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DOES LIPOPROTEIN-X (LP-X) ACT AS A SUBSTRATE FOR THE LECITHIN: CHOLESTEROL ACYLTRANSFERASE (LCAT)?

H. WENGELER* AND D. SEIDEL

Medizinische Universitätsklinik (Ludolf-Krehl-Klinik) University of Heidelberg, Bergheimerstr. 58, 69 Heidelberg, (W. Germany)

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SUMMARY

Lecithin:cholesterol acyltransferase (LCAT) activity has been studied in normals and in jaundiced patients, who showed the abnormal lipoprotein-X (LP-X) in their plasma. There was no difference in LCAT activity, expressed as μmole of [26-^{14}C]cholesterol ester/ml/h $\times 10^{-2}$ whether LP-X was removed or added to the enzyme assay system, nor did the isolated LP-X act as substrate, when incubated with a partially purified LCAT preparation. These findings led to following conclusions:

1. LP-X, in particular its free cholesterol content, does not act as substrate for the LCAT enzyme.

2. Therefore in patients with liver disease the free cholesterol transported by LP-X should not be taken into consideration for the calculation of the LCAT activity in these patients.

INTRODUCTION

In 1935 Sperry[1] demonstrated an enzyme in normal human serum, capable of esterifying cholesterol *in vitro*. Later this enzyme was characterized[2,3] as lecithin:cholesterol acyltransferase (LCAT) acting upon circulating lipoproteins and catalyzing the transfer of fatty acids from the β position of lecithin to the 3-β-OH group of free cholesterol. Although there is only indirect evidence for the liver origin of LCAT[4–9] it seems probable that the liver might be connected with the production of this enzyme, since the ratio of free to ester cholesterol is often abnormal in hepatic disorders. Subsequent studies[10–18] correlating LCAT activity with liver diseases demonstrated dramatic changes in the activity of this enzyme in many liver patients. However, some controversy exists with respect to LCAT activity in patients suffering from obstructive jaundice[10–18]. The percentage of esterification was found to be significantly lower in obstructive jaundice than in parenchymal disease, but there seemed to be no corresponding reduction in LCAT activity[12,17]. Thus changes in LCAT activity may

* Present address: Universitäts Frauenklinik, Maistr. 11, 8 München, W. Germany.

be only partially responsible for the fall in the cholesterol ester:free cholesterol ratio in liver disease and cannot account for its whole magnitude. A combined determination of LCAT and the abnormal plasma lipoprotein (LP-X) characterizing obstructive jaundice[19,20] demonstrated that the low cholesterol ester:free cholesterol ratio in various liver diseases is due in part to the presence of LP-X with its unique protein lipid composition[19] and in part to a diminished LCAT activity[18]. Recently it has been suggested[13] that LP-X, because of its high content of phospholipids and unesterified cholesterol, may be an unusually good substrate for the LCAT enzyme.

To prove or disprove this suggestion is not only of theoretical interest but also of practical significance with regard to the calculation of LCAT activity in jaundiced patients, showing LP-X in their plasma, because the LCAT activity is usually measured by the ability of serum to esterify labelled cholesterol *in vitro*. If LP-X would be a good substrate for LCAT one would have to take the amount of free cholesterol transported by LP-X into consideration.

Further studies using lipoprotein-X as substrate and determining LCAT activity in the plasma from patients with obstructive jaundice after removal of LP-X from this plasma were needed to clarify the situation of LCAT activity in cholestasis. This is the subject of the present study.

MATERIAL AND METHODS

All patients were hospitalized at the medical clinic, University of Heidelberg, for either extrahepatic biliary obstruction ($n = 3$) or hepatitis with cholestasis ($n = 5$). Diagnoses were confirmed by operation and/or liver biopsy. Controls ($n = 2$) were healthy volunteers with normal values of total bilirubin, SGOT, SGPT, alkaline phosphatase and GGT.

The activity of the LCAT enzyme was measured according to Glomset and Wright[21] with some previously described modifications[14,18]. Human albumin (Behring Werke, Marburg, Germany) which was used for the stock solution was first delipidized in ethanol–ether = 3:2 (v/v) at $-20°$ in order to make the albumin able to accept all lysolecithin which is a product of the LCAT reaction[2,3]. The plasma used as substrate for lecithin and the human albumin stock solution (pH 7.6; 0.02 M phosphate buffer) were incubated separately twice for 30 min at 58° in order to destroy trace amounts of LCAT activity. LCAT activity is expressed as μmoles [26-^{14}C]cholesterol ester/ml/h $\times 10^{-2}$. All samples were run in duplicate and their agreement was always more than 94%.

Lipoprotein-X was isolated as described by Seidel *et al.*[19] and the LP-X in whole serum was determined according to Seidel[22].

The heparin–$MnCl_2$ precipitation was performed according to Burstein[23]. After precipitation the filtrate and the patients' serum were dialysed against 0.02 M phosphate buffer pH 7.6; 0.9% NaCl for 12 h at 4°.

RESULTS AND CONCLUSIONS

From the obtained data presented in the table it is apparent that there is no difference in LCAT activity in whole serum and in the serum after removal of very low and low density lipoproteins by heparin precipitation in any of the subjects stud-

ied, although their initial values varied. Since LP-X is in the low density fraction and is precipitated by heparin and $MnCl_2$ [19], this indicates that the LCAT enzyme does not react with the LP-X. This has also been demonstrated for the normal low density and very low density lipoproteins[24]. If the free cholesterol transported by LP-X would have been a substrate for LCAT, one should have seen an increase in LCAT activity in this assay system after removal of LP-X.

On the other hand, when isolated intact LP-X—in an amount similar to that found in cholestasis—is added to the assay system (see Table I), there is also no change in LCAT activity. If LP-X would be a good substrate for LCAT, there should be a drop in LCAT activity measured by the assay system used.

TABLE I

LCAT ACTIVITY IN WHOLE SERUM AND IN SERUM FRACTIONS

	Group I		*Group II*			*Group III*				
	1	*2*	*1*	*2*	*3*	*1*	*2*	*3*	*4*	*5*
Whole serum	7.0	6.5	7.6	8.0	8.5	3.1	2.0	1.8	2.7	4.0
Heparin filtrate	6.9	6.6	7.6	7.9	8.3	3.1	2.1	1.6	2.7	4.0
Heparin filtrate+LP−X*	6.9	6.5	7.6	8.0	8.4	3.1	2.3	1.7	2.7	3.9
Whole serum+LP−X*	6.9	6.4	7.6	8.0	8.4	3.0	2.1	1.8	2.7	3.9

LCAT activity is expressed in μmole[26-^{14}C]cholesterol ester/ml/h $\times 10^{-2}$. Group I = normals ($n = 2$) (LP-X neg), group II = patients with extrahepatic biliary obstruction ($n = 3$) (LP-X pos), group III = patients with acute hepatitis with cholestasis ($n = 5$) (LP-X pos).
Each vertical column represents the data obtained in one subject. Heparin filtrate: filtrate after heparin–$MnCl_2$ precipitation of whole serum.
* The final concentration of LP-X added to the heparin filtrate fraction or to serum was 150 mg/100 ml which is within the range found in plasma of cholestatic patients.

In control studies neither the isolated intact LP-X preparation nor the dissolved heparin–$MnCl_2$ precipitate from the plasma of our patients with LP-X in their plasma revealed any LCAT activity, nor did these fractions act as substrate, when incubated with a partially purified active LCAT preparation[25], which is devoid of any plasma lipoproteins. Phospholipids in the used concentration did neither affect the assay system.

From the results presented in this study we would like to conclude that:

1. LP-X, in particular its free cholesterol content, does not act as substrate for the LCAT enzyme.

2. Therefore, in patients with liver disease, the free cholesterol transported by LP-X, should not be taken into consideration for the calculation of LCAT activity in these patients.

This is important because it has been suggested that a combined determination of LP-X, as the most specific blood chemical parameter to demonstrate or exclude cholestasis[26,27], and the determination of the LCAT enzyme should open the possibility of differentiating between intrahepatic cholestasis and extrahepatic biliary obstruction[18].

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