Inducing apolipoprotein A-I synthesis to reduce cardiovascular risk: from ASSERT to SUSTAIN and beyond

Belinda A. Di Bartolo, Daniel J. Scherer, Stephen J. Nicholls

South Australian Health and Medical Research Institute, University of Adelaide, Adelaide, Australia

Corresponding author:
Stephen Nicholls
South Australian Health
and Medical Research
Institute
PO Box 11060. Adelaide
SA, 5001, Australia
Phone: +61-8-8128-4510
E-mail: stephen.nicholls@
sahmri.com

Abstract

Increasing attention has focused on efforts to promote the biological activities of high-density lipoproteins (HDL) in order to reduce cardiovascular risk. Targeting apolipoprotein A-I (apoA-I), the major protein carried on HDL particles, represents an attractive approach to promoting HDL by virtue of its ability to increase endogenous synthesis of functional HDL particles. A number of pharmacological strategies that target apoA-I, including upregulation of its production with the bromodomain and extraterminal (BET) protein inhibitor RVX-208, development of short peptide sequences that mimic its action, and administration as a component of reconstituted HDL particles, have undergone clinical development. The impact of these approaches on cardiovascular biomarkers will be reviewed.

Key words: apolipoprotein A-I, atherosclerosis, risk factors, clinical trials, lipids.

Introduction

Randomized controlled trials have consistently demonstrated that lowering levels of low-density lipoprotein cholesterol (LDL-C) reduces cardiovascular event rates in the primary and secondary prevention settings [1–4]. As a result, LDL-C lowering, primarily with statins, has become central to all therapeutic strategies designed to reduce cardiovascular risk. Despite their widespread use, there remains a considerable residual risk of cardiovascular events [5]. This supports the need to develop novel therapeutic approaches to further reduce cardiovascular risk in the statin-treated patient.

What we know about high-density lipoproteins

High-density lipoproteins (HDL) circulate as a heterogeneous population of particles differing in size, shape and composition of both protein and lipid species. The mature HDL particle is spherical, containing a core of esterified cholesterol, surrounded by a surface layer comprising phospholipid, free cholesterol and a range of apolipoproteins. A number of lines of evidence support the concept that HDL are cardioprotective [6, 7]. Population studies demonstrate an inverse relationship between HDL-C levels and cardiovascular risk, regardless of the level of athero-

AMS

genic lipid parameter [8–11]. In clinical trials of intensive lipid lowering, this inverse association continues to be observed and is primarily driven by high cardiovascular risk being observed at low HDL-C levels. Animal studies have shown that interventions that target HDL via transgenic expression of its major proteins (apoA-I, apoA-II) or by direct intravenous infusions have a favorable impact on both the size and histologic composition of atherosclerotic plaque [12–14].

The principal biological activity of HDL appears to be its central role in the promotion of reverse cholesterol transport, the process by which excess cholesterol is removed from peripheral tissues. High-density lipoprotein acts as the preferred acceptor for free cholesterol that undergoes efflux from cells, via a range of trans-membrane pathways facilitated by ATP binding cassette A1 (ABCA1), ATP binding cassette G1 (ABCG1) and scavenger receptor-BI (SR-BI) [15, 16]. Following efflux to the surface of the HDL particle, cholesterol undergoes esterification by the factor lecithin:cholesterol acyltransferase (LCAT), enabling cholesterol to be stored within the HDL particle core. This maintains a relatively low concentration of cholesterol on the particle surface, enabling ongoing cholesterol efflux activity, and results in a spherical particle of increasing size and cholesterol content. The lipid within HDL is ultimately delivered to the liver via the SR-BI receptor or by LDL particles following cholesteryl ester transfer protein (CETP) mediated exchange from HDL.

Additional studies have demonstrated that HDL possess biological activities beyond their role in lipid mobilization. These include favorable influences on inflammatory, oxidative, thrombotic and apoptotic pathways implicated in the pathogenesis of atherosclerosis [17]. Central to these activities is the demonstration that HDL increases the bioavailability of nitric oxide via a direct impact on endothelial nitric oxide synthase activity [18]. The demonstration that these functional activities occur in animals with low cholesterol levels suggests that they are not secondary to the cholesterol efflux activity of HDL [7]. More recently, evidence has revealed considerable heterogeneity with regard to HDL functionality [19–22]. Whether this reflects the variety of circulating HDL particles in its protein cargo or the biological activity of pathways that have been reported to impair HDL function remains to be determined [23]. Recent reports that functional assays of HDL that measure cholesterol efflux or anti-oxidant activity independently predict cardiovascular risk have provided further support for the importance of HDL quality, as opposed to quantity, in determining cardiovascular risk [24–28].

Therapeutic strategies to promote high-density lipoproteins

Current approaches to lipid modification have modest effects on HDL. Lifestyle measures have been demonstrated to raise HDL-C by up to 10%, with the greater effects observed in patients who lose abdominal adiposity [29]. Statins raise HDL-C by 3–15% in addition to their LDL-C lowering properties [30–32]. Modestly raising HDL-C by 7.5% has been reported to independently associate with the ability of statins to slow progression of coronary atherosclerosis and to reduce cardiovascular event rates. Fibrates increase HDL-C by 5–20%, with evidence that increasing the circulating concentration of small HDL particles was the strongest predictor of a beneficial effect of gemfibrozil on cardiovascular events [33]. Niacin is the strongest HDL-C raising agent, with early evidence of a beneficial effect on cardiovascular events in the pre-statin era [34] and having a favorable impact on vascular disease in serial imaging [35–37]. Difficulty with tolerance, limiting the ability to use sufficiently high doses in clinical practice, has stimulated efforts to develop novel approaches to its administration that will improve tolerance. However, large clinical trials have failed to demonstrate cardiovascular benefit of these efforts to administer extended release forms of niacin in statin-treated patients [38]. CETP inhibitors are currently undergoing development by virtue of their ability to substantially raise HDL-C levels much more than niacin [39]. However, enthusiasm for this approach has been attenuated by observations from clinical trials that early CETP inhibitors are associated with adverse clinical effects or have no cardiovascular benefit at all [40, 41]. Ongoing clinical trials of potent CETP inhibitors will ultimately determine whether this approach has any clinical potential.

Increasing apoA-I synthesis

While considerable attention has focused on raising HDL-C levels by disrupting physiological lipoprotein remodeling factors, in parallel there has been immense interest in simply turning on endogenous HDL production by increasing hepatic synthesis of apoA-I. Production of apoA-I by the liver would be rapidly combined with phospholipid to form nascent, discoidal HDL particles that enter the circulation and carry out its biological activities. Despite this interest, developing an agent that selectively upregulates hepatic synthesis of apoA-I has proven elusive.

RVX-208 is a bromodomain and extraterminal (BET) protein inhibitor, which has been developed as a selective means to increase endogenous synthesis of apoA-I. BET proteins have been demonstrated to repress the genetic sequence

encoding for apoA-I, preventing expression. RVX-208 has been demonstrated to selectively bind to the BET family member BRD4, which competes for the apoA-I site bound by the endogenous ligand, acetylated lysine. This results in induction of apoA-I mRNA expression with evidence of increasing systemic levels of apoA-I and lipid-depleted pre-β HDL particles. This is associated with increasing systemic cholesterol efflux capacity in non-human primate models [42] and reduced atherosclerotic lesion formation in hyperlipidemic apoE knockout mice [43].

The ApoA-I Synthesis Stimulation Evaluation in Patients Requiring Treatment for Coronary Artery Disease (ASSERT) study investigated the effect of administration of RVX-208 in 299 statin-treated patients with coronary artery disease. After 12 weeks of treatment, RVX-208 produced a modest dose-dependent increase in apoA-I by up to 5.6% and HDL-C by up to 8.3%. This was largely driven by an increase in the circulating concentration of larger, cholesterol-enriched HDL particles by up to 21.1%, which would be consistent with the generation of nascent HDL particles which subsequently facilitate cholesterol mobilization, resulting in an increase in HDL size [44]. Elevations in liver transaminases, without accompanying increases in bilirubin, were demonstrated with increasing RVX-208 doses.

The Study of Quantitative Serial Trends in Lipids with Apolipoprotein A-I Stimulation (SUSTAIN) was performed with a view to characterizing the longer term lipid effects of RVX-208 100 mg twice daily administration for 24 weeks in patients treated with low or moderate doses of atorvastatin or rosuvastatin and without baseline liver enzyme abnormalities [45]. Consistent with the earlier ASSERT study, modest, albeit statistically significant effects were observed with regard to percentage changes in HDL-C, apoA-I and large HDL particles in the RVX-208 treatment group compared with placebo. Similar increases in ALT (11.4% vs. 0%) and AST (5.7% vs. 0%) greater than three times the upper limit of normal were observed with RVX-208, with all such increases observed in the first 12 weeks, quickly decreased with study drug cessation and did not recur in the patients who subsequently completed treatment as per protocol (Table I).

The impact of RVX-208 administration on coronary atherosclerosis was subsequently investigated in the ApoA-I Synthesis Stimulation and Intravascular Ultrasound for Coronary Atheroma Regression Evaluation (ASSURE) study. Three hundred and twenty-three patients with angiographic coronary artery disease and low HDL-C levels were treated with RVX-208 100 mg or placebo twice daily for 26 weeks and underwent evaluation of

Table I. Mean (*median) percentage change in lipid and inflammatory parameters from baseline to week 24 with RVX-208 and placebo in SUSTAIN

Parameter	Placebo	RVX-208	*P*-value
Intent-to-treat analysis:			
HDL-C	−4.96%	+2.84%	0.0003
ApoA-I*	−0.56%	+4.03%	0.0015
LDL-C*	−1.45%	−1.55%	0.54
Triglycerides*	−1.20%	+1.30%	0.75
ApoB*	−3.33%	+4.34%	0.85
Large HDL	−5.49%	+8.11%	0.01
CRP	−13.98%	−14.37%	0.50
Per protocol analysis:			
HDL-C	−4.88%	+4.27%	< 0.0001
ApoA-I*	−1.59%	+6.00%	0.0004
LDL-C*	−0.86%	−3.52%	0.21
Triglycerides*	−5.22%	+0.62%	0.67
ApoB*	+2.00%	−0.51%	0.39
Large HDL	−5.74%	+16.02%	0.004
CRP	−13.24%	−14.83%	0.26

Apo – apolipoprotein, CRP – C-reactive protein, HDL – high-density lipoprotein, LDL – low-density lipoprotein.

progression of coronary atherosclerosis with serial intravascular ultrasonography. There were similar increases in apoA-I by 10.6% and 12.8% and in HDL-C by 9.1% and 11.1% with placebo and RVX-208 respectively. This was associated with similar reductions in percent atheroma volume (by 0.30% and 0.40% with placebo and RVX-208 respectively) and total atheroma volume (by 3.8 mm^3 and 4.2 mm^3 with placebo and RVX-208 respectively) in both groups. While reductions in atheroma burden were consistent with the changes in HDL-associated parameters, the similar findings in both treatment groups reflected a similar lipid benefit in placebo-treated patients, which may have reflected lifestyle modification in these patients.

As a result, early data from clinical trials in patients with established coronary artery disease reveal modest favorable changes in HDL-associated parameters with RVX-208, suggesting a potentially beneficial effect on the generation of nascent HDL particles and stimulation of lipid transport. The unusually beneficial lipid changes in the placebo group in ASSURE prevent meaningful assessment of the true impact of RVX-208 on coronary atherosclerosis. Post hoc pooled analysis of the ASSURE and SUSTAIN studies suggested potentially fewer cardiovascular events in patients treated with RVX-208, which is now being developed as a BET inhibitor with potential cardioprotective effects beyond HDL. Further understanding of the potential cardiovascular impact of RVX-208 is being undertaken in a phase 3 clinical trial (BETonMACE) and will ultimately require larger clinical trials.

Short mimetic peptides

Interest has also focused on the synthesis of short peptides that form an amphiphatic helix and share considerable functional properties with apoA-I. When produced with D-type amino acids, which are resistant to gastric hydrolysis, these peptides can be administered orally and therefore represent an additional apoA-I targeted approach to treatment of cardiovascular disease [46]. Early preclinical studies of these peptides demonstrated favorable effects on cholesterol efflux and inflammatory pathways, which was associated with an atheroprotective impact in mouse models of atherosclerosis [47, 48]. Unfortunately, to date no such peptides have advanced in clinical development to the point where they can achieve therapeutically active concentrations in the circulation.

Infusional approaches involving apoA-I

A number of groups have investigated the impact of directly infusing forms of apoA-I either as a pro-protein or as part of reconstituted HDL particles [49]. Such approaches, in principle, represent an alternative approach to directly admin-istering nascent HDL, which can then carry out its biological activities *in vivo*. Early studies demonstrated that infusing pro-apoA-I increased excretion of fecal sterol, a surrogate marker of reverse cholesterol transport [50]. Infusing reconstituted HDL has favorable effects on animal models of atherosclerosis and vascular injury [12–14], while in humans it improves endothelial function in addition to favorable effects on cholesterol efflux and LCAT activity [51, 52]. Serial coronary imaging with intravascular ultrasound in patients with a recent acute coronary syndrome revealed that infusing reconstituted HDL containing either wild-type apoA-I or its genetic variant, apoA-I$_{Milano}$, over a course of approximately 6 weeks promoted rapid regression of coronary atherosclerosis [53, 54]. Challenges with producing these preparations in sufficiently large quantities have prevented these infusions from proceeding to large clinical outcome trials. Accordingly, their ultimate cardiovascular impact remains to be established.

Conclusions

Given that apoA-I is the major protein carried on HDL particles and contributes to the potentially beneficial functional properties, there remains considerable interest in developing therapies that target its levels and activity. All of these approaches demonstrate varying degrees of promise, which remains to be fully characterized in large clinical trials.

Conflict of interest

The authors declare no conflict of interest.

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