CSL112 Enhances Biomarkers of Reverse Cholesterol Transport After Single and Multiple Infusions in Healthy Subjects

Andreas Gille, Rachael Easton, Denise D’Andrea, Samuel D. Wright, Charles L. Shear

Objective—The ability of apolipoprotein A-I (apoA-I) to transport cholesterol from atherosclerotic plaque is thought to underlie its inverse correlation with cardiovascular risk. To gauge the potential of infused apoA-I to transport cholesterol, we quantified cholesterol transport markers in human subjects infused with a novel formulation of apoA-I (CSL112).

Approach and Results—CSL112 was infused into human subjects in single (57 subjects) and multiple (36 subjects) ascending dose trials. Pharmacokinetic and biomarker assessments were conducted before and after infusions. CSL112 caused an immediate, up to 3-fold elevation of apoA-I and subsequent movement of tissue cholesterol into plasma. Cholesterol appeared first as unesterified cholesterol in the high-density lipoprotein (HDL) fraction and was promptly esterified by lecithin cholesterol acyltransferase. HDL cholesterol increased up to 81±16.5%. Underlying this movement of cholesterol was an immediate and strong rise in the ability of plasma to promote cholesterol efflux from cells ex vivo. CSL112 had its greatest impact on the fraction of efflux mediated by ATP-binding cassette transporter A1 (ABCA1), a cholesterol transporter induced in cholesterol-loaded tissues such as plaque. ABCA1-dependent efflux capacity increased ≤630±421% and total efflux capacity by ≤192±40%. In keeping with this finding, we observed a profound rise in very small HDL, also known as preβ1-HDL, the preferred substrate for ABCA1. Very small HDL increased ≤3596±941%. Elevations in apoA-I, cholesterol efflux, and very small HDL were dose-proportional over a wide range. No significant changes in atherogenic lipids were observed at any dose.

Conclusions—Infusion of CSL112 elevates the ability of plasma to withdraw cholesterol from cells. Preferential elevation of ABCA1-dependent efflux may target atherosclerotic plaque for cholesterol removal, making CSL112 a promising candidate therapy for acute coronary syndrome. (Arterioscler Thromb Vasc Biol. 2014;34:2106-2114.)

Key Words: acute coronary syndrome ■ CSL112 ■ reverse cholesterol transport

Atherosclerotic cardiovascular disease represents an enormous and increasing cause of global mortality.1 Cardiovascular events occur as a consequence of rupture or erosion of vulnerable atherosclerotic plaque2 and subsequent thrombotic events. Cholesterol is a defining and necessary component of atherosclerotic plaque, and cholesterol content correlates with the propensity to plaque rupture.3-6 Therapeutic strategies that either reduce plaque lipid volume or stabilize vulnerable plaques represent areas of intense clinical development.

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A potential therapeutic strategy that may enhance removal of plaque cholesterol is infusion of HDL or apoA-I.8-10 The infused HDL is expected to accept cholesterol from tissues and reduce the size, cholesterol content, and instability of atherosclerotic lesions. Infusion of complexes of recombinant apoA-I Milano and phospholipids demonstrated a reduction in atheroma volume by intravascular ultrasound after 5 once-weekly (1W) doses in patients with acute coronary syndrome (ACS).11 A further study of reconstituted HDL (CSL111) reported similar intravascular ultrasound findings and additionally showed significant changes in the plaque characterization index and coronary score.12 Both studies showed significant reduction in plaque size versus pretreatment levels, but neither study demonstrated significance versus placebo. In a different vascular bed, a single infusion of a reconstituted HDL preparation reduced femoral plaque lipid by >50% in 5 to 7 days.13 Taken together, this evidence suggests that infusion of HDL may be a viable strategy for increasing RCT and cholesterol efflux from atherosclerotic plaques.
CSL112 is a novel formulation of apoA-I, the chief protein component of HDL, purified from human plasma and reconstituted with phosphatidylcholine to form HDL particles suitable for infusion. The production, physical characteristics of CSL112, and the pharmacokinetic and biomarker profile in rabbits have recently been described. CSL112 is a diskoid particle containing 2 copies of apoA-I and ≈110 molecules of phosphatidylcholine. CSL112 was designed to provide improved particle uniformity and an improved safety profile compared with a previous formulation termed CSL111. The safety characteristics of CSL1112 and improved safety characteristics of CSL112 have been previously described. CSL112 is also designed to optimize cholesterol efflux by ATP-binding cassette transporter A1 (ABCA1), a transporter induced by excess cellular cholesterol and present in atherosclerotic plaque. A detailed description of CSL112 pharmacokinetic in a multiple ascending dose (MAD) phase 1 study has been described. Here, we report for the first time the effects of CSL112 on key biomarkers of RCT after single and multiple intravenous infusions in healthy adult subjects.

Materials and Methods
Materials and Methods are available in the online-only Supplement.

Results
Demographics and Baseline Characteristics
In the single ascending dose (SAD) study, 58 subjects were randomized to receive CSL112 or placebo in a 3:1 ratio, 57 received a dose of study drug, and 56 completed the study. One subject was withdrawn before dose administration and another was withdrawn after dose administration because of an adverse event. Thirty-six subjects were enrolled in the MAD study and randomized to receive CSL112 or placebo in a 3:1 ratio in each of the 3 dose groups: 3.4 g 1W, 6.8 g 1W, or 3.4 g twice weekly (2W). Subject characteristics for both studies are summarized in Table 1. All randomized subjects received ≥1 dose of study drug, and 35 subjects completed the study. Study drug was administered as a 2-hour intravenous infusion (at a constant rate) in both studies.

Pharmacokinetics of ApoA-I
The apoA-I administered in CSL112 is human protein, and thus endogenous and exogenous apoA-I cannot be distinguished with the apoA-I assay. Pharmacokinetic parameters (Table 2) were, therefore, calculated for baseline-adjusted apoA-I to reflect administered apoA-I and only for those subjects who received CSL112 (SAD, n=42; MAD, n=27). Endogenous apoA-I levels at baseline were in the expected range for healthy adults in both the SAD and the MAD studies (1.20±0.17 g/L [n=57], 1.23±0.23 g/L [n=36]). For a more detailed description of apoA-I and phosphatidylcholine pharmacokinetics, see Easton et al.15

After the infusion of CSL112, an immediate increase in apoA-I plasma concentrations was observed (Figure 1). Regardless of dose, peak plasma concentrations of apoA-I were reached at the end of infusion (tmax, ≥2 hours) followed by a biphasic decline. The majority of the infused apoA-I was present in the circulating plasma compartment at the end of infusion.

For both studies, apoA-I levels remained above baseline for ≥3 days (Figure 1). For doses >70 mg/kg in the SAD study, apoA-I levels remained above baseline for ≥5 days (Figure 1A). At doses that yield acceptable signal/background for ≥24 hours (≥40 mg/kg in the SAD study), half-life could be calculated for the majority of the subjects in each dose group and the range for these groups was from 39.8 to 99.5 h in the SAD study. Based on the trough levels over time, there was no accumulation with 1W infusions (Figure 1B) and only a small degree of accumulation with 2W infusions (Figure 1C). Selected baseline-adjusted apoA-I pharmacokinetic results for the SAD and MAD studies are presented in Table 2, and a more comprehensive overview of MAD study pharmacokinetic data are provided in Easton et al.15

CSL112 Infusion Mobilizes Cholesterol From Cells and Tissues
Plasma Total Cholesterol
After a single infusion of CSL112 in the SAD study, total cholesterol in plasma increased in a dose-dependent manner from a mean baseline level of 4.45±0.91 mmol/L (n=57) by a maximum of 0.88±0.39 mmol/L (135 mg/kg dose group). In general, total cholesterol peaked between 4 and 24 hours, with a time frame that clearly lagged that of apoA-I (Figure 2A). Total cholesterol in plasma represents the sum of unesterified and esterified cholesterol on all lipoproteins present in plasma. The rapid increase in total cholesterol is consistent with a mobilization of cholesterol from cells and tissues, and the delayed peak is consistent with the time needed for movement of the cholesterol from tissue into plasma.

Mobilized Cholesterol Is Found in the HDL Fraction
HDL Cholesterol
In the SAD and MAD studies, infusion of CSL112 caused a rapid and sustained elevation in HDL cholesterol (HDL-C) levels (Figure 2B–2D) but no change in non-HDL cholesterol (Figure 2E). This suggests that tissue cholesterol moved first to the HDL fraction. The time course for HDL-C showed a peak within 24 to 48 hours, and levels remained elevated for ≥72 hours with gradual return to baseline (Figure 2B). This pattern was seen in both studies (Figure 2B–2D).
The concentration of HDL-C progressively increased in the 6.8 g 1W group before each subsequent weekly infusion. Of note, the apoA-I concentration did not increase before each subsequent weekly infusion; there was no accumulation in the 3.4 g 1W group.

Dose proportionality was observed for baseline-adjusted area under the effect curve (AUEC) for time point zero to the time point 72 hours (AUEC\(_{0-72}\)) of HDL-C after the first and last 1W infusion of CSL112. Unfortunately, the last infusion of the 2W CSL112 group did not include a 48-hour time point, and no analysis was performed. A summary of the baseline-adjusted HDL-C AUEC\(_{0-72}\) and apoA-I exposure (AUC\(_{0-72}\)) for the first and last infusion of CSL112 in the MAD study is shown in Table 3.

With the higher doses of CSL112 (6.8 g 1W or 3.4 g 2W), there was some evidence of accumulation during the study: the concentration of HDL-C measured before each infusion gradually increased during the investigation (Figure 2C and 2D).

Table 1. Summary of Demographics and Baseline Characteristics, All Enrolled Subjects in the SAD and MAD Studies

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo</th>
<th>CSL112 5 mg/kg</th>
<th>CSL112 10 mg/kg</th>
<th>CSL112 40 mg/kg</th>
<th>CSL112 70 mg/kg</th>
<th>CSL112 135 mg/kg</th>
<th>Placebo*</th>
<th>CSL112 3.4 g 1W</th>
<th>CSL112 3.4 g 2W</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y (SD)</td>
<td>28.1 (8.8)</td>
<td>27.5 (8.2)</td>
<td>27.5 (8.7)</td>
<td>26.8 (7.9)</td>
<td>25.1 (8.8)</td>
<td>26.0 (10.9)</td>
<td>25.6 (8.4)</td>
<td>22.5 (2.9)</td>
<td>26.3 (8.1)</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td>Male</td>
<td>6 (40)</td>
<td>5 (83.3)</td>
<td>6 (85.7)</td>
<td>4 (50)</td>
<td>4 (57.1)</td>
<td>5 (62.5)</td>
<td>5 (55.6)</td>
<td>9 (100)</td>
</tr>
<tr>
<td>Female</td>
<td>4 (60)</td>
<td>3 (50)</td>
<td>1 (16.7)</td>
<td>1 (14.3)</td>
<td>4 (50)</td>
<td>3 (42.9)</td>
<td>3 (37.5)</td>
<td>4 (44.4)</td>
<td>0</td>
</tr>
<tr>
<td>Weight, kg (SD)</td>
<td>76.4 (17.3)</td>
<td>94.9 (34.0)</td>
<td>86.7 (31)</td>
<td>84.2 (20.6)</td>
<td>85.3 (19.8)</td>
<td>78.9 (18.3)</td>
<td>79.4 (18.0)</td>
<td>73.0 (10.6)</td>
<td>80.3 (16.5)</td>
</tr>
<tr>
<td>BMI, kg/m² (SD)</td>
<td>25.7 (3.8)</td>
<td>30.9 (9.6)</td>
<td>26.7 (7.7)</td>
<td>26.1 (4.5)</td>
<td>28.6 (6.5)</td>
<td>27.1 (5.5)</td>
<td>27.5 (6.1)</td>
<td>24.1 (2.1)</td>
<td>26.0 (5.9)</td>
</tr>
<tr>
<td>ApoA-I, g/L (SD)</td>
<td>0.77 (0.28)</td>
<td>0.77 (0.18)</td>
<td>0.64 (0.19)</td>
<td>0.72 (0.12)</td>
<td>0.66 (0.20)</td>
<td>0.60 (0.10)</td>
<td>0.67 (0.12)</td>
<td>0.71 (0.18)</td>
<td>0.72 (0.23)</td>
</tr>
<tr>
<td>HDL-C, mmol/L (SD)</td>
<td>1.47 (0.28)</td>
<td>1.41 (0.24)</td>
<td>1.44 (0.41)</td>
<td>1.32 (0.27)</td>
<td>1.43 (0.38)</td>
<td>1.48 (0.34)</td>
<td>1.36 (0.27)</td>
<td>1.28 (0.22)</td>
<td>1.32 (0.30)</td>
</tr>
</tbody>
</table>

1W indicates once weekly; 2W, twice weekly; apoA-I, apolipoprotein A-I; apoB, apolipoprotein B; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL, low-density lipoprotein; MAD, multiple ascending dose; and SAD, single ascending dose.

A linear relationship of HDL-C AUEC\(_{0-72}\) and apoA-I exposure (AUC\(_{0-72}\)) was observed over the entire dose range of CSL112 in the SAD study (data not shown). Similarly, there was also a linear relationship of baseline-adjusted HDL-C AUEC\(_{0-72}\) and apoA-I exposure (AUC\(_{0-72}\)) for the first and last infusion of CSL112 in the MAD study (Table 3).

CSL112 Supports Lecithin Cholesterol Acyltransferase–Mediated Esterification of Cholesterol

In the SAD study, the rapid rise in HDL-C seen at the 2-hour point was accounted for mainly by a rise in HDL unesterified cholesterol (HDL-UC; Figure 3). This finding is consistent with the fact that cholesterol only exits tissues as unesterified cholesterol and with the well-known role of HDL as the principal acceptor of tissue cholesterol. The HDL-UC levels

Table 2. Combined SAD/MAD ApoA-I PK Data (Baseline Adjusted)

<table>
<thead>
<tr>
<th>PK Parameter</th>
<th>MAD Study CSL112 Dose Group</th>
<th>MAD Study CSL112 Dose Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.4 g 1W</td>
<td>6.8 g 1W</td>
<td>3.4 g 2W</td>
</tr>
<tr>
<td>First Infusion</td>
<td>Last Infusion</td>
<td>Last Infusion</td>
</tr>
<tr>
<td>(t_{1/2}), h</td>
<td>36.4 (43.1)</td>
<td>92.8 (90.1)</td>
</tr>
<tr>
<td>(C_{\text{max}}), g/L</td>
<td>2.05 (0.14)</td>
<td>1.55 (0.34)</td>
</tr>
<tr>
<td>(AUC_{0-\text{last}}), g.h/L</td>
<td>119.93 (18.98)</td>
<td>21.80 (24.44)</td>
</tr>
</tbody>
</table>

Values are shown as mean (SD) together with number of subjects included in each analysis. 1W indicates once weekly; 2W, twice weekly; apoA-I, apolipoprotein A-I; \(AUC_{0-\text{last}}\) area under the plasma concentration time curve from time point zero (before dosing) to the last time point above baseline; \(C_{\text{max}}\), maximum plasma concentration; MAD, multiple ascending dose; PK, pharmacokinetic; SAD, single ascending dose; and \(t_{1/2}\), terminal elimination half-life.

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that elevation in efflux capacity explains the rise in HDL-C observed in both SAD and MAD studies.

Total cholesterol efflux represents the sum of ABCA1-independent and -dependent cholesterol efflux at each time point (Figure 4). In the SAD study, CSL112 produced a 2.9-fold increase in total cholesterol efflux (Figure 4A) and a 2.6-fold increase in the MAD study (Figure 4B).

Infusion of CSL112 caused marked elevations in both ABCA1-dependent and ABCA1-independent cholesterol efflux activity (upper and lower bars respectively of Figure 4). The increases in cholesterol efflux activity were sustained above baseline (ie, before dosing) for >24 hours (and for 168 hours in the SAD study at doses ≥40 mg/kg CSL112). The largest effect of CSL112 was observed for ABCA1-dependent cholesterol efflux (Figure 4, upper bars), which was elevated by ≤5.8-fold and 6.3-fold in the SAD and MAD studies, respectively.

The AUEC values for cholesterol efflux correlate closely with those for apoA-I, indicating the dependence of efflux on exposure to apoA-I. There was no evidence for saturation of the AUEC for cholesterol efflux with the apoA-I exposures achieved by any dose level in the 2 studies.

**Dramatic Generation of Very Small HDL (preβ1-HDL) Explains Preference for the ABCA1-Dependent Pathway**

The ABCA1 transporter shows strong substrate selectivity. Large species of HDL such as HDL do not serve as acceptors. Rather, ABCA1 preferentially transports cholesterol to small, poorly lipidated HDL species such as very small HDL (HDL-VS). To pursue an explanation for the preferential elevation of the ABCA1-dependent portion of cholesterol efflux, we measured HDL-VS levels by ELISA using an anti–preβ1-HDL antibody. Infusion of CSL112 in the SAD study showed a sharp elevation in HDL-VS levels. The increase was up to 36-fold; the levels increased from 11.1 μg/mL predose to 400.6 μg/mL after 2 hours (Figure 5A). Peak HDL-VS levels were reached at 2 hours after infusion for all doses except the 135 mg/kg dose which peaked at 8 hours (Figure 5A). Because no measurements were recorded for any dose between 2 and 8 hours, the peak levels of HDL-VS could occur at any time in this interval. HDL-VS accounted for up to 20% (105 mg/kg dose group) of the total apoA-I at its peak, which declined to baseline levels of ≤5% by 48 hours (SAD study).

Similarly in the MAD study, infusion of CSL112 caused a dramatic rise in HDL-VS, which peaked at the end of the infusion (2 hours) and remained elevated 24 hours after the start of infusion (last assessment in the MAD study; Figure 5B and 5C). At higher doses of CSL112 in the SAD study, HDL-VS levels remained elevated at 72 hours (Figure 5A). Baseline-adjusted AUEC for HDL-VS shown in Table 3 indicates that HDL-VS production increased with increasing doses. However, in the SAD study, HDL-VS levels achieved at 2 hours were saturated at 40 mg/kg and from 70 mg/kg decreased with increasing doses. Nevertheless, at later time points HDL-VS levels were related to dose in a continuous manner. In addition, HDL-VS AUEC behaved in a dose-linear fashion: there was a linear relationship between apoA-I AUC and HDL-VS AUEC (data not shown). These data indicate that there is a temporal lag in HDL-VS production at higher doses, but overall

**Dramatic Increase of Cholesterol Efflux Capacity Explains Cholesterol Mobilization**

To pursue an explanation for the redistribution of cholesterol from tissues to plasma, we measured the capacity of plasma to support efflux of cholesterol from cells ex vivo. After infusion of CSL112, we observed a sharp rise in total cholesterol efflux capacity that peaked at 2 hours and returned to baseline by 72 hours. This time course is consistent with the hypothesis
HDL-VS production is dose-linear and not saturated. Finally, it was observed that HDL-VS AUEC₀–2₄ after the last infusion was greater than after the first infusion. The difference between first and last HDL-VS AUC was small in the 3.4 g 1W group, larger in the 3.4 g 2W group, and largest in the 6.8 g 1W group. Unlike the case with HDL-C, the elevated HDL-VS AUC on repeat administration of CSL112 cannot be accounted for by elevation of trough levels of apoA-I before the last infusion. An explanation for this small effect is not evident at this time.

We have previously shown that CSL112 and HDL-VS migrate differently on gradient gel electrophoresis. In keeping with this difference, the antibody used for the preβ₁-ELISA reacts little if at all with CSL112 (Figure 5D; CSL112 alone). However, reactivity is observed when plasma is incubated at 37°C for 1 hour with CSL112. The baseline levels in plasma HDL-VS were slightly reduced during this incubation (Figure 5D, plasma alone). CSL112 spiked to plasma and incubated at 0°C did not change the plasma baseline concentration of HDL-VS. These data suggest that HDL-VS is generated by a remodeling process in vivo and is consistent with observations of remodeling made in vivo in rabbits and ex vivo in human plasma. In addition, we have shown that HDL-VS species generated after infusion of CSL112 runs in the pre-β₁ position by 2-dimensional gel electrophoresis (data not shown).

We also observed a good correlation ($r^2=0.54$; SAD study) between HDL-VS levels and ABCA1-mediated efflux, implying that the preferential increase in ABCA1-dependent cholesterol efflux observed is because of the dramatic effect of CSL112 on HDL-VS generation.

**CSL112 Does Not Increase Proatherogenic Lipids**

No changes in the levels of the lipid markers, apolipoprotein B (apoB) and triglycerides, were observed in the CSL112 SAD study (data not shown). Similarly, in the MAD study, CSL112 did not increase levels of apoB or triglycerides in any of the dosing groups with either 1W or 2W dosing (Figure 6). The mean baseline plasma apoB levels were 0.70±0.20 g/L (n=57) in the SAD study and 0.70±0.16 g/L (n=36) in the MAD study and did not change after CSL112 infusion. Triglycerides were transiently elevated 12 hours after infusion of either CSL112 or placebo and coincided with the timing of meals. There was no evidence that the capacity for clearance of apoB-containing lipoprotein particles was exceeded during this study even with the associated increase in RCT resulting from CSL112 infusion.

**Discussion**

Coronary events are caused by rupture of cholesterol-rich atherosclerotic plaque. The ability of apoA-I to transport cholesterol out of cholesterol-loaded tissues offers a potential means for reducing risk of an ischemic occlusion. The cholesterol-removing activity of apoA-I may be particularly
important in treatment of patients with ACS in whom cholesterol has already accumulated to high levels in the artery wall. ACS patients experience an extremely high rate of recurrent ACS in the period immediately after an index ACS: in the year after ACS, nearly half of the recurrent events were recorded in ACS patients experience an extremely high rate of recurrent ACS in the period immediately after an index ACS: in the year after ACS, nearly half of the recurrent events were recorded in the first 30 days.\textsuperscript{18} Statins, which lower low-density lipoprotein cholesterol in 1 week (reviewed in Diditchenko et al).\textsuperscript{14} These elevations are of particular note given that neither statins nor niacin elevates cholesterol caused by CSL112 infusion appeared as unesterified cholesterol but not cholesterol ester is known to be raised substantially (by several SDs) above baseline (Figure 4). Here, we show that infusion of CSL112 causes an immediate rise in apoA-I (C\textsubscript{max} 2 hours) to levels that depending on the dose double or even triple endogenous levels of apoA-I (Figure 1). Like endogenously produced apoA-I, the infused apoA-I seems to have some but limited access to the extravascular space (volume of distribution range, 5.6–9.7 L)\textsuperscript{15} and to have clearance properties similar to endogenous apoA-I. Infusion of CSL112 caused movement of tissue cholesterol into the plasma as documented by the rise in total plasma cholesterol (Figure 2A), and the pathway of this movement seems consistent with the normal steps documented in the literature: unesterified cholesterol but not cholesterol ester is known to move to HDL before further transport or metabolism.\textsuperscript{30} In keeping with this pattern, the elevated plasma cholesterol caused by CSL112 infusion appeared as unesterified cholesterol (Figure 3) and appeared in the HDL fraction (Figure 2B–2D).

The first step of movement of cholesterol from tissues, cholesterol efflux from cells to HDL, can be modeled ex vivo with cholesterol-loaded cells. Using an assay identical to the one used here, Khera et al\textsuperscript{7} have shown that total cholesterol efflux capacity by CSL112 infusion appeared as unesterified cholesterol but not cholesterol ester is known to be raised substantially (by several SDs) above baseline (Figure 4). Here, we show that infusion of CSL112 causes an immediate rise in apoA-I (C\textsubscript{max} 2 hours) to levels that depending on the dose double or even triple endogenous levels of apoA-I (Figure 1). Like endogenously produced apoA-I, the infused apoA-I seems to have some but limited access to the extravascular space (volume of distribution range, 5.6–9.7 L)\textsuperscript{15} and to have clearance properties similar to endogenous apoA-I. Infusion of CSL112 caused movement of tissue cholesterol into the plasma as documented by the rise in total plasma cholesterol (Figure 2A), and the pathway of this movement seems consistent with the normal steps documented in the literature: unesterified cholesterol but not cholesterol ester is known to move to HDL before further transport or metabolism.\textsuperscript{30} In keeping with this pattern, the elevated plasma cholesterol caused by CSL112 infusion appeared as unesterified cholesterol (Figure 3) and appeared in the HDL fraction (Figure 2B–2D).
transporter induced by excess cellular cholesterol and present in atherosclerotic plaque. This preferential use of ABCA1 in man is consistent with observations in human plasma ex vivo and with rabbits in vivo and suggests CSL112 will draw cholesterol preferentially from cholesterol-rich tissue such as atherosclerotic plaque, in which ABCA1 is upregulated. Finally, we noted in the MAD study that the levels of HDL-C increased with each successive weekly infusion, even in the absence of accumulation of apoA-I. One potential explanation of this phenomenon could be a time-dependent elevation in ABCA1 on cells of the subjects which could favor cholesterol exit from cells. Precedent has been provided by studies in which elevated apoA-I increased half-life of macrophage ABCA1 both in vitro and in mice.

A physical explanation for the preferential use of the ABCA1 pathway is found in the striking elevation of HDL-VS caused by infusion of CSL112. The poorly lipidated HDL-VS, also known as preβ1-HDL, is the preferred substrate for ABCA1 and has been postulated to play a critical role in removing cholesterol from the artery wall. Waksman et al. tested this hypothesis by remodeling HDL in patient plasma ex vivo with a proprietary procedure and then reinfusing the plasma with elevated HDL-VS. Intravenous ultrasound measurements in treated monkeys and humans showed a pattern of reduced plaque size. In this setting, the total amount of apoA-I circulating in patients was not increased. These data suggest that elevation of HDL-VS levels, even without changes in plasma apoA-I, may be sufficient to reduce plaque. By extension, coupling strong HDL-VS elevation with net elevation of plasma apoA-I should provide even greater effects on plaque.

After the efflux of cholesterol from cells to HDL, the next step of cholesterol transport involves esterification of the cholesterol by the enzyme lecithin cholesterol acyltransferase. Esterification dramatically enhances the cholesterol-carrying capacity of HDL and contributes to RCT. We observed that CSL112 caused a striking rise in the cholesteryl ester content in HDL after the infusion of CSL112. The rise in HDL-EC was accompanied by a quantitatively similar fall in UC (Figure 2F), strongly suggesting direct esterification of cholesterol in HDL. Strong elevation of cholesterol esterification
by CSL112 is consistent with measurements in human plasma spiked with CSL112 and plasma taken from rabbits infused with CSL112 in which a strong elevation of esterification was observed.\textsuperscript{14} We further observed that with doses of CSL112 of 70 mg/kg or larger, the levels of HDL-EC continued to rise for 24 hours after infusion (Figure 2F). This continuous rise occurs despite the opposing processes of export of cholesteryl ester from HDL via cholesteryl ester transfer protein and export of cholesteryl ester from HDL via SRB1 (scavenger receptor B1) in the liver. We interpret the prolonged rise in HDL-EC to suggest continuing efflux of UC from the tissues throughout at least the first 24 hours after infusion.

We further observed that infusion of CSL112 was not accompanied by changes in either triglycerides or apoB (Figure 6). These 2 plasma constituents are considered proatherogenic, and elevations would be undesirable for an agent intended to reduce plaque size or instability.

A key limitation of this study is that it was conducted in healthy volunteers that likely have few or no atherosclerotic lesions. The rise in plasma cholesterol thus represents movement from tissues other than plaque. An additional limitation is that our assay of cholesterol efflux from cells ex vivo does not account for reverse flow of cholesterol from plasma into cells, and none of our assays measure the net flux of cholesterol from tissues. Confirmation of the beneficial effects of CSL112 on patients with active plaque thus awaits trials of clinical outcome.

In summary, we have shown here that infusion of CSL112 in healthy subjects caused an immediate elevation of apoA-I and a rapid rise in all measured parameters of cholesterol transport: increased cholesterol efflux capacity, increased cholesterol mobilization from tissues, and increased cholesterol esterification. For all of these parameters, the magnitude of the increase is not only faster but also larger than responses caused by any currently available therapy.
The ultimate culprit of atherothrombotic events is the buildup of cholesterol in the arterial wall. By preference for a cholesterol transporter pathway, CSL112 is designed to remove cholesterol selectively from tissues expressing ABCA1 such as atherosclerotic plaque. The removal of cholesterol may stabilize plaques rendering them less likely to erode or rupture. CSL112 also possesses strong anti-inflammatory properties\(^1\) that may synergistically stabilize vulnerable plaque. These properties encourage further study of CSL112 as a treatment to reduce the high risk of coronary events in the weeks after ACS.

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### Disclosures

All authors are employees of CSL Ltd or CSL Behring.

### References


### Significance

Rapid reduction of atherosclerotic plaque lipid volume is a novel therapeutic strategy aimed at decreasing the high risk of early recurrent cardiovascular events in acute coronary syndrome. Apolipoprotein A-I, the active component of high-density lipoprotein, is responsible for the antiatherosclerotic properties of high-density lipoprotein and promotes removal of plaque cholesterol by reverse cholesterol transport. CSL112 is a novel formulation of apolipoprotein A-I, purified from human plasma. Here, we report that single and multiple infusions of CSL112 in healthy subjects cause a rapid, up to 3-fold, elevation of apolipoprotein A-I concentrations in plasma. Infusion was accompanied by a striking increase in very small high-density lipoprotein (preβ1-high-density lipoprotein), the preferred substrate for ABCA1, a transporter present in atherosclerotic plaque. CSL112 infusion induced a robust increase in cholesterol efflux from cells, mediated by ABCA1. These findings point to the potential of CSL112 to remove cholesterol from plaque and thus reduce the possibility of an atherothrombotic event.
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Supplementary Materials

CSL112 Enhances Biomarkers of Reverse Cholesterol Transport Following Single and Multiple Infusions in Healthy Subjects

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Figure I. Subject disposition

A) SAD Study

Of the 204 subjects screened, 13 were screened twice.

Dose Group A1 = 5 mg/kg CSL112 infusion or placebo infusion
Dose Group A2 = 15 mg/kg CSL112 infusion or placebo infusion
Dose Group A3 = 40 mg/kg CSL112 infusion or placebo infusion
Dose Group A4 = 70 mg/kg CSL112 infusion or placebo infusion
Dose Group A5 = 105 mg/kg CSL112 infusion or placebo infusion
Dose Group A6 = 135 mg/kg CSL112 infusion or placebo infusion
B) MAD Study

Subjects Screened
n=93

Subjects Not Randomized
n=57
- Subjects not required (n=20)
- Screen failure (n=37)

Subjects Randomized
n=36

Received CSL112
n=27
- 9 in Treatment Group 1
- 9 in Treatment Group 2
- 9 in Treatment Group 3

Withdrawn from Study
n=1
- Withdrawal of Consent

CSL112 Subjects Completing Study
n=26

Received Placebo
n=9
- 3 in Treatment Group 1
- 3 in Treatment Group 2
- 3 in Treatment Group 3

Placebo Subjects Completing Study
n=9

Treatment Group 1 = 3.4 g CSL112 per infusion once weekly or placebo infusion once weekly
Treatment Group 2 = 6.8 g CSL112 per infusion once weekly or placebo infusion once weekly
Treatment Group 3 = 3.4 g CSL112 per infusion twice weekly or placebo infusion twice weekly
Materials and Methods

Study design

The safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of intravenous (IV) infusions of CSL112 were assessed in two phase 1 studies, a single ascending dose (SAD) study (NCT01129661) and a multiple ascending dose (MAD) study (NCT01281774) conducted in healthy adult subjects at single centers in Australia.

The SAD study was a randomized, double-blind, placebo-controlled investigation performed at Royal Adelaide Hospital, Adelaide, Australia. The MAD study was a randomized, placebo-controlled, sponsor-unblinded study performed at Q-Pharm, Brisbane, Australia.

The primary objective of both studies was to evaluate the safety and tolerability of escalating doses of CSL112 after single or multiple IV infusions, together with an evaluation of the PK of apolipoprotein A-I (apoA-I) after single and multiple IV infusions of CSL112, including plasma apoA-I concentration without baseline correction, and the PD of biomarkers linked to CSL112. This manuscript focuses on the biomarker data.

Inclusion and exclusion criteria

The main inclusion criteria were: male or female healthy subjects aged between 18 and less than 55 years, weighing at least 45 kg in the SAD study (50 kg in the MAD study), capable of understanding the purposes and risks of the study, and able to provide written informed consent. Subjects were excluded from the study if they had significant medical conditions, any clinically relevant abnormal laboratory values, history of coagulopathy, hypotension, evidence of renal impairment, evidence of substance or alcohol abuse or were unable to comply with the study protocol.

Both studies were reviewed and approved by the Bellberry Human Research Ethics Committee, an Independent Ethics Committee. Subjects provided written informed consent before any study-specific assessments were performed. The studies were conducted in accordance with standards of Good Clinical Practice, as defined by the International Conference on Harmonisation, the principles outlined in the Declaration of Helsinki and all applicable national and local regulations.

Randomization

In both studies subjects were randomized to receive either CSL112 or placebo in the approximate ratio of 3:1 (active:placebo).

Demographics and baseline characteristics

The disposition of subjects in both studies is shown in Supplemental Figure I. In the SAD study, the majority of the subjects were male (63.2%) and of white race (94.7%) with a median age of 23 years, and median height and weight of 1.75 m and 78.5 kg, respectively. The majority of the subjects included in the MAD study were male (63.9%) and of white race (97.2%), with a median age of 23 years. Subject characteristics for both studies are summarized in Table 1. In the MAD study, the 3.4 g once weekly CSL112 group (n=9) were all male; the uneven distribution of males across the groups may confound data interpretation relating to dose-response relationships and gender.

Study product, dose and administration
The composition and characterization of CSL112 has been described in detail (1). Briefly, CSL112 is apoA-I purified by cold ethanol precipitation from human pooled plasma (CSL Behring) (2, 3) and reconstituted with soy PC using the sodium cholate dialysis method of Matz & Jonas (4) with modifications (1, 2). The product was subjected to sterile filtration (0.22 µm) and lyophilization and produced disc-shaped particles with high batch-to-batch consistency. The principal differences between CSL112 and a previously described formulation (CSL111) are lower levels of PC (55 moles per mole of apoA-I), lower levels of residual cholate (0.03 g per g of apoA-I) and greater particle uniformity in CSL112.

Lyophilized vials of CSL112 were dissolved in sterile water for injection and dosed based on the protein content. In both studies, the placebo comprised 0.9% sodium chloride solution for injection. In both studies CSL112 or placebo were administered intravenously via a 2 h infusion. The study product was physically masked to maintain blinding. Unblinded site personnel prepared and administered the study product.

In the SAD study subjects were stratified by body weight and received either placebo or one of six different CSL112 doses: 5, 15, 40, 70, 105 and 135 mg/kg.

In the MAD study subjects received placebo or CSL112 either once- (1W) or twice-weekly (2W) for 4 weeks: 3.4 g or 6.8 g 1W CSL112 or placebo (a total of 4 infusions); 3.4 g 2W CSL112 or placebo (a total of 8 infusions).

**ApoA-I pharmacokinetic assessment**

ApoA-I was assessed at a specialty lipid laboratory (Pacific Biomarkers, Seattle, WA, USA) by an immunonephelometric method run on Roche Modular P. In both studies blood samples for PK assessment were collected at time points selected to capture the peak and expected decline in apoA-I plasma concentration. The PK samples in the SAD study were collected on days 1-4, 6, 8 and 11. In the MAD study, blood samples for PK analysis were taken on days 1-4, 6, 8, 9, 15, 21-25 and 27 for 1W dosing groups and on days 1-4, 8, 11, 15, 18, 22, and 25-28 for the 2W dosing group. Validation of PK bioanalysis has been described in detail (5).

PK parameters for baseline corrected plasma concentrations of apoA-I were determined after single and multiple IV infusions of CSL112 and included area under the plasma concentration time curve from time point zero (before dosing) to the last time point above baseline (AUC₀⁻last), maximum plasma concentration (Cₘₐₓ) and half-life (t₁/₂).

**Pharmacodynamic biomarkers**

The following biomarkers were assessed in both the SAD and MAD studies; apoA-I, phosphatidylcholine (PC), ABCA1-dependent, ABCA1-independent and total cholesterol efflux capacity, HDL-VS (very small (6); also known as PreBeta1-HDL), apolipoprotein B (apoB), triglycerides (TG), total cholesterol (TC) and HDL-cholesterol (HDL-C). HDL-unesterified cholesterol (HDL-UC) and HDL-esterified cholesterol (HDL-EC) were measured in the SAD study only. Cholesterol efflux assays were performed in apoB-depleted serum samples using J774 macrophages at Vascular Strategies LLC, Plymouth Meeting, PA, USA as previously described (7). Total and ABCA1-independent cholesterol efflux, with and without cAMP induction respectively, were assayed. ABCA1-dependent efflux was calculated as the difference between total and ABCA1-independent efflux. Plate-to-plate variability was monitored with control samples and was within acceptability criteria. Efflux data were not normalized. All other PD biomarkers were assessed in plasma samples at Pacific Biomarkers, Seattle, WA, USA. HDL-VS was preserved by 21-fold dilution in 50% sucrose before freezing and measured by
enzyme-linked immunosorbent assay for PreBeta1-HDL (Sekisui Medical Co, Tokyo, Japan). HDL was separated by the polyethylene glycol (PEG) precipitation method. All cholesterol and triglycerides were measured by standard enzymatic methods.

Validation of PD assays was conducted ahead of the assessment of clinical samples to ensure accuracy of reported concentrations and activities in the presence of CSL112. Briefly, plasma samples were collected from five subjects who had a range of lipid phenotypes including two normolipidemic, one with high HDL-C, and two with low HDL-C and high TG. Samples included plasma or serum spiked with controls or CSL112 at various concentrations (v/v, 89.3/10.7). Specifically, samples were spiked with saline control, sucrose control, or 0.1, 0.8 and 2.8 mg/mL CSL112. Samples were incubated for 1 h at 37°C or in some instances at 0°C and then kept frozen at -70°C prior to analysis. Comparison of biomarker assessments in the absence and presence of CSL112 indicated no significant interference of CSL112 in the HDL-precipitation procedure by PEG or the assessments of biomarkers.

In the studies no food was provided for 8 hours before and after dosing and all data shown was obtained in fasting samples. During the SAD study, blood samples for assessing TC, HDL-UC, HDL-EC, apoB and TG were collected at 0 h [before infusion], 1, 2, 4, 8, 24, 48, 72 and 168 h. HDL-VS and cholesterol efflux capacities were assessed in blood samples taken (at 0 h [before infusion], 2, 8, 24, 48, 72 and 168 h.

For the measurement of apoB, samples were taken at the same time points irrespective of 1W or 2W dosing, i.e. before dosing on days 1, 8, 15, 22 and on day 28. Samples for measurement of cholesterol efflux and HDL-VS were taken on day 1 (before dosing, at 2, 4 and 8 h), day 2, day 22 (before dosing and 2, 4 and 8 h) and day 23 in the 1W dosing groups. For the 2W dosing group these samples were collected on day 1 (before dosing and at 2, 4 and 8 h), day 2, day 25 (before dosing and 2, 4 and 8 h) and day 26.

**Pharmacodynamic Parameters**

PD parameters were calculated from biomarker concentrations and activities. The following PD parameters were assessed: area under the effect curve (AUEC) for time point zero to the time point of the last quantifiable concentration/activity (AUEC0-last), maximum biomarker concentration/activity (Rmax), time to reach maximum concentration/activity (Tmax).

**Statistical analysis**

The sample size was not based on formal power calculations. A sample size of nine subjects on CSL112 and three on placebo per group was chosen as the smallest number that would allow an adequate early clinical evaluation of safety and PK. In the SAD study 42 subjects received CSL112 and 15 subjects received placebo. In the MAD study, 27 subjects received CSL112 and 9 subjects received placebo.

Subjects were randomized by an individual not directly involved in the analysis of study results. A randomization block size of four (3 CSL112:1 placebo) was used to ensure the balance between dosing groups was maintained.

The data analysis involved descriptive statistics because there was no formal hypothesis to be tested during the study. The placebo group was not included in the PK analysis. In the PD analyses the placebo groups from each study were combined to form a single placebo group.

The baseline value for all analyses was the last value recorded before dosing on day 1, i.e. before administration of study medication. PK and PD baseline correction involved subtracting
the baseline value from the value obtained from each sample collected after study medication administration.

SAS Version 9.2 (SAS Institute Inc., Cary, NC) was used for the analysis of study data. Non-compartmental PK analysis was performed using model 202 (constant infusion) in WinNonlin™ Enterprise Version 5.2 (Pharsight Corporation, Montreal, Canada). Non-compartmental PD analysis of biomarkers was performed using Model 220 (baseline effect) in WinNonlin™ Enterprise Version 5.2.

References


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