

Mechanism of HDL remodeling induced by CSL112

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Introduction

CSL112 is apoA-I, the active component of HDL, purified from human plasma and reconstituted to form lipoprotein particles suitable for infusion. It is being developed as a novel means to rapidly stabilize atherosclerotic lesions and reduce the risk of recurrent events after an acute coronary syndrome. CSL112 is designed to optimize cholesterol efflux by the ATP-binding cassette A1 (ABCA1) transporter present in macrophages of atherosclerotic plaques. We have recently demonstrated that infusion of CSL112 into humans and animals causes a strong and immediate increase in capacity of plasma to efflux cholesterol via ABCA1. This elevation is due in part to a rapid remodeling of CSL112 resulting in a very large (>30-fold) increase in plasma levels of lipid-poor apoA-I (pre-β1 HDL), the preferred substrate for ABCA1^{1,2}. In parallel to the rise in lipid-poor apoA-I, CSL112 also causes an increase in particle size of plasma HDL2 and HDL3 subclasses suggesting that CSL112 induces remodeling of plasma lipoproteins.

Objective

We sought the mechanistic basis for elevation of pre-β1 HDL and ABCA1-dependent cholesterol efflux upon infusion of CSL112.

Methods and Results

CSL112 and HDL labeling

CSL112 particles contain two molecules of apoA-I and 110 molecules of phosphatidylcholine¹. Biotinylation of CSL112 or HDL3 was performed as described by Lund-Katz *et al.*³. For fluorescence imaging, CSL112 was labeled with DyLight 488 Amine-Reactive Dye (Pierce). The labeling procedure yielded one to two biotin or DyLight 488 molecules per apoA-I molecule in CSL112 or HDL particles.

In vitro incubations, lipoprotein isolation

LDL ($d = 1.019\text{--}1.055$ g/mL), HDL2 ($d = 1.065\text{--}1.055$ g/mL) and HDL3 ($d = 1.13\text{--}1.21$ g/mL) were isolated from cryo-depleted plasma (CSL Behring) by sequential ultracentrifugation⁴. Incubation of CSL112 with individual lipoproteins (1 mg/mL) was carried out in PBS or PBS supplemented with 5% BSA. Three products of HDL remodeling were purified from HDL3 and CSL112 mixtures by sequential ultracentrifugation: large HDL (Fr 1; $d = 1.12\text{--}1.18$ g/mL), lipid-poor apoA-I (Fr 2; bottom fraction, $d = 1.225$ g/mL), small HDL (Fr 3; $d = 1.19\text{--}1.22$ g/mL). HDL particle size distribution was assessed by 4–30% non-denaturing polyacrylamide gradient gel electrophoresis (NGGE).

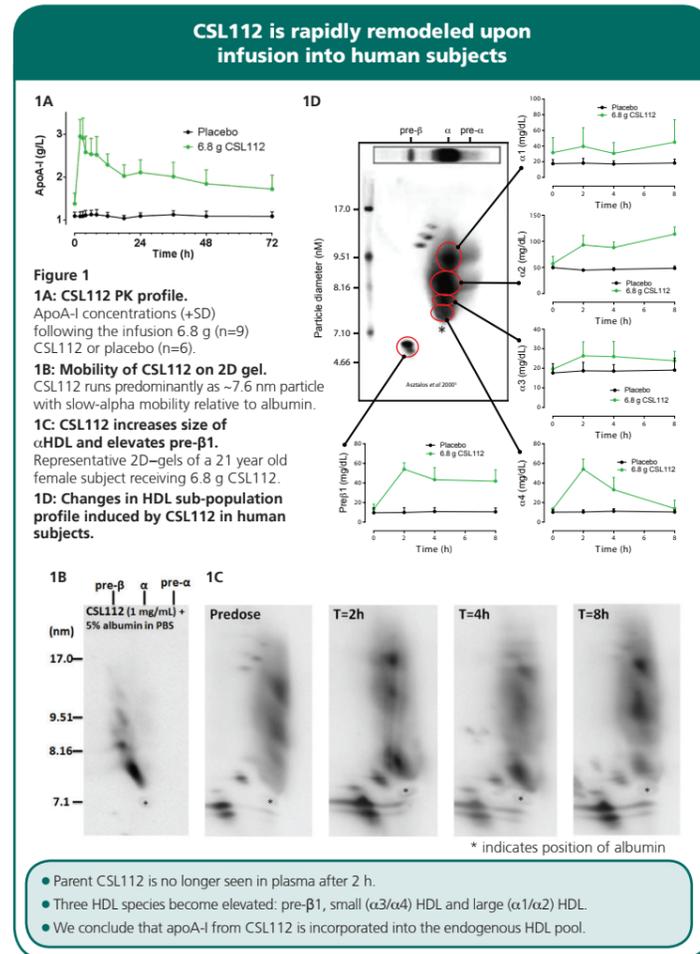
C-efflux

The capacity of the HDL to efflux cholesterol was assessed using [³H]cholesterol-loaded RAW264.7 macrophages. Before initiation of efflux, RAW264.7 cells were loaded with free [³H]cholesterol for 36 h, and stimulated with 0.3 mM 8Br-cAMP for 16 h to up-regulate ABCA1. Efflux was promoted by incubating the [³H]cholesterol-labeled RAW264.7 cells with each individual acceptor for 6 h. Difference in efflux between stimulated and non-stimulated cells is taken as a measure of ABCA1-mediated efflux.

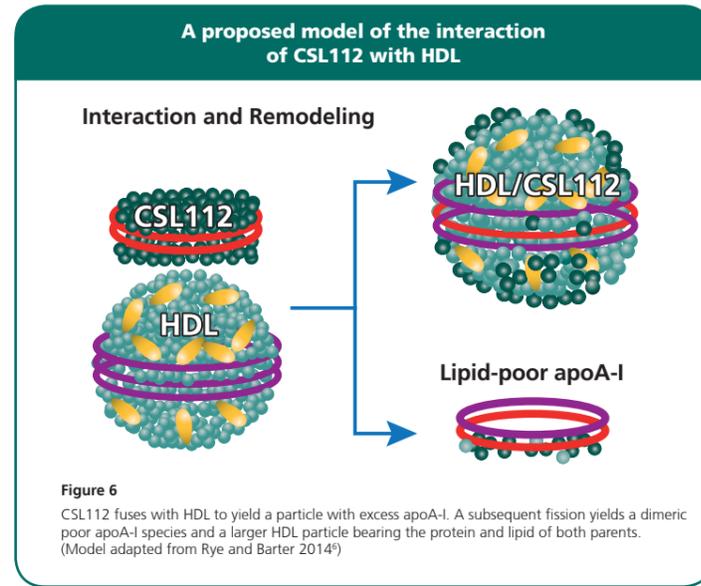
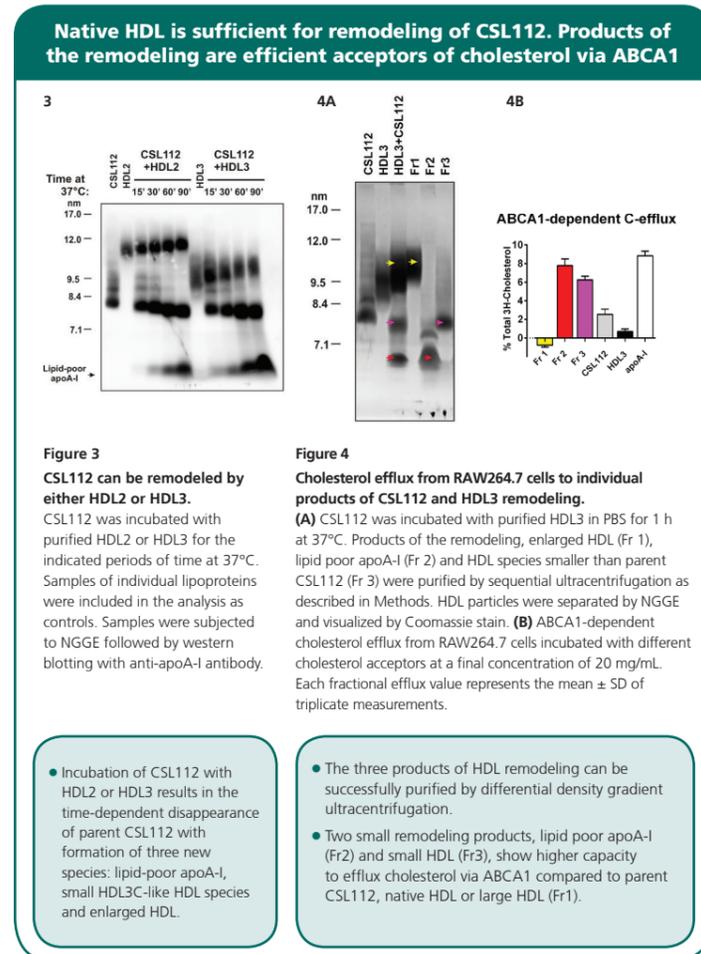
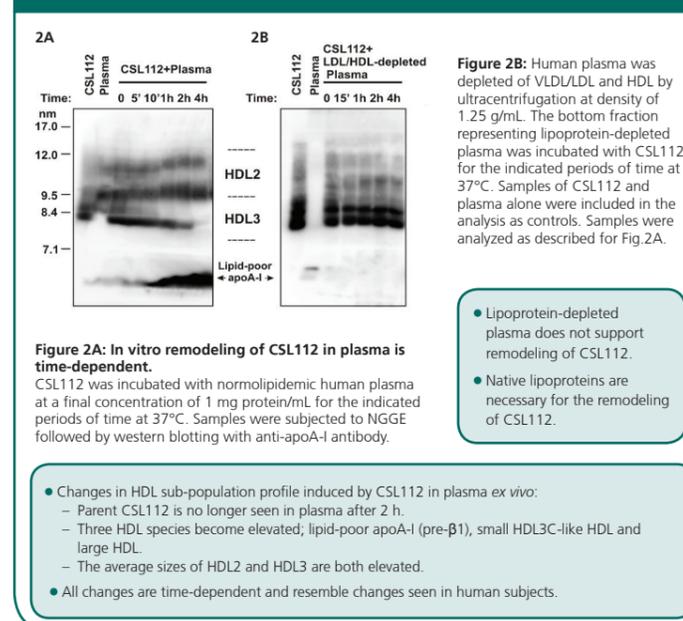
Studies in human subjects

HDL remodeling was studied in selected healthy subjects receiving infusions of 6.8 g of CSL112 or placebo (n=4 per group) from a completed phase 1 clinical trial (NCT01281774). Plasma samples were obtained at multiple time-points for assessment of apoA-I PK by nephelometry (Pacific Biomarkers) and 2D-gel (3–35% concave gradient) electrophoresis (Boston Heart Diagnostics)⁵.

Western blotting of 2D-gradient gels was conducted using polyclonal anti-apoA-I and dilute anti-albumin antibodies. Signal intensities of HDL subfractions were quantified by densitometry and expressed as percentages of the total combined signal measured (100% per blot). HDL subfraction concentrations were then calculated as the measured percentage of the apoA-I concentrations for matching time points.



Remodeling of CSL112 in plasma ex vivo resembles that in vivo: native lipoproteins are necessary for the remodeling



Conclusions

- CSL112 is remodeled upon infusion into patients or addition to plasma *in vitro* to form lipid-poor apoA-I (pre-β1 HDL).
- Endogenous HDL is necessary and sufficient for the remodeling of CSL112 in plasma. Plasma enzymes do not significantly alter the course of remodeling. Lipid-poor apoA-I formed during this interaction is generated from both CSL112 and HDL.
- We postulate that CSL112 spontaneously fuses with plasma HDL donating protein and lipid components with subsequent release of the lipid-poor apoA-I. Thus the lipid-poor apoA-I is formed by a natural process similar to that which occurs during shrinkage of large HDL particles.
- Products of the HDL remodeling induced by CSL112 show much higher capacity to efflux cholesterol via ABCA1 compared to the original CSL112 and HDL. This finding provides a biochemical basis for the strong elevation of ABCA1-dependent cholesterol efflux and lipid-poor apoA-I observed upon infusion of CSL112 into human subjects.
- These properties make CSL112 a promising candidate for rapidly removing cholesterol from cells in the atherosclerotic plaque.

References

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Disclosures

Authors are employees of CSL Behring. Presentation includes investigational uses of drugs.

