Infusional high-density lipoproteins therapies as a novel strategy for treating atherosclerosis

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Submitted: 27 August 2015
Accepted: 18 November 2015

Arch Med Sci 2017; 13, 1: 210–214
DOI: 10.5114/aoms.2016.60941

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Abstract

High-density lipoproteins (HDL) have received considerable interest as a target for the development of novel anti-atherosclerotic agents beyond conventional approaches to lipid lowering. While a number of approaches have focused on modifying remodeling and expression pathways implicated in the regulation of HDL levels, an additional approach involves simply infusions of delipidated HDL. Several groups have advanced HDL infusions to clinical development with intriguing signs suggesting potentially favorable impacts at the level of the artery wall. The findings of early studies of infusional HDL therapies will be reviewed.

Key words: high density lipoprotein, risk factors, lipids, atherosclerosis, clinical trials, imaging.

Introduction

The unequivocal clinical benefit of lowering levels of low-density lipoprotein cholesterol (LDL-C) with statins has had a profound impact on cardiovascular outcomes in the patient with established coronary artery disease [1–4]. However, despite their widespread use there continues to be a considerable rate of cardiovascular events in the statin-treated patient [5]. This ongoing cardiovascular risk emphasizes the need to develop novel therapeutic strategies that will produce incremental risk reduction. Increasing interest has focused on pharmacological strategies that target the functional properties of high-density lipoproteins (HDL), the putative protective lipid fraction in plasma.

Evidence supporting a protective role for HDL

Following the early reports that patients with myocardial infarction had lower levels of HDL-C on ultracentrifugation, a number of lines of evidence have accumulated supporting a potentially protective role of HDL in cardiovascular disease [6, 7]. Population studies consistently demonstrate an inverse relationship between HDL-C levels and the prospective risk of cardiovascular events, which continues to be observed regardless of the level of atherogenic lipid factors [8–11]. Animal studies have demonstrated that targeting HDL via direct infusion or transgenic expression of its major proteins results in a favorable effect on both the size and histologic composition of atherosclerotic plaque [12–14]. HDL

possess a number of functional properties that may contribute to these favorable effects on the artery wall. In addition to their well-characterized roles in promoting cholesterol efflux and reverse cholesterol transport, HDL have also been demonstrated to exert favorable effects on inflammatory, oxidative, apoptotic and thrombotic pathways implicated in atherosclerosis [15]. The degree to which these non-lipid transporting activities are related to the ability of HDL to promote the bioavailability of nitric oxide is uncertain. More recent investigations have focused on the functional quality of HDL, which may differ between various HDL subspecies and in a range of clinical settings such as inflammation, smoking, renal dysfunction and diabetes [16–22]. Accordingly, there is interest not only in raising HDL-C, but more importantly in promoting functional forms of HDL [23, 24].

Current approaches to targeting HDL

Existing approaches to management of lipids have modest effects on HDL-C levels [25]. While lifestyle modification is central to all approaches to cardiovascular prevention, the HDL-C raising effect is modest, predominantly occurring in the setting of weight loss. Statins and fibrates both raise HDL-C by up to 20% at most, with evidence from outcome and imaging trials suggesting that these HDL-C changes independently associate with their benefit [26–29]. While niacin is the most effective HDL-C raising agent currently used in clinical practice, with early evidence of benefit prior to the advent of statins and a favorable influence on disease progression on imaging [30–32], it has failed to demonstrate widespread benefit in the contemporary era of the statin-treated patient.

Cholesteryl ester transfer protein (CETP) inhibition demonstrated potential interest due to its HDL cholesterol raising properties [33]. However, even with substantial increases in HDL cholesterol and LDL cholesterol lowering in statin treated patients, torcetrapib (the first potent CETP inhibitor) resulted in adverse cardiovascular effects [34–36]. Since then the development of another CETP inhibitor (dalcetrapib, a modest CETP inhibitor) has demonstrated no protective effect in patients with a recent acute coronary syndrome (ACS), even though there was evidence of improved cholesterol efflux activity [37–39]. Further studies are assessing other CETP inhibitors (evacetrapib and anacetrapib) in high vascular risk patients [40–42]. Accordingly, there is considerable interest in developing novel approaches to targeting HDL. One particular area of interest involves the concept of infusing delipidated forms of HDL, which may have the potential for rapid onset of action of lipid transporting and other biological activities of HDL.

Infusing HDL containing apoA-I$_{Milano}$

ApoA-I$_{Milano}$ (AIM) is a genetic point mutation of apoA-I, resulting in an arginine to cysteine substitution at position 173. This mutation results in formation of AIM homodimers, with functional studies demonstrating favorable effects on cholesterol efflux, in addition to inflammatory and thrombotic mediators of atherosclerosis [43]. The variant was first identified in a cohort of northern Italian individuals, with evidence of low apoA-I and HDL-C levels, who appeared to be protected from cardiovascular disease. *Escherichia coli* based expression systems enabled generation of large quantities of recombinant AIM to produce reconstituted HDL particles in combination with phospholipid. These complexes were demonstrated to exert beneficial effects in animal models of balloon injury and atherosclerosis [44]. The combination of beneficial effects on endothelial function, aortic cholesterol content and platelet aggregation suggested potentially favorable effects in the setting of acute ischemic syndromes. Further studies in *ex vivo* and animal models of ischemia reperfusion demonstrating beneficial effects of AIM:phospholipid complexes on the extent of ischemic injury suggested potential additional benefits beyond direct effects on the artery wall [45–47].

A seminal proof-of-concept study demonstrated that administration of AIM:phospholipid (ETC-216) exerted favorable effects on atherosclerotic plaque burden in patients with a recent acute coronary syndrome (ACS) [48]. Fifty-seven patients with an ACS in the preceding 2 weeks underwent intravascular ultrasonography within a coronary artery at baseline and following a treatment period in which they received intravenous infusions of saline or ETC-216 containing 15 or 45 mg/kg AIM weekly for 5 weeks. Significant reductions in percent atheroma volume by 1.06% ($p = 0.02$) and total atheroma volume by 14.3 mm^3 ($p < 0.001$) were observed in ETC-216 treated patients, with no differences between the two active treatment groups. These changes were observed despite no discernible change in HDL-C levels at steady state. In fact, a greater degree of regression was observed in the lower dose group. Whether this reflects some degree of saturation of cholesterol mobilization, favoring efficacy at a lower dose, remains uncertain. Further analysis demonstrated the greatest degree of regression in the segments that contained the greatest amount of plaque at baseline and that regression appeared to associate with reverse remodeling of the artery wall [48, 49]. All of these changes were associated with the observation that ETC-216 was well tolerated. The major challenge subsequent to this landmark study has been the ability to produce sufficiently large quantities in a form that can be employed

in larger clinical trials. These limitations appear to have been overcome, and clinical development of HDL complexes containing AIM has recently continued.

Infusing HDL containing wild-type apoA-I

While the demonstration of rapid plaque regression with ETC-216 in humans provided an important proof-of-concept validation of potential benefits for HDL in humans, many in the field have been uncertain to what degree the specific protein composition influenced the results. While some investigators have reported that AIM possesses superior protective properties compared with wild-type apoA-I, others have not [50]. The effect of rHDL (reconstituted HDL) on Atherosclerosis-Safety and Efficacy (ERASE) study investigated the impact of infusing particles containing apoA-I (CSL-111) on coronary atherosclerosis [51]. One hundrd and eighty-three patients underwent serial intravascular ultrasonography to compare the effects of four weekly infusions of either saline or CSL-111 at a dose of 40 or 80 mg/kg. The higher dose of CSL-111 was discontinued due to the early appearance of liver enzyme elevations that prohibited ongoing evaluation of that dose [51]. While significant reductions in total atheroma volume by 3.4% ($p < 0.001$) or 5.3 mm^3 ($p < 0.001$) were observed in the CSL-111-treated patients compared with baseline, these changes failed to meet statistical significance compared with placebo [51]. Additional measures of plaque reflecting plaque characterization and quantitative coronary angiography did demonstrate favorable benefits with CSL-111 compared with placebo [51]. Safety evaluation demonstrated that the lower dose of CSL-111 was associated with well-tolerated increases in liver enzymes. These findings suggest a potential benefit with HDL infusions containing apoA-I and support the concept that the administration of lipid-depleted HDL is beneficial in patients with coronary disease. Similar challenges with producing large quantities of agent have slowed down clinical development, although a reformulation (CSL-112) is now undergoing safety evaluation in a large study of patients following myocardial infarction (AEGIS-I).

Infusing HDL containing sphingomyelin

An alternative approach to developing reconstituted HDL particles is to modify its lipid components. The clinical development program for CER-001 has focused on the generation of negatively charged HDL complexes that contain wild-type apoA-I in combination predominantly with sphingomyelin. This is based on observations that sphingomyelin and negative charge may favorably influence the extent and duration of cholesterol mobilization [52]. Early studies in LDL receptor knockout mice demonstrated that administration of CER-001 enhanced reverse lipid transport and decreased the size of atherosclerotic plaque [53]. In the Can HDL Infusions Significantly Quicken Atherosclerosis Regression (CHI-SQUARE) study, 507 patients with acute coronary syndromes and angiographic coronary artery disease were randomized to treatment with 6 weekly infusions of placebo or CER-001 at a dose of 3, 6 or 12 mg/kg and underwent evaluation of the change in atheroma burden with serial coronary intravascular ultrasonography. Primary analysis of the change in total atheroma volume revealed reductions of 2.71 mm^3 in the placebo group and by 3.13, 1.50 and 3.05 mm^3 in the 3–12 mg/kg groups, respectively, failing to meet statistical significance [50]. A subsequent analysis performed by another core laboratory, which evaluated anatomically matched arterial segments, demonstrated reductions in total atheroma volume by 2.85 mm^3 with placebo and by 4.76, 3.34 and 2.61 mm^3 with increasing doses of CER-001 [50]. Per protocol analysis demonstrated a greater degree of regression of total atheroma volume at the 3 mg/kg dose, which was significantly greater than changes observed in the placebo group (–6.28 vs. –3.63 mm^3, $p = 0.03$) [54]. Accordingly, a potential signal for regression at the lowest dose of CER-001 was observed. This is currently undergoing confirmation in a repeat imaging trial that will directly compare the effects of CER-001 3 mg/kg vs. placebo.

Autologous HDL infusions

An alternative approach has emerged, which involves selective delipidation of a patient's own HDL, which subsequently undergoes autologous reinfusion. In a pilot study, 28 patients with an acute coronary syndrome and at least one nonobstructive native coronary artery were randomized to receive 7 weekly infusions of either delipidated HDL or plasma [55]. Serial intravascular ultrasonography demonstrated numerically greater regression in the HDL-infused patients, although this failed to meet statistical significance [55]. This approach is currently undergoing evaluation in a larger, more appropriately powered imaging study.

Summary

Several lines of evidence favor the protective properties of HDL, with evidence from imaging studies of potentially early changes in plaque burden in patients following an acute coronary syndrome. However, to date the efficacy of these agents has only been demonstrated on surrogate

endpoints in imaging studies, and therefore the field is greatly in need of more definitive studies that investigate their effects on clinical outcomes. Each of these strategies requires ongoing validation before it can proceed to a large outcome trial, which will be required before these agents can be used in clinical practice.

Conflict of interest

The authors declare no conflict of interest.

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