Targeting high-density lipoproteins: Update on a promising therapy

Les HDL comme cible thérapeutique : état des lieux

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Summary Numerous epidemiological studies have demonstrated the atheroprotective roles of high density lipoproteins (HDL), so that HDL is established as an independent negative risk factor. The protective effect of HDL against atherosclerosis is mainly attributed to their capacity to bring peripheral excess cholesterol back to the liver for further elimination into the bile. In addition, HDL can exert other protective functions on the vascular wall, through their anti-inflammatory, antioxidant, antithrombotic and cytoprotective properties. HDL-targeted therapy is thus an innovative approach against cardiovascular risk and atherosclerosis. These pleiotropic atheroprotective properties of HDL have led experts to believe that ”HDL-related therapies” represent the most promising next step in fighting against atherosclerosis. However, because of

Abbreviations: ABCA1, Adenosine triphosphate binding cassette A1; ABCG1, Adenosine triphosphate binding cassette G1; apo, Apolipoprotein; ATP, Adenosine triphosphate; CETP, Cholesteryl ester transfer protein; CVD, Cardiovascular disease; HDL, High-density lipoprotein; HDL-C, High-density lipoprotein cholesterol; LCAT, Lecithin cholesterol acyl transferase; LDL, Low-density lipoprotein; LDL-C, Low-density lipoprotein cholesterol; LXR, Liver X receptor; P2Y\textsubscript{13}, Purinergic receptor 13; PPAR, Peroxisome proliferator-activated receptor; RCT, Reverse cholesterol transport; SR-BI, Scavenger receptor class B type I; VLDDL, Very-low-density lipoprotein.

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the heterogeneity of HDL functions, targeting HDL is not a simple task and HDL therapies that lower cardiovascular risk are NOT yet available. In this paper, an overview is presented about the therapeutic strategies currently under consideration to raise HDL levels and/or functions. Recently, clinical trials of drugs targeting HDL-C levels have disappointingly failed, suggesting that HDL functions through specific mechanisms should be targeted rather than increasing per se HDL levels. © 2013 Elsevier Masson SAS. All rights reserved.

Background

Cardiovascular pathologies are now considered as the biggest scourge of our modern society; during 2008, nearly 30% of deaths worldwide were due to cardiovascular diseases (CVDs) [1]. At the origin of most cardiovascular pathologies, atherosclerosis is a deleterious phenomenon responsible for coronary artery diseases, peripheral vascular diseases and strokes. This inflammatory process is characterized by alteration of the arterial wall, followed by lipid infiltration leading to thickening of the atheroma plaque and then, ultimately, to its rupture with the formation of a thrombus. Cholesterol deposit is crucial and two main lipoproteins are able to transport cholesterol in the plasma: while increased low-density lipoprotein cholesterol (LDL-C) concentration is among the main risk factors for CVDs, high-density lipoprotein cholesterol (HDL-C) concentration is inversely related to atherosclerosis severity. Therefore, these two variables are included in systematic routine measurements to screen dyslipidaemias in the general population, but also to set therapeutic objectives. Lowering LDL-C, known to be responsible for cholesterol deposition in the vessel wall, has always been one of the most attractive targets of lipid-lowering drugs. Indeed, statin therapy has a beneficial effect on the atherosclerotic process and a 12% reduction in overall mortality has been observed for each mmol/L decrease in LDL-C concentration (40 mg/dL) [2]. Unfortunately, the residual risk remains important, which emphasizes the need to find new targets to achieve further benefits. Among all possibilities, raising HDL-C concentration has appeared as a most promising strategy. Indeed, epidemiological studies have shown that a 0.03 mmol/L (1 mg/dL) reduction in HDL-cholesterol concentration is associated with a 2–3% increase in cardiovascular risk [3]. And, while a concentration < 1 mmol/L (40 mg/dL) is considered as an independent risk factor for CVD, it has recently been shown that 33% of men and 40% of women treated for dyslipidaemia in Europe display low concentrations of HDL-C [4]. A low HDL-C concentration is therefore very common and strategies aiming at raising the plasma concentration could be promising. In the first part of this review, we will focus on the different protective effects of HDL before addressing treatments that are currently used or under development.

High-density lipoprotein protective mechanisms

Reverse cholesterol transport

Classically, the main HDL atheroprotective function is ‘reverse cholesterol transport’ (RCT), a process whereby excess cell cholesterol is taken up from peripheral (and vascular foam) cells and is delivered to the liver for further elimination into the bile. To have a better understanding of this process, it is important to be reminded of the structure of HDLs: these are nanoparticles composed of a lipid moiety (free and esterified cholesterol, triglycerides, phospholipids, lysosphingolipids) and a protein part, including the major apolipoprotein A-I (apoA-I) and various enzymes, each one being responsible for numerous beneficial effects. Briefly, after synthesis by the liver, apoA-I acquires phospholipids and free cholesterol through efflux of cellular cholesterol by active (adenosine triphosphate [ATP] binding cassette A1 and G1 [ABCA1, ABCG1]) and passive (scavenger receptor class B type I [SR-BI]) transporters, to form...
pre-β HDL. Then, several enzymes carried by HDL itself lead to the formation of mature HDL. Lecithin cholesterol acyl transferase (LCAT) esterifies free cholesterol, leading to migration of esterified cholesterol into the particle core, generating a continuous gradient of free cholesterol from cells towards HDL. After plasma remodelling, HDL-C is taken up by the liver through SR-BI and is eliminated into the bile as free cholesterol or as biliary acids after metabolism.

Another putative pathway involved in RCT has also been evoked. Demonstrated in mice, it is responsible for the uptake of the whole HDL particle and involves activation of the membrane ecto-F$_1$-ATPase by apoA-I [5]. The generated adenosine diphosphate further activates the purinergic receptor P2Y$_{13}$, which in turn stimulates endocytosis of the entire HDL particle [6]. Besides this general process, an alternate route exists in humans for the delivery of HDL-C back to the liver, involving cholesteryl ester transfer protein (CETP); this pathway is quantitatively important in normolipaemic conditions. CETP is combined with HDL lipoprotein and mediates transfer of esterified cholesterol towards very-low-density lipoprotein (VLDL) and LDL in exchange for triglycerides. This transfer protein is therefore responsible for a decrease in HDL-C concentration but also accounts for an enrichment of LDL particles in cholesterol, allowing cholesterol elimination by the liver through the LDL receptor. This indirect route that is quantitatively important in humans is absent in rodents, which questions the relevance of murine models for studies on lipoprotein metabolism.

The whole RCT process is therefore physiologically important as it allows removal of excess cholesterol from the artery wall and from atherosclerotic plaques.

**Other atheroprotective effects of high-density lipoprotein**

Beyond this main atheroprotective mechanism, HDL exerts pleiotropic functions that protect against atherosclerosis (Fig. 1). Indeed, as detailed in different reviews [7,8], HDL can protect endothelium by different mechanisms: by stimulating endothelial cell nitrite oxide and prostacyclin production, HDLs promote better regulation of vascular structure and tone, and thus display antithrombotic and antiaggregating properties [4]. Sphingosine-1-phosphate, a major lysosphingolipid associated with HDL particles, also promotes endothelial survival via activation of its specific receptor. HDLs are also able to decrease endothelial apoptosis induced by tumour necrosis factor-α and growth factor deprivation by several mechanisms and, particularly, through pathways triggered by SR-BI activation, involving proapoptotic factors Bcl-2-associated death promoter and Bcl-2-associated X protein [4] or by apoA-I-mediated ecto-F$_1$-ATPase activation [9]. Furthermore, HDLs present potent antioxidative properties due to numerous enzymes carried by these lipoproteins, such as paraoxonase, platelet-activating factor-acetyl hydrolase, LCAT or glutathione selenoperoxidase, which degrade oxidized lipids and therefore prevent LDL oxidation, which is a key determinant of atherogenesis. Apolipoproteins (A-I, A-II, A-IV, E or J) also display antioxidative properties and also have an anti-inflammatory impact. Moreover, HDLs exhibit an anti-infectious role against bacteria and parasites. They protect against endotoxaemia by accelerating bile clearance of gram-negative bacteria due to their binding to membrane lipopolysaccharides, but they also show specific lytic

![Figure 1](https://example.com/fig1.png)

**Figure 1.** Pleiotropic protective effects of high-density lipoprotein (HDL). Green text indicates pleiotropic effects. Red text indicates HDL components or mechanisms responsible for these biological effects. Apo: apolipoprotein; ATP: adenosine triphosphate; BAD: Bcl-2-associated death promoter; BAX: Bcl-2-associated X protein; ICAM: intercellular adhesion molecule; LDL: low-density lipoprotein; NO: nitric oxide; PAF-AH: platelet-activating factor-acetyl hydrolase; S1P: sphingosine-1-phosphate; SAA: serum amyloid A protein; SR-BI: scavenger receptor class B type I; VCAM: vascular cellular adhesion molecule.
activity against *Trypanosoma brucei brucei*, the sleeping sickness parasite [10]. In addition, HDLs seem to be important in cellular immunity, through macrophage expression of inflammatory chemokines, such as monocyte chemoattractant protein-1. HDLs also promote humoral immunity by modulating activation of the complement system [11].

**High-density lipoprotein under inflammatory conditions**

HDLs are complex particles, which are continuously remodelled. Systemic inflammation associated with oxidative stress induces structural and compositional modifications. These abnormal HDLs are considered dysfunctional, with loss of their normal properties. Indeed, it has been shown that acute phase proteins, such as serum amyloid A, can displace apoA-I from HDL, causing a negative impact on cholesterol efflux capacity. Modification of HDL composition is also deleterious because of enrichment in triglycerides at the expense of cholesterol esters. Finally, a decrease in antioxidative properties is related to decreased activities of paraoxonase, platelet-activating factor-acetyl hydrolase and LCAT [12]. Furthermore, phospholipase A2, either lipoprotein associated or secreted, has been involved in the inflammatory reaction occurring during atherogenesis. HDLs, due to their anti-inflammatory effects, might counteract actions of phospholipase A2. However, clinical trials using specific phospholipase A2 inhibitors did not show significant effects on HDL or LDL concentrations, as shown by Mohler et al. [13]. Hence, in HDL particles, pro- and anti-inflammatory properties are in subtle equilibrium and some authors have proposed an ‘anti-inflammatory index’ to quantify HDL properties. This approach assesses either LDL-induced monocyte chemotaxis [14] or dichlorofluorescein oxidation [15], with and without HDL. A ratio between these two conditions allows separation into two groups: a ratio below 1 indicates that HDLs are anti-inflammatory and, conversely, a ratio greater than 1 indicates a proinflammatory profile for HDL. In 2003, a study reported that this index could be more useful than a single measurement of HDL-C for evaluating coronary artery patients: among 26 patients, 77% presented an inflammatory index above 1, while only 11% had a low HDL-C concentration [16]. Unfortunately, these techniques have not been introduced in routine measurements so far, probably because of a lack of standardization.

Modification of HDL composition during acute or chronic inflammation leads to functional alterations; this emphasizes the need to evaluate HDL composition and functions, rather than simple measurement of HDL-C concentration. Therefore, therapies improving HDL functionality could be a more promising bet compared with therapies that only increase concentration.

**Pharmacological therapies**

**Niacin (nicotinic acid)**

Niacin, also known as nicotinic acid or vitamin B3, is a physiological precursor of nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate, two coenzymes involved in oxidoreductive reactions and energy metabolism. An inadequate nutritional intake leads to pelagra, an old disease characterized by dermatitis, dementia and diarrhoea. Vitaminic potential is demonstrated with milligram doses, but at a pharmacological dose of approximately 1.5–2 g per day, niacin is one of the most potent agents available for increasing HDL-C concentration. Niacin also reduces all proatherogenic lipids and lipoproteins, including total cholesterol, triglycerides, VLDL, LDL and lipoprotein(a). Different potential mechanisms underlying the antidyshlipidaemic effects of niacin have been recently extensively reviewed [17,18] and are summarized in Fig. 2 and Table 1. Beyond its lipid-modifying activity, niacin has also been shown to exert other potential antiatherosclerotic effects, in part through mechanisms involving its receptor (hydroxycarboxylic acid receptor 2 [also called GPR109A]) on immune cells as well as through direct and indirect effects on the vascular endothelium [18]. In accordance with these pleiotropic potentially beneficial actions of niacin in CVDs, its therapeutic use has been considered for decades in the prevention and treatment of atherosclerosis, but negative outcomes of recent clinical trials – discussed below – have led to questions about its efficacy.

Initially, an immediate release form of niacin was used, but was associated with frequent flushes due to activation of hydroxycarboxylic acid receptor 2 on epidermal Langerhans cells as well as keratinocytes, and subsequent formation of prostaglandins D2 and E2. Different pharmaceutical formulations have been developed: an extended-release form (Niaspan®), which causes fewer flushes because of its lower absorption rate; an extended-release form combined with laropiprant, a prostaglandin D2 antagonist (Cordaptive™, also called Tredaptive™); and niacin combined with simvastatin (Simcor®) or lovastatin (Advicor®). Two large publicized clinical trials have been recently designed to evaluate whether adding these modern niacin formulations to statin therapy provides incremental benefit over statin therapy alone in terms of cardiovascular primary events in patients with established CVD: AIM-HIGH (Atherosclerosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides and Impact on Global Health Outcomes) and HPS2-THRIVE (Heart Protection Study 2-Treatment of High density lipoprotein to Reduce the Incidence of Vascular Events).

AIM-HIGH, which compared the Niaspan®/simvastatin combination with simvastatin alone in about 3500 patients was stopped before the planned end because of a lack of cardiovascular reducing effects and because of a non-significant trend towards an increase in ischaemic strokes in the treated group. However, as explained by Nicholls [19], several limitations may balance the negative findings of this study, including higher doses of simvastatin and greater use of ezetimibe (22% vs. 10%) in the statin only arm compared with in the statin/niacin arm, as well as the fact that the statin only group actually received a low dose of niacin (up to 200 mg) to mimic flushes and did have an increase in HDL-C (+9.8%). Finally, the study was stopped after only 36 months, which might have been too early to observe benefits in patients with baseline LDL-C concentrations of only 71 mg/dL.

Concerning HPS2-THRIVE, which evaluated the Cordaptive™/statin combination versus statin alone in more than 25,600 patients, the trial was recently stopped after only 3.9 years because of serious adverse events — not
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Figure 2. Possible explanations for the effects of nicotinic acid on lipoprotein profile and protective mechanisms. apo: apolipoprotein; ATP: adenosine triphosphate; CE: cholesterol ester; CETP: cholesteryl ester transfer protein; DGAT: diacylglycerol acyltransferase; HCAR: hydroxycarboxylic acid receptor; HDL: high-density lipoprotein; LDL: low-density lipoprotein; MCP: monocyte chemoattractant protein; P2Y13: purinergic receptor 13; TG: triglycerides; VCAM: vascular cellular adhesion molecule; VLDL: very-low-density lipoprotein.

detailed yet — in the treated arm. The primary endpoints (i.e., reduction in heart attacks and strokes) were also not met and a statistical increase in non-fatal side effects was even reported in the niacin-treated group [20]. However, a detailed subgroup analysis, especially of those subgroups most likely to benefit from niacin therapy (i.e., patients with low HDL-C concentration), might yield more insight into this trial. Moreover, despite these disappointing extended-release niacin trials (AIM-HIGH and HPS-THRIVE), a recent meta-analysis of 11 clinical trials, including AIM-HIGH, in 9959 subjects, showed that niacin therapy was associated with a significant reduction in major CVD and coronary heart disease events [21], which might partly rescue niacin’s reputation as an effective preventive therapy.

In conclusion, better understanding of niacin’s actions and targets might help to better design combination therapy and new treatment strategies for atherosclerosis.

Cholesteryl ester transfer protein inhibitors

Given the proatherogenic potential of CETP, which favours transfer of cholesterol from HDL to LDL, synthetic inhibitors were developed to restore a favourable lipoprotein profile by increasing HDL-C concentration and lowering LDL-C concentration. Torcetrapib (Pfizer, New York, USA) was the first molecule to be designed as an inhibitor of CETP lipid transfer functions. Evaluated in phase III (the ILLUMINATE study), its development was stopped due to a global overall mortality in patients treated with torcetrapib, although HDL-C concentration had increased. Subsequent analyses showed that this negative effect was due to activation of the renin-angiotensin-aldosterone system, increasing blood pressure, but also to direct vascular endothelium toxicity [33].

After this failure, new drugs were still developed: dalcetrapib (Hoffman-La Roche Inc, Basel, Switzerland), evacetrapib (Eli Lilly and Co, Indianapolis, USA) and anacetrapib (Merck & Co, Whitehouse station, USA). Concerning dalcetrapib, several studies have been scheduled (dal-VESSEL and dal-PLAQUE), but the phase III dal-OUTCOME study was stopped in May 2012 due to a lack of significant results. The safety of evacetrapib has been demonstrated in a phase II study involving 398 patients [25]; a phase III study began in October 2012 to evaluate its efficacy and safety (versus placebo) in participants with high-risk vascular disease (the ACCELERATE study). Lastly, two phase III trials are currently underway for anacetrapib: the DEFINE trial (Determining the Efficacy and Tolerability of CETP Inhibition with Anacetrapib) was completed by the end 2012 and the REVEAL HSP-3 TIMI-55 trial (Randomized Evaluation of the Effects of Anacetrapib through Lipid Modification), which includes 30,000 coronary patients treated with statins, will end in 2017.

Reconstituted high-density lipoprotein

Intravenous administration of liposomic complexes containing human proapolipoprotein A-I (the secreted form of apoA-I) in four patients with familial hypercholesterolaemia showed a > 30% stimulation of faecal excretion of cholesterol and biliary acids [34]. This increase in RCT was further confirmed in the first clinical trial using purified human apoA-I and synthetic phospholipids [35]. Purified apoA-I was then combined with soya phophatidylcholines and the product was called CSL-111. These reconstituted HDLs were tested
in the ERASE trial (Effect of rHDL on Atherosclerosis Safety and Efficacy) in patients with acute coronary syndrome [36]. Although CSL-111 produced a 3.4% volume reduction in the atherosclerotic plaque after a weekly perfusion over 1 month, its development has been stopped because of liver toxicity. Second generation products have emerged, such as CSL-112 (CSL Laboratories, Victoria, Australia) and CER-001 (Cerenis Therapeutics Labège, France), which are currently being tested in acute coronary syndrome patients (a phase II study) and in patients with familial hypercholesterolaemia (for CER-001).

In the early 1980s, apoA-I Milano was discovered in Northern Italy (Limone sul Garda) in three members of a family presenting with significant hypertriglyceridaemia associated with low HDL-C concentrations (0.07–0.14 g/L), without any manifestation of atherosclerosis [37]. This abnormal apoA-I differs from the native form by a substitution at position 173, where arginine is replaced by cysteine, leading to loss of stability and decreased affinity for lipids, explaining the accelerated degradation of free apoA-I by the kidneys. However, it has since been demonstrated that this mutation allows formation of apoA-I Milano homodimers and that this particular apoA-I displays a higher capacity for cellular cholesterol efflux and has a potent antioxidant activity, therefore preventing phospholipid oxidation [38,39]. Reconstituted HDL containing apoA-I Milano dimers and synthetic phospholipids (named ETC-216 and then MDCO-216; The Medicines Company, Parsippany, USA) have shown beneficial effects in patients with acute coronary syndrome: weekly perfusions over 5 weeks led to a 4.2% decrease in volume of the atherosclerotic plaque [26].

Despite encouraging results, reconstituted HDL therapies have not yet emerged. However, rapid regression of a condition that has developed over years was observed after only a few injections, which indicates the potential value of this therapeutic approach.

Mimetic peptides

ApoA-I is a major atheroprotective protein due to its antioxidant properties and its capacity to capture excess cellular cholesterol, notably from macrophages. However, this molecule is difficult and expensive to produce, which has motivated the synthesis of smaller oral ingestible apoA-I mimetic peptides. Among them, synthesis of octadecapeptide called 18A has emerged; although they do not present any homology with apoA-I, they are able to mimic its functional activity and have identical binding capacity. In this family, peptides 4F and 5F appear to be the most effective. The 4F peptide binds oxidized phospholipids with higher affinity than apoA-I [40] and oral administration of D-4F leads to a decrease in the HDL inflammatory index [41]. The levorotatory enantiomer called L-4F (or APL180; Novartis, Basel, Switzerland), which is degraded in the intestine and thus cannot be orally administered, has been tested via intravenous infusion for 7 days or via subcutaneous injection for 28 days. Unfortunately, despite modification of the lipoprotein profile, the HDL inflammatory index did not improve and authors observed a trend toward elevated C-reactive protein [27].

Delipidated high-density lipoprotein

Thanks to a selective delipidation method allowing conversion of large lipigated HDL to lipid-poor HDL, RCT was found to be enhanced [42]. A clinical study, the LS-001 (Lipid Sciences Selective Delipidation Trial), has evaluated the effect of serial autologous infusions of delipidated HDL in 14 patients with acute coronary syndrome. Although the results showed its harmlessness, no significant improvement was seen in atheroma volume despite a non-significant decrease [43].

Peroxisome proliferator-activated receptor agonists (α and γ glitazars)

Peroxisome proliferator-activated receptors (PPARs) are nuclear transcription receptors involved in lipid and carbohydrate homeostasis; they are composed of three isoforms (α, γ and β/δ). After activation, PPARs heterodimerize with the retinoid X receptor and further bind to a specific DNA region called peroxisome proliferator response element, localized on target genes.

The first agonists synthesized, commonly known as fibrates, targeted the α isofrom, which is mainly expressed in liver, heart and muscle. After binding, expression of numerous genes is up-regulated, including genes encoding apoA-I, apoA-II, ABCA1 and acyl-CoA synthetase, the last of which promotes fatty acid oxidation. Other genes, such as fibrinogen, cyclo-oxygenase 2, vascular cell adhesion molecule and C-reactive protein, are down-regulated, thus enabling an overall protective effect. Indeed, fibrates improve lipoprotein plasma profile and insulin sensitivity, depress inflammation and clotting and enhance vasomotor reactivity [28]. As summarized by Chapman, triglycerides are reduced by 30–50%, LDL by 15–20% and HDL-C rises from 5% to 15% [44]. Many clinical studies have assessed the impact of fibrates on clinical outcomes in primary and secondary prevention (the Helsinki Heart Study, the VA-HIT study, the BIP study, the FIELD study, etc.). Despite a 34% reduction in major coronary events at 5 years, as shown by the Helsinki Heart Study [44], fibrates have failed to reduce overall mortality in the general population. They are potentially useful in specific populations and selective subgroups: for instance, fenofibrate and bezafibrate could be useful for diabetes or metabolic syndrome and gemfibrozil could be useful for patients with dyslipidaemia [45].

The γ isoform, highly expressed in adipose tissue and muscle, regulates glucose homeostasis and insulin sensitivity, in addition to its role in lipid metabolism and inflammation. Thus, agonists called glitazones have been designed to be used in type 2 diabetes mellitus. Unfortunately, due to side effects (bladder cancer for pioglitazone, increased cardiovascular risk for rosiglitazone and peripheral oedema for both), this family of molecules is no longer marketed in France.

Thus, the general idea was to create dual agonists that are able to impact lipid and carbohydrate metabolism by targeting the two isoforms, α and γ. New drugs were therefore designed, called glitazars, which have positive effects on lipid metabolism due to activation of PPAR-α in the liver and insulin-sensitizing effects, as a
consequence of PPAR-γ activation, making possible their use in metabolic syndrome \[46\], a condition associated with low HDL. Due to side effects (haematological disturbance, serum creatinine increase, etc.) \[45\], most molecules were given up on, except for aleglitazar (Hoffmann-La Roche Inc., Basel, Switzerland). During a 16-week administration period, its safety and positive impact on lipoprotein profile was shown in type II diabetics in the phase II SYNCHRONY study \[47\]. The maximum effect on HDL-C concentration was seen with a 150 \(\mu\)g dose (placebo-adjusted increase of 20.7%, 13.2–28.2) and was responsible for a greater effect than 45 mg of pioglitazone. The same dose led to a decrease in triglycerides (−43.4%, −27.4 to −59.4) and LDL-C (−15.5%, −5.4 to −25.6). Glycated haemoglobin decreased in a dose-dependant manner. Furthermore, according to an investigator update from Roche’s website, AleNepho (a phase IIb study) has also demonstrated the renal safety of this molecule in type II diabetes patients with stage 3 chronic renal disease. A phase III study (AleCardio), involving patients with type 2 diabetes hospitalized for acute coronary syndrome, began in 2009 and results are expected in 2015.

Other targets

Liver X receptor agonists

Liver X receptors (LXRs) belong to a nuclear receptor family involved in lipid homeostasis. Activated by oxysterols, derived from cholesterol degradation or from dietary intake, LXRs consist of two isoforms (\(\alpha\) and \(\beta\)). While LXR\(\alpha\) is highly expressed in liver, intestine, kidney, spleen and adipose tissue, LXR\(\beta\) is ubiquitously expressed, but at lower concentrations. Considered as intracellular sensors, they can activate transcription of many genes involved in RCT, such as \(ABCA1\), \(ABCG1\) and \(CETP\), and, at the same time, they depress expression of genes involved in intestinal cholesterol absorption, such as Niemann-Pick C1-Like 1 (\(NPC1L1\)). However, as LXRs also stimulates the synthesis of fatty acids and triglycerides in the liver, its agonists have lipogenic effects. This emphasizes the need to synthesize LXR\(\beta\)-specific agonists, which is currently difficult to achieve due to a high sequence homology between the two isoforms (80% homology sequence). Besides, a positive effect on \(CETP\) transcription was also noted, which could confer fewer beneficial effects in humans than those observed in species lacking \(CETP\), such as mice.

Stimulation of apolipoprotein A-I transcription

RVX-208 (Resverlogix Corp., Calgary, Canada) was the first bromo and extra terminal (BET) bromodomain (an epigenetic regulator) inhibitor to be used in therapeutics. This oral inducer of apoA-I synthesis was first evaluated over 12 weeks in 225 patients from the ASSERT study (ApoloA-I Synthesis Stimulation Evaluation in Patients Requiring Treatment for Coronary Artery Disease) \[48\]. ApoA-I changes did not reach statistical significance, although a trend was observed, but higher increases could require chronic treatment. HDL-C concentrations increased from 3.2% to 8.3% according to dose and transient elevations of liver transaminases were seen. Two phase II studies are under way: SUSTAIN (The Study of Quantitative Serial Trends in Lipids with ApoA-I Stimulation), which is evaluating its safety and efficacy on blood lipids; and ASSURE (The ApoA-I Synthesis Stimulation and Intravascular Ultrasound for Coronary Atheroma Regression Evaluation), which is studying its impact on atherosclerotic plaques. A press release on the Resverlogix website (August 28, 2012) states that after 24 weeks of treatment in the SUSTAIN study, there was a statistical increase in HDL-C, apoA-I and large HDL particle concentrations without any further increase in transaminase concentrations. Results of the ASSURE study are expected in 2013.

Modulation of reverse cholesterol transport

With the aim of increasing HDL-C concentration, researchers tried to find new drugs targeting HDL uptake and catabolism by the liver. In this regard, Masson et al. tested a novel SR-BI inhibitor — ITX5061 — in mice and humans \[29\]. The use of ITX5061 in hypertriglyceridaemic patients led to an increase in HDL-C concentration of about 20% without modifying LDL-C or triglyceride concentrations. Furthermore, ITX5061 was responsible for a reduction in early atherosclerotic lesions in the aortic arch in mice fed an atherogenic diet. However, the beneficial effect of inhibition of SR-BI, which participates in hepatic HDL-C uptake and cellular cholesterol efflux, is questionable, as this would rather impair RCT.

Conclusion

The concept of HDL-therapy, considered as one of the most promising strategies for the treatment of atherosclerosis, is not yet established (as summarized in Table 1). Epidemiological studies had given hope that a simple increase in HDL-C would reduce cardiovascular risk. However, HDL metabolism is quite complex and is still not completely understood. The termination of the CETP inhibitor dalcetrapib, the lack of effect of niacin in the AIM-HIGH and HPS2-THRIVE clinical trials \[19,20\] and evidence for a lack of association with cardiovascular risk in genome-wide association studies of HDL genes \[30\] have all raised questions about the HDL hypothesis and have cast many doubts about the relevance of increasing HDL-C concentration per se.

One argument to explain the HDL controversy is that HDL-C concentration is a poor measure for targeted intervention. HDL-C concentration is considered a surrogate for the efficiency of cholesterol efflux from tissues. However, given that macrophage-derived cholesterol represents only a minor proportion of the cholesterol transported by HDL particles, this may be an inadequate measure. Moreover, HDL-C concentration is a static measurement, and does not take into account the dynamics of the HDL particle population and HDL functionality, which might differ depending on the metabolic status of individuals. For instance, patients with type 2 diabetes display a higher catabolic rate of HDL-ApoA-I \[49\] and HDL from coronary patients does not have endothelial anti-inflammatory effects \[50\], illustrating the need to identify more precisely the patient subgroups that should benefit from personalized HDL therapies.

In this context, although there is still an urgent need to better understand the molecular mechanisms underlying multiple regulation downstream HDL action, other therapeutic strategies aiming to improve some critical steps...
Table 1  Summary of high-density lipoprotein therapeutic options, targets and main effects.

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<th>Drugs</th>
<th>Targets</th>
<th>Positive impacts</th>
<th>Negative impacts</th>
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<tr>
<td>Niacin (nicotinic acid)</td>
<td>✚ Inhibition of DGAT-2 (final enzyme of TG synthesis in the liver) [17,18]</td>
<td>✚ Plasmatic TG concentration</td>
<td>✚ Lack of cardiovascular reducing effect in secondary prevention trials (AIM-HIGH and HPS2-THRIVE) [19,20] but a recent meta-analysis involving 11 clinical trials showed a significant reduction in major cardiovascular diseases and coronary heart disease events [21]</td>
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<td></td>
<td>✚ Activation of HCAR-2 (fewer free fatty acids delivered by the adipocyte) [18]</td>
<td>✚ Expression of the β chain of F1-ATPase on the hepatocyte cell surface [17]</td>
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<td>✚ CETP transcription in animals [22]</td>
<td>✚ VCAM-1 and MCP-1 expression (mechanism?) [18]</td>
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<td></td>
<td>✚ Fibrinogen and PAI-1 expression (mechanism?) [23]</td>
<td>✚ CETP transcription in animals [22]</td>
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<td>✚ Activation of HCAR-2 (Langerhans cells and macrophages): prostaglandin D2 and E2 synthesis (activation of their respective receptors in dermal capillaries) [18]</td>
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<td>✚ Antithrombotic</td>
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<td>✚ Skin flushes (dose related, poor compliance)</td>
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<tr>
<td>Inhibitors of CETP</td>
<td>✚ Inhibition of CETP (anacetrapib, dalcetrapib, evacetrapib, torcetrapib)</td>
<td>✚ Plasma HDL-C concentration (anacetrapib and evacetrapib &gt; 130%, torcetrapib ≈ 70%, dalcetrapib ≈ 30%) [24]</td>
<td>✚ For torcetrapib: increased blood pressure and aldosterone production</td>
</tr>
<tr>
<td></td>
<td></td>
<td>✚ Plasma LDL-C concentration [24]</td>
<td>✚ For torcetrapib: increased blood pressure and aldosterone production</td>
</tr>
<tr>
<td></td>
<td></td>
<td>✚ Safety for evacetrapib [25]</td>
<td>✚ For torcetrapib: no significant reduction in cardiovascular adverse events</td>
</tr>
<tr>
<td></td>
<td></td>
<td>✚ Phase III anacetrapib clinical trials ongoing</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>✚ Modification of lipoprotein profile</td>
<td></td>
</tr>
<tr>
<td>Reconstituted HDL</td>
<td>✚ Mimic HDL properties</td>
<td>✚ Decrease in volume of atherosclerotic plaque (apoA-I Milano) [26]</td>
<td></td>
</tr>
<tr>
<td>(liposomes with purified apoA-I or apoA-I Milano)</td>
<td></td>
<td>✚ Second generation products currently being tested in ACS and familial hypercholesterolaemia</td>
<td></td>
</tr>
</tbody>
</table>
**Table 1 (Continued)**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Targets</th>
<th>Positive impacts</th>
<th>Negative impacts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mimetic peptides (octapeptides)</td>
<td>✓ Mimic apoA-I properties</td>
<td>✓ Modification of lipoprotein profile [27]</td>
<td>✓ No modification of HDL inflammatory index [27]</td>
</tr>
<tr>
<td>PPAR α and γ agonists (glitazars)</td>
<td>✓ PPARα activation; + transcription of apoA-I and apoA-II, + transcription of VCAM-1 and fibrinogen [28]</td>
<td>✓ Improvement in lipoprotein profile</td>
<td>✓ Toxicity of first generation drugs</td>
</tr>
<tr>
<td></td>
<td>✓ PPARγ activation of genes involved in glucose homeostasis</td>
<td>✓ Insulin-sensitizing effects</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>✓ Current phase III aleglitazar clinical trial in type 2 diabetes</td>
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</tr>
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<tr>
<td></td>
<td></td>
<td>✓ Current phase III aleglitazar clinical trial in type 2 diabetes</td>
<td></td>
</tr>
<tr>
<td>LXR agonists</td>
<td>✓ LXR α and β; + expression of genes involved in RCT and + expression of genes involved in intestinal cholesterol absorption</td>
<td></td>
<td>✓ Lipogenic effects</td>
</tr>
<tr>
<td>BET bromodomain inhibitors</td>
<td>✓ Epigenetic regulation: stimulation of apoA-I transcription</td>
<td>✓ apoA-I and HDL concentrations [29]</td>
<td>++ Hepatic HDL-C uptake could lead to detrimental accumulation with impairment of their functions</td>
</tr>
<tr>
<td>Modulators of reverse cholesterol transport</td>
<td>✓ Inhibition of SR-BI (BLT-1, ITX-5061, ML278, ML279)</td>
<td>✓ Two phase II studies underway</td>
<td>✓ HDL-C (?) could lead to + in pleiotropic activities of HDL</td>
</tr>
<tr>
<td></td>
<td>✓ Activation of P2Y$_13$ (AR-C69931MX, ct1007900)</td>
<td>✓ Hepatic HDL-C uptake [31]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>✓ RCT [31]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>✓ Atherosclerosis regression in animal models [32]</td>
<td></td>
</tr>
</tbody>
</table>

apo: apolipoprotein; ACS: acute coronary syndrome; ATP: adenosine triphosphate; BET: bromo and extra terminal; CETP: cholesteryl ester transfer protein; DGAT: diacylglycerol acyltransferase; HCAR: hydroxycarboxylic acid receptor; HDL: high-density lipoprotein; HDL-C: high-density lipoprotein cholesterol; LDL: low-density lipoprotein; LDL-C: low-density lipoprotein cholesterol; LXR: liver X receptor; MCP: monocyte chemoattractant protein; P2Y$_13$: purinergic receptor 13; PAI: plasminogen activator inhibitor; PPAR: peroxisome proliferator-activated receptor; RCT: reverse cholesterol transport; SR-BI: scavenger receptor class B type I; TG: triglycerides; VCAM: vascular cell adhesion molecule; VLDL: very-low-density lipoprotein.
of HDL metabolism and HDL pleiotropic beneficial functions (i.e., antioxidant, anti-inflammatory) have emerged. For instance, among recent findings, the newly discovered HDL endocytosis pathway, involving ecto-F1-ATPase and the P2Y13 receptor, could be an interesting target for increasing HDL liver uptake [5,6,31]. Indeed, ct1007900, a P2Y13 receptor agonist, has recently been tested in animal models, demonstrating potential beneficial effects on atherosclerotic plaques [32].

In conclusion, the residual risk persisting under statin treatment means that more research is needed on potential therapeutic approaches and that, despite numerous setbacks, HDL should be targeted by new drugs addressing specific mechanisms.

Disclosure of interest

Jean Ferrières has received grants from and undertaken educational activities for AstraZeneca, MSD Chibret, Novartis and Servier. Meyer Elbaz is involved in the REVEAL study (MSD) and the ACCELERATE study (Lilly). The other authors (Céline Verdier, Laurent O. Martinez, Annelise Genoux, Bertrand Perret) declare that they have no conflicts of interest concerning this article.

References

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[38] Bielicki JK, Oda MN. Apolipoprotein A-I(Milano) and apolipoprotein A-I(Paris) exhibit an antioxidant activity distinct from that of wild-type apolipoprotein A-I. Biochemistry 2002;41:2089–96.


