCAT, lecithin:cholesterol acyltransferase (EC 2.3.1.43).

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precursors to a nonrecirculating rat liver perfusion and found that the major apoproteins secreted were arginine-rich protein (highest specific activity), apo B, and apo C with most of the radioactivity of nascent HDL in arginine-rich protein and apo C. It has also been shown that HDL fractions of patients with hereditary LCAT deficiency contain bilayer discs and arginine-rich protein (4,5).

In a recent study (6), we reported that patients with acute alcohol-induced liver injury (alcoholic hepatitis) have an extreme LCAT deficiency, very low plasma cholesteryl ester concentrations, and HDL of abnormal lipid composition which appears by electron microscopy as bilayer stacked discs. In the present study, we have further characterized the HDL from several subjects with alcoholic hepatitis and find that it is similar in composition and ultrastructure to the nascent HDL isolated from rat liver perfusates. Further, it is a preferred substrate for human LCAT.

MATERIALS AND METHODS

Six patients with alcoholic hepatitis were selected for study on the basis of clinical and laboratory findings previously described (6). They were admitted to the Clinical Research Center and studied throughout the course of their illness until recovery (4-12 weeks). All blood samples for lipoprotein studies were collected in EDTA following a 14 hour fast. Dithionitrobenzoic acid was added to all samples to inhibit LCAT activity. Lipoprotein density classes were isolated by sequential ultracentrifugation and lipid and total protein content determined as previously described. HDL fractions studied represent $d > 1.063$, < 1.21.

Lipoproteins were solubilized by boiling in 1% sodium dodecyl sulfate for one minute and apoproteins separated by polyacrylamide gel electrophoresis, essentially according to the method of Laeammle (7) using 5% stacking gel and 15% running gel. Apoprotein composition was estimated by densitometry of the Coomassie Blue stained gels without correction for differences in dye binding between different apoproteins.

Arginine-rich protein was isolated from alcoholic hepatitis HDL by gel filtration on Sephadex G-200 and preparative electrophoresis. Analytical electrophoresis of the preparation revealed a single band corresponding to arginine-rich protein of normal VLDL. Amino acid analyses were performed through the courtesy of Drs. A.H. Kang and J.M. Seyer, Veterans Administration Hospital, Memphis, Tennessee.

LCAT activity was assayed essentially as described by Hamilton et al (1). HDL fractions equivalent to 5μg of unesterified cholesterol were equilibrated with [^{14}C] cholesterol and assayed in a total volume of 0.2 ml using a normal plasma fraction of $d > 1.225$ as enzyme source. Blanks were run in which no enzyme was added and also in which no HDL source was added and were subtracted from the results reported.

TABLE 1

Percent Composition of HDL from Patients with Alcoholic Hepatitis on Admission and Following Recovery

	Unesterified Cholesterol	Cholesteryl Esters	Triglycerides	Phospholipid	Total Protein
Admission n=6	13.7 ± 1.1 #	1.5 ± 0.4	11.3 ± 2.9	45.8 ± 3.5	27.8 ± 2.2
Recovery n=4*	4.4 ± 4.0	18.0 ± 2.6	5.1 ± 4.4	27.4 ± 4.2	43.1 ± 2.8
P**	< .0001	< .0001	.36	< .001	< .0001

Values in the Table are the mean percentages of each component found in all patients ± 1 standard error of the mean.
* Of six patients admitted to the study, two did not recover.
** Probability of difference between mean of admission and recovery groups.

RESULTS

The composition of HDL from patients with alcoholic hepatitis was significantly different from that of HDL following recovery at which time HDL was essentially normal (Table 1). Thus, unesterified cholesterol was elevated, cholesteryl esters were nearly absent, phospholipids were elevated, and total protein was strikingly low for an HDL fraction. Triglycerides were not significantly elevated.

Apoprotein analysis (Table 2) of HDL isolated from patients with alcoholic hepatitis revealed the presence of unusually large amounts of arginine-rich protein and a deficiency or near absence of apo AI, normally the predominant apoprotein in HDL. In addition, the gels invariably contained one or more as yet unidentified protein bands of higher molecular weight, not ordinarily seen in normal HDL and designated in Table 2 as other protein. The gel conditions were selected to obtain maximal separation between arginine-rich protein and apo AI and did not adequately separate apo C from apo AII.

TABLE 2

Soluble Apoprotein Content of HDL from Patients with Alcoholic Hepatitis on Admission and Following Recovery

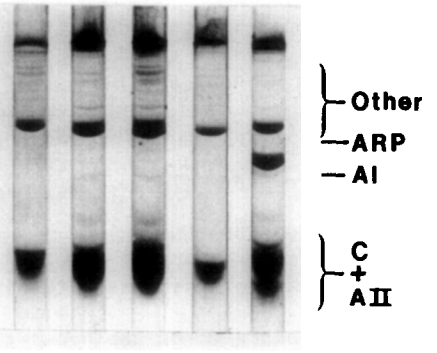
	APOPROTEIN			
	AI	C + AII	Arginine-Rich Protein	Other
Admission n=6	35.4 ± 8.9 #	15 ± 7.7	36.7 ± 3.3	11.2 ± 2.0
Recovery n=4	80.4 ± 4.3	14.3 ± 4.6	2.4 ± .9	3.0 ± 2.3
P*	< .0001	.92	< .0001	< .001

Values in the Table are mean percentages of dye bound by each apoprotein ± 1 standard error as determined by densitometric scanning of gels.

* Probability of difference between mean of admission and recovery.

In order to establish whether the large amounts of arginine-rich protein in the HDL fractions were similar to or identical with arginine-rich protein normally found primarily in VLDL, co-electrophoresis of the abnormal HDL with normal VLDL was performed at a wide range of proportions of the two lipoproteins. In every instance, only a single band corresponding to arginine-rich protein was obtained (Figure 1). In this patient apo AI was not detectable in HDL. The amino acid composition of arginine-rich protein isolated from HDL from one subject was very similar, though not identical, to that of arginine-rich protein from normal VLDL reported by other investigators (8,9,10). This similarity was best demonstrated by a mean arginine content of 104 parts per thousand by two analyses. Glutamic acid was the major amino acid present, leucine and alanine were next highest and approximately equal, and ½ cystine was absent; all characteristic features of arginine-rich protein.

Negatively stained preparations of all of the HDL fractions were examined by electron microscopy. In every case, the HDL obtained from the patients



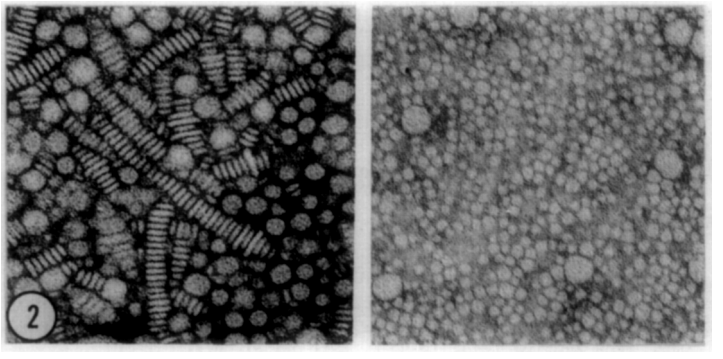


Figure 1. Polyacrylamide gel electrophoresis of HDL from alcoholic hepatitis and normals and normal VLDL. The apoproteins are identified on the right side of the figure. ARP=arginine-rich protein; A, 27µg alcoholic hepatitis HDL protein; B, 57µg normal VLDL protein; C, A mixed with B; D, 1/2 A mixed with 1/5 B, E, 16µg normal HDL protein mixed with 1/2 B.

Figure 2. Electron micrographs of negatively stained preparations of HDL from a patient with severe alcoholic hepatitis (left) and following recovery (right). Magnification=148,000.

when first studied consisted primarily of rouleaux of stacked bilamellar discs (Figure 2), approximately 150-240 Å in diameter with an edge width of 30-50 Å. These preparations were practically indistinguishable in appearance from electron micrographs of rat nascent HDL (1). Following recovery, all of the patients HDL fractions appeared by electron microscopy as normal spherical HDL.

Since it had been established (1) that rat nascent HDL was a preferred substrate for purified LCAT, we prepared a lipoprotein-free preparation of LCAT from normal plasma by ultracentrifugation. The fraction of $d > 1.225$ was dialyzed and used as partially purified LCAT. This preparation contained only

traces of free cholesterol and showed insignificant LCAT activity when incubated alone with [^{14}C] cholesterol. The nascent HDL isolated from alcoholic hepatitis plasma was a better substrate for LCAT than either normal HDL or HDL obtained from the same patients following recovery (Table 3).

DISCUSSION

The HDL isolated from the plasma of patients with alcoholic hepatitis who have an extreme LCAT deficiency appears to be analogous to the nascent HDL that accumulates in rat liver perfusates under conditions in which LCAT is inhibited. The characteristic apoprotein of nascent HDL in rat perfusates and human plasma appears to be arginine-rich protein. The HDL fraction of normal circulating rat plasma contains significant amounts of arginine-rich protein which is not detectable in normal human HDL. These differences could be explained if human nascent HDL metabolism (by LCAT) is a more efficient process than that in rats or conversely, if the production of nascent HDL is much more rapid in the rat.

In both humans and rats, under conditions of severe LCAT deficiency, much greater quantities of arginine-rich protein accumulate in the HDL fraction than are normally found in human VLDL or rat VLDL plus HDL. This fact along with the suggestion of others (10) that arginine-rich protein is transferred to VLDL during the course of metabolism suggests that the main pathway of arginine-rich protein into plasma from liver is via nascent HDL. If such is the case, it is likely that the arginine-rich protein found in normal human plasma VLDL is mainly derived from nascent HDL during the course of lipoprotein metabolism.

An important question which remains to be answered is whether or not apo AI is a component of nascent HDL or whether it enters plasma by a different pathway and becomes associated with the HDL fraction as a result of LCAT activity. Nascent HDL isolated from rat liver perfusates may well be a mixture of nascent HDL rich in arginine-rich protein, and normal HDL which contains primarily apo AI. An examination of electron micrographs presented by Hamilton et al (1) tends to confirm this since some normal spherical HDL appeared together with the chains of bilayer discs. The relative proportions of arginine-rich

TABLE 3

Activity of LCAT* on HDL

Source of Plasma HDL		
Alcoholic Hepatitis	Recovery	Control
.089**	.020	.027

* Expressed as μg [^{14}C] cholesterol esterified per hour per ml of incubation medium.

** Each value is the mean of duplicate determinations.

protein and apo AI in the nascent HDL of subjects with alcoholic hepatitis represent mean values of six patients. In fact, we have actually observed a wide range of relative proportions of arginine-rich protein and apo AI in nascent HDL. Some patients (Figure 2) have nascent HDL containing no detectable apo AI but relatively large amounts of peptides in the apo AII and apo C bands in addition to arginine-rich protein. These findings suggest that pure human nascent HDL does not contain apo AI. This supports the proposal of Hamilton et al (1) that normal rat serum HDL rich in apo AI is probably derived from the interaction of nascent HDL with either LCAT and apo AI or a complex of apo AI and LCAT.

Arginine-rich protein accumulates in VLDL of rabbits fed a high cholesterol diet (8), a condition which might lead to the necessity for transport of ncreased amounts of cholesterol. Our results also suggest that a probable physiological role of arginine-rich protein ARP is the transport of unesterified cholesterol and/or cholesteryl esters in plasma. We always found a large quantity of arginine-rich protein associated with the presence of excess unesterified cholesterol. This occurred with any level of plasma cholesterol, and the same relationship appears to hold true in the rat (1).

Barter and Connor (11) concluded that there are two pools of esterified cholesterol in human HDL following injection of labeled mevalonic acid. They

speculate that a small, rapidly metabolized, pool represents nascent HDL and is the source of cholesteryl esters in VLDL, LDL, and a larger pool of HDL which turns over more slowly. Human alcoholic hepatitis provides a unique opportunity to investigate nascent HDL which accumulates as a result of a reversible metabolic block in lipoprotein metabolism. The present results in man, as well as those in animal investigations, suggest that circulating normal plasma HDL, rich in apo AI and cholesteryl esters, represents a metabolic end-product of lipoprotein metabolism formed in the plasma compartment rather than a secretory product of the liver or intestine.

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