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Commentary

HDL-cholesterol: Is it really good? Differences between apoA-I and HDL

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ABSTRACT

Since the very first report showing the regression of established atherosclerotic lesions by means of high-density lipoprotein cholesterol (HDL-C) plasma fraction, much information has been generated about the protective role of HDL-C in atherosclerosis. Nonetheless, this positive point of view about HDL has been nearly surpassed since modern informations concerning torcetrapib have appeared. Disappointment was palpable when its pivotal morbidity-and-mortality clinical trial, ILLUMINATE, was abruptly stopped due to excess mortality amongst the group randomized to receive torcetrapib. In this work we will try to put things in perspective.

Lowering low-density lipoprotein cholesterol (LDL-C) levels with statins is a proven strategy for reducing the cardiovascular disease (CVD) risk. Despite the impressive benefits of statins, there remain a significant proportion of treated patients in which cardiovascular events are not prevented. Low HDL-C levels are an important independent risk factor for CVD. There is a need to develop suitable therapies to reduce this residual risk through HDL-C related mechanisms. Therefore, we will first review HDL-C pathways and we will subsequently state the new pharmacological approaches to HDL-C metabolism.

1. Vessel wall lipid deposition in atherosclerosis

Cholesterol accumulation plays a central role in atherogenesis. Low-density LDL-C penetrates the vessel wall following endothelial dysfunction (the early phenomenon in atherosclerosis). It binds to the proteoglycans of the subendothelial space, where it undergoes an oxidative process. Oxidized cholesterol is highly toxic, and as part of a mechanism of defense, it is phagocytosed by the vessel wall macrophages. The presence of the oxidized lipids triggers a series of proinflammatory reactions via different mediators, perpetuating the activation and recruitment of monocytes-macrophages and inflammatory cells. Macrophages, by engulfing the lipid material, become foam cells. Failure of macrophages to remove cholesterol from the vessel wall promotes its apoptotic death, releasing cholesterol to the vessel wall and, more importantly, inflammatory substances like tissue factor and metalloproteases (enzymes able to digest the matrix scaffold), making atherosclerotic lesions more prone to rupture (the so-called vulnerable plaque). Secondary changes may occur in the underlying media and adventitia, particu-

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larly in advanced disease stages. Lesions progress to fibroatheroma by developing a cap of smooth muscle cells and collagen.

Therefore, the excess of vessel wall unesterified cholesterol is the pivotal phenomenon in atherosclerosis. Glomset coined the term ''reverse cholesterol transport'' (RCT) in 1968 [4] to describe the process by which peripheral cholesterol is returned to the liver for excretion in the bile and ultimately the feces. RCT is believed to be one of the main explanations in HDL-C atheroprotective effect.

2. Metabolism of HDL-C

HDL is a class of heterogeneous lipoproteins containing approximately equal amounts of lipid and protein. HDL particles are characterized by high density (>1.063 g/mL) and small size (5–17 nm). When separated by agarose gel electrophoresis, HDLs exhibit either α, pre-β, or γ migration. α-Migrating HDLs are mature, spherical particles that account for the major proportion of HDLs in plasma; a minor subpopulation of α-HDLs consists of large, spherical particles containing apoE and phospholipids (PL). Pre-β HDLs represent either discoidal particle consisting of apolipoprotein A-I (apoA-I) complexed with PL and free cholesterol (FC) or monomolecular, lipid-poor apoA-I.

Most of apoA-I, the predominant HDL protein, migrates in agarose gels with α-electrophoretic mobility and is designated α-LpA-I. This fraction accounts for almost all of the cholesterol quantified in the clinical laboratory as HDL-C. α-HDL can be further fractionated by density using ultracentrifugation into two major subfractions, HDL2 ($1.063 < d < 1.125$ g/mL) and HDL3 ($1.125 < d < 1.21$ g/mL. Approximately 5–15% of apoA-I in human plasma is associated with particles with pre-β-electrophoretic mobility [5].

These different properties of different types of molecules all named ''HDL-cholesterol'' highlights the fact that not all HDL-particles are equal, and their function (and therefore their role in atherosclerosis) is different from each other.

Fig. 1 shows a schematic view of the HDL metabolism and fate.

3. Synthesis of HDL-C

apoA-I is present on the majority of HDL particles and constitutes about 70% of the apolipoprotein content of HDL particles; thus, plasma apoA-I concentrations correlate closely with plasma HDL-C. apoA-II is the second most abundant apolipoprotein of HDL, but its physiologic role has not yet been fully defined; anyway, both apolipoproteins are required for normal HDL biosynthesis. HDL also contains a variety of other proteins, including apoA-IV, apoC-I, apoC-II, apoC-III, apoD, apoE, apoJ, apoL-I, apoM, serum amyloid A proteins, ceruloplasmin, transferrin, and enzymes such as LCAT, PON1, and PAF-AH/Lp-PLA2 [6].

As expected, gene deletion of apoA-I results in extremely low levels of HDL-C in mice [7]. Atherosclerosis-prone mice lacking apoA-I develop significantly increased atherosclerosis [8]. Hepatic overexpression of apoA-I significantly raises HDL-C levels and inhibits the progression of and even regresses atherosclerosis in mice [9,10]. Thus, upregulation of endogenous apoA-I expression is widely considered one of the most promising approaches in HDL-related therapies. However, in vivo studies of HDL metabolism in human populations have shown that clearance of apoA-I, rather than its production

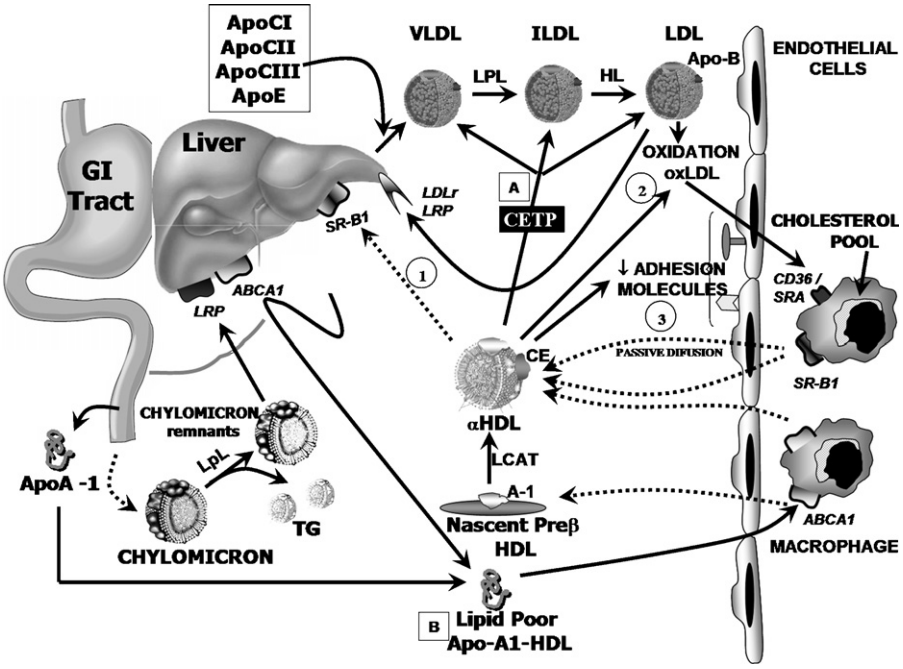

Fig. 1 – Schematic view of cholesterol metabolism and reverse cholesterol transport. See text for further details. Taken from Choi et al. [3].

rate, is the most important determinant of plasma HDL-C and apoA-I concentrations [11].

apoA-II constitutes approximately 20% of HDL protein, is present in two-thirds of HDL particles in humans and is synthesized only in the liver [12]. Gene deletion of apoA-II in mice markedly reduces HDL-C levels, suggesting that apoA-II is also required for normal HDL metabolism. apoA-II plasmatic levels are mainly determined by synthesis rather than by its catabolic rate [13].

The liver [14] and the intestine [15] have been known for 30 years to be both capable of synthesizing and secreting apoA-I, but the relative contribution of both organs was just recently elucidated. In 1999, the molecular basis of Tangier disease was found to be loss-of-function mutations in both alleles for the gene encoding the ATP binding cassette transporter A1 (ABCA1) [16–18]. Tangier disease causes virtually undetectable levels of HDL-C and very low levels of apoA-I that are confined to pre-β-HDL, as well as cholesterol accumulation in peripheral macrophage-enriched tissues. ABCA1 is a ubiquitously expressed cellular lipid transport protein that promotes efflux of PL and FC from cells to lipid-poor apoA-I (pre-β-HDL). ABCA1 is required for normal "lipidation" of lipid-poor apoA-I, and in its functional absence, apoA-I is rapidly catabolized and does not reach maturity [19]. Since then, using liver- or intestine-specific ABCA1 knockout (KO) mice, we know that the liver synthesize 70–80% of apoA-I [20] while the intestine is responsible for 30% of apoA-I [21], both tissues being also primarily responsible of the lipidation of newly secreted lipid-poor apoA-I via ABCA1. This nascent HDL-C (also called lipid-poor apoA-I, pre-β mobility) generally contains 2 copies of apoA-I per particle and lipids account (FC and PL) for less than a 10% of its mass [22].

4. Acquisition of cholesterol by nascent HDL-C

The initial lipidation of nascent HDL-C can occur through a different number of mechanisms [23], which results in the formation of discoidal pre-β-HDL particles.

4.1. *Aqueous diffusion*

Efflux of free cholesterol via aqueous diffusion occurs with all cell types but is inefficient. This passive mechanism involves a simple diffusion process, the flux of cholesterol being bidirectional, with the direction of net flux driven by cholesterol gradient. It is a long-duration process (it occurs in the time scale of hours).

4.2. *ABCA1-mediated FC efflux*

In contrast to aqueous diffusion FC flux, the movement of FC by ABCA1 is unidirectional, occurring only from cells to lipid-poor apolipoproteins.

Cholesterol-enriched fibroblasts and macrophages from patients with Tangier disease lacked the ability to release both phospholipid and FC to lipid-free lipolipoproteins but that efflux to mature HDL was normal [24]. ABCA1 also mediates the lipidation of apoA-I and the formation of nascent HDL (as seen previously).

ABCA1 is expected to serve an antiatherogenic function. In humans, there is an association between increased arterial wall thickness and impaired ABCA1-mediated FC efflux [25]. ABCA1 knockout mice have an extremely low HDL-C phenotype similar to that of humans with Tangier disease [26]. Macrophage-specific deficiency of ABCA1 caused a minimal reduction of HDL-C levels in mice, but it resulted in significantly increased atherosclerosis [27,28]. ABCA1 overexpression in liver increased HDL-C levels [29] and in macrophages and liver was associated with protection against atherosclerosis [30,31].

4.3. *SR-B1*

Similar to aqueous diffusion but in clear contrast to ABCA1-mechanism, SR-BI-mediated efflux occur to PL-containing acceptors (HDL-C and lipidated apolipoproteins), and the flux of cholesterol is bidirectional (direction of net flux depending on cholesterol gradient). SR-BI, a member of the CD36 family, also mediates the selective uptake of other lipoprotein lipids, including cholesteryl esters (CE), PL and triglycerides (TG), thus promoting depletion of HDL-C core lipids [32].

SR-BI-deficient mice fed a Western diet have increased lipid deposition and atherosclerosis in the aorta [33]. Furthermore, in mice, SR-BI deficiency on the background of apoE deficiency results in increased early atherosclerosis [34] and markedly accelerated atherosclerosis and mortality [35] and on the background of LDL receptor deficiency results in increased atherosclerosis [36]. Moreover, bone marrow transplantation from SR-BI-deficient mice into LDL receptor-deficient or apoE-deficient [37] mice results in increased atherosclerosis, consistent with a protective role of macrophage SR-BI.

4.4. *ABCG1/ABCG4*

Recently, ABCG1 was identified as promoting an alternative cholesterol efflux pathway from macrophages [38,39] to mature HDL, not to lipid-poor apoA1. In contrast to ABCA1, ABCG1 promotes macrophage efflux to mature HDL particles, which represent a much larger proportion of plasmatic HDL-C and apoA-I than the small pool of lipid-poor apoA-I. ABCG1-KO mice demonstrate macrophage lipid accumulation, and their macrophages have impaired cholesterol efflux to mature HDL [39]. The importance of ABCG1 in humans remains to be determined.

5. Maturation and remodelling of HDL-C

These nascent HDL particles then suffer an intravascular process of maturation and remodelling [40] through several enzymatic reactions.

5.1. *Lecithin-cholesterol acyltransferase (LCAT)*

Cells efflux unesterified FC, which associates with HDL in the plasma. LCAT catalyzes the transfer of 2-acyl groups from lecithin to FC, generating CE and lysolecithin [4]. CE is more hydrophobic than FC and moves to the core of the lipoprotein particle, allowing the formation of the larger mature HDL particle [41].

LCAT is critical for normal HDL metabolism, because its absence results in the inability to generate mature HDL particles with normal CE cores. LCAT-deficient mice and humans [42] have extremely low HDL-C and apoA-I levels due to rapid catabolism.

LCAT overexpression in rabbits resulted in increased HDL-C levels and reduced atherosclerosis [43]. However, in mice data are conflicting because its effect on atherosclerosis is still not clear [44,45], due to the absence of CETP in mice. LCAT-heterozygous subjects (fish-eye disease) seem to have increased carotid intima-media thickness compared with family controls [46] suggesting that a reduction in LCAT activity in humans may be proatherogenic. Notwithstanding, more studies are required to determine its effect.

5.2. *Cholesteryl ester transfer protein (CETP)*

CETP is a hydrophobic glycoprotein made by liver and adipose that circulates in the plasma bound to lipoproteins. CETP promotes the transfer of CE from HDL to apoB-containing lipopoteins (VLDL, chylomicrons, LDL) in exchange for TG and the transfer of TG from VLDL and LDL to HDL in exchange for CE [47,48], thus resulting in the migration of CE back into the LDL fraction. RCT is completed by transfer of CE back to the liver via the LDL-R. The net effect of CETP action on HDL is depletion of CE and enrichment with TG, with an overall net reduction in the size of the HDL particle. CETP mass in plasma is not the rate-limiting factor that determines CE distribution between the slowly catabolized LDL and HDL, whereas it is rate limiting in TG transfer between HDL and the rapidly metabolized VLDL.

5.3. *Phospholipid transfer protein (PLTP)*

PLTP transfers surface PL from VLDL and chylomicrons to HDL during TG lipolysis and accounts for most of the PL transfer in human plasma. PLTP remodels HDL particles into larger HDL particles by particle fusion, subsequently releasing lipid-poor apoA-I. Targeted disruption of PLTP in mice results in 60% reduction in HDL and apoA-I levels [49] because of enhanced clearance of HDL particles. Human PLTP transgenic mice have increased levels of pre-β1 HDL, apoA-I and PL [50]. The role of PLTP in human pathophysiology has yet to be elucidated.

5.4. *Lipoprotein lipase (LPL)*

LPL is secreted by many tissues of the body, particularly metabolically active adipose tissue and muscle. It is bound in the luminal surface of endothelial cells as homodimers to heparan sulfate proteoglycans and can be released by administration of heparin. LPL has predominantly TG lipase activity with minor phospholipase activity. It is the principal enzyme responsible for the hydrolysis of TG, transforming large TG-rich particles (VLDL, chylomicrons) into smaller TG-depleted remnant lipoproteins. Redundant surface lipid (FC, PL) and apolipoproteins are subsequently transferred from chylomicrons to HDL, increasing HDL-C and apoA-I plasmatic levels.

Plasma HDL-C concentrations correlate with postheparin plasma LPL activity [6]. Pharmacological upregulation of LPL elevates HDL [51] whereas LPL-KO mice [52] or antibody-mediated inhibition of LPL in monkeys [53] results in low HDL-C levels through an increase in HDL-C catabolic rate.

5.5. *Hepatic lipase (HL)*

Hepatic lipase (HL) is a enzyme synthesized by hepatocytes with both TG lipase and phospholipase A1 activity [54]. HL has greater activity against HDL than VLDL or chylomicrons and converts large HDL particles into smaller HDL remnants and lipid-poor apoA-I. HL is most effective in hydrolyzing HDL if the HDL is TG enriched; hypertriglyceridemic conditions (such as insulin-resistant states), HL increases apoA-I catabolism and decreases HDL levels [6].

Mice with targeted disruption of the HL gene have a mild elevation of plasma HDL, more marked if they are fed a cholesterol-rich diet [54]. Overexpression of HL in mice and rabbits results in marked reductions in HDL-C and reductions in HDL size [55]. Inhibition of HL is expected to slow apoA-I catabolism and increase apoA-I and HDL-C levels.

5.6. *Endothelial lipase (EL)*

EL is a recently described member of the TG lipase gene family that hydrolyzes HDL PL. Although it shows considerable homology with both LPL, EL appears to have relatively more phospholipase activity than TG lipase activity and appears to have a greater preference for HDL over apoB-containing lipoproteins.

EL overexpression in mice reduces HDL-C and apoA-I levels [56] due to increased renal catabolism. Conversely, gene deletion [57] of EL results in increased HDL-C and apoA-I levels. Human mutations in EL gene seem to be associated to subjects with high HDL-C levels [58]. Therefore, inhibition of EL seems an attractive therapeutic approach in atherosclerosis.

5.7. *Secretory phospholipase A2 (sPLA2)*

The sPLA2 family consists of low-molecular-weight secreted phospholipases exhibiting sn-2 phospholipase activity, with the ability to hydrolyze HDL PL. Transgenic overexpression of sPLA2 group IIA in mice results in reduced levels of HDL-C, reduction in HDL size and faster HDL catabolism [59].

6. HDL catabolism

Clearance of apoA-I is most important in determining HDL-C and apoA-I levels than production rate. Kidney, liver, and steroidogenic tissues are major sites of HDL catabolism. Studies in animals [60] established that one-third of apoA-I is catabolized by the kidneys, and the rest is catabolized by the liver. Clearance of HDL may happen in two ways.

6.1. *Holoparticle*

HDL and apoA-I endocytosis and lysosomal degradation of the whole particle (including apoA-I) are known to occur in

both liver and kidney. Lipid-poor apoA-I is filtered at the glomerulus and then catabolized by in the apical part of proximal renal tubular epithelial cells via the cubilin/megalin [61].

The rate-limiting step in the apoA-I renal catabolism is glomerular filtration rather than tubular catabolism. Mature HDL seems to be too large to be filtered: both Tangier disease and LCAT deficiency causes poorly lipidated apoA-I that is rapidly catabolized not being large enough to escape glomerular filtration.

6.2. *Selective cholesterol uptaken (selective removal of cholesterol and other lipids from the particle, without uptake of the whole particle)*

The best understood mechanism of HDL-C liver uptake is mediated through hepatic SR-BI [62]. SR-BI is also highly expressed in adrenal gland and ovary. It is capable of mediating selective uptake of other lipids, with the highest uptake constants for CE and FC and lower uptake constants for PL and TG.

Overexpression of SR-BI reduces HDL-C and apoA-I plasma concentrations [63] because of accelerated clearance. Hepatic SR-BI expression in mice [64] resulted in increased macrophage RCT (despite reduced plasma HDL-C levels); SR-BI deficiency was associated with markedly increased atherosclerosis and reduced RCT despite increased HDL-C levels [35]. It seems to exist an inverse relation of hepatic SR-BI expression to atherosclerosis [33–35].

HDL-derived cholesterol is suggested to be more directly shunted toward the bile than other pools of hepatic cholesterol [65]. HDL-UC can be directly excreted into the bile or converted to bile acids (the rate-limiting enzyme for bile acid synthesis is 7α-hydroxylase) before biliary excretion [22]. Two ATP-binding cassette (ABC) transporters, ABCG5 and ABCG8 are half-transporters that work together as heterodimers at the apical membranes of hepatocytes and have been proved to limit sterol absorption and to promote biliary sterol excretion in humans. Transgenic mice overexpression of ABCG5 and ABCG8 promotes biliary cholesterol secretion [66]. Conversely, genetic deficiency of ABCG5 or ABCG8 causes sitosterolemia [67], characterized by reduced biliary sterol excretion and elevated plasma and tissue cholesterol and plant sterol levels. Biliary and intestinal receptors are revised in an excellent article [68].

There is avid bile reabsorption in the terminal ileum through the intestinal bile acid transporter (50–80% of biliary cholesterol is reabsorbed). Intestinal epithelial cells express ABCG5 and ABCG8, so this reabsorbed cholesterol can be again reexcreted into the intestinal lumen.

7. Other atheroprotective effects of HDL-C beyond RCT

HDL has other atheroprotective effects beyond RCT (antioxidant, anti-inflammatory, antithrombotic compound and it improvement in endothelial function) which escape the scope of this revision but are clearly explained in other works [7,69].

8. New strategies for HDL-C increase

Since the early experimental demonstrations of the beneficial role of HDL in atherosclerosis [1,70,71], HDL remains as a tantalizing therapeutical target. The National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III guidelines [72] recognize low HDL-C (<40 mg/dL) as one of the 5 major CVD risk factors, component of the metabolic syndrome, component of the Framingham scoring system and as a potential target for therapeutic intervention. Furthermore, an increasing number of experts believe that low HDL-C may warrant treatment in a wider range of patients. Therefore, new HDL-related therapies are under development.

9. Liver receptor X (LXR)

LXRα and LXRβ were recently cloned based on sequence homology with other receptors. Originally considered ''orphan'' nuclear receptors, we know nowadays their natural ligands are oxysterols. LXRα is highly expressed in the liver and at lower levels in adrenal glands, intestine, adipose, macrophages, lung, and kidney, whereas LXRβ is ubiquitously expressed (LXRα the dominant isoform in liver). LXRs are ligand-dependent transcription factors that form permissive heterodimers with the retinoid X receptor (RXR); i.e., the complex can be activated by ligands of either partner. LXRs reside within the nucleus while bound to corepressors; when ligands bind to LXR, a conformational change happens and the LXR/RXR heterodimers are able to bind to LXR-responsive elements (LXREs) in DNA [73].

LXR has an overall atheroprotective effect [74,75]. LXR agonists increase HDL levels and net cholesterol secretion by enhancing expression of ABCA1, ABCG1, ABCG5/ABCG8 and 7α-hydroxylase gene (the rate-limiting enzyme in bile acid synthesis). They also improve glucose tolerance in animal models (by decreasing hepatic glucose output, repressing hepatic gluconeogenesis, increasing expression of glucose transport GLUT4 and enhancing glucose-dependent insulin secretion). Both LXR isoform possess anti-apoptotic and anti-inflammatory mechanisms (decrease in iNOS, COX-2, IL-6, IL-1β, MMP9 and MCP-1, through NF-KB inhibition).

Macrophage-specific loss of LXRs resulted in a marked increase in lesion size [76]. Synthetic LXR agonist treatment inhibited atherosclerosis progression [73,77], and even regressed atherosclerosis in both LDL-R and apoE KO mice [78]. Enthusiasm has been tempered by the development of hypertriglyceridemia and fatty liver, partly due to LXR-mediated upregulation of hepatic SREBP-1c [74] that elevates triglyceride levels in the liver. There are two alternative strategies for sidetracking this adverse effect, either LXR modulators that are relatively selective for specific tissues (macrophage over liver) or developing isoform-specific LXRs ligands (since LXRβ may induce RCT without the hepatic complications which are attributed to LXRα). One selective LXR agonist has been reported to induce less hepatic steatosis [79].

10. Cholesteryl ester transfer protein (CETP) inhibitors

Mutations in the CETP gene, causing CETP protein deficiency, were demonstrated to be the genetic base of the high HDL-C levels in Japanese individuals with familial hyperalphalipoproteinaemia [80,81]. These mutation are relatively frequent (up to 7% of the general population of Japan, up to 27% of people in the Omagari region), and they result in both no measurable CETP activity and markedly raised HDL-C levels (up to 165 + 39 mg/dL) because of accumulation of CEs in the HDL fraction, with associated increases in apoA-I and apoA-II due to reduced catabolic rates [82].

However, epidemiological studies did not tell a compelling story. A study population in Omagiri City found those living past the age of 80 were less likely to have CETP deficiency, and CETP deficiency was associated with increased CHD [83]. The Honolulu Heart Study showed that men of Japanese descent who were heterozygous for these mutations had a 50% increased risk of CHD compared with men with similar HDL levels but without the mutation [84].

Rabbit atherosclerosis models suggested an antiatherosclerotic effect [85]. JTT-705 was the first CETP inhibitor to be tested, showing an increase in HDL-C up to 34% [86]. Torcetrapib, the next CETP inhibitor, showed promising results in phase I [87] studies. In fact, a phase II, single-blind study in 19 subjects [90] showed HDL-C increases of 46% (torcetrapib 120 mg/day) up to 106% (twice-daily torcetrapib 120 mg/day).

Despite these impressive effects, results from recent clinical trials have proscribed this drug from the therapeutic armamentarium. ILLUMINATE trial [2] demonstrated an increased risk of cardiovascular events and death from any cause in the torcetrapib arm. Moreover, there was also excess congestive heart failure, revascularization procedures, an increase of 5.4 mmHg in systolic blood pressure, a decrease in serum potassium, and increases in serum sodium, bicarbonate, and aldosterone. As a result all torcetrapib clinical trials were halted.

Imaging studies have obtained congruent results (lack of atherosclerosis regression under torcetrapib). ILLUSTRATE trial showed no difference on the progression of coronary atherosclerosis (using IVUS) in 1,188 patients with CAD [88] between torcetrapib and torcetrapib + atorvastatin. RADIANCE I trial [89] confirmed there was no effect of torcetrapib treatment on carotid intima-media thickness in 850 patients with heterozygous familial hypercholesterolemi. RADIANCE II [90] was a randomized double-blind trial which studied the effect of torcetrapib on carotid atherosclerosis progression in 683 patients with mixed dyslipidaemia; primary endopoint was neutral at 24 months and secondary endpoints were against torcetrapib ($p = 0.46$) despite the impressive increases in HDL-C.

Much has been discussed about these disappointing results. The particular concern is if the deletereous effect of torcetrapib resides in its intrinsic properties (failure of the molecule) or the whole concept of CETP inhibition is erroneous (failure of the mechanism).

10.1. *Failure of the mechanism hypothesis*

Unfortunately, not only does CETP inhibition induces quantitative changes in lipidic profile, but it also seems to cause qualitative changes in HDL particles, altering their functionality. CETP protein deficiency appears to favour accumulation of larger, CE-rich HDL particles, which have less ability to promote cholesterol efflux from macrophages [91] than apoA1-lipid-poor HDL. Most of the HDL CE ultimately excreted in the bile was transferred from HDL to apoB-containing lipoproteins before uptake by the liver [92], which implies inhibiting CETP blocks CE excretion into the bile. Thus, its major failure was likely due to producing HDL-C elevation without RCT augmentation. HDL-C produced by CETP inhibitors may be dysfunctional.

10.2. *Failure of the molecule hypothesis*

This hypothesis defends the toxic effect lies on torcetrapib alone, not in the approach of increasing HDL-C levels through CETP inhibitors. In the ILLUSTRATE trial, torcetrapib was associated with an increase of 4.6 mmHg in mean systolic blood pressure; moreover, about 4% of subjects experienced blood pressure elevations over 15 mmHg. The hypertensive effect of torcetrapib is probably not related to the drug's mechanism, since such an effect is not seen in patients with a genetic CETP deficiency; in addition, other classes of CETP inhibitors do not cause this side effect. Although the increase in blood pressure seems modest, it may be indicative of an underlying adverse effect of torcetrapib, such as activation of the renin–angiotensin system. In fact, since torcetrapib decreases in serum potassium, and increases serum sodium, bicarbonate, and aldosterone, it has been hypothesised it may have intrinsecal off-target adrenal toxicity, although further analyses are needed to interpret this relation. This cornerstone point must be elucidated because, if true, would allow the development of other CETP inhibitors; anacetrapib, another CETP inhibitor, has been found to increase HDL-C without affecting blood pressure [93]. However, prudence is necessary because torcetrapib and anacetrapib are in the same structural class, and because the effect of torcetrapib on systolic blood pressure was only found in large long-term studies.

11. apoA-I_{Milano}

In 1985 Sirtori and Franceschini studied a small group of people in the little city of Limone sul Garda, near Lake Como, Italy, who shared a common lipidic profile consisting in very low HDL-C and apoA-I levels, elevated TG and surprisingly low cardiovascular event risk [94,95]. They were identified to be carriers of a mutation characterized by an arginine-173 to cysteine-173 substitution leading to the formation of homodimers and heterodimers with wild type apoA-II in the carriers. All these individuals seem to be descendant of a couple traced through church records to 1780 (Giovanni Pomaroli and Rosa Giovanelli) and are heterozygotes for the mutant allele. These low rates of cardiovascular events have

suggested the theory of apoA-I_{Milano} having improved functionality compared with the wild type apoA-I [96].

Repeated administration of the recombinant apolipoprotein A-I_{Milano}/PL substantially reduces ileofemoral atherosclerosis in cholesterol-fed rabbits subjected to balloon injury [97]. At high doses, it promotes the regression of aortic atheromatosis in cholesterol-fed apolipoprotein E-null mice, despite severe hypercholesterolemia [98], decreasing lipid and macrophage content, thus promoting a more stable plaque-phenotype. Furthermore, it reduces the proapoptotic effect of oxysterols on vascular smooth muscle cells, promoting a more stable plaque [99], reduces neointimal thickening because of its anti-inflammatory effects [100], improves endothelial function and was cardioprotective in a model of left anterior descending artery ischemia and reperfusion [101].

These findings have been replicated in humans. apoA-I_{Milano} administered intravenously in weekly injections for 5 weeks in acute coronary syndrome patients produced significant regression of atherosclerosis (-4,2% from baseline) as measured by IVUS [102]; although promising, these data require confirmation in clinical trials with ''hard'' endpoints (morbimortality).

Our group has replicated these interesting findings, adding some new explanations [103]. In a rabbit model of atherosclerosis, apoA-I_{Milano} treatment caused plaque regression (5% plaque volume regression, as assessed by MRI) and strong signs of plaque stabilization: reduction in plaque macrophage density and a significant down-regulation in gene–protein expression of tissue factor, MCP-1, and COX-2, as well as marked decrease in gelatinolytic activity and significant upregulation of COX-1.

12. Conclusions

HDL as a therapeutic target for atherosclerotic disease seems a plausible and attractive strategy towards treating CVD. However, it is critical to understand that not all HDL-C particles are equal. The fate of the lipid-poor nascent HDL/apoA-I is to remove cholesterol from extrahepatic tissues and bring it back to the liver, while the effects of the end-stage, lipid full spherical HDL particle are not completely understood. Equally important is the fact that raising HDL by different mechanism will not have the same effects. The recent failure of the CETP inhibitor torcetrapib should not be taken as a failure of HDL-raising therapy for cardiovascular disease. Rather it should be taken as proof that the quality of the HDL raised and not the quantity will render the beneficial effects.

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