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LIPID ABNORMALITIES AND CARDIOVASCULAR PREVENTION (G DE BACKER, SECTION EDITOR)

Targeting High Density Lipoproteins in the Prevention of Cardiovascular Disease?

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Abstract Recent studies involving HDL-raising therapeutics have greatly changed our understanding of this field. Despite effectively raising HDL-C levels, niacin remains of uncertain clinical benefit. Synthetic niacin receptor agonists are unlikely to raise HDL-C or have other beneficial effects on plasma lipids. Despite the failure in phase 3 of 2 CETP inhibitors, 2 potent CETP inhibitors that raise HDL-C levels by >100 % (and reduce LDL-C substantially) are in late stage clinical development. Infusions of recombinant HDL containing 'wild-type' apoA-I or apoA-I Milano, as well as autologous delipidated HDL, all demonstrated promising early results, and remain in clinical development. A small molecule that causes upregulation of endogenous apoA-I production is also in clinical development. Finally, upregulation of macrophage cholesterol efflux pathways through agonism of liver X receptors or antagonism of miR-33 remains of substantial interest. The field of HDL therapeutics is poised to transition from the 'HDL-cholesterol hypothesis' to the 'HDL flux hypothesis' in which the impact on flux from macrophage to feces is deemed to be of greater therapeutic benefit than the increase in steady-state concentrations of HDL cholesterol.

Keywords Lipids · HDL · Reverse cholesterol transport · Niacin · GPR109A · Cholesteryl ester transfer protein · CETP · Anacetrapib · Torcetrapib · Dalcetrapib · Evacetrapib · ApoA-I · Recombinant HDL · RVX-208 · Liver X receptor · miR-33 · Cardiovascular disease

Introduction

Despite aggressive LDL-reducing therapy, there remains substantial residual cardiovascular risk [1, 2]. The strong inverse association of plasma levels of high density lipoprotein (HDL) cholesterol with coronary artery disease (CAD) led to the development of the "HDL cholesterol hypothesis" that intervention to raise HDL cholesterol will result in reduced risk of CAD. The most popular mechanistic theory underlying the HDL cholesterol hypothesis has been the concept of "reverse cholesterol transport"; that HDL promotes cholesterol efflux from arterial macrophage foam cells and transports it to the liver for biliary excretion [3]. Recent discoveries have provided new insights into the complex metabolic and anti-atherosclerotic pathways of HDL. However, recent studies – including 2 randomized, placebo-controlled intervention trials [4••, 5] and a large genetic association analysis [6••] – call for a careful re-examination of the HDL cholesterol hypothesis. Here we review the current status of HDL-targeted therapies in the context of a re-evaluation of the HDL cholesterol hypothesis.

Niacin and Niacin Receptor Agonists

Nicotinic acid, or niacin, is the most effective HDL-raising drug currently on the market. In addition to raising HDL-C levels, niacin reduces triglycerides (TG), LDL-C, and lipoprotein(a)

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[Lp(a)]. New published clinical data involving niacin and its effects on lipids over the last 2 years has primarily involved studies of combination therapy as well as with co-administration with laropiprant, an antagonist of the prostaglandin receptor that mediates niacin-induced flushing. In a multiple-arm study in hyperlipidemic patients, the addition of extended-release niacin (ER-niacin) to the combination of ezetimibe/simvastatin significantly improved multiple lipid parameters compared with ezetimibe/simvastatin alone [7]. Metabolic syndrome patients randomized to ER-niacin in combination with laropiprant lowered TG and LDL-C and increased HDL-C significantly compared with placebo [8]. The combination of niacin/laropiprant plus a statin compared with doubling of the statin dose showed that combination treatment was associated with a significantly greater decrease in TG and LDL-C and a significantly greater increase in HDL-C [9]. An analysis of 4 trials of niacin/laropiprant revealed that this combination led to significantly larger decreases in TG, LDL-C, non-HDL-C, apoB, and Lp(a), and significantly greater increases in HDL-C and apoA-I compared with placebo or active comparator [10].

There has been considerable work on the molecular mechanisms of niacin action, both regarding its lipid and atherosclerosis effects as well as its cutaneous side effects, over the last several years. The discovery of the niacin receptor GPR109A created substantial excitement with regard to a better understanding of niacin's molecular mechanisms of action. Activation of GPR109A on skin cells such as Langerhans cells and keratinocytes promotes synthesis of prostaglandin D2 (PGD_2) and prostaglandin E2 (PGE_2), which subsequently induce cutaneous capillary vasodilation by binding to DP1 and EP2/4 receptors [11–13]. Co-administration of the DP1 antagonist laropiprant significantly reduces but does not eliminate niacin-induced skin symptoms [14]. It is also clear that activation of GPR109A on adipocytes mediates suppression of lipolysis and release of free fatty acids [15].

Nevertheless, recent evidence strongly indicates that activation of GPR109A does not mediate niacin's effects on plasma lipid and lipoprotein concentrations. Studies in a partially "humanized" mouse model showed that niacin reduces TG and LDL-C levels even when the GPR109A receptor is genetically deleted [16••]. Even more compelling are data in humans using synthetic agonists of GPR109A. Administration of several synthetic GPR109A agonists in humans effectively suppressed lipolysis and plasma free fatty acids but had minimal to no effect on TG, LDL-C, or HDL-C levels [16••, 17]. These results suggest that niacin modulates plasma lipids through mechanism(s) independent of its receptor GPR109A, and have brought into serious question the wisdom of developing synthetic GPR109A agonists as a therapeutic strategy. Interestingly, however, activation of GPR109A by niacin mediates certain anti-inflammatory effects such as macrophage recruitment into atherosclerotic plaques and the peritoneum. Provocatively, administration of niacin to atherosclerosis-prone mice decreased atherosclerotic plaques despite having minimal effects on cholesterol levels; this effect was dependent on expression of GPR109A in hematopoietic cells [18]. These results suggest that niacin may inhibit atherogenesis through activation of its receptor in macrophages or other hematopoietic cells and independently of effects on plasma lipid levels.

While niacin clearly improves all major lipid fractions, the major question has been whether it provides additional cardiovascular benefit when added to a statin, particularly in patients with low levels of HDL-C, and CAD. A randomized clinical trial in the pre-statin era in men with hypercholesterolemia and CAD indicated clinical benefit with reduction in cardiovascular event rates [19, 20]. Studies using vascular imaging measures suggested a benefit of combined simvastatin plus niacin on angiographic coronary disease and on carotid intimal medial thickness [21, 22]. Indeed, in 2010 2 published meta-analyses of randomized controlled trials of niacin concluded that niacin therapy was associated with significant reduction in major coronary events, stroke, and overall cardiovascular events, and led to the regression of coronary atherosclerosis and carotid intima thickness [23, 24].

Two trials were launched several years ago to test the incremental benefit of niacin added to a statin in patients with CAD and low HDL-C on cardiovascular events. The Atherothrombosis Intervention in Metabolic Syndrome With Low HDL/High Triglycerides: Impact on Global Health Outcomes (AIM-HIGH) trial randomized 3414 statin-treated CAD patients with low HDL-C to niacin vs placebo. The trial was halted early for futility: while niacin modestly but significantly increased HDL-C levels, there was no difference between the 2 groups in terms of CV events [4••]. The Heart Protection Study 2 - Treatment of High density lipoprotein to Reduce the Incidence of Vascular Events (HPS2-THRIVE) trial is a much larger trial with an arguably more appropriate comparative effectiveness design [25], and is scheduled to report by the end of 2012 [26, 27]. Thus, at the current time the clinical benefit of niacin, particularly with regard to the benefit of its HDL-raising properties, is uncertain.

Cholesteryl Ester Transfer Protein (CETP) Inhibitors

The cholesteryl ester transfer protein (CETP) exchanges cholesteryl esters primarily from HDL for triglycerides primarily from VLDL [28]. Genetic CETP deficiency causes marked elevation in HDL-C levels, leading to the concept

that pharmacologic inhibition of CETP would raise HDL-C levels and, according to the "HDL cholesterol hypothesis," reduce cardiovascular risk. The first CETP inhibitor to be tested in humans, torcetrapib, did substantially increase HDL-C levels [29]. However, in a large clinical outcome trial, subjects randomized to torcetrapib had increased cardiovascular events and total mortality compared with those allocated to placebo [30]. This outcome has been widely attributed to off-target effects of torcetrapib, including increased aldosterone production and raising of blood pressure [31, 32].

The next CETP inhibitor to enter a phase 3 clinical outcome trial was dalcetrapib. Less potent than torcetrapib, it increased HDL-C by an average of about 25 %–30 % in clinical trials [33–35]. The dal-PLAQUE trial tested the effect of dalcetrapib on atherosclerotic plaques using arterial positron-emission tomography (PET)/computed tomography (CT) and magnetic resonance imaging (MRI). It focused largely on ruling out an adverse torcetrapib-like effect and found no adverse effects on vessel wall/arterial inflammation – but little evidence of beneficial effects [36]. The dal-VESSEL trial tested the effect of dalcetrapib on vascular function and found no effect, adverse or beneficial, on NO-dependent endothelial function, inflammation, or oxidative stress [37]. Finally, the dal-OUTCOMES trial was a phase 3 outcomes trial testing the effect of dalcetrapib on cardiovascular events. It was terminated early due to futility; no details have yet been reported. This result would appear to challenge the simplistic HDL cholesterol hypothesis that raising HDL-C will reduce cardiovascular risk, and also poses additional questions about CETP inhibition as a therapeutic strategy.

However, at least 2 CETP inhibitors, both more potent than dalcetrapib, remain in clinical development. Anacetrapib inhibits CETP by forming a tight reversible bond [38]. At a dose of 150 mg daily it not only raises HDL-C by>100 % but also reduces LDL-C by about 40 % and Lp(a) by up to 50 % [39, 40]. In a phase 2b trial, 589 dyslipidemic patients were administered anacetrapib, atorvastatin, or placebo in various combinations with anacetrapib causing substantial increases in HDL-C and apoA-I and reductions in LDL-C and apoB [41•]. In a detailed study of lipoprotein subfractions, anacetrapib increased large HDL particles enriched with CE, apoA-I and apoC-III [42]. There are also some data suggesting that anacetrapib may improve HDL function and reverse cholesterol transport. HDL from subjects who received 300 mg anacetrapib daily for 8 weeks promoted greater cholesterol efflux from foam cells than HDL from subjects given placebo [43]. A detailed study in hamsters showed that anacetrapib promoted macrophage-specific reverse cholesterol transport (RCT) compared with placebo [44].

The Determining the Efficacy and Tolerability of CETP Inhibition with Anacetrapib (DEFINE) trial randomized 1623 patients with CAD or at high-risk for CAD who had achieved LDL-C treatment goals with statin therapy to 100 mg of anacetrapib or placebo [45, 46•]. Anacetrapib treatment resulted in a 138 % increase in HDL-C, a 40 % reduction in LDL-C, and a 36 % decrease in Lp(a) [46•]. After 76 weeks of follow-up, no increases in blood pressure, serum aldosterone levels, or cardiovascular events were observed. The Randomized Evaluation of the Effects of Anacetrapib Through Lipid-modification (REVEAL) trial is now enrolling and will randomize 30,000 subjects with CAD, cerebrovascular atherosclerotic disease, or peripheral artery disease to anacetrapib 100 mg, or placebo to formally assess the impact on cardiovascular events. The estimated study completion date is January 2017.

Evacetrapib is another potent CETP inhibitor in clinical development. Administration of evacetrapib in a 12-week randomized trial of 398 dyslipidemic patients in doses of 30–500 mg daily as monotherapy increased HDL-C from 54 %–129 % and decreased LDL-C from 14 %–36 %. Addition of evacetrapib 100 mg daily to statin therapy produced similar HDL-C increases and yielded further LDL-C reductions [47•]. A large phase III clinical outcomes trial is apparently planned to determine the effect of evacetrapib in reducing cardiovascular events [48].

In summary, the first CETP inhibitor (torcetrapib) to enter phase 3 increased CV events and mortality due most likely to off-target effects. The second CETP inhibitor (dalcetrapib) to enter phase 3 failed due to lack of efficacy in reducing cardiovascular risk, with details still unknown. There is a theoretical case to be made that CETP inhibition may not be an optimal mechanism to target HDL. However, at least 2 potent CETP inhibitors (anacetrapib and evacetrapib) are still in clinical development. In addition to raising HDL-C levels considerably more than dalcetrapib, they also substantially reduce LDL-C and Lp(a) levels. Thus, even if the HDL-C raising is of marginal benefit, these potent CETP inhibitors may reduce CV risk due to their effects on LDL-C and Lp(a).

Infusions of apoA-I-Containing Recombinant HDL Particles

ApoA-I is the most abundant protein in HDL. Lipid-poor apoA-I is effective at promoting cholesterol efflux from macrophages by serving as the preferred "acceptor" of cholesterol from the adenosine triphosphate binding cassette transporter 1 (ABCA1) transporter. Animal studies are strongly supportive of the concept that overexpression or injection of apoA-I can reduce or even regress atherosclerotic plaque. Thus there is interest in the concept of infusing apoA-I-containing recombinant HDL particles in humans. Small clinical studies using coronary imaging support the

concept of intravenous apoA-I infusion for reducing cardiovascular risk [49–51, 52•].

One approach uses apoA-I purified from human plasma and complexed with phosphatidylcholine derived from soybean, a preparation often termed "recombinant HDL" (rHDL) [51, 53]. A randomized, placebo-controlled study involved the administration in 145 patients with acute coronary syndrome of 4 weekly infusions of this type of rHDL (termed CSL-111). Serial intravascular ultrasound (IVUS) was used to assess the impact on coronary atherosclerosis. Infusion of rHDL was found to reduce atheroma volume by 3.4 % compared with baseline, although this was not significantly different than placebo [51]. Another study in patients with lower extremity peripheral artery disease utilized a single CSL-111 infusion followed by percutaneous superficial femoral artery revascularization 5-7 days following CSL-111 infusion. There were significant reductions in lipid content and endothelial adhesion molecule expression in plaque excised by atherectomy [54]. Among patients with diabetes, infusion of CSL-111 increased HDL-C up to 40 %, inhibited ex vivo platelet aggregation, and reduced monocyte activation and neutrophil adhesion [55, 56]. A reformulated version of CSL-111, called CSL-112, has been reported in preclinical studies to provide greater cholesterol efflux capacity as well as reduced hepatotoxicity compared with CSL-111, and is currently in clinical development [57].

ApoA-I Milano is a naturally-occurring mutation in apoA-I that has a cysteine to arginine substitution at amino acid 173. It is associated with very low levels of HDL-C, but despite this is not associated with increased atherosclerotic disease, giving rise to the concept that this mutant apoA-I may actually be more anti-atherogenic [58, 59]. Recombinant apoA-I Milano complexed with phospholipid has therefore also been studied for its effects on atherosclerosis in animal models and in humans. In mice and rabbits it has been shown to reduce atherosclerosis [60–63], though not to a greater extent than wild-type rHDL [64–67]. In a human study in subjects with CAD, 5 weekly doses of apoA-I Milano rHDL or placebo were administered and coronary atheroma was investigated by IVUS at the beginning and end of the study. Total atheroma volume was significantly reduced compared with baseline but no significant difference was observed with placebo [49]. ApoA-I Milano-containing rHDL infusion is still being investigated.

Another approach to apoA-I infusion utilizes autologous delipidated HDL [52•]. A device was invented that involves collection of plasma by apheresis over 1.5–2 hours followed by selective removal of lipids from HDL using organic solvents. The delipidated HDL is subsequently reinfused over 1 hour. In a non-human primate study this approach resulted in a significant reduction in aortic atheroma volume by IVUS [68]. In a clinical study of 28 patients with acute coronary syndrome, 7 weekly infusions of autologous delipidated HDL decreased total atheroma volume significantly from baseline though not from the control group [52•].

Upregulation of Endogenous apoA-I Production

Upregulation of endogenous apoA-I is conceptually highly attractive. RVX-208 is a synthetic small molecule that increases the transcription of the apoA-I gene. In a monkey model, administration of the compound RVX-208 over approximately 2 months significantly increased plasma apoA-I levels up to 60 % in a dose-dependent manner [69]. A small human study showed a significant increase in plasma apoA-I levels of 10 %, as well as augmentation of cholesterol efflux capacity [69]. In a Phase 2 trial of RVX-208, 299 statin-treated CAD patients were randomized to placebo or 3 different treatment doses for 12 weeks. While there was an increase in apoA-I levels compared with baseline, there was no statistically significant change in apoA-I compared with placebo [70•]. An ongoing phase 2b trial of RVX-208 involves 172 statin-treated patients randomized to placebo or RVX-208 100 mg twice daily for 24 weeks [71]. In another phase 2b trial, the effect of RVX-208 on coronary atherosclerosis is being assessed by IVUS [72]. It will be of substantial interest to determine whether this approach has beneficial effects on coronary disease.

Enhancing Macrophage Cholesterol Efflux Through Upregulation of ABC Transporters

Liver X receptors (LXRs) are nuclear receptors that act as cholesterol sensors and regulate expression of genes involved in cholesterol metabolism. Activation of LXRs by natural and synthetic agonists has been demonstrated to promote mobilization of intracellular cholesterol, increase macrophage cholesterol efflux via macrophage ABCA1 and ABCG1, and augment intestinal HDL generation [73–75]. Dyslipidemic hamsters treated with the LXR agonist GW3965 had increased macrophage-to-feces RCT, but also increased TG and LDL-C [76]. In a rabbit model of atherosclerosis, combination therapy with the LXR agonist LXR-623, and simvastatin induced plaque regression, while simvastatin alone, and LXR monotherapy at low and medium doses attenuated plaque progression [77].

Therapeutic development of LXR agonists has been hindered by hepatic steatosis and increased plasma triglyceride concentrations reported in preclinical studies of these drugs [78]. Dissociating LXR efficacy and toxicity might be possible owing to the differential effects of LXR agonism by receptor isoform and by tissue-specific effects. Administration of a nonselective LXR agonist to LXRα-deficient mice stimulated

macrophage ABCA1 expression and cholesterol efflux without inducing fatty liver and with minimal upregulation of hepatic triglyceride synthesis [79]. Liver-specific LXRα knockout mice have increased atherosclerosis and decreased RCT. Interestingly, synthetic LXR agonist rescue in these mice still led to anti-atherogenic activity despite the lack of hepatic LXRalpha; it also did not increase plasma TG but still increased plasma HDL [80]. The LXR agonist AZ876 decreased atherosclerotic lesion size in APOE*3 Leiden mice at low dose without increasing TG or causing liver steatosis; at high dose, lesion size was decreased to an even greater degree and also there were decreased cytokine levels/vessel wall inflammatory markers, but with the addition of increased plasma TG [81].

A second approach to LXR agonist development might be to selectively activate intestinal LXR. An intestine-specific LXRα/β agonist, GW6340, promoted macrophage-specific reverse cholesterol transport, augmenting the fecal excretion of radiolabeled sterol by 52 % via increased intestinal HDL production and intestinal excretion of HDL-derived cholesterol [82].

Another mechanism for increasing ABCA1 and ABCG1 expression is through the microRNA miR-33. MicroRNAs are short non-coding sequences of RNA that inhibit gene expression by binding to complementary 3' untranslated regions of mRNAs and causing translational repression and/or mRNA destabilization [83]. MiR-33 is encoded within an intron of the gene encoding the sterol regulatory element binding transcription factor 2 (SREBF2) and suppresses macrophage and hepatocyte expression of ABCA1 and ABCG1, thus reducing circulating HDL-C levels and macrophage efflux to apoA-I [84]. Silencing of miR-33 with an antisense oligonucleotide (ASO) was associated with greater macrophage and hepatocyte expression of ABCA1 and increased HDL-C levels. In a mouse model of atherosclerosis, administration of an ASO to miR-33 significantly increased HDL-C, promoted macrophage-specific reverse cholesterol transport, and induced atheroma regression [85••]. In a non-human primate model of dyslipidemia, subcutaneous delivery of anti-miR-33 ASO over a 12-week period increased HDL-C up to 50 % [86•]. Greater macrophage cholesterol efflux was observed following incubation of foam cells with serum obtained from treated monkeys compared with equivalent volumes of serum isolated from control monkeys, correlating with the HDL-C levels in the 2 groups. Thus, anti-miR-33 therapy is another potential approach to promoting macrophage cholesterol efflux and RCT.

Conclusions

Recent events have brought into question the simple "HDL cholesterol hypothesis" that raising HDL cholesterol levels will reduce cardiovascular risk. The HPS2-THRIVE trial with niacin and the trials with CETP inhibitors anacetrapib and evacetrapib will provide the next wave of critical clinical data of HDL-directed strategies in large contemporary cohorts managed with aggressive medical therapy. It may be time to modify the "HDL cholesterol hypothesis" to the "HDL flux hypothesis": Intervention to promote cholesterol efflux and reverse cholesterol transport will reduce CAD risk. Clinical outcomes studies of interventions that promote cholesterol efflux and reverse cholesterol transport are ultimately required to test this hypothesis.

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