Familial lecithin:cholesterol acyltransferase deficiency: First-in-human treatment with enzyme replacement

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BACKGROUND: Humans with familial lecithin:cholesterol acyltransferase (LCAT) deficiency (FLD) have extremely low or undetectable high-density lipoprotein cholesterol (HDL-C) levels and by early adulthood develop many manifestations of the disorder, including corneal opacities, anemia, and renal disease.

OBJECTIVE: To determine if infusions of recombinant human LCAT (rhLCAT) could reverse the anemia, halt progression of renal disease, and normalize HDL in FLD.

METHODS: rhLCAT (ACP-501) was infused intravenously over 1 hour on 3 occasions in a dose optimization phase (0.3, 3.0, and 9.0 mg/kg), then 3.0 or 9.0 mg/kg every 1 to 2 weeks for 7 months in a maintenance phase. Plasma lipoproteins, lipids, LCAT levels, and several measures of renal function and other clinical labs were monitored.

RESULTS: LCAT concentration peaked at the end of each infusion and decreased to near baseline over 7 days. Renal function generally stabilized or improved and the anemia improved. After infusion, HDL-C rapidly increased, peaking near normal in 8 to 12 hours; analysis of HDL particles by various methods all revealed rapid sequential disappearance of preβ-HDL and small α-4 HDL and appearance of normal α-HDL. Low-density lipoprotein cholesterol increased more slowly than HDL-C. Of note, triglyceride routinely decreased after meals after infusion, in contrast to the usual postprandial increase in the absence of rhLCAT infusion.

CONCLUSIONS: rhLCAT infusions were well tolerated in this first-in-human study in FLD; the anemia improved, as did most parameters related to renal function in spite of advanced disease. Plasma lipids transiently normalized, and there was rapid sequential conversion of small preβ-HDL particles to mature spherical α-HDL particles.

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Introduction

Lecithin:cholesterol acyltransferase (LCAT) is a plasma enzyme that catalyzes the production of cholesteryl esters (CEs) from free cholesterol (FC) and phosphatidylcholine (lecithin).1 In humans, about 90% of CE in plasma is
synthesized by LCAT mainly in high-density lipoprotein (HDL). It is believed that newly formed CE accumulates in the core of HDL particles, resulting in the maturation of HDL particles from small discoidal particles to mature, spherical α-HDL. In humans, the resulting CE in mature HDL are then directly removed by the liver (minor route) or transferred to apolipoprotein B–containing lipoproteins by CE transfer protein (CETP; major route) and cleared via the classical hepatic low-density lipoprotein (LDL) receptor pathway, originally described by Glomset as reverse cholesterol transport. 1

Inherited mutations in the gene for LCAT result in 2 autosomal recessive forms of LCAT deficiency. Patients with a total loss of LCAT activity are classified as having familial LCAT deficiency (FLD) and have a marked decrease in HDL cholesterol (HDL-C) levels (<10 mg/dL), plasma CE <25% of total cholesterol (TC; normal >70%), mild-to-severe hypertriglyceridemia, lipoprotein-X (Lp-X) in plasma, corneal opacities, normochromic normocytic anemia, and progressive renal disease. 2-5 FLD patients often develop proteinuria as young adults and then go on to develop nephrotic syndrome and end-stage renal disease typically in their 40s and 50s. 6 There is no effective treatment except for dialysis or renal transplantation, and the disease can rapidly reoccur in the transplanted kidney. 7-11 Renal disease may develop secondary to the appearance of Lp-X, which is a vesicular-like abnormal lipoprotein particle rich in phospholipid (PL) and FC that accumulates in the kidney. 12 Patients with fish-eye disease have a partial LCAT deficiency with some residual LCAT activity. 1,8 These patients are relatively asymptomatic with no Lp-X or renal disease but have reduced HDL-C and corneal opacities.

FLD patients have an abnormal distribution of HDL subfractions; most of their plasma apoA-I is found in small, disc-shaped, poorly lipidated preβ-HDL particles and α-4 HDL particles containing PL and FC. 13 Interestingly, patients with LCAT deficiency do not have a markedly increased risk for cardiovascular disease in most studies, 1,14 likely because they also have low levels of LDL cholesterol (LDL-C) because of the decreased formation of CE on HDL, which are normally transferred from HDL to LDL by CETP.

Recently, recombinant human LCAT (rhLCAT; ACP-501) was shown to be safe in a phase I study of subjects with stable cardiovascular disease 15 (ClinicalTrials.gov NCT01554800) and is being developed as a potential therapy for acute coronary syndrome. In this report, we describe the first-in-human use of enzyme replacement therapy (ERT) with rhLCAT in a patient with FLD and its effect on lipoprotein metabolism and hematologic and renal function.

Methods

Study design

This single-center study was approved by the National Heart, Lung and Blood Institute, Institute Review Board, before patient recruitment. The subject provided informed consent before participation in the study. The study was conducted after Food and Drug Administration review under an Investigational New Drug 117100 as an Expanded Access Protocol. This is a first-in-human study of ACP-501 (rhLCAT) in a subject with FLD.

The subject was administered 1-hour intravenous infusions of rhLCAT (ACP-501) during a dose escalation optimization phase (0.9, 3.0, and 9.0 mg/kg over 22 days; Supplementary Fig. 1), followed by a maintenance phase of 10 infusions of each of the 2 higher doses weekly or biweekly over 7 months (Supplementary Table 1). A detailed Methods section is available in the Supplementary data.

Statistics

Summary statistics were reported as percent change or fold change from prestudy levels or preinfusion baseline levels.

Results

Demographics of subject

A 52-year-old man with FLD and end-stage renal disease previously described 16 was enrolled in an expanded access use protocol (IND 117,100) to determine whether dialysis could be avoided or delayed. Over the 31 months before inclusion, the patient’s renal function rapidly declined (creatinine increasing from 2.5 to 5.6 mg/dL), necessitating the placement of a fistula in his arm in anticipation of dialysis within weeks. Baseline labs included: blood urea nitrogen (BUN) 159 mg/dL, creatinine 5.6 mg/dL, estimated glomerular filtration rate (eGFR) 13 mL/min/1.73 m², 24-hour urine protein 2307 mg, hemoglobin (HGB) 8.2 g/dL, hematocrit (HCT) 24.7%, TC 80 mg/dL, LDL-C 46 mg/dL, HDL-C <5 mg/dL, and triglyceride (TG) 147 mg/dL. See full clinical history in Supplementary data.

Summary of safety of rhLCAT

Over the 8-month course of rhLCAT (ACP-501) therapy (Supplementary Fig. 1), the patient received a total of 23 infusions that were well tolerated by the patient. There were no infusion site reactions or infusion toxicities. Other than favorable changes in creatinine, BUN, cystatin C, HGB, and HCT, as summarized in the following sections, there were no other clinically meaningful shifts in clinical laboratory parameters or physical examination during the study. There were 3 adverse events (AEs) (atrial fibrillation, a mild viral syndrome, and elective hemodialysis at the completion of the study) that were not attributed to ACP-501.

Atrial fibrillation, which occurred 72 hours after receiving the third dose of rhLCAT, was classified as an serious adverse event (SAE). The patient had a long history

 achievable HDL cholesterol (HDL-C) levels (<10 mg/dL), plasma CE <25% of total cholesterol (TC; normal >70%), mild-to-severe hypertriglyceridemia, lipoprotein-X (Lp-X) in plasma, corneal opacities, normochromic normocytic anemia, and progressive renal disease. 2-5 FLD patients often develop proteinuria as young adults and then go on to develop nephrotic syndrome and end-stage renal disease typically in their 40s and 50s. 6 There is no effective treatment except for dialysis or renal transplantation, and the disease can rapidly reoccur in the transplanted kidney. 7-11 Renal disease may develop secondary to the appearance of Lp-X, which is a vesicular-like abnormal lipoprotein particle rich in phospholipid (PL) and FC that accumulates in the kidney. 12 Patients with fish-eye disease have a partial LCAT deficiency with some residual LCAT activity. 1,8 These patients are relatively asymptomatic with no Lp-X or renal disease but have reduced HDL-C and corneal opacities.

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Atrial fibrillation, which occurred 72 hours after receiving the third dose of rhLCAT, was classified as an serious adverse event (SAE). The patient had a long history
of atrial fibrillation, since the age of 27 years. Atrial fibrillation has not been previously reported to be associated with FLD, and hence given the patient’s history this event was viewed to be unrelated to the patient’s underlying lipid disorder or to the rhLCAT treatment. The patient presented with palpitations typical of his previous atrial fibrillation after a vigorous walk. He had a heart rate of 115 bpm and was stable and failed to convert with intravenous diltiazem as he had in the past. He had a controlled pulse rate throughout from 90 to 130. The patient was treated electively by cardioversion and amiodarone treatment and remained in normal sinus rhythm for the remainder of the study. Amiodarone is known to increase blood creatinine levels resulting in a slight increase in the creatinine, but the BUN and cystatin C remained stable for the remainder of the study.17

The subject presented with a viral syndrome consisting of chills and fatigue that started on day 65, the day before dose 10 infusion. He developed a low-grade fever before the infusion and became febrile to 39.9°C before normalizing in 3 days. This was associated with a drop in the HDL-C, apoA-I, CE, and LDL-C and worsening of his 24-hour urine protein, which took about 4 weeks for his labs to recover.

The infusions appeared to stabilize renal function and delayed imminent dialysis by 8 months. After supplies of rhLCAT (ACP-501) became limited, the subject elected to begin hemodialysis, as per the recommendation of his nephrologist.

Pharmacokinetics of rhLCAT

Peak concentrations of rhLCAT were observed at the completion of the 1-hour infusion (Fig. 1A). Peak LCAT mass increased with increasing dose, during the optimization phase and maintenance phase. The peak LCAT mass remained relatively constant over time for the 9.0 mg/kg and for the 3.0 mg/kg doses during the maintenance phase (Fig. 1B).

Effect of rhLCAT on lipid parameters

Lipid parameters for the optimization phase are shown in Figure 2 and lipid parameters over the duration of the entire study are shown in Figures 3 and 4.

HDL-C was <5 mg/dL at screening and increased immediately after rhLCAT infusion. HDL-C peaked 8 to 12 hours after infusion in the optimization phase after the 3.0- and 9.0-mg/kg doses, with a minimal response at 0.9 mg/kg (Fig. 2A). HDL-C peaked later at 12 to 24 hours after most infusions in the maintenance phase and weekly infusions resulted in a higher peak HDL-C than biweekly (Fig. 3A). The peak HDL-C on the 9.0-mg/kg dose increased after repeated weekly doses (dose 5–9), with measurable HDL-C 7 days after infusion (dose, 7–9) before the viral syndrome on day 65.

LDL-C was very low (46 mg/dL) at screening, which is characteristic of FLD. In contrast to HDL-C, the rise in LDL-C after rhLCAT infusion was slower and peaked later. LDL-C increased after a 2- to 4-hour delay before reaching peak levels by 2 to 3 days (Figs. 2B and 5E). LDL-C increased in a dose-dependent manner and remained above the preinfusion baseline 7 days after the 3.0 and 9.0 mg/kg doses in the optimization phase (Fig. 2B). Weekly infusions resulted in higher peak LDL-C and higher day 7 levels than biweekly in the maintenance phase (Fig. 3B). LDL-C reached near normal levels of 70 mg/dL at peak and peak levels plateaued on the 9.0 mg/kg dose after repeated weekly infusions before the viral syndrome.

Plasma CE increased immediately in a dose-dependent manner initially paralleling HDL-C appearance but peaked later at 24 hours and then remained above the preinfusion baseline in all 3 doses in the optimization phase (Fig. 2C). Peak CE increased over 2-fold in the 3.0-mg/kg dose and
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Figure 2  Optimization phase–lipoproteins and lipids. Dose optimization phase included the 0.9 mg/kg dose (black) observed for 3 days, then a 3.0 mg/kg dose (blue) observed for 7 days, and then a 9.0 mg/kg dose (red) observed for 7 days. The rhLCAT infusion was given intravenously over 1 hour starting at time 0. Samples were taken at 0, 0.5, 1, 2, 4, 6, 8, 12, and 24 hours and then a fasting daily sample for up to 7 days. Atrial fibrillation occurred 3 days after the 9.0 mg/kg dose. The subject fasted for 12 hours before and during the infusion, and then resumed his regular diet. (A) HDL-C, (B) LDL-C, (C) plasma CE, (D) percent CE, (E) plasma TC, (F) plasma FC, (G) TG, (H) plasma PL, (I) ApoA-I, and (J) ApoB. CE, cholesteryl ester; FC, free cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; rhLCAT, recombinant human lecithin:cholesterol acyltransferase; TC, total cholesterol; TG, triglyceride.

almost 3-fold in the 9.0-mg/kg dose. After the 0.9-mg/kg dose, peak plasma CE increased about 1.5-fold despite only a minimal change in HDL-C. During the maintenance phase, peak CE increased more than 3-fold after repeated weekly doses on the 9.0-mg/kg but less on the biweekly 3.0-mg/kg doses (Fig. 4A). Peak and nadir levels were always higher than baseline, during the weekly 9.0-mg/kg rhLCAT infusion.

The %CE peak occurred rapidly after infusion, reaching normal levels of about 70%. During the optimization phase,
the time to peak %CE occurred rapidly (8–24 hours) and plateaued for 2 days in a dose-dependent manner (Fig. 2D). The %CE plateaued at 73% CE in the 9.0-mg/kg dose compared with the 19% CE prestudy level. The peak %CE levels increased to 70% or more after all infusions of the weekly 9.0-mg/kg dose, during the maintenance phase maintaining near normal %CE levels through 7 days (Fig. 3C). Peak and nadir %CE remained above 50% on the 9.0-mg/kg dose during doses 6 to 10 before the viral syndrome. Biweekly infusion of 3.0-mg/kg resulted in a lower %CE peak of about 50% and nadir of about 30% but still successive infusions resulted in a 1.5- to 3-fold increase in %CE.

ApoA-I increased in a dose-dependent manner over 2 days then plateaued through 7 days as opposed to the lipid changes in the optimization phase (Fig. 2I). ApoA-I increased by about 45% and 95% compared with the prestudy levels after the 3.0-mg/kg and 9.0-mg/kg doses, respectively. In the maintenance phase, apoA-I rapidly increased in parallel with the appearance of HDL-C and increased in a dose-dependent manner (Fig. 4D). ApoA-I
increased by more than 50% from baseline in the 9.0-mg/kg dose, during weekly infusions vs the biweekly infusion and tended to plateau with successive doses. ApoA-I decreased during the viral syndrome.

ApoB levels approached normal levels and then plateaued (9.0-mg/kg dose) similar to the pattern of apoA-I as opposed to the lipid changes. During the optimization phase, apoB also gradually increased in a dose-dependent manner after the 0.9-mg/kg and 3.0-mg/kg doses but remained elevated at the 9.0 mg/kg dose (Fig. 2J). ApoB rapidly increased in a dose-dependent manner in the 9.0-mg/kg and 3.0-mg/kg dose in the maintenance phase (Fig. 4E). ApoB plateaued at normal levels during the 9.0-mg/kg dose and remained stable with successive doses. ApoB levels were disturbed after the viral syndrome at day 65 and transiently increased after the start of dialysis at dose 19 on day 178.

The overall effect of infusion of rhLCAT on lipid parameters was to convert a dyslipidemic profile characteristic of FLD to a nearly normal lipoprotein profile. TC
increased similar to LDL-C and CE but of smaller magnitude due both to an increase in CE and a decrease in FC. During the optimization phase, TC increased in a dose-dependent manner and the peak TC increased 39% from prestudy levels in the 9.0-mg/kg dose (Fig. 2E); this increase was less than the increase in CE because FC decreased. TC increased in a dose-dependent manner in the maintenance phase and the peak TC plateaued after repeated doses at 9.0 mg/kg and remained below 120 mg/dL. (Fig. 4B).

Concomitantly, FC rapidly decreased in a dose-dependent manner and remained below the preinfusion baseline after all 3 doses in the optimization phase (Fig. 2F). Nadir FC was 49% below the preinfusion baseline and 60% below the prestudy level after the 9.0-mg/kg dose. FC rapidly decreased to about 50% of baseline by 12 to 24 hours, and the nadir was consistently 20 mg/dL after all 9.0-mg/kg infusions in the maintenance phase (Fig. 3D).

Plasma PL decreased rapidly, in a dose-dependent manner, by 24% after the 3.0-mg/kg and 63% after the 9.0-mg/kg dose compared with prestudy levels in the optimization phase (Fig. 2H). PL remained decreased for 3 days and 6 days after the 3.0 and 9.0 mg/kg doses, respectively. The PL trough decreased about 33% and plateaued after repeated doses at 9.0 mg/kg in the maintenance phase (Fig. 4C).

TG increased postprandially in the patient in the absence of rhLCAT infusion, as expected. The subject fasted for 12 hours before and during all infusions and then had his regular diet; TG remained unchanged for over 12 hours after the 0.9-mg/kg dose in the optimization phase. Of major interest, TG rapidly decreased postprandially by about 65% after every 9.0-mg/kg dose and by 40% after every 3.0-mg/kg dose in the optimization phase, and did not return to baseline for 3 to 5 days (Fig. 2G).

Similarly, TG rapidly and routinely decreased postprandially by about 65% after every 9.0-mg/kg dose and by 40% after every 3.0-mg/kg dose in the maintenance phase and did not increase to baseline for 3 to 5 days (Fig. 3E).

**Effect of rhLCAT on lipoprotein particles**

Lipoprotein particle composition and changes in HDL subpopulation distribution after rhLCAT infusion were sequentially evaluated by a variety of methods, including fast protein liquid chromatography (FPLC) (Fig. 5A and B), nuclear magnetic resonance (NMR; Fig. 5C and D), native 1-dimensional gel electrophoresis (Fig. 6), and native–native 2-dimensional gel analysis of apoA-I–containing HDL subpopulations (Fig. 7). Formation of normal-sized α-HDL particles from small preβ-HDL particles and small disoidal α-HDL after rhLCAT infusion was evident by all methods used (Figs. 5–7). Small nascent HDL particles were converted to larger CE-rich HDL-sized particles (Fig. 5A and B). The increase in TC was due to the formation of normal levels of LDL-CE and HDL-CE because FC decreased. Small preβ-HDL particles and FC-rich α-4 HDL, the major substrate of rhLCAT, rapidly disappeared during the 24 hours after infusion, the time of maximal LCAT mass and CE formation. Changes in the distribution of apoA-I on HDL subfractions (Figs. 6B and A)
7) paralleled the appearance of CE on α-HDL (Fig. 5A and B). α-3 HDL was immediately generated after rhLCAT infusion (Figs. 6 and 7). Larger α-2 HDL appeared at 4 to 6 hours and α-1 HDL appeared at 12 to 24 hours, coinciding with maximal concentrations of HDL-C. HDL particles detected by NMR (Fig. 5C and D) correlated with the appearance and disappearance of HDL particles on 1-dimensional and 2-dimensional gels (Figs. 6 and 7) and HDL-C levels. Small HDL, the substrate of rhLCAT, detected by NMR at baseline paralleled α-4 HDL, which initially disappeared and then reappeared after 12 to 24 hours (Figs. 5C, D and 6A). Large HDL appeared at 4 hours when HDL-C was first measurable at 2 mg/dL. Large HDL peaked at 8 hours concomitantly as HDL-C peaked at 23 mg/dL (Fig. 5C and D) and the disappearance of large HDL, the product of rhLCAT, paralleled the slow decline of HDL-C, and α-1 HDL over 192 hours (Figs. 6 and 7).

Lp-X disappearance was indirectly assayed by changes in FPLC analysis of the PL-rich particles (Fig. 5A and B) and by plasma PL concentration (Figs. 2H and 4C). Lp-X–like particles were identified by FPLC as PL-rich particles across broad size ranges at baseline (Fig. 5A) and were absent at 24 hours corresponding with the rapid decrease of plasma PL. In addition, large (~17 nm) FC/PL-rich particles abundant at time 0 (Fig. 6A), corresponding in size to the Lp-X particles (Fig. 5A), began to disappear immediately after LCAT infusion and were gone 2 hours after LCAT infusion. They gradually reappeared at 120 hours and were back to near baseline levels at 240 hours.

Changes in renal parameters on rhLCAT

Renal parameters, including serum creatinine and BUN, eGFR, cystatin C, spot urine protein, and 24-hour urine protein, were collected throughout the study (Table 1). During the optimization phase, 24-hour urine protein improved overall (2307 to 1843 mg/24 h). Twenty-four hour urine protein was somewhat variable but initially remained stable (2498 mg/24 h) compared with baseline at the 9.0 mg/kg rhLCAT dose, which was usually administered once a week (Table 1). Before receiving dose 10, the subject had developed a viral-like symptom (chills and fatigue). Over the next few days, the patient became febrile and the 24-hour proteinuria transiently worsened for several weeks. This was associated with a drop in the baseline HDL-C, apoA-I, CE, and LDL-C. Thereafter, his proteinuria appeared to improve with treatment and continued to improve (1347 mg/24 h) even when the patient was started on the 3.0-mg/kg dose, which was usually administered at biweekly intervals. Random spot urine protein largely paralleled the 24-hour urine protein excretion but varied considerably, depending on the total urine volume.

The BUN showed a modest improvement during the dose optimization phase (159–97 mg/dL) and during the maintenance phase on both the 9.0-mg/kg and 3.0-mg/kg dose (Table 1). The creatinine slightly improved compared with baseline by approximately 12%, during the dose optimization phase (5.56–4.88 mg/dL). However, 72 hours after dose 3, the subject developed atrial fibrillation, requiring the initiation of amiodarone. Amiodarone is well known...
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<th>Cystatin-C (mg/L)</th>
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GFR, estimated glomerular filtration rate; HCT, hematocrit; HGB, hemoglobin.
*Amiodarone started 72 hours after infusion for atrial fibrillation.
†Amiodarone dose doubled.
‡Viral infection.
§Missed 3 doses (6 weeks) of Darbepoetin alfa.
◆Dialysis started 1 week prior.
Changes in hematology parameters on rhLCAT

The effect of rhLCAT on anemia was determined by HGB and HCT from prestudy through the end of treatment (Table 1). There was a significant upward trend in both parameters through the 9.0-mg/kg dose in the maintenance phase, and the patient noted an improvement in exercise tolerance. Compared with baseline, the HGB increased (8.2–10.1 g/dL) by 2 g/dL or an approximate 25% increase in the first 4 weeks. The HGB and HCT improved transiently, but returned to baseline during a period of 6 weeks (dose 12–14) when darbepoetin alfa was not available to the subject. However, the HGB and HCT again returned to near maximum levels over 4 weeks when darbepoetin alfa was restarted when the 3.0-mg/kg dose was administered biweekly.

Discussion

We report the first-in-human results of rhLCAT in a patient with FLD. rhLCAT therapy was safe and well tolerated over an 8-month period and produced normal HDL subfractions and near-normal HDL-C levels. Several features of rhLCAT make it particularly amenable as an ERT, particularly its relatively low plasma concentration and its effectiveness for 1 to 2 weeks after infusion. Moreover, rhLCAT does not need to be targeted to any specific tissue or organelle because it catalyzes its reaction in the plasma compartment, thus making it easier to deliver and monitor.

CE, the product of the LCAT reaction, is the lipid parameter that is most closely related to the biochemical effect of rhLCAT (ACP-501). The low levels of CE in FLD result in low HDL-C, thus making this lipoprotein parameter also useful to follow. During the optimization phase, a dose-dependent increase in CE, %CE, and HDL-C and a decrease in FC was observed as would be predicted based on the enzyme reaction catalyzed by LCAT. Interestingly, apoA-I and apoB also increased with each dose but quickly plateaued, which is probably a consequence of the longer half-life of the newly formed HDL and LDL particles. In the maintenance phase, peak CE and HDL-C levels were sustained from one infusion to the next and were always greater than baseline levels throughout the 9.0-mg/kg maintenance phase and higher levels were achieved with weekly vs biweekly infusions. The %CE in the 9.0-mg/kg dose increased from 19% to a normal level of >70%, peaking at 24 hours, and the %CE nadir increased slightly with successive infusions.

rhLCAT consistently had a major effect on lowering plasma TG levels, suggesting a previously unrecognized role for LCAT in TG metabolism. TG concentrations decreased by more than 40% to 65% after every infusion and remained low for up to 4 to 5 days. Normally, TG increases after a meal, but the patient’s TG always decreased postprandially after the rhLCAT infusion. A potential mechanism for the rapid and sustained drop in TG could be the redistribution of apoA-II and apoC-III, inhibitors of lipoprotein lipase, from very low-density lipoprotein to newly formed HDL after rhLCAT.

Results from this study also enabled us to observe for the first time in humans the sequential formation of HDL from small preβ-HDL particles and small discoidal α-4 HDL to the normal formation of larger α-HDL particles. Small PL-rich particles were converted to normal CE-rich HDL-sized particles. CE increased in both HDL and LDL and HDL-FC decreased. Interestingly, CE and HDL-C increased in parallel for 6 hours starting immediately after infusion, whereas there was a 2- to 4-hour delay in the increase of LDL-C (Fig. 5E). The low LDL-C levels observed at baseline increased to near normal levels by 1 to 2 days (Fig. 2B). This was consistent with the initial formation of CE by LCAT mostly occurring on HDL followed by the transfer of HDL-CE to LDL by CETP.

Sequential HDL particle formation was also measured by NMR, and the results closely paralleled the formation of larger HDL particles on 1D and 2D gels. Small HDL paralleled the disappearance and reemergence of α-4 HDL (Fig. 5C and D). Large HDL appearance and later disappearance coincided with HDL-C concentration. These findings support the use of NMR lipoprotein particle analysis as a method to follow HDL maturation in future studies.

The possibility of preventing or reversing some of the pathologic features of FLD by rhLCAT treatment was supported by the change observed in the subject’s anemia. The mechanism for hemolysis in FLD is possibly due to the lack of exchange of cholesterol between red blood cells and HDL. The 4-week delay in the improvement of the HGB was likely due to the time it took for the generation of newly synthesized red blood cells that were then protected by normal HDL formed after rhLCAT infusion.

The cause of renal disease in FLD is not known, but the low levels of CE correlate with the presence of Lp-X, which is implicated in the pathogenesis of the renal disease. This is supported by the fact that fish-eye disease patients, which have some residual LCAT activity and have normal %CE, do not form Lp-X and do not develop renal disease. We had no direct measurement of Lp-X but have indirect evidence supporting its disappearance after rhLCAT. We observed an
immediate 50% decrease in PL that was sustained over 7 days, when CE was also increased, along with the rapid disappearance of abnormal PL particles spread across the FPLC consistent with the disappearance of PL-rich Lp-X particles. Importantly, the sustained increased %CE above baseline, particularly on the 9.0-mg/kg dose, suggests that sufficient CE levels may be formed from the rhLCAT treatment to prevent Lp-X formation.

The main objective of this study was to determine if the renal disease in FLD can be stabilized or even reversed by rhLCAT (ACP-501) therapy. At presentation, the patient already had advanced renal disease, and it was uncertain whether the loss in renal function would be reversible. As shown in Table 1, the rhLCAT treatment appeared to slightly improve the subject’s renal function or at least stabilized it, which up until the time of the rhLCAT treatment was rapidly worsening. This will have to be carefully assessed in future clinical trials, but it may be necessary to start rhLCAT therapy at an earlier stage in the disease process to be more effective. Fortunately, FLD patients typically first present with proteinuria, and it can take as long as 20 to 30 years before end-stage renal disease develops. Hence, if the diagnosis is made early enough, the development of significant renal disease could possibly be prevented by rhLCAT treatment.

In summary, the beneficial changes in clinical, biochemical, and lipoprotein parameters in the first report of an FLD patient treated by ERT are encouraging and support continued development of rhLCAT therapy.

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Rebecca Bakker-Arkema, Bruce J. Auerbach, Brian R. Krause, and Reynold Homan were employees of AlphaCore Pharma, LLC (now owned by AstraZeneca PLC). Rebecca Bakker-Arkema is currently employed by MedImmune, LLC, Gaithersburg, MD.

Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.jacl.2015.12.007.

References

