LCAT : FROM STRUCTURE TO CLINICAL SIGNIFICANCE

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INTRODUCTION

Lecithin : cholesterol acyltransferase (LCAT ; EC 2.3.1.43) is a plasma glycoprotein enzyme of the serine esterase type which is synthesized and secreted by hepatocytes and has a key role in the metabolism of cholesterol especially in reverse cholesterol transport (Efflux) from the peripheral tissues to the liver [1].

Although it is composed of a single polypeptide unit of about 66 Kd [2] LCAT catalyses sequential reactions. It mediates the transfer of the sn-2 acyl group from phosphatidylcholine (PC) to the 3-hydroxyl group of cholesterol with the production of lyso-PC and cholesteryl ester (CE), which requires both phospholipase (PL) and acyl transferase activities. However, the phospholipase activity of LCAT has some particularities : it is Ca2+ independent, is not absolutely specific for the sn-2 position as are other PLA2, it is also responsible for the PC-lyso PC acyltransferase exchange reaction, it is inhibited by PC containing eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) in the sn-2 position [3-5]. Furthermore LCAT catalyses the transacylation of intact CE [6]. LCAT activity is dependent on the composition of its physiological substrates, high density lipoprotein (HDL) and the major apolipoprotein (apo) component, apo A-I, which is required for optimal activation of the enzyme.

LCAT is responsible for the synthesis of virtually all of the CE in plasma lipoproteins in man. Immunologically reactive LCAT protein has been found on low-density lipoprotein (LDL) [7], however, it is debatable that this LCAT is active. LCAT derived CE on HDL are subsequently transferred by cholesterol ester transfer protein (CETP) to acceptors among the major plasma lipoprotein classes (LDL and very low density lipoprotein (VLDL)) in exchange for triglyceride [8]. Thus LCAT appears both as a "pump" of cell-cholesterol and the origin of a transfer cascade of CE in plasma lipoproteins. However, plasma cholesterol metabolism is much more complex and the detailed analysis of LCAT, CETP as other factors within HDL subfractions are required to understand what regulates the flow of cholesterol through plasma lipoproteins [9]. Major compositional abnormalities of plasma lipoproteins would therefore be expected in cases of LCAT deficiency.

To date the familial LCAT deficiency which was firstly described by Norum and Gjone [10], has now been identified in more than 50 patients from approximately 30 families. Massive corneal opacities presenting as arcus lipoide which appear in the third decade of life are observed in all patients and are associated with one or more of the following clinical symptoms : normochronic hemolytic anaemia (target cells, stomatocytes, schyzocytes, anulocytes), focal and segmental glomerulosclerosis (renal
insufficiency, proteinuria, hematuria, uremia), and in a small number of cases premature atherosclerosis. Partial LCAT deficiency or Fish-Eye disease (FED) which was first described by Carlson [11] has presently been identified in about 10 patients who show early arcus lipoides and corneal clouding without any other clinical symptom and a total lack of premature atherosclerosis. In intermediary forms of LCAT deficiency which resemble FED, abnormal osmotic fragility of erythrocytes has been reported [12]. Familial LCAT deficiency, FED and intermediary forms are all biochemically characterized by the impairment of cholesterol esterification with drastic decreases in HDL cholesterol (HDLc) concentration and anomalies in lipoprotein composition (decrease in normal HDL, increase in fast migrating HDL, increase of triglycerides in LDL) and decreases of apo A-I and apo A-II caused by the lack and/or dysfunction of plasma LCAT [13]. LCAT activity is also reduced in some HDL deficiencies which are not genetically related to anomalies of the LCAT gene eg Tangier disease, apo A-I/A-IV/C-III deficiency, apo A-I/C-III deficiency, and in the case of single base deletion in codon 202 of apo A-I (truncated apo A-I) [14]. Corneal opacifications have been observed in these secondary forms of LCAT activity deficiency. Paradoxically, it has been found, in a peculiar Japanese case, that a marked hyper-HDL2-cholesterolaemia was associated with premature corneal opacity [15]. The variability of the clinical phenotypes corresponding to these patients with primary or secondary LCAT deficiency suggest that the disease is caused by different mutations in the LCAT gene and/or defects in the genes of LCAT activating proteins.

GENE STRUCTURE AND FUNCTIONAL REGIONS OF LCAT

Following the report of the protein sequence from amino acid (AA) analysis [17] the AA and mRNA sequence of human LCAT has been determined from cDNA and genomic cloning experiments [16]. A probable linkage of the LCAT locus in man to the alpha haptoglobin locus on chromosome 16 was first described by Teisberg and Gjone [18]. Azoulay et al [19] refined the assignment of the LCAT gene to 16 q 22. The 1550 base LCAT mRNA can be detected in liver and Hep G2 cells [16]. Preliminary data on the human LCAT gene promoter has been reported [20]. The mature protein was found to contain 416 AA residues (AAR) with a calculated polypeptide molecular weight of 47090. A hydrophobic leader sequence of 24 AA represents the signal peptide [16]. The gene is divided into six exons spanning about 4200 bp. Exon five codes for AA homologous to the interfacial active site of several lipases (a hydrophobic hexapeptide identical with the interfacial binding segment of the active site of pancreatic lipase and lingual lipase), and also codes for an amphipatic alpha helix resembling the carboxyl terminus of apo E.

A number of functional regions have been studied: (i) the active site and the location of the two essential sulphydryl groups among the six CYS residues of the protein, (ii) the sites of disulfide linkage, (iii) the location of the carbohydrate side chains, and (iv) the site of protein-protein interaction with apolipoprotein A-I.

(i) - SER, HIS and CYS residues have been known for a long time as active residues as their chemical modification inhibits the activity of the enzyme [3]. The putative active site of LCAT including SER 181 as the acyl acceptor was identified through its homology with other serine-type esterases [21]. A covalent catalytic mechanism of action for LCAT in which SER 181 and HIS 180 residues mediate PC cleavage and both CYS residues 31 and 184 mediate cholesterol esterification was proposed [22]. Site directed mutagenesis experiments demonstrated that the serine residue at position 181 when replaced by either ALA, GLU or THR gave rise to an enzyme product that was normally secreted by transfected CHO cells but had no detectable catalytic activity [23]. Paradoxically, the double mutant in which CYS-31 and CYS-184 had both been replaced with GLY residues was fully active in the synthesis of cholesteryl-esters [24]. The catalytic mechanism of LCAT on LDL has been described as the ability of LCAT to catalyse acyl