Safety and Tolerability of ACP-501, a Recombinant Human Lecithin:Cholesterol Acyltransferase, in a Phase 1 Single-Dose Escalation Study

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Rationale: **Low high-density lipoprotein-cholesterol (HDL-C) in patients with coronary heart disease (CHD) may be caused by rate-limiting amounts of lecithin:cholesterol acyltransferase (LCAT). Raising LCAT may be beneficial for CHD, as well as for familial LCAT deficiency, a rare disorder of low HDL-C.**

Objective: **To determine safety and tolerability of recombinant human LCAT infusion in subjects with stable CHD and low HDL-C and its effect on plasma lipoproteins.**

Methods and Results: **A phase 1b, open-label, single-dose escalation study was conducted to evaluate safety, tolerability, pharmacokinetics, and pharmacodynamics of recombinant human LCAT (ACP-501). Four cohorts with stable CHD and low HDL-C were dosed (0.9, 3.0, 9.0, and 13.5 mg/kg, single 1-hour infusions) and followed up for 28 days. ACP-501 was well tolerated, and there were no serious adverse events. Plasma LCAT concentrations were dose-proportional, increased rapidly, and declined with an apparent terminal half-life of 42 hours. The 0.9-mg/kg dose did not significantly change HDL-C; however, 6 hours after doses of 3.0, 9.0, and 13.5 mg/kg, HDL-C was elevated by 6%, 36%, and 42%, respectively, and remained above baseline ≤4 days. Plasma cholesteryl esters followed a similar time course as HDL-C. ACP-501 infusion rapidly decreased small- and intermediate-sized HDL, whereas large HDL increased. Pre–β-HDL also rapidly decreased and was undetectable ≤12 hours post ACP-501 infusion.**

Conclusions: **ACP-501 has an acceptable safety profile after a single intravenous infusion. Lipid and lipoprotein changes indicate that recombinant human LCAT favorably alters HDL metabolism and support recombinant human LCAT use in future clinical trials in CHD and familial LCAT deficiency patients.**

Clinical Trial Registration: **URL: http://www.clinicaltrials.gov. Unique identifier: NCT01554800.**

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Key Words: acute coronary syndrome ■ cardiovascular diseases ■ cholesterol ■ cholesterol, HDL ■ lecithin cholesterol acyltransferase deficiency ■ lecithin cholesterol acyltransferase

Lecithin:cholesterol acyltransferase (LCAT) is a plasma enzyme secreted by the liver.[1–3] It catalyzes the production of cholesteryl ester (CE) from free cholesterol and phosphatidylcholine (lecithin). In humans, ≈90% of CE in plasma is formed by LCAT, and the reaction mostly occurs on high-density lipoproteins (HDL; the so called α-LCAT activity), and to a lesser extent on apoB-containing particles (β-LCAT activity). The esterification of cholesterol by LCAT helps maintain HDL levels by promoting the maturation of small discoidal forms of HDL (pre–β-HDL and α_4-HDL) into larger spherical forms of HDL ($\alpha_{1\text{-}3}$-HDL), which have a longer half-life.[4] In humans, most HDL-CEs are eventually transferred in exchange for triglycerides to very low-density lipoproteins, intermediate-density lipoproteins, and low-density lipoproteins (LDL) by CE transfer protein (CETP).[5]

Mutations in the human LCAT gene are rare and result in either familial LCAT deficiency (FLD) or Fish Eye Disease.[6] The former is characterized by extremely low HDL-C, corneal opacities, anemia, and progressive renal disease, which eventually requires either dialysis or renal transplantation. Despite their low HDL-C levels, FLD patients do not seem to have a marked increased risk of coronary heart disease (CHD), most

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Nonstandard Abbreviations and Acronyms	
CE	cholesteryl esters
CETP	cholesteryl ester transfer protein
FLD	familial LCAT deficiency
LCAT	lecithin:cholesterol acyltransferase
RCT	reverse cholesterol transport
rHDL	reconstituted high-density lipoprotein
rhLCAT	recombinant human LCAT
TC	total cholesterol

likely because they typically also have low LDL-C. There is no effective therapy for FLD, but in LCAT-knockout mice, it has been shown that intravenous administration of recombinant human LCAT (rhLCAT) can rapidly correct the lipid abnormalities in this disorder.[7]

Besides its role in the pathogenesis of FLD, LCAT has long been proposed to play a role in reverse cholesterol transport (RCT),[8] the pathway for removal of excess cellular cholesterol, and hence may also be important in protecting against the development of CHD.[9] In the first step of RCT, free cholesterol effluxes from foam cells in plaque by the ABCA1 transporter to plasma acceptors, such as pre–β-HDL and other small forms of HDL. The conversion of free cholesterol on HDL to CE increases the capacity of HDL to remove additional cholesterol and maintains the gradient for cholesterol efflux from cells.[10] This concept is consistent with the findings of low LCAT activity and increased pre–β-HDL in patients with CHD,[5,11] but there are also contradictory data on the relationship between HDL, LCAT, and CHD,[1,12] particularly from recent large-scale GWAS.[13]

It has been postulated that a therapy that could rapidly remove plaque cholesterol would stabilize vulnerable plaques in patients with acute coronary syndrome and reduce the likelihood of additional ischemic events.[14] One such promising approach has been the intravenous infusion of reconstituted high-density lipoprotein (rHDL) particles containing phospholipid and either the apoA-I variant, apoA-I_{Milano},[15] or native apoA-I.[16] The intent is to provide additional pre–β-HDL-like particles to rapidly promote cholesterol efflux from atherosclerotic lesions. A possible limitation of this approach may be that pre–β-HDL levels are often already elevated in patients with CHD, together with low levels of large, CE-rich HDL (α_1-HDL),[17,18] and therefore additional pharmacologic elevation of pre–β-HDL particles without optimizing the other steps of the RCT pathway, such as the esterification of cholesterol by LCAT, may be less effective. In fact, in preclinical animal models, transient increased expression of LCAT by either infusion of the recombinant protein[7] or by adenoviral expression[19] can in itself substantially raise HDL-C and may therefore increase RCT. Although adenoviral overexpression of LCAT in mice expressing human apoA-I and CETP was not found to increase the fecal excretion of cholesterol,[20] transgenic overexpression of hLCAT in rabbits that express endogenous CETP has nonetheless been shown to protect against diet-induced atherosclerosis.[21] Similarly, intravenous administration of rhLCAT to wild-type rabbits has also been shown to protect against atherosclerosis.[22]

Overall, the above-combined clinical observations and preclinical animal data created the impetus for us to undertake the development of rhLCAT as a potential therapy for CHD and FLD. We report here a first-in-human clinical trial in which we assessed the safety and tolerability of a single infusion of rhLCAT (designated ACP-501) in subjects with stable CHD and low HDL-C. The pharmacokinetics of ACP-501 and its effect on the pharmacodynamics of HDL-C and HDL subpopulations were assessed, as well as its impact on HDL function.

Methods

Study Subjects

Between May and August 2012, 16 subjects were enrolled in this study (ClinicalTrials.gov identifier: NCT01554800) at the National Institutes of Health. Nonsmoking men and women with a body mass index between 18 and 35 kg/m^2 and a total body weight ≥50 kg (110 lbs) were eligible if they were between the ages of 30 and 85 years and had a history of stable (>60 days) CHD, as determined by either previous myocardial infarction, previous revascularization (eg, percutaneous coronary intervention and coronary artery bypass graft), angiographically proven coronary atherosclerosis, or noninvasive (eg, magnetic resonance imaging and computed tomography) evidence of CAD. Female subjects of child-bearing potential (neither pregnant nor breast feeding) were required to use birth control during study conduct. Chronic concomitant medications must have been stable for at least 2 months before screening. Inclusion criteria also required that HDL-C was <50 mg/dL for men and <55 mg/dL for women to select for patients who may potentially show a greater effect from the ACP-501 treatment. Exclusion criteria are shown in Online Table I. The study protocol, amendments, and subject informed consent documents were approved by the Institutional Review Board of the National Heart, Lung, and Blood Institute.

Clinical Study Design

A phase 1b, open-label, single-dose escalation design was used to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of 4 doses of ACP-501 (0.9, 3.0, 9.0, and 13.5 mg/kg) in each of the 4 dosing cohorts (n=4). A total of 23 subjects consented to participate, of whom 16 subjects were treated with ACP-501. Subjects remained on their standard medical care for the duration of the trial. Subjects were admitted (day 0) to the National Institutes of Health Clinical Center ≈12 hours before the infusion start time to allow for the collection and assessment of entry criteria. Study subjects then received a single-dose intravenous infusion (day 1) and remained an inpatient for at least 24 hours after the infusion. During the follow-up phase, subjects were seen at an outpatient clinic on the mornings of day 3, day 4, day 5, day 7, and day 28. Blood samples for safety and pharmacokinetics and pharmacodynamics measurements were obtained at hours 0, 1, 6, 12, 24, and 48 (morning of day 3), 72 (morning of day 4), 96 (morning of day 5), 168 (morning of day 8), and on day 28 after the start of the ACP-501 infusion (day 1, hour 0). Only 1 subject was initially treated in each cohort to determine the safety and tolerability of ACP-501 before exposing additional subjects. After a review of vital signs, ECGs, clinical laboratory tests, and adverse event (AE) data from each cohort by a Safety Review Group, subjects in the following cohort received the next higher dose of ACP-501.

Clinical Study End Points

The primary end point was safety and tolerability of a single dose of ACP-501, as determined by treatment emergent AE reporting, which reflected clinically significant changes in clinical chemistries, hematology, vital signs, physical examination, and ECGs, as well as infusion site reactions and toxicities. Secondary end points included the pharmacokinetics profile of ACP-501 after a single dose, and the pharmacodynamics effects of ACP-501, as assessed by plasma biomarkers, such as changes in HDL-C and CE, as well as other lipid and lipoprotein assays (HDL subfractions, pre–β-HDL, non–HDL-C,

LDL-C, total cholesterol [TC], triglycerides, apoB, apoA-I, apoA-II, TC/HDL-C, and cholesterol efflux).

Additional Methods

ACP-501 formulation and administration, detailed analytical methods, cholesterol efflux and ex vivo LCAT activity methods, and statistical analysis are included in the Methods in the Online Data Supplement.

Results

Subject Baseline Characteristics

Subject demographic and other baseline characteristics are summarized in the Table. Overall, the majority of subjects were men (87.5%) and white (75.0%), with a median age of 67 years (range, 60–77 years). At baseline, all subjects had a normal or slightly elevated LCAT mass, with a median value of 8.02 µg/mL (reference range, 5–11 µg/mL). The mean baseline HDL-C was 37 mg/dL. Because it is one of the potential target groups for ACP-501 therapy, all patients were selected for a positive history of CHD, and the majority also had at least 3 of the following concurrent medical conditions: hypertension, diabetes mellitus, arthritis, and dyslipidemia. Subjects were on an average of 7 concomitant medications (not including herbal and vitamin supplements), with all but 1 subject receiving ≥1 lipid-altering medications (13/16 on statins, 7/16 on fish oil, and 5/16 on niacin).

Safety Profile and Tolerability of ACP-501

All 16 subjects were able to receive their full dose of ACP-501, diluted with 270 mL of normal saline, by intravenous infusion over a 1-hour period. During 28 days of follow-up, there were no serious AEs, or severe AEs, or any infusion site reactions, such as localized redness, pain, swelling in the area, or increased warmth. All clinically significant changes noted in the physical examination, ECGs, vital signs, urinalysis, clinical chemistries, and hematology laboratories were reported as AEs. A total of 8 AEs (Online Table II) occurred in 7 subjects. Two AEs, both mild rashes, were considered to be possibly related to the ACP-501 treatment. In both cases, the rash spontaneously resolved without treatment. The other 6 AEs, which included dehydration during a heat wave, hypertriglyceridemia after alcohol ingestion, and hyperglycemia, were considered to be clinically insignificant and unrelated to the drug treatment. No subject withdrew from the study because of an AE.

ACP-501 Pharmacokinetics

Pharmacokinetics parameters for ACP-501 were calculated after a single intravenous infusion of ACP-501 at doses of 0.9, 3.0, 9.0, or 13.5 mg/kg administered over 1 hour. The upper dose was based on the no-observed adverse effect level of 60 mg/kg for ACP-501 as determined in a nonhuman primate toxicology study. Peak concentrations of ACP-501 were detected 1 hour after the start of the infusion and showed multiexponential decay (Figure 1A; Online Table III). The assay used to measure ACP-501 mass (rhLCAT) also measures endogenous LCAT, but the individual subject baseline LCAT levels, which was on average ≈8 µg/mL (Table), were subtracted from the result shown in Figure 1A. Peak ACP-501 levels were significantly higher than baseline LCAT levels, even for the lowest dose. The vast majority of ACP-501 was cleared from the circulation by 2 days and was estimated to have a terminal half-life of ≈42 hours, based on analysis of the 9.0- and 13.5-mg/kg cohort data. Peak exposure (C_{max}) and systemic plasma exposure (area under the curve $[AUC]_{0–48}$, AUC_{0-t}, and $AUC_{0-\infty}$) generally increased in proportion to the dose and to the measured C_{max} at each dose (median, 8.6, 32.2, 107, and 163 µg/mL for 0.9, 3.0, 9.0, and 13.5 mg/kg, respectively) (Online Tables III and IV).

Effect of ACP-501 on HDL-C Levels

The effect of ACP-501 on lipoproteins was first assessed by examining its effect on HDL-C. A dose–response relationship was observed between the dose of ACP-501 and the peak HDL-C levels, which typically occurred between 12 and 24 hours after the start of the infusion. When expressed as maximum % change from baseline, ACP-501 at the 0.9-, 3.0-, 9.0-, or 13.5-mg/kg dose increased HDL-C by 21%, 19%, 48%, and 44% over the initial 4 days post infusion, respectively. The mean % change from baseline in the 9.0- and 13.5-mg/kg ACP-501 dose groups were similar and statistically greater than that in the 0.9-mg/kg low-dose group (P=0.0020 and P=0.0061, respectively). An overall positive linear relationship was observed (Figure 1B) between maximum change from baseline in HDL-C in absolute units and maximum change from baseline in ACP-501 mass, with a Pearson correlation coefficient of 0.7909 (P=0.0003). Overlap, however, was observed for some patients in the 9.0- and 13.5-mg/kg doses for both the maximum change from baseline in HDL-C and in ACP-501 mass post infusion.

The mean absolute change from baseline in HDL-C versus study time through the day 7 visit is illustrated in Figure 2A. HDL-C began to increase 1 hour post infusion in both the 9.0- and 13.5-mg/kg dose groups, and 6 hours post infusion in the 3.0-mg/kg group. In contrast, only a relatively small change in HDL-C occurred for the 0.9-mg/kg dose group at the later time points. For the 9.0- and 13.5-mg/kg dose groups, the increase in HDL-C peaked between 6 and 24 hours post infusion. A dose–response for increase in AUCs (AUC_{0-48}, AUC_{0-96}, and AUC_{0-168}) of HDL-C was also observed across the 0.9-, 3.0-, and 9.0-mg/kg groups, with the AUCs of the 13.5-mg/kg dose similar to those of the 9.0-mg/kg dose (Online Table IV).

Effect of ACP-501 on Plasma CE

Similar to HDL-C, statistically significant mean % changes in maximum plasma CE of 10%, 11%, 19%, and 22% were observed at doses of 0.9, 3.0, 9.0, and 13.5 mg/kg, respectively. The mean % changes in the 9.0- and 13.5-mg/kg ACP-501 groups were statistically significant versus the low-dose group (P=0.0273 and P=0.0077, respectively). As shown in Figure 2B, the mean absolute change from baseline in plasma CE started to increase by 1 hour post infusion in the both 9.0- and 13.5-mg/kg ACP-501 dose groups, peaked between 6 and 24 hours and then gradually decreased over several days. Smaller changes in CE were observed at the lower doses, but during the initial 48 hours post infusion, the AUCs for plasma CE increased proportional to the dose (Online Table V).

Table. Demographic and Other Baseline Characteristics

	ACP-501 Dose, mg/kg				
Characteristic	0.9 (n=4)	3.0 (n=4)	9.0 (n=4)	13.5 (n=4)	Total (n=16)
Age, y					
Mean (SD)	66.3 (2.4)	68.3 (7.9)	70.5 (6.8)	65.3 (2.9)	67.6 (5.4)
Median	67.0	68.5	72.0	66.5	67.0
Min–max	63–68	60–76	61–77	61–67	60–77
Sex, n (%)					
Men	3 (75.0)	4 (100)	3 (75.0)	4 (100)	14 (87.5)
Women	1 (25.0)	0 (0.0)	1 (25.0)	0 (0.0)	2 (12.5)
Race, n (%)					
White	3 (75.0)	4 (100)	2 (50.0)	3 (75.0)	12 (75.0)
Asian	1 (25.0)	0 (0.0)	1 (25.0)	1 (25.0)	3 (18.8)
Black	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	1 (6.3)
Ethnicity, n (%)					
Not Hispanic or Latino	4 (100)	4 (100)	4 (100)	4 (100)	16 (100)
Height, cm					
Median	166.4	178.3	172.4	177.6	175.2
Min–max	162.0–175.9	166.5–181.9	167.5–177.4	174.5–184.8	162.0–184.8
Weight, kg					
Median	81.2	93.0	82.4	79.1	83.9
Min–max	75.6–85.1	76.9–95.3	78.5–94.2	66.8–96.2	66.8–96.2
BMI, kg/m^2					
Median	28.8	28.8	28.8	25.4	28.1
Min–max	27.1–30.2	27.7–29.6	25.2–30.8	21.4–28.2	21.4–30.8
LCAT mass, μg/mL					
Median	7.9	7.5	8.4	8.2	8.0
Min–max	6.5–11.9	6.9–8.6	6.3–13.5	6.2–8.9	6.2–13.5
HDL-C, mg/dL					
Mean (SD)	40.5 (6.6)	37.8 (4.3)	35.3 (4.6)	34.8 (3.4)	37.0
Median	38.0	38.5	35.0	34.0	…
Min–max	36–50	32–42	30–41	32–39	32–50
LDL-C, mg/dL					
Mean (SD)	72.5 (50.0)	54.3 (5.4)	71.8 (14.1)	97.0 (44.2)	73.9
Median	51.5	55	74	99	
Min–max	41–146	47–60	54–85	41–149	41–149
TG, mg/dL					
Mean (SD)	130.8 (60.3)	95.3 (25.5)	147.3 (71.3)	182.0(139.7)	138.9
Median	135	98	130	151	
Min–max	60–194	66–119	87–242	49–378	49–378
VLDL-C, mg/dL					
Mean (SD)	26.0 (12.2)	19.0 (5.3)	29.3 (14.1)	36.5 (28.1)	27.7
Median	26.5	19.5	26.0	30.0	…
Min–max	12–39	13–24	17–48	10–76	10–76
TC, mg/dL					
Mean (SD)	138.0 (52.3)	111.0 (9.0)	138.8 (16.0)	166.3 (53.9)	138.5
Median	119	108	135	182	…
Min–max	101–214	104–124	124–161	92–209	92–214

BMI indicates body mass index; HDL-C, high-density lipoprotein-cholesterol; LCAT, lecithin:cholesterol acyltransferase; LDL-C, low-density lipoprotein-cholesterol; TC, total cholesterol; TG, triglycerides; and VLDL-C, very low-density lipoprotein.

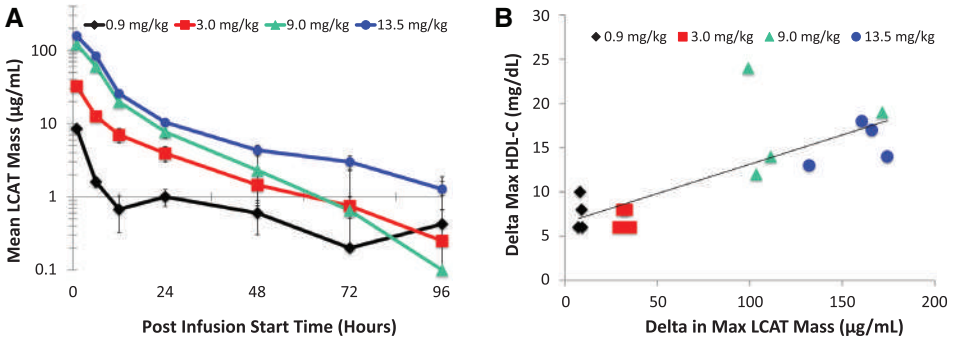

Figure 1. Plasma kinetic decay curve of lecithin:cholesterol acyltransferase (LCAT). A, ACP-501 was intravenously infused over a 1-h time point, and total LCAT mass was measured at the indicated time points by an ELISA that measures both recombinant human LCAT (ACP-501) and endogenous LCAT. Results represent the mean (n=4) ±1 SEM for each dose cohort. The baseline LCAT mass for each patient at time 0 was subtracted from subsequent time points. **B**, Dose–response relationship between ACP-501 dose and change in high-density lipoprotein-cholesterol (HDL-C). The maximum HDL-C concentration change from baseline that occurred any time after the infusion was plotted against the plasma level of total LCAT mass after completion of infusion. Results are shown for individual subjects for the indicated dose cohorts.

Effect of ACP-501 on Other Lipid and Lipoprotein Parameters

A full lipid profile (TC, TG, LDL-C, apoB, apoA-I, apoA-II, and TC/HDL-C) was also assessed at time points up to and including day 7. There was an ≈10–15-mg/dL increase in apoA-I in the 9.0- and 13.5-mg/kg dose groups, which remained partially elevated when compared with baseline up to day 8 (Figure 3A). As shown for the 9.0-mg/kg dose cohort (Figure 3B), changes in TC for the first 24 hours after infusion closely paralleled changes observed in HDL-C. Later when HDL-C started to decrease, however, TC still remained elevated. LDL-C showed a slight transient decrease immediately after ACP-501 infusion and then later increased by day 3 by ≈10 to 15 mg/dL before decreasing again back to baseline along with TC. All other dose cohorts (both higher and lower) also showed a slight drop in LDL-C immediately after the infusion and then a later increase in LDL-C but only back to baseline or only slightly above baseline by day 3 (Online Figure I). There was no apparent ACP-501 infusion effect on TG, apoB, and apoA-II throughout the 7 days post infusion for each dose group. Finally, the atherogenic profile of subjects, as defined by TC/HDL-C ratio, was decreased throughout the 7 days post infusion with a maximum decrease of ≈22% within the initial 24 hours post infusion for the both 9.0- and 13.5-mg/kg dose groups.

In Online Figure II, we also measured the effect of ACP-501 treatment on HDL and LDL particle counts, as determined by nuclear magnetic resonance (NMR). In contrast to HDL-C, ACP-501 seemed to have only a minimal effect on HDL-P. A small increase of ≈10% to 15% was observed in HDL-P for time points after 48 hours, for the 2 highest dose cohorts, but this change did not reach statistical significance. No major changes were also observed in LDL-P after ACP-501 treatment.

Effect of ACP-501 on HDL Subfraction Distribution

Infusion of ACP-501 caused a rapid conversion of small HDL subfractions to larger HDL subfractions (Figure 4A–4C), which is consistent with the known effect of LCAT on HDL maturation. Within 12 hours of infusion for the 9.0- and 13.5-mg/kg dose (1) small- and intermediate-sized HDL subfractions decreased by ≈8% and 13% for the 9.0- and 13.5-mg/kg dose, respectively, and (2) a corresponding 10% and 15% increase was observed in large-sized HDL subfractions. HDL subfraction distribution returned to baseline by 168 hours. Similar changes in the small, medium, and large HDL subfraction distribution after ACP-501 treatment (9.0- and 13.5-mg/kg dose) were detected by NMR analysis (Online Table VI).

Analysis of HDL subfractions by nondenaturing gel electrophoresis showed a similar pattern (Figure 4D). Pre–β-HDL, which is not detectable by the Lipoprint tube gel or NMR

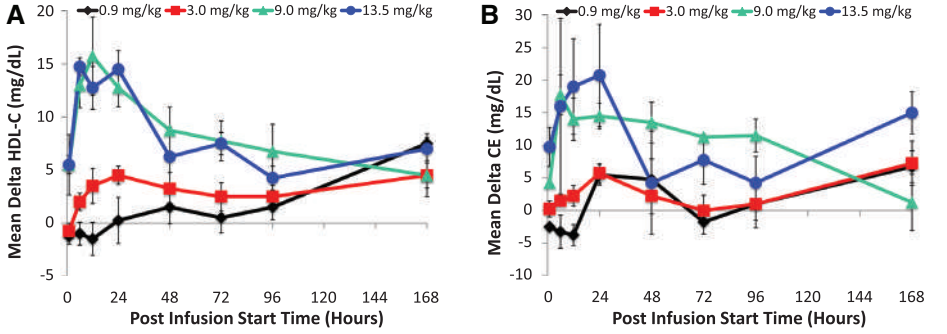

Figure 2. Time course for changes in high-density lipoprotein-cholesterol (HDL-C) and cholesteryl ester (CE) after ACP-501 infusion. A, Mean absolute change from baseline in plasma HDL-C through day 7. **B**, Mean absolute change from baseline in plasma CE through day 7. Results represent mean (n=4) ±1 SEM for each dose cohort.

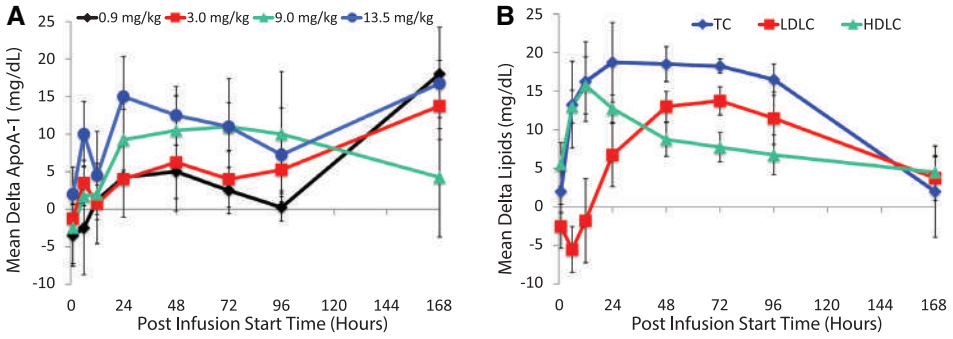

Figure 3. Time course for changes in apoA-I and cholesterol levels after ACP-501 infusion. **A**, Mean absolute change from baseline in apoA-I levels over time through day 7. Results represent mean (n=4) ±1 SEM for each dose cohort. **B**, Mean absolute change from baseline for indicated lipids is shown through day 7. Results represent mean (n=4) ±1 SEM for the 9.0-mg/kg dose cohort.

methods used above, disappeared 1 hour after the infusion, and then reappeared at 12 hours at slightly higher levels. An increase in the larger spherical HDL subfractions ($\alpha_{1\text{-}3}$-HDL) occurred by 6 to 12 hours, and a decrease in the discoidal α_4-HDL subspecies was evident during this time. By 168 hours, the HDL subfraction distribution was similar to baseline.

Effect of ACP-501 on Ex Vivo Cholesterol Efflux and LCAT Activity

The effect of ACP-501 infusion on ex vivo HDL-mediated cholesterol efflux is shown for the 9-mg/kg dose cohort in Figure 5. Serum was treated with polyethylene glycol to remove non-HDL lipoproteins and incubated with J774 macrophages not stimulated with cAMP to measure non–ABCA1-dependent cholesterol efflux (Figure 5A) and with J774 macrophages stimulated with cAMP to induce ABCA1 for the measurement of global cholesterol efflux (Figure 5B).[23] The ABCA1-dependent component of cholesterol efflux (Figure 5C) was calculated by subtracting the non–ABCA1-dependent cholesterol efflux from the global efflux results. Non–ABCA1-dependent cholesterol efflux increased by ≈20% when compared with baseline after ACP-501 treatment and remained elevated for several days. Similar results were seen in global cholesterol efflux, but the increase was slightly larger and at peak levels it was ≈30% higher than baseline. This is consistent with an increase in ABCA1-dependent cholesterol efflux from the ACP-501 treatment, but the results for ABCA1-dependent cholesterol efflux were highly variable and the change was not statistically significant. We also consistently observed a small and transient (<12 hours) decrease in ABCA1-dependent cholesterol efflux (P=0.06) after ACP-501 treatment, which corresponded to the time points when

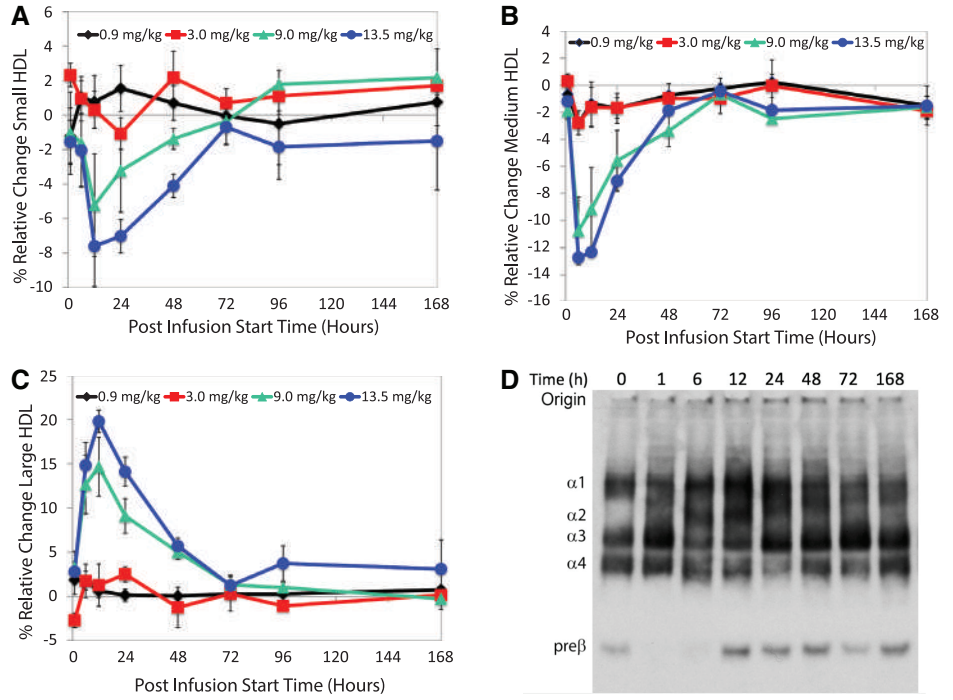

Figure 4. Time course for changes in high-density lipoprotein (HDL) subfraction distribution after ACP-501 infusion. Mean % change from baseline in small (**A**), medium (**B**), and large (**C**) HDL subfractions over time, determined by Lipoprint electrophoresis followed by staining with Sudan Black. Results represent mean (n=4) ±1 SEM for each dose cohort. **D**, Nondenaturing gel electrophoresis of serum from a patient receiving 9.0-mg/kg ACP-501 collected at the indicated time points and immunoblotted for apoA-I.

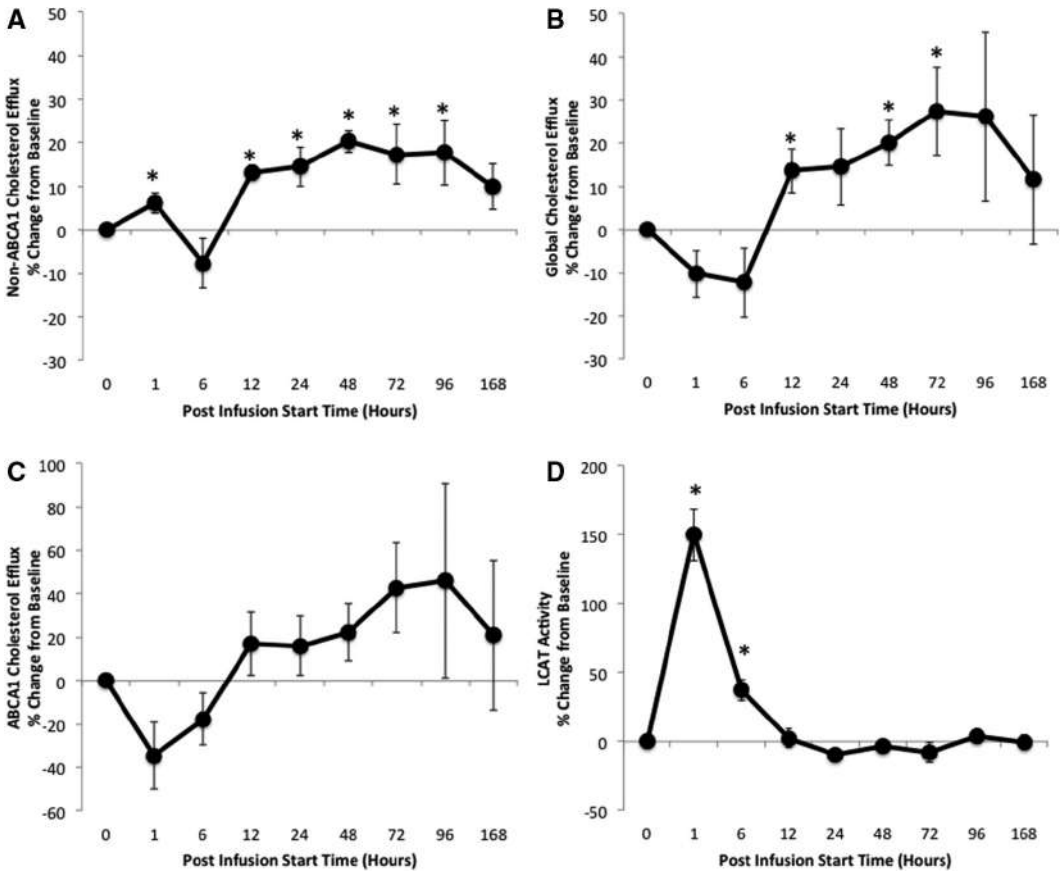

Figure 5. Time course for changes in high-density lipoprotein (HDL)-mediated cholesterol efflux and lecithin:cholesterol acyltransferase (LCAT) activity after ACP-501 infusion. A, Mean % change from baseline in non–ABCA1-dependent cholesterol efflux from J774 cells not stimulated with cAMP. **B**, Mean % change from baseline in global cholesterol efflux from J774 cells stimulated with cAMP. **C**, Mean % change from baseline in ABCA1-dependent cholesterol efflux. **D**, Mean % change from baseline in cholesterol esterification during the ex vivo cholesterol efflux study from J774 cells not stimulated with cAMP. Results represent mean (n=4) ±1 SEM for the 9-mg/kg dose cohort. Mean absolute values at baseline ±1 SEM were the following: non-ABCA1 cholesterol efflux (4.35±0.44% per 4 h), Global cholesterol efflux (8.14±1.79% per 4 h), ABCA1 cholesterol efflux (3.79±1.71% per 4 h), LCAT activity (15.6±3.0% per 4 h). *P<0.05 compared with baseline.

pre–β-HDL levels were low after the treatment (Figure 4D). The percent of cholesterol esterified during the efflux assay was also monitored from the cells not treated with cAMP as a measure of LCAT activity. Samples collected within the first 6 hours after the infusion of ACP-501 showed a marked increase in the percent of effluxed cholesterol in the media that was esterified, but this parameter returned to baseline thereafter (Figure 5D).

Discussion

Because this is the first study investigating the infusion of rhLCAT (ACP-501) in human subjects, the finding that ACP-501 had an acceptable safety profile was the most important outcome of this study. All patients were able to complete the study, and no serious AEs were observed. In addition, a dose-proportional increase in HDL-C was found after infusion of ACP-501, consistent with the known effect of LCAT on raising HDL-C by catalyzing the esterification of cholesterol. Overall, these 2 findings support the future development of ACP-501.

In terms of pharmacokinetics and pharmacodynamics, most of the findings were expected. Peak levels of LCAT mass occurred shortly after the ACP-501 infusion and began to return to baseline by 96 hours and eventually reached basal levels at 168 hours consistent with the known half-life of LCAT. ACP-501 infusion produced a dose-related increase in HDL-C AUC up to the 9.0-mg/kg dose. Depending on baseline values, doses of ACP-501 higher than 9.0 mg/kg increased HDL-C on average between 36% and 42% and as much as 67% in 1 patient. Similarly, the total amount of plasma CE was also increased. It was of interest that the pharmacodynamics response (HDL-C and plasma CE) persisted beyond the pharmacokinetics (ACP-501 levels), suggesting that changes related to the remodeling of HDL by the infused rhLCAT persisted for several days. The formation of larger HDL with a longer half-life could potentially explain these observations.[4] There was also a modest rise of apoA-I in the 3.0-, 9.0-, and 13.5-mg/kg dose groups that lasted over the course of 7 days. For the 9.0-mg/kg dose of ACP-501, a delayed and transient increase in LDL-C was also observed after HDL-C peaked and then started to decline. One possible interpretation of these findings is that CE in HDL was transferred to LDL via CETP.[24] This change, however, was variable and was not seen in either the lower or higher dose cohorts (Online Figure I). No significant or consistent changes with ACP-501 treatment were also observed in any of the dose cohorts for either apoB

or LDL-P. Both of these alternative markers of LDL have been shown in several studies to be better than LDL-C as a cardiovascular disease risk marker,[25] but the possible effect of ACP-501 in changing LDL-C and the role of CETP will have to be monitored carefully in future studies.

On the basis of the known effect of LCAT on HDL metabolism,[8,26–29] the infusion of ACP-501 most likely raised HDL-C by enhancing its ability to act as a cholesterol sink. Assuming a 70-kg adult, the mean increase of 15 mg/dL for HDL-C with the 9.0- and 13.5-mg/kg dose of ACP-501 (Figure 2) should correspond to ≈470 mg of cholesterol being mobilized by the treatment. Although the tissue source of this cholesterol was not investigated, red blood cells are likely to be an important source of free cholesterol as they contain an abundant pool of free cholesterol[30] that is accessible to LCAT. In FLD patients, free cholesterol is significantly enriched on RBCs, likely contributing to their anemia; additional studies to determine the effect of ACP-501 treatment on anemia and RBC half-life will help to clarify this issue. The overall change in cholesterol mobilization seen in this study is comparable with what has been observed in clinical trials involving the infusion of rHDL, which have shown promise in early stage clinical trials.[14–16] Larger HDL particles were also formed after ACP-501 infusion presumably as a consequence of CE enrichment at the expense of the pre–β-HDL, which again is consistent with the known effects of LCAT on HDL maturation. Radiotracer studies in humans have shown that the vast majority of esterified cholesterol is eventually delivered to the liver in one of the last steps in RCT.[24]

In addition to modulating the lipid content of HDL, ACP-501 also seemed to affect its ability to promote cholesterol efflux from cells, using an in vitro cell-based assay (Figure 5). Using LDL-depleted serum, ACP-501 treatment caused a modest increase when compared with baseline in non-ABCA1 cholesterol efflux, which persisted for several days and is consistent with previous studies.[20] An even larger increase of ≈30% compared with baseline was observed in global cholesterol efflux, which also persisted for several days. This is consistent with an increase in ABCA1-dependent cholesterol efflux from the ACP-501 treatment, but the results were more variable and did not reach statistical significance. We also observed a slight transient decrease in ABCA1-dependent cholesterol efflux from macrophages following ACP-501 treatment. The acute drop in ABCA1-dependent cholesterol efflux corresponded to the time period when pre–β-HDL was depleted from the serum (Figure 4D). Because the macrophage cells in the in vitro assay were stimulated to induce the expression of ABCA1, this is consistent with a mechanism whereby ACP-501 enhanced cholesterol efflux and the maturation of HDL in vivo, thus depleting pre–β-HDL and producing large HDL with lower ability to remove cholesterol by ABCA1 during the in vitro cholesterol efflux assay.[31] This result is also consistent with previous studies showing that serum from LCAT-deficient patients with high pre–β-HDL levels had an increase in the in vitro cholesterol efflux from cells expressing ABCA1.[32] We also observed that more of the effluxed cholesterol, during the in vitro cholesterol efflux study, was esterified after the ACP-501 treatment, which would also be predicted to enhance net cholesterol efflux from cells.[10,24] Additional studies, however, will be needed to fully understand how these in vitro findings relate to in vivo cholesterol efflux and the overall effect of LCAT on the RCT pathway.

Most of the evidence from previous animal models supports the rationale for stimulating RCT and reducing atherosclerosis by increasing the amount of LCAT. LCAT transgenic rabbits have high HDL-C levels and are protected from diet-induced atherosclerosis.[21] Furthermore, wild-type rabbits treated with rhLCAT are also protected against diet-induced atherosclerosis.[22] Studies using adenoviral gene transfer have demonstrated increased HDL-C in monkeys,[19] enhanced biliary cholesterol excretion in hamsters,[33] and cholesterol unloading from preestablished atherosclerotic lesions in rabbits.[34] Importantly, these are all animal species that, like humans, transport most of their plasma cholesterol in LDL and express CETP. Without CETP, there would be predicted to be a block in the transport of CE to the liver, thus causing the accumulation of CE in HDL (dysfunctional HDL), as occurs in C57Bl/6 mice overexpressing LCAT[7,35] and possibly in humans treated with CETP inhibitors. Coexpression of CETP in LCAT transgenic mice corrects dysfunctional HDL and reduces atherosclerosis.[36] In another mouse study, adenoassociated viral LCAT expression increased fecal cholesterol excretion from radiolabeled macrophages implanted into the peritoneal cavity of LCAT-knockout mice but did not do so from wild-type mice that also expressed CETP.[20] Transgenic rabbits lacking the LDL receptor were also not protected against atherosclerosis when they overexpressed LCAT, which suggests that the delivery of cholesterol transferred by CETP to LDL and its subsequent uptake by hepatic LDL receptors may be a critical step in this pathway. ACP-501 may perhaps work synergistically with statins, and other therapies, such as proprotein convertase subtilisin/kexin type 9 inhibitors, that upregulate the LDL receptor.

Recent data from human studies also suggest that LCAT may be rate-limiting in patients with CHD although some contradictory studies have also been described. Participants in the current study had normal LCAT mass and this criteria, along with LCAT activity and pre–β-HDL, were not used to select patients, but it has been previously shown that low LCAT activity and high pre–β-HDL are associated with CHD.[5] For example, LCAT activity was lower in subjects with CHD in the Copenhagen City Heart Study.[11] In this study, pre–β-HDL levels as measured by ELISA were also markedly higher in the CHD group, even in those without dyslipidemia, than in the control group matched for traditional lipid markers. These data confirmed earlier work on subjects with CHD, using electrophoresis to measure pre–β-HDL.[17] Moreover, in earlier studies, the severity of CHD (ie, number of diseased coronary vessels) was correlated with LCAT activity.[37] The increase in pre–β-HDL levels in CHD has been ascribed to reduced levels of LCAT, resulting in delayed conversion of pre–β-HDL and α_4-HDL to larger spherical forms of HDL.[35] Others, however, have reported increased LCAT mass in patients with metabolic syndrome,[38] but this could perhaps be a compensatory response. A recent GWAS examining polymorphisms in LCAT associated with low HDL-C also failed to show an association with cardiovascular outcomes.[13] It is important to note, however, that the acute infusion of a large dose of rhLCAT is

a nonphysiologic intervention, and thus it may be difficult to discern the effect of this type of treatment on atherosclerosis from any type of observational study in humans.

In summary, the current study supports the future investigation of rhLCAT in phase 2a multiple-dose trials at weekly doses. In addition to CHD, future clinical trials may also be aimed at treating FLD patients, based on the previous demonstration that rhLCAT can reverse the lipid and lipoprotein abnormalities in LCAT-knockout mice.[7] rhLCAT may ameliorate the anemia and, even more importantly, the life-threatening renal complications that develop in this disorder.[3,6] Key outstanding questions for rhLCAT therapy include the following: (1) the immunogenic potential of this protein, (2) the optimal dosing regimen, and (3) the optimal biomarkers for efficacy. In regard to CHD, it will be important to determine in future studies whether rhLCAT in humans can enhance RCT or whether it can also positively affect one of the many other proposed antiatherogenic functions of HDL.[9] It will also be important to identify biomarkers that may predict a good response to the therapy, such as low LCAT activity, low HDL-C levels, and high pre–β-HDL.

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Novelty and Significance

What Is Known?

- Decreased high-density lipoprotein-cholesterol (HDL-C) levels are associated with increased risk for atherosclerosis.
- Lecithin:cholesterol acyltransferase (LCAT) catalyzes plasma free cholesterol to cholesteryl ester, converting small HDL to large HDL in the proposed second step of reverse cholesterol transport.
- In patients with coronary heart disease (CHD), the levels of small HDL enriched in free cholesterol are elevated and HDL-C is decreased, suggesting that LCAT availability may be rate limiting for reverse cholesterol transport.
- LCAT is also defective in familial LCAT deficiency and fish eye disease and accounts for the clinical manifestations of these 2 genetic disorders.

What New Information Does This Article Contribute?

- Recombinant human LCAT (rhLCAT) was tested as a novel therapeutic agent and was found to be safe and well tolerated in patients with CHD and low HDL-C, with no clinically meaningful changes in safety labs or serious adverse events.
- Plasma concentrations of rhLCAT, HDL-C, and cholesteryl ester increased and pre–β-HDL rapidly decreased after rhLCAT infusion.
- Small HDL was converted to antiatherogenic larger HDL.
- rhLCAT is the first-in-class therapy that stimulates the rate-limiting second step in HDL reverse cholesterol transport, thus offering a new mechanism to increase HDL.

rhLCAT was developed as a new treatment to raise HDL-C in patients with CHD and low HDL-C and for patients with familial LCAT deficiency, a rare orphan disorder of extremely low HDL-C. We conducted a first-in-human phase 1b, open-label, single intravenous infusion, dose-escalation of 4 doses of rhLCAT in patients with stable CHD and low HDL-C to evaluate safety, tolerability, pharmacokinetics, and pharmacodynamics. rhLCAT was safe and well tolerated with no infusion site reactions. HDL-C was unchanged at the lowest no-effect dose but was elevated by 6%, 36%, and 42% by 6 hours after increasing doses and remained above baseline for at least 4 days. Free cholesterol (the substrate of LCAT) was reduced after rhLCAT infusion but later reappeared while cholesteryl ester (the product) paralleled the rapid appearance of HDL-C. After infusion of rhLCAT, small- and intermediate-sized HDL particles decreased, whereas the larger more atheroprotective HDLs were formed. LCAT seems to be a rate-limiting step in the pathway of HDL maturation in patients with CHD with low HDL-C. The lipid and lipoprotein changes indicate that rhLCAT favorably alters HDL metabolism and support rhLCAT use in future clinical trials in CHD and familial LCAT deficiency patients.

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Safety and Tolerability of ACP-501, a Recombinant Human Lecithin:Cholesterol Acyltransferase, in a Phase 1 Single-Dose Escalation Study

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SUPPLEMENTAL MATERIAL

Safety and Tolerability of ACP-501, a Recombinant Human Lecithin:Cholesterol Acyltransferase, in a Phase 1 Single-Dose Escalation Study

ONLINE METHODS

ACP-501 Formulation and Administration

The drug product, ACP-501, a 5.3 mL preservative-free solution of rhLCAT, was dissolved in 25 mM sodium phosphate, 100 mM sodium chloride, and 2% (20 mg/mL) glycerol at pH 7.2, and supplied in 5 mL clear glass vials at a concentration of 9.9 mg/dL. rhLCAT was purified from conditioned media from a CHO cell line stably transfected with the human LCAT cDNA in a procedure similar to what has been previously been described.[1] It was over 99% pure as tested by gel electrophoresis, sterile and endotoxin free. It was stored frozen at -80^{o} C at the study site, thawed and diluted with normal saline to obtain a total volume of 270 mL and infused over a 1-hour period. When stored frozen at -80^{o} C, ACP-501 was found to be stable in terms of LCAT activity for at least 6 months.

Analytical Methods

Clinical chemistries, hematology, urinalysis, and lipid profile were analyzed by the clinical laboratory at the NIH Clinical Center on a Vista analyzer, using Siemen reagents. LDL-C was calculated by the Friedewald equation. Auxiliary lipids (total cholesterol [TC], free cholesterol [FC], and cholesteryl esters [CE] by enzymatic methods), HDL-C (dextran sulfate precipitation method), and LCAT mass were analyzed by Pacific Biomarkers, Inc. (PBI, Seattle, WA). HDL-C values provided by PBI were chosen for the PK and PD analysis, because they were standardized to the CDC reference method. The LCAT mass, which measures both rhLCAT and endogenous LCAT, was determined with a validated enzyme-linked immunosorbent assay with a reference range of 5-11 ug/mL (Daiichi Fine Chemical Co Japan (distributed by ALPCO). The HDL subfraction distribution in serum was obtained by Sudan Black B staining and polyacrylamide gel electrophoresis with the Lipoprint® system (Quantimetrix, Redondo Beach, CA). Single dimension native gel electrophoresis (nondenaturing TBE gradient minigels) and Western blotting were performed, as previously described for the determination of preβ-HDL.[2]

Cholesterol Efflux and Ex Vivo LCAT Activity

Serum HDL (apoB-depleted serum) was prepared from individual serum samples by precipitation of apoB-containing lipoproteins with polyethylene glycol (20%, v/v, in glycine buffer, pH 7.4). Non-ABCA1 cholesterol efflux and global cholesterol efflux was measured for 4 hours from J774 mouse macrophage stimulated to express ABCA1 to apoB-depleted serum (added at 2.8%, v/v), as previously described.[3] For determination of ex vivo LCAT activity, the proportion of [^{3}H]-cholesterol effluxed from J774 cells to serum that was esterfied during the 4 hour period was measured after the separation of free and esterified cholesterol by thin layer chromatography, as previously described.[3]

Statistical Analysis

The full analysis set (FAS) was defined as all subjects who were treated; all efficacy and safety analyses were performed on the FAS. Baseline values were defined as the last valid assessment prior to the first administration of investigational drug. Statistical comparisons of PD parameters were made among doses of ACP-501, using an analysis of covariance (ANCOVA) model that included investigational infusion dose as a factor and baseline PD parameter measurement as a covariate. No adjustments were made for multiple comparisons, because of

the nature of the study. All statistical tests were conducted at a significance level of 0.05 and were performed using SAS software (version 9.3, SAS Institute Inc., Cary, North Carolina).

ONLINE TABLES

Online Table I: Study Exclusion Criteria

Subjects who met any of the following criteria were not eligible for entry into the study:

1. Myocardial infarction, stroke, or coronary intervention/revascularization procedure within 6 months prior to dosing
2. Chronic heart failure (> New York Heart Association Class II)
3. Ventricular tachyarrhythmias
4. Uncontrolled type 2 (hemoglobin A1c > 8.5%) or type 1 diabetes mellitus
5. History of febrile illness within 5 days prior to dosing
6. History of regular alcohol consumption exceeding 10 drinks per week
7. Twelve (12)-lead electrocardiogram (ECG) demonstrating QTc > 500 milliseconds at screening
8. Blood donation of approximately 1 pint (500 mL) within 56 days prior to dosing
9. Treatment with an investigational drug within 28 days prior to dosing
10. Known hypersensitivity to heparin or IV infusion equipment, plastics, adhesive or silicone or known history of hypotension or infusion site reactions with IV administration
11. Other severe acute or chronic medical or psychiatric condition or laboratory abnormality that may have increased the risk associated with trial participation or investigational product administration or may have interfered with the interpretation of trial results and, in the judgment of the investigator, would make the subject inappropriate for entry into this trial
12. Total bilirubin > 2.0 × upper limit of normal (ULN); creatinine ≥ 2.0 mg/dL; aspartate aminotransferase (AST) or alanine aminotransferase (ALT) > 1.5 × ULN; alkaline phosphatase > 1.5 × ULN; or hemoglobin < 11 g/dL (< 110 g/L)

Online Table II: Listing of All Treatment-emergent Adverse Events

Dose Group (mg/kg)	SID	MedDRA SOC/ Preferred Term	Start Day	Stop Day	Inten-sity	Outcome	Action Taken	Related to ACP-501	SAE
0.9	01-002	Skin and subcutaneous tissue disorders/ Rash	D2	D45	Mild	Recovered without sequelae	None	Possibly	No
	01-003	Metabolism and nutrition disorders/ Dehydration	D8	D9	Mild	Recovered without sequelae	None	Definitely not	No
9.0	01-004	Metabolism and nutrition disorders/ Dehydration	D3	D8	Mild	Recovered without sequelae	None	Definitely not	No
	01-009	Metabolism and nutrition disorders/ Dehydration	D3	D8	Mild	Recovered without sequelae	None	Definitely not	No
	01-019	Metabolism and nutrition disorders/ Dehydration	D8	D14	Mild	Recovered without sequelae	None	Definitely not	No
13.5	01-018	Skin and subcutaneous tissue disorders/ Rash	D1	D2	Mild	Recovered without sequelae	None	Possibly	No
		Metabolism and nutrition disorders/ Hyperglycemia	D2	D5	Mild	Recovered without sequelae	None	Definitely not	No
	01-022	Metabolism and nutrition disorders/ Hypertriglyceri-demia	D3	D4	Mild	Recovered without sequelae	None	Unlikely	No

SID = subject identification
MedDRA SOC = Medical Dictionary for Regulatory Activities System Organ Class

Online Table III: Summary of PK Parameters for ACP-501

Parameter	Dose of ACP-501 (mg/kg)			
	0.9 (n = 4)	3.0 (n = 4)	9.0 (n = 4)	13.5 (n = 4)
C_{max} (µg/mL)				
n	4	4	4	4
Mean (SD)	8.50 (0.95)	32.3 (2.81)	121 (33.9)	158 (18.3)
Median	8.6	32.2	107	163
Min – max	7.30 – 9.40	29.1 – 35.6	99.2 – 172	132 – 174
%CV	11.2	8.72	27.9	11.6
T_{max} (hours)				
n	4	4	4	4
Mean (SD)	1.00 (0.00)	1.00 (0.00)	1.01 (0.02)	1.10 (0.20)
Median	1.00	1.00	1.00	1.00
Min – max	1.00 – 1.00	1.00 – 1.00	1.00 – 1.05	1.00 – 1.40
%CV	0.00	0.00	2.5	18.2
Terminal $t_{½}$ (hours)				
n	0	0	3	4
Mean (SD)	NA *	NA *	44.3 (19.6)	40.8 (37.4)
Median	NA	NA	42.7	23.7
Min – max	NA – NA	NA – NA	25.5 – 64.7	19.1 – 96.5
%CV	NA	NA	44.4	91.7
AUC_{0-48} (µg-hr/mL)				
n	4	4	4	4
Mean (SD)	66.9 (19.1)	318 (103)	1040 (212)	1404 (98.7)
Median	62.0	276	1030	1380
Min – max	50.7 – 92.9	251 – 471	803 – 1290	1320 – 1530
%CV	28.6	32.4	20.4	7.0
AUC_{0-t} (µg-hr/mL)				
n	4	4	4	4
Mean (SD)	177 (102)	365 (133)	1170 (323)	1620 (199)
Median	138	316	1180	1580
Min – max	107 – 324	265 – 561	789 – 1520	1420 – 1890
%CV	57.5	36.5	27.7	12.3
$AUC_{0-\infty}$ (µg-hr/mL)				
n	0	0	3	4
Mean (SD)	NA *	NA *	1340 (269)	1690 (312)
Median	NA	NA	1370	1590
Min – max	NA – NA	NA – NA	1050 – 1590	1430 – 2140
%CV	NA	NA	20.2	18.5

Mean, median, and Min-max had baseline subtracted.
* Plasma levels were too low to calculate the PK parameter.

Online Table IV: Change from Baseline in AUCs of HDL-C Following ACP-501 Infusion

	Dose of ACP-501 (mg/kg)			
	0.9 (n = 4)	3.0 (n = 4)	9.0 (n = 4)	13.5 (n = 4)
AUC_{0-48} (mg-hr/dL)				
Mean (SD)	0.40 (144)	161 (24.0)	561 (134)	546 (161)
Median (min, max)	11.8 (-186, 164)	156 (141, 189)	509 (471, 758)	581 (339, 681)
AUC_{0-96} (mg-hr/dL)				
Mean (SD)	48.4 (266)	289 (117)	933 (281)	851 (269)
Median (min, max)	83.8 (-306, 332)	303 (136, 417)	876 (684, 1290)	947 (459, 1050)
AUC_{0-168} (mg-hr/dL)				
Mean (SD)	372 (348)	542 (234)	1340 (588)	1260 (397)
Median (min, max)	479 (-126, 656)	591 (244, 741)	1330 (756, 1950)	1390 (675, 1560)

Online Table V: Change from Baseline in AUCs of Plasma CE Following ACP-501 Infusion

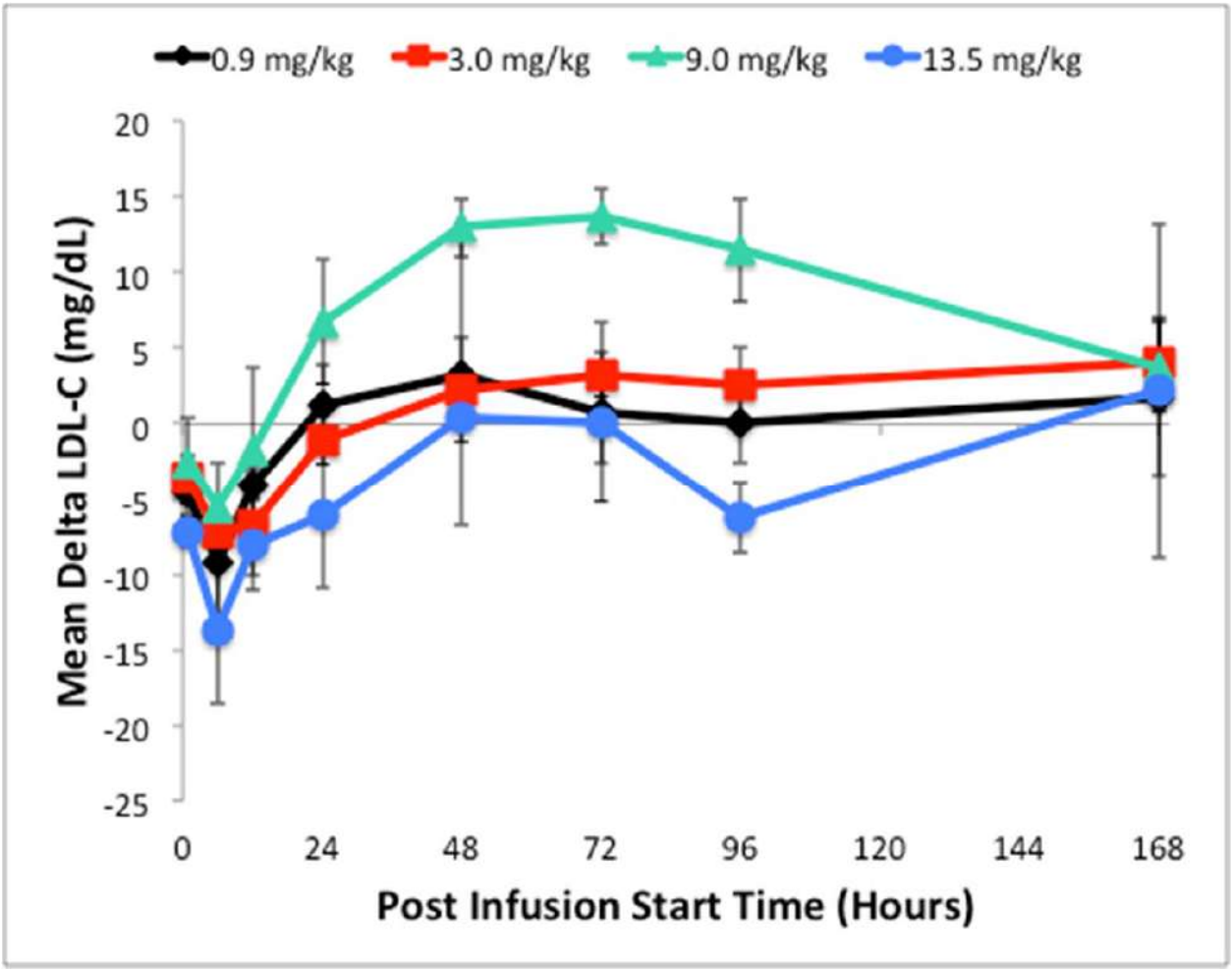
	Dose of ACP-501 (mg/kg)			
	0.9 (n = 4)	3.0 (n = 4)	9.0 (n = 4)	13.5 (n = 4)
AUC_{0-48} (mg-hr/dL)				
Mean (SD)	98.1 (30.7)	159 (63.8)	657 (131)	708 (678)
Median (min, max)	95.5 (65.0, 137)	165 (93.0, 215)	642 (532, 812)	569 (57.0, 1640)
AUC_{0-96} (mg-hr/dL)				
Mean (SD)	125 (181)	199 (301)	1230 (169)	996 (1098.67)
Median (min, max)	173 (-115, 269)	292 (-219, 430)	1170 (1102, 1470)	869 (-207, 2450)
AUC_{0-168} (mg-hr/dL)				
Mean (SD)	404 (514)	496 (787)	1690 (494)	1690 (1460)
Median (min, max)	479 (-223, 881)	724 (-579, 1110)	1550 (1280, 2360)	1280 (405, 3780)

Online Table VI HDL Lipoprotein profile by NMR

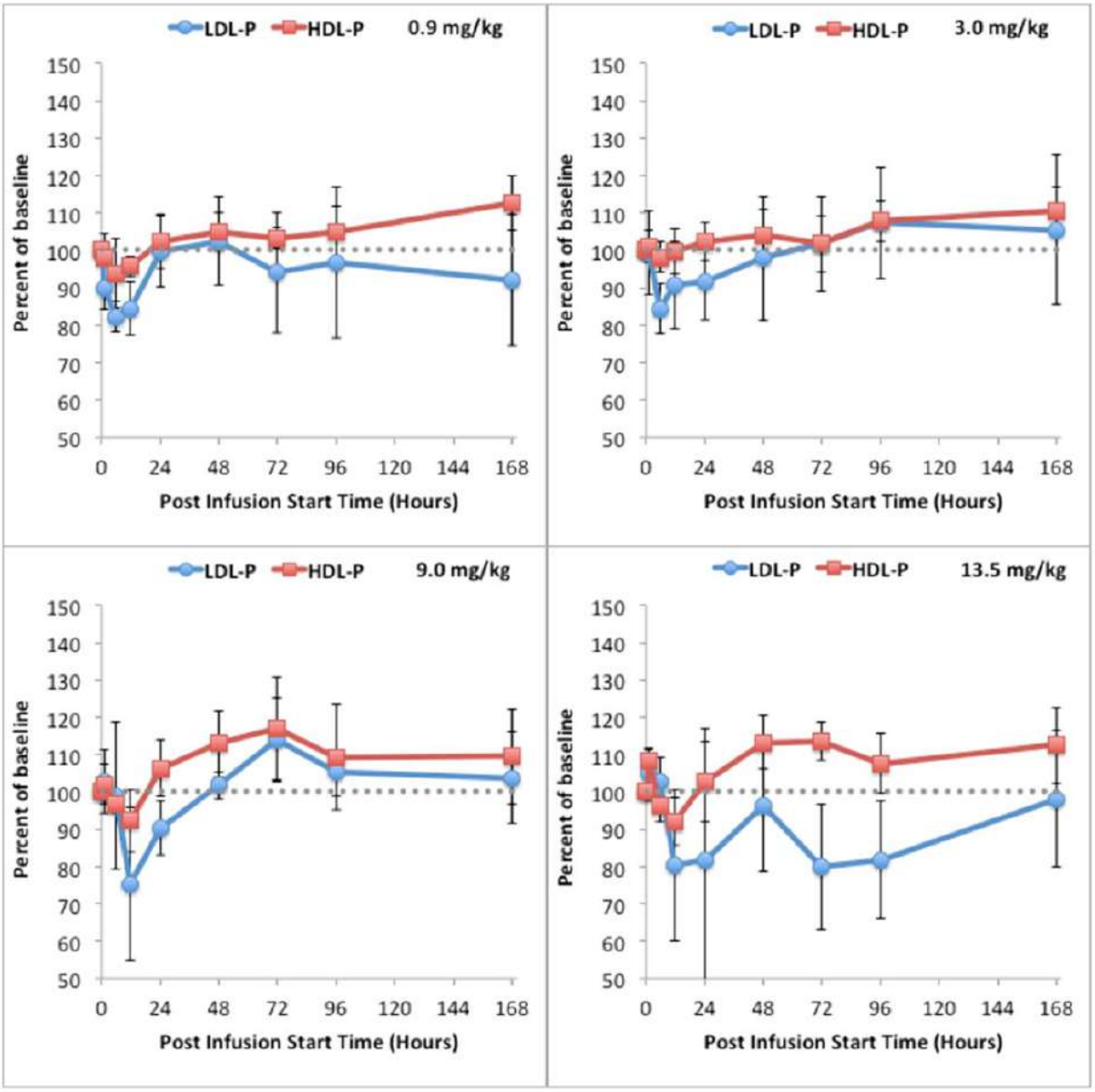
	0 h	1 h	6 h	12 h	2 D	3 D	4D	5D	8D
0.9 mg/kg dose group lipoprotein particle concentration									
Small HDL, µmol/L	15.9 ± 5.8	18.8 ± 4.3	17.1 ± 5.2	14.9 ± 4.6	18.4 ± 3.5	14.2 ± 3.2	16.0 ± 3.6	14.7 ± 5.3	16.4 ± 7.5
Medium HDL, µmol/L	9.8 ± 1.6	5.8 ± 2.0	7.2 ± 3.2	9.5 ± 3.4	7.4 ± 4.4	13.2 ± 5.2	12.9 ± 2.2	12.1 ± 6.5	13.2 ± 6.3
Large HDL, µmol/L	3.7 ± 0.5	3.7 ± 0.7	3.1 ± 0.3	3.7 ± 0.2	4.3 ± 0.5	3.6 ± 0.7	3.5 ± 0.1	4.1 ± 0.3	4.0 ± 0.7
Lipoprotein particle size									
HDL, nm	9.2 ± 0.1	9.4 ± 0.5	9.0 ± 0.3	9.2 ± 0.2	9.1 ± 0.2	9.1 ± 0.1	9.1 ± 0.0	9.1 ± 0.1	9.2 ± 0.2
3.0 mg/kg dose group lipoprotein particle concentration									
Small HDL, µmol/L	15.0 ± 4.2	18.4 ± 2.7	15.0 ± 4.3	11.2 ± 4.5	14.8 ± 4.1	16.0 ± 3.9	16.5 ± 3.0	15.4 ± 5.2	17.0 ± 4.6
Medium HDL, µmol/L	7.4 ± 4.5	4.8 ± 2.3	8.1 ± 2.8	10.7 ± 3.5	7.2 ± 3.8	7.2 ± 5.4	6.1 ± 2.9	8.7 ± 5.1	7.4 ± 3.4
Large HDL, µmol/L	4.0 ± 1.5	3.5 ± 1.7	3.0 ± 0.8	4.5 ± 1.0	4.9 ± 1.3	4.4 ± 1.2	4.3 ± 1.9	4.6 ± 1.5	5.0 ± 1.9
Lipoprotein particle size									
HDL, nm	9.1 ± 0.4	9.0 ± 0.2	8.9 ± 0.1	9.3 ± 0.3	9.3 ± 0.2	9.2 ± 0.3	9.2 ± 0.2	9.2 ± 0.3	9.3 ± 0.3
9.0 mg/kg dose group lipoprotein particle concentration									
Small HDL, µmol/L	15.9 ± 3.2	18.6 ± 1.2	13.2 ± 2.0*	8.8 ± 2.4*	14.8 ± 2.6	16.1 ± 2.9	15.9 ± 2.1	17.5 ± 1.2	17.3 ± 6.6
Medium HDL, µmol/L	5.5 ± 0.3	3.3 ± 1.8	8.0 ± 1.8	7.8 ± 2.2	4.1 ± 0.7	5.6 ± 1.8	7.3 ± 3.2	4.2 ± 3.3	4.9 ± 3.2
Large HDL, µmol/L	3.0 ± 1.6	3.0 ± 1.4	2.8 ± 2.8	5.4 ± 2.7[a]	6.1 ± 2.5*	5.5 ± 2.2*	4.6 ± 1.5*	4.9 ± 1.4*	4.7 ± 2.0
Lipoprotein particle size									
HDL, nm	9.2 ± 0.2	8.9 ± 0.1	9.0 ± 0.6	9.6 ± 0.5	9.6 ± 0.5	9.3 ± 0.3	9.3 ± 0.3	9.2 ± 0.3	9.4 ± 0.3
13.5 mg/kg dose group lipoprotein particle concentration									
Small HDL, µmol/L	18.2 ± 2.1	23.7 ± 1.6*	15.4 ± 1.0	10.2 ± 2.0*	12.0 ± 5.9	13.9 ± 5.4	17.7 ± 3.9	18.9 ± 4.8	21.5 ± 2.1
Medium HDL, µmol/L	7.7 ± 5.1	3.6 ± 1.4	8.1 ± 2.7	7.6 ± 2.6	8.7 ± 2.4	13.1 ± 7.4	9.1 ± 5.7	7.0 ± 4.5	5.3 ± 2.3
Large HDL, µmol/L	3.3 ± 1.6	3.0 ± 1.6	3.4 ± 1.3	6.8 ± 1.5*	7.4 ± 1.8*	5.4 ± 2.0*	4.6 ± 1.5*	4.5 ± 1.6*	4.3 ± 1.3
Lipoprotein particle size									
HDL, nm	9.1 ± 0.4	8.9 ± 0.5	9.0 ± 0.4	9.8 ± 0.5	9.8 ± 0.4	9.3 ± 0.4	9.4 ± 0.4	9.4 ± 0.5	9.4 ± 0.5

Data in means ± SD. Dose group n = 4. 2-sided Student's t test. *p ≤ 0.05 from baseline.

Diameters are as follows: small HDL (7.3-8.2 nm); medium HDL (8.2-9.4 nm), and large HDL (9.4-14.0 nm)



Online Figure I. Time course for changes in LDL-C after ACP-501 infusion. Mean absolute change from baseline in plasma LDL-C through day 7. Results represent mean (N=4) ±1 SEM for the each dose cohort.



Online Figure II. Time course for changes in HDL and LDL particle counts after ACP-501 infusion. Mean percent change from baseline in HDL-P and LDL-P through day 7. Mean HDL-P and LDL-P at baseline were 27 umol/L and 839 nmol/L, respectively. Results represent mean (N=4) ±1 SEM for each dose cohort.

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