Recombinant human LCAT normalizes plasma lipoprotein profile in LCAT deficiency

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Background and aim: Lecithin:cholesterol acyltransferase (LCAT) is the plasma enzyme responsible for the formation of most of the CE in human plasma[1]. Mutations in the LCAT gene leads to two rare disorders, familial LCAT deficiency (FLD) and fish-eye disease (FED), both characterized by severe hypoalphalipoproteinemia associated with several lipoprotein abnormalities[2]. Clinical manifestations in FLD cases include corneal opacity, anemia, and renal disease that eventually progresses to end-stage renal disease. FED cases do not have clinical manifestations of the disease except for the corneal opacity[2]. There is no specific treatment for genetic LCAT deficiency. Early successful attempts to correct the biochemical LCAT deficient phenotype through the infusion of normal plasma provide the basis for the development of enzyme replacement therapy as a therapeutic strategy for LCAT deficiency[3]. In the present study, recombinant human LCAT was expressed and tested for its ability to correct the lipoprotein profile in plasma from subjects carrying loss of function mutations in the *LCAT* gene.

Methods: Human rLCAT was produced in human embryonic kidney 293 cells using the plasmid transient transfection technique and purified from cell medium by chromatography; plasma derived human LCAT and rhLCAT were run on SDS-PAGE and immunoblotted in order to compare their electrophoretic mobility and antibody reactivity. The activity of LCAT and rhLCAT was measured using reconstituted HDL as a substrate. rhLCAT ability to correct the lipoprotein profile was evaluated incubating equal aliquots of LCAT deficient plasma [4] with rhLCAT or saline. Plasma levels of total cholesterol, unesterified cholesterol, HDL-C, pre-β HDL, Apolipoprotein A-I (apoA-I) and apoB were then determined. Plasma lipoprotein profile was analyzed by FPLC.

Results: Human rLCAT showed the same antibody reactivity and electrophoretic mobility of plasma derived human LCAT. Protein sequence was identified for 87% and the purified protein had a high specific activity.

As expected, plasma total cholesterol levels did not change during incubation with rhLCAT. Plasma CE in FLD plasma was absent at the baseline and markedly increased after incubation with rhLCAT (+210%), while unesterified cholesterol levels were decreased (-30%). These modifications were less pronounced in control plasma. HDL-C levels doubled in FLD plasma and slightly increased in control plasma. On the contrary, non-HDL-cholesterol decreased in all plasma samples . HDL subclass distribution in FLD plasma, was normalized after incubation with rhLCAT. Concomitantly, the small discoidal preß and α-migrating particles disappeared. The analysis of plasma lipoproteins by FPLC confirmed the normalization of the lipoprotein profile after incubation of FLD plasma with rhLCAT, with the conversion of abnormal phospholipid-rich particles in normally sized LDL.

Conclusions: Altogether, the present results show that rhLCAT can normalize the lipid/lipoprotein profile in FLD plasma. Since the excess of unesterified cholesterol and the abnormal lipoprotein particles have been implicated in the occurrence of renal disease in FLD subjects, the correction of the lipid profile, not achievable with any available treatment, is thus the first goal of future cures for this rare disease.

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