CETP Pharmacological Manipulation Transforming Human into Mouse: Is this Still a Viable Hypothesis?

Enrique Morales-Villegas*1, Gualberto Moreno-Virgen1 and Philip J. Barter

1Centro de Investigación Cardiometabólica de Aguascalientes SA de CV-W Hospital MAC-CMQ, Aguascalientes, México; 2Heart Research Institute, Sydney, Australia

Abstract: Manipulating the action of the Cholesteryl Ester Transfer Protein -CETP- and transforming the human lipid phenotype into one resembling the mouse lipid phenotype, in order to reduce susceptibility to atherosclerosis, is a hypothesis based on at least three lines of scientific evidence summarized within the introduction of this article. The following aspects related to the pharmacological manipulation of the CETP are discussed within the present article: a) CETP as a controversial protein involved in heterotypic and homotypic transport of neutral lipids between different lipoproteins; b) CETP as a protein involved in atherogenic dyslipidemia associated with the insulin resistance syndrome and c) pharmacological manipulation of the CETP by using "second generation" drugs -dalcetrapib, anacetrapib and evacetrapib- focusing on the results of Phase IIb and Phase III studies published up to May 2012. The article concludes with a review of the strengths, weaknesses, opportunities and current controversy on the HDL-centric versus LDL-centric theories.

Keywords: Anacetrapib CETP, CETP, cholesterol efflux, dalcetrapib, evacetrapib, HDL-C, inhibition, LDL-C, torcetrapib, RCT.

INTRODUCTION: THE THREE LEVELS OF EVIDENCE

The mouse is an animal species that lacks CETP activity, and is characterized by high levels of apo-AI and HDL-C, with reversal of the relationship between LDL-C and a ratio LDL-C/HDL-C <1 as well as is characterized by resistance to the development of atherosclerosis [1]. This seminal observation led to the hypothesis of CETP inhibition as an antiatherosclerosis strategy [2].

The native phenotype of the mouse is modified by manipulating its genome with the "inclusion" of the CETP gene from primates or humans. The modified genotype induces CETP expression in mice, with decreased levels of apo-AI and HDL-C, LDL-C/HDL-C ratio >1, and susceptibility to the development of atherosclerosis, especially when a high-fat diet is administered [3]. This phenomenon is associated with the HDL-dependent inhibition of the RCT via SR-B1, the main route of RCT in the mouse, and with an increased transfer of cholesteryl esters from HDL to apoB100-containing lipoproteins, potentially atherogenic; both processes mediated by CETP [4-5].

Unlike the mouse, the rabbit is a species exhibiting CETP overexpression and a high susceptibility to develop atherosclerosis. In this species, inhibition of CETP by "anti-sense" oligonucleotides [6], monoclonal antibodies [7-8], and CETP-inhibitors [9], consistently increase the levels of apoAI and HDL-C, and provides atherogenic protection [6-9].

Humans contain the CETP gene located at 16q12-q21 in its genome. In our species, CETP expression is inversely associated to the amount of HDL; the higher the expression of CETP, the lower the amount of HDL and vice versa [2]. In the era of human genome analysis, several mutations -polymorphisms- in the CETP gene have been identified; such polymorphisms are closely associated with a wide range in protein expression, from decline to absence in their concentration and activity, with variable levels of apoAI and HDL. In the classic Honolulu Heart Study, Zhong reported that genetic CETP deficiency associated with the D442G mutation had a cardioprotective effect only when the level of HDL was between 60mg/dl and 80mg/dl; this finding has been called the U curve [10]. Subsequently, several analyzes have examined the association between CETP activity and the incidence of cardiovascular diseases [11-19]. Results of these analyzes have been controversial. Neutral, positive and negative associations have been reported; however, a relationship between a low activity of the CETP and a lower incidence of cardiovascular events, in subpopulations of subjects with hypertriglyceridemia, stands out as a constant [15, 17].

Together, the three types of evidence referred to, keep in force the therapeutic hypothesis that CETP pharmacological manipulation is a potential strategy to reduce cardiovascular events associated with atherosclerosis. Therefore, once it is confirmed that the negative results with the CETP inhibitor torcetrapib are related to effects unassociated to the inhibition of the CETP -aldosterone and cortisol secretion with L-type calcium channels activation- [20, 21], this hypothesis is currently the subject of an extensive research in Phase II and Phase III trials. These trials include over 50,000 subjects with cardiovascular disease by atherosclerosis and/or subjects at a high risk for its development, and some results have already been published.
This review represents an update on the results already published of dalcetrapib, program canceled for lack of efficacy on May 7, 2012 -see below-, anacetrapib and evacetrapib, and on the status of ongoing studies. All trials explore the feasibility of a strategy aimed, with a pharmacological approach, to simulate the phenotype of the mouse in humans, and thereby reduce the residual cardiovascular risk in the presence of an optimal LDL-lowering therapy.

**CETP: A CONTROVERSIAL PROTEIN**

CETP is a hydrophobic protein, encoded by the gene located at the 16q12-q21 position with 16 exons. This gene is expressed mainly but not exclusively in hepatocytes [22, 23]. The CETP is included among the lipid-transfer proteins and is part of the proteome of HDL lipoproteins, especially preβ-HDL and HDL-3, in a ratio of 1 CETP molecule per 500 HDL molecules. The three-dimensional structure of the CETP is formed by alpha-helices and beta-sheets forming a "hydrophobic tunnel" which transfers neutral or hydrophobic lipids -cholesterol esters and triglycerides- between various lipoproteins [24].

CETP is usually described as a protein facilitating the exchange of cholesterol esters and triglycerides between HDL and LDL-VLDL [25, 26]. It has been proposed that this mechanism originally described by Schwartz in 1981 [27], aims to accelerate, by means of an "indirect route", the reverse cholesterol transport. In other words, CETP favors the elimination of this lipid via LDL-R by transferring cholesterol esters from HDL to LDL. Since the LDL half-life is shorter than the HDL half-life, this elimination route of cholesterol esters is faster than elimination via the hepatic SR-B1 and/or LRP-1. This transfer mechanism now is called heterotypic transfer of cholesteryl ester [24]. It should be noted that, although this mechanism represents an indirect route of RCT, and therefore is potentially anti-atherogenic, cholesteryl ester transfer to apoB100-containing lipoproteins, represents potentially and paradoxically a pro-atherogenic mechanism, especially in dyslipidemia with high levels of TRL -see below- (Fig. 1).

More recently, it has been described that CETP also has the function of transferring cholesteryl ester among different HDL subtypes. By the action of CETP, the HDL-3c transfers cholesteryl ester towards the HDL-2b [28, 29]. This mechanism facilitates two potentially anti-atherogenic processes: first, the "maturation" by the anterograde conversion of HDL-3c into HDL-2b, and secondly, the "recycling" by the retrograde conversion of HDL-3 into preβ-HDL. With equal HDL levels, the increase in preβ-HDL fraction is the variable with greater association to the increase in the efflux of cholesterol from the macrophage to HDL [30, 31]. This fact is explained by the high affinity of this lipoprotein for the transporter ABC-A1. This mechanism of cholesteryl ester transfer among HDL subtypes is called homotypic cholesteryl transfer, and is considered potentially anti-atherogenic because it provides a cycle that, in an anterograde manner, leads to maturation of the HDL and, in a retrograde manner, increases the formation of preβ-HDL, the major cholesteryl ester mobilizing fraction of the macrophage towards the HDL (Fig. 2).

**CETP: PHARMACOLOGICAL MANIPULATION**

**Heterotypic and Homotypic Manipulation**

Blockade of heterotypic cholesterol transfer with drugs such as torcetrapib -IC50 14±0.7-, anacetrapib -IC50 13±1.4nmol/l- or CP800569 results in: a) reduction of cholesteryl ester-rich VLDL/LDL; b) increase in the anterograde

---

Fig. (1). Reverse cholesterol metabolism involving heterotypic cholesterol transport between HDL2 and LDL. It is noted that while the transfer of cholesterol from HDL to LDL "accelerates" the RCT via LDLR, the potentially atherogenic LDL "pool" is also increased.
conversion of HDL-3c into HDL-2b, c) inhibition of the “indirect route” of RCT via LDL-R. The first effect without controversy is favorable since it reduces the lipoprotein “pool” with apoB100, potentially atherogenic [32-38]; the second effect, also observed in subjects with genetic CETP deficiency, determines a significant increase in HDL-2, substrate for the remodeling action of the PLTP, and the formation of apoE-rich and apoAI deficient HDL, whose functionality to remove and eliminate cholesterol, is still controversial [39-43]; the third effect has not been studied well enough, according to some authors, inhibition of the “indirect route” of RCT could have a pro-atherogenic effect [44, 45]. In turn, blockade of the homotypic cholesterol transfer results in: a) reduction in the retrograde conversion of HDL-3 into preβ-HDL with a potential negative effect on the RCT via SR-B1, a phenomenon demonstrated by Niesor in vivo, in a hamster model [46, 47] (Fig. 3).

**Pure Heterotypic Manipulation**

Blockade of heterotypic transfer without affecting homotypic transfer with drugs such as dalcetrapib results in: a)
reduction of cholesterol ester-rich VLDL/LDL, b) conservation of the retrograde conversion of HDL-3 into pre-β-HDL and the anterograde maturation of HDL-3 into HDL-2; c) inhibition of the "indirect route" of RCT via LDL-R. As previously discussed, the first effect, without controversy is favorable [32-38]; the second effect could be convenient, especially because it determines the formation of pre-β-HDL, as confirmed by the increased expression of the pre-β-HDL band in several studies, including studies with dalcetrapib, but not with anti-CETP antibodies or with inhibitors such as torcetrapib or anacetrapib [46]; this mechanism may explain the aforementioned increase in the efflux of cholesteryl ester from macrophage to HDL and stools [47-49]; the third effect could be offset by the increase referred to on the RCT via SR-B1 [46, 47] (Fig. 4).

Thus, besides demonstrating the efficiency - efficacy, safety, tolerance and reduction of cardiovascular events- of the CETP manipulation, research with anacetrapib, evacetrapib and dalcetrapib, will enable us to better define which transfer route contributes the most to the potential cardioprotective effect of the CETP manipulation.

**CETP: ROLL IN MIXED DYSLIPIDEMIA**

In the presence of insulin resistance, the incidence of mixed dyslipidemia -total cholesterol >200mg/dl + triglycerides >150mg/dl-, whose prevalence in Mexico in the adult population is 18.3% [50], is closely associated with increased expression, concentration and activity of the CETP [51]. In a lipid environment characterized by TRL with CETP over-expression, this protein transfers cholesteryl ester from HDL towards VLDL, IDL and LDL, this in exchange for a counter-transfer of triglycerides of the VLDL and IDL towards HDL [51]. This heterotypic transfer of neutral lipids favors increased catabolism of the HDL and the conversion of TRL into small dense cholesterol ester-rich LDL -LDL pattern ZG-. The HDL, by reducing their cholesteryl ester content and by increasing their triglyceride content, constitute substrates for hydrolytic action of several lipases, particularly the HL and the EL; hydrolysis of triglycerides contained in HDL, transforms this lipoprotein into an immature HDL, eagerly catalyzed in the liver and/or kidney. This mechanism explains in a 50% the low levels of HDL in the insulin resistance syndrome. In parallel, the VLDL and IDL with "residual" triglycerides and with a high content of cholesteryl ester, being hydrolyzed by the LPL and the HL, is transformed into a LDL with high cholesteryl ester content [51]. This dyslipidemia profile is proatherogenic, at least by the following mechanisms: a) lipotoxic effect of free fatty acids -Wang’s lipotoxic theory-; b) infiltration of apoB100-containing lipoproteins -Zilversmit’s infiltrative theory- and c) HDL reduction [52] (Fig. 5).

**INCREASED HDL AND PHARMACOLOGICAL MANIPULATION OF CETP**

The pharmacological increment in HDL is still a subject of clinical research, particularly for three reasons. a) the basic and clinical evidence on the beneficial role of HDL in the atherorregression and other cardiovascular and metabolically favorable biological actions [53]; b) the classic epidemiological evidence on the reverse relationship between the spontaneous level of HDL and the incidence of cardiovascular events, the more spontaneous HDL, the fewer cardiovascular events [54, 55]; c) the controversial evidence of residual risk -HDL-dependent- in primary and secondary prevention in the presence of LDL-lowering therapy [56-59].
three lines of evidence sustain interest in the research of pharmacological strategies to increase HDL levels.

However, it is worth mentioning that the strength of such evidences is being challenged by other recent evidences. The classic epidemiological evidence linking the reverse relationship between spontaneous HDL and cardiovascular events [54, 55], has been recently challenged by researchers that performed two Mendelian randomization analyses of single nucleotide polymorphisms associated with HDL-C, in order to determine whether carriers of the HDL-raising genetic variant had a lower risk for heart disease than non-carriers. In this paper, authors concluded “some genetic mechanisms that raise plasma HDL-C do not seem to lower risk of myocardial infarction. These data challenge the concept that raising of plasma HDL-C will uniformly translate into reduction in risk of myocardial infarction” [60].

Regarding the role of HDL in the atheroregression, the programs ILLUMINATE, ILLUSTRATE and RADIANCE with torcetrapib called into question the soundness of the hypothesis, since even with highly significant elevations of HDL ->50%, there was no atheroregression at a coronary and/or carotid level and the cardiovascular morbidity-mortality was 25% higher with torcetrapib than with placebo [59]. Although this result may have several explanations -see below-, the data generated controversy and extreme precaution. With regard to residual risk in secondary prevention in the presence of a statin therapy, the original TNT sub-analysis published by Barter, showed that the residual risk is inversely proportional to the HDL values -non-apo-AI adjusted- on-treatment with HMGCoAR inhibitors; the lower the residual HDL, the greater the likelihood of a subsequent cardiovascular event [56]. However, the most recent TNT sub-analysis, published by Mora, after a multivariable analyses did not found any significant HDL-dependent residual risk on-statin treatment [61]. Studies ACCORD-Lipids [62] and AIM-HIGH [63], both with some design flaws, were unable to prove that the use of fenofibric acid and niacin, respectively, was better than placebo in reducing cardiovascular events in subjects with cardiovascular disease, optimal therapy with statins, mean LDL-C of 60mg/dl and optimal concomitant treatment -anti-platelet aggregation, CNS inhibition, RAAS inhibition, etc., again, data generating controversy and skepticism [64].

The aforementioned make this cardiovascular therapeutic area one, if not the most interesting, and controversial area of the decade, with the universal goal of demonstrating that after optimal doses of statins and LDL-C <70mg/dl, the addition of a HDL promoter significantly reduces the residual risk.

**DALCETRAPIB, ANACETRAPIB AND EVACE-TRAPIB**

By omitting the extensively reviewed history of torcetrapib, whose failure according to Barter may be explained by one or a combination of the following mechanisms [65]: a) the *in vitro and in vivo* evidence that torcetrapib activates the Renin Angiotensin Aldosterone -RAA- system regardless of its inhibitory action of the CETP -off-target effect- b) the potential pro-atherogenic effect of inhibition of indirect reverse cholesterol transport route; c) the possibility of an increase in non-functional or dysfunctional HDL; d) the possibility that, in the presence of LDL-C "physiological" levels of <70mg/dl, the increasing HDL effect was minimal.

**Effect on Lipids, Biomarkers, Bioimages, and Cardiovascular Events**

The CETP blockade percentage is directly related to the increase in HDL, the greater the blockade, the greater the number of HDL. On average, the CEPT blockade percentage
for dalcetrapib [66, 67], anacetrapib [68] and evacetrapib [69] is 37.2%, 90% and 70% with increases in HDL-C of 25.5%, 129% and 70%, and LDL-C reductions of 5%, 40% and 22%, respectively. These three molecules have shown a lack of activating effect of the RAAS [66-69] (Fig. 2).

**Dalcetrapib**

This molecule was the first in its class to publish its phase II results [70]. On average, dalcetrapib at a dose of 600mg shows a 30% reduction of the CETP activity, with a mean HDL increase between 30% and 35% with no significant change in LDL and a good safety profile, specifically with no increase in blood pressure [70-71]. These reports led to the development of a research program called “dalcetrapib HDL Evaluation, Atherosclerosis and Reverse cholesterol Transport” -dal-HEART-, whose purpose was to demonstrate the reduction of cardiovascular events through the CETP manipulation with increased concentration and functionality of the HDL [72]. This program was cancelled by the sponsor for futility on May 7, 2012, based on results of the 2nd interim analysis of the project dal-OUTCOMES-1 [73]. Dal-HEART program included 6 studies, of which to date only the dal-PLAQUE and dal-VESSEL studies have been published [66-67].

**Dal-VESSEL Study [67]**

Dal-VESSEL was a safety study with dalcetrapib to assess changes in arterial endothelial function. The primary endpoint of the dal-VESSEL was to assess the flow-dependent brachial vasodilator reserve and the ambulatory blood pressure over a period of 24 hours; the secondary endpoints were changes in lipid profiles, inflammation biomarkers, -13 biomarkers-, insulin, serum electrolytes, concentration, CETP activity and cardiovascular events record in the dal-HEART program.

In 476 randomized subjects at risk for or with coronary artery disease, LDL-C <100mg/dl on statin and/or ezetimibe therapy and HDL-C <50mg/dl, was compared in a double-blind manner, dalcetrapib 600mg versus placebo.

In a non-inferiority design of dalcetrapib versus placebo, the primary endpoint of the study was met. The mean baseline value of the brachial vasodilator reserve -change in the arterial diameter, comparing the baseline value with the third minute measurement, after five minutes of arterial occlusion-, was 4.1% in the placebo group and 4% in the torcetrapib group, with no significant change noted at 12 and 36 weeks of treatment. The baseline and mean value of ambulatory blood pressure was 125/74mmHg in the placebo group and 128/75mmHg in the dalcetrapib group, with no significant change noted at 36 weeks of treatment; for both evaluations no significant differences were observed between the prespecified groups. Placebo-corrected dalcetrapib, increased HDL and apoA1 up to 31% and 10% respectively, reduced CETP activity up to 56% and decreased triglycerides, LDL and apo B100 up to 14%, 4% and 5% respectively; all these values were statistically significant. No significant differences were noted in serum electrolytes, creatinine and glucose/insulin ratio between groups. Inflammation biomarkers, except for Lp-PLA2, were not significantly different between both groups; Lp-PLA2 -protein associated with HDL- was significantly increased in the dalcetrapib group, this change was associated to the increase in HDL according to the authors. Adverse events and pre-specified cardiovascular events were similar, with no significant differences observed between both groups.

The conclusion of this study is that dalcetrapib did not affect endothelial vascular function, this conclusion is based on the absence of changes in the endothelium-dependent brachial vasodilator response and the absence of an increase in ambulatory blood pressure. One observation of this study was that, although the primary non-inferiority endpoint was met, the lack of benefit with dalcetrapib in all parameters evaluated is intriguing, including inflammation biomarkers. The most likely explanation proposed by the authors is the elevation of only 31% in the HDL values.

---

**Comparison between CETP inhibitors and modulator**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dalcetrapib 600mg</th>
<th>Torcetrapib 60mg</th>
<th>Anacetrapib 150mg</th>
<th>Evacetrapib 100mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>↓ CETP</td>
<td>37.2%</td>
<td>≥80%</td>
<td>90%</td>
<td>70%</td>
</tr>
<tr>
<td>↑ HDL</td>
<td>26.5%</td>
<td>62%</td>
<td>129%</td>
<td>95%</td>
</tr>
<tr>
<td>↓ LDL</td>
<td>-5%</td>
<td>-11%</td>
<td>-40%</td>
<td>-22%</td>
</tr>
<tr>
<td>↑ Blood Pressure</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>↑ Aldosterone</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

*Fig. (6). Comparison between dalcetrapib, torcetrapib, anacetrapib and evacetrapib.*
Dal-PLAQUE Study [66]

Dal-PLAQUE was a safety study with dalcetrapib to assess changes in arterial structure. The primary endpoint of the dal-PLAQUE design, a pioneer of its kind, was the evaluation by MRI and by PET with 18-FDG of the structural and inflammatory changes in the carotid arterial wall and the ascending aorta. The MRI, as a non-ionizing method, allowed to measure in a non-invasive manner the following parameters: diameter and lumen area, diameter and vessel area, and wall thickness and area; on the other hand, the PET, as the 18 FGD is eagerly taken up by inflammatory cells, is a method that detects vascular areas with inflammatory activity. As part of the dal-HEART program, the secondary endpoints of the dal-PLAQUE study were similar to those of the dal-VESSEL study.

In 130 subjects at risk for or with coronary artery disease, LDL-C <100mg/dl on statin therapy, triglycerides ≤ 400mg/dl and an artery/blood ratio of ≥ 1.6 in the 18-FDG uptake, was compared in a double-blind manner, dalcetrapib 600mg versus placebo.

In a non-inferiority design of dalcetrapib versus placebo, the primary endpoint of the study was met. As assessed by MRI, the total vascular area, the wall area, the wall thickness, and the total wall area/vascular area values at 24 months of treatment were not statistically different between the dalcetrapib group and the placebo group. A significant trend towards a reduced total vascular area in the dalcetrapib group was observed; this finding probably implied the regression of positive remodeling of the arterial segments evaluated. As assessed by PET, in the index segments, no statistically significant differences were noted in the degree of vascular inflammation measured by the 18-FDG uptake at 6 months of treatment. The clinic blood pressure measurement was not different between groups. Placebo-corrected dalcetrapib, increased HDL and apoA1 up to 26.9% and 6.8% respectively, reduced CETP activity up to 55.5%, while triglycerides, LDL and apoB100 were not significantly different between both groups; the CRP paradoxically increased 33.3% in the dalcetrapib group, with no changes observed in the placebo group. As in the dal-VESSEL study, Lp-PLA2 was significantly increased in the dalcetrapib group, this change was associated to the increase in HDL according to the authors. Adverse events and pre-specified cardiovascular events were similar, with no significant differences noted between both groups.

The conclusion of this study is that dalcetrapib does not favor athero-progression or carotid-aortic vascular inflammation, and there is a trend towards a benefit in both vascular parameters correlated with early and sustained reduction of HDL. As for dal-VESSEL, the hypothesis of the absence of vascular damage by dalcetrapib is confirmed, although no benefit in the evaluated parameters by dalcetrapib and a paradoxical elevation of CRP were observed. The most likely explanation proposed by the authors for these findings is the limited follow-up at 24 months and the dissociation already reported by other investigators between the intra-arterial inflammation and systemic biomarkers.

Anacetrapib

This molecule has been studied in phases I and II, and at the end of 2010 the DEFINE phase III study was reported [68]. This was a medium-term efficacy and safety study with anacetrapib in subjects at high cardiovascular risk ≥20% as assessed by Framingham or equivalent, or with coronary artery disease documented more than three months prior to enrollment, with LDL-C between 50mg/dl and 100 mg/dl on statin therapy, HDL-C <60mg/dl and TG ≤400mg/dl. The primary endpoint of the study was the change in the lipid profile - LDL primary endpoint and HDL secondary endpoint- and in the safety parameters: vital signs with automated blood pressure measurement at the clinic, serum electrolytes, aldosterone, liver enzymes and CPK, among the most important; furthermore, the combo of cardiovascular death, nonfatal myocardial infarction, nonfatal stroke and hospitalization for unstable angina was evaluated. In the double-blind phase with anacetrapib 100mg versus placebo, 1,623 subjects were enrolled; 55% with cardiovascular disease and 45% at high cardiovascular risk.

The mean baseline LDL-C and HDL-C values were 81mg/dl and 41mg/dl, respectively. The lipid profile assessment at 24 weeks of treatment showed a reduction in LDL of 81mg/dl to 45mg/dl in the anacetrapib group versus 82mg/dl to 77mg/dl in the placebo group - 39.8% reduction with p < 0.001-; the HDL increased from 41mg/dl to 101mg/dl in the anacetrapib group versus 40mg/dl to 46mg/dl in the placebo group -38.1% increase with p <0.001-. Apoprotein B, non-HDL cholesterol, Lp-a and TG were significantly decreased, whereas apoAI was significantly increased. These lipid profile changes were maintained during the 76 weeks of treatment. Safety assessments, including adverse events, vital signs, serum electrolytes, liver enzymes, CPK, aldosterone etc., showed no differences between the anacetrapib and placebo groups. The pre-specified combo of cardiovascular events had an incidence of 2% in the anacetrapib group and of 2.6% in the placebo group -HR 0.76, with 95% CI 0.39 to 1.45 and p 0.04-.

Thus it is concluded that the DEFINE study provides a 94% certainty in the prediction that anacetrapib will not increase the incidence of cardiovascular events in a similar way to torcetrapib. These results sustain the clinical research with CETP manipulation, although there are questions left unanswered on the functionality of HDL and the impact on cardiovascular events in a study "ad-hoc".

In order to answer this last question, the REVEAL-HPS3-TIMI-55 [74], study was designed and recently initiated, in which in a double-blind, randomized and placebo-compared manner, anacetrapib100mg will be evaluated in 300,000 subjects with cardiovascular disease or diabetes mellitus with coronary atherosclerosis and optimal therapy with atorvastatin -LDL-C <70mg/dl-, being the primary endpoint the assessment of the combo: cardiovascular death, nonfatal myocardial infarction and coronary revascularization.

Evacetrapib

This molecule has been studied in phases I and II, and at the end of 2011, Nicholls reported the double-blind, placebo-
The primary endpoint of this study was to evaluate the change in LDL and HDL at 12 weeks of treatment. In subject with a mean LDL-C of 144.3mg/dl and a mean HDL-C of 55.1mg/dl, evacetrapib as monotherapy dose-dependently increased the HDL level from 53.6% to 128.8% -30mg/dl to 60mg/dl- from baseline, compared with a decrease of -3% in the placebo group -p<0.001- and reduced the LDL level from 13.6% to 35.9% -20.5mg/dl to 51.4mg/dl below the baseline value compared with an increase of 3.9% in the placebo group -p<0.001-.

In combination with statins, evacetrapib 100mg increased the HDL level from 78.5% to 88.5%, compared with statin alone -p<0.001-, and reduced the LDL level from -11.2% to 13.9% compared with statins -p<0.001-. No serious adverse events observed were reported.

The conclusion of this efficacy study was that evacetrapib compared with placebo, as monotherapy or in combination with statins, significantly increases the HDL level and reduces the LDL level.

CONCLUSIONS: LDL, HDL OR BOTH?

The theory that states that cardiovascular diseases associated with atherosclerosis are mainly associated to supra-physiological numbers of LDL has many positive evidences in favor. Unlike this theory, the LDL-centric hypothesis has still some controversial evidences and a big challenge, showing that the pharmacological increase in HDL is a strategy that provides an additional benefit over the benefits already demonstrated by the classic and emerging lowering LDL therapies.

The solid cornerstones of the LDL-centric theory can be summarized in 5 as follows: a) the evidence of the directly proportional relationship between high rates of cholesterol and the incidence of cardiovascular disease [75-77]; b) the evidence of subjects with Familial Hypercholesterolemia, who in the absence of any other factors of vascular endothelial injury, develop atherosclerosis and cardiovascular events at ages as short as 18 months [78]; c) the evidence that increased numbers of LDL lead to its transformation into oxidized LDL -epitope of DAMPs and PAMPs-, and thus initiates an inflammatory process of vascular endothelial activation, dysfunction and structural changes called atherosclerosis [79]; d) the evidence of the use of HMGCoA-R inhibitors as a strategy that by reducing the gap between the LDL level of the population "normal value 130mg/dl" and the physiological LDL level "25mg/dl to 50mg/dl" reduces the risk of cardiovascular diseases associated with atherosclerosis -1 mg reduction in LDL by a 0.55% risk reduction- regardless of baseline LDL value [80-82]; e) the new therapeutic and epidemiological evidences showing that sub-populations of subjects with different polymorphisms determining low levels of LDL since their birth, have per 1mg/dl reduction in LDL, a 1.42% reduction in risk -2.5 times greater protection than the achieved with the use of statins in adulthood*. This observation demonstrates the maximum benefit of having low LDL levels from birth and throughout life, and proposes, among other questions, the behavioral and/or early pharmacological approach, aimed to maintain low levels of LDL since childhood. In parallel, new reducing LDL strategies, such as the anti-sense inhibition or by monoclonal antibodies of the serine protease PCSK9, have recently demonstrated the achievement of LDL levels <70mg/dl in 100% of treated subjects [83-86]. This emergent strategy opens up new treatment options for LDL reduction, atherosclerosis and cardiovascular disease, particularly in those intolerant subjects with suboptimal response or those refractory to HMGCoA-R inhibitors.

*Data presented by Ference, B in the ACC 61st Annual Scientific Meeting, Chicago III, March 26, 2012.

In a testing scheme similar to the one previously mentioned, and in accordance with was laid down in this article, the HDL-centric theory, has the following profile in May 2012: a) the classic epidemiological evidence [54, 55] of the reversely proportional relationship between high rates of spontaneous HDL and the incidence of cardiovascular disease was -before Voight et al. paper [60]- the less controversial evidence; b) unlike the evidence with LDL, subjects with mutations determining low levels of HDL do not show consistently an increased incidence in cardiovascular diseases associated with atherosclerosis. Moreover some of them, with low levels of HDL, have an incidence of cardiovascular disease associated with atherosclerosis below the average, i.e. apoAI-Milano [86]. On the other hand, as it was widely referred to, those mutations with CETP polymorphisms, determining an increase in HDL, have a variable protective cardiovascular effect, with a probable differential benefit among subjects with and without mixed dyslipidemia [10-19, 60]; c) the evidence of the HDL involvement in reverse cholesterol transport and other protective metabolic and vascular endothelial effects represent solid evidence [53]. This knowledge has been increased with new advances in HDL biology, important advances are especially reviewed recently by Rosenson et al. [87] and involves new data regarding: a) the multiple components in RCT -macrophage wall efflux, non-macrophage wall efflux, non-arterial wall efflux, lipoprotein transport, hepatic cholesterol uptake, direct excretion into small intestine and fecal excretion-; b) the evidence demonstrating that HDL-C is not necessarily an adequate surrogate for macrophage cholesterol efflux and c) the evidence postulating that fecal excretion of cholesterol is not necessarily a prerequisite for assessing the cholesterol efflux from the arterial wall. However, that strength varies between the different HDL-boosting strategies. This article has reviewed the evidence with CETP pharmacological manipulation, other strategies such as the use of niacin [88-92], fibrates [93-95], ACAT inhibitors [96-98], HDL infusion, apoA1 infusion (native, Milano, mimetics or regulators) [99-103] etc., have been largely investigated and reviewed by other authors; d) unlike the evidence with LDL, at present time and as discussed throughout this article, no HDL increasing strategy has been shown to reduce the incidence of cardiovascular disease associated with atherosclerosis in subjects receiving optimal treatment at a cardiovascular risk according to the current guidelines; certainly this is a big challenge for this decade which have become more difficult to accomplish by the recent interruption of the dal-HEART
program; e) finally the HDL-modifying strategies have new aspects dictated by the emerging Pharmacology based on Systems Biology, in which the interaction of genome, proteome, metabolome, and phenotype and their modification by a disease or a pathological phenome should be considered as an interconnected network that will suffer from modifications by the action of a drug; the desired modification is the attenuation of the pathology, however there are unexpected or undesired changes to the system - off-target effects- which are not possible to anticipate based on the classic Pharmacology [104, 105]. Based on the Systems Pharmacology, the study of their role in complete biological systems, such as the cholesterol efflux analysis, it is likely that it will allow dissecting those HDL subpopulations that are to be optimized, as well as identifying those subpopulations of subjects to be treated [106, 107].

**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-FDG</td>
<td>18-Fluorodeoxyglucose</td>
</tr>
<tr>
<td>ABC-A1</td>
<td>ATP Binding Cassette A1</td>
</tr>
<tr>
<td>ABC-G1</td>
<td>ATP Binding Cassette G1</td>
</tr>
<tr>
<td>ACAT</td>
<td>Acyl-Coenzym-A:cholesterol acyl-Transf erase</td>
</tr>
<tr>
<td>Apo-AI</td>
<td>Apoprotein A1</td>
</tr>
<tr>
<td>CETP</td>
<td>Cholesteryl Ester Transfer Protein.</td>
</tr>
<tr>
<td>CPK</td>
<td>Creatine Phospho Kinase</td>
</tr>
<tr>
<td>CRP</td>
<td>C - reactive protein</td>
</tr>
<tr>
<td>DAMP</td>
<td>Damage Associated Molecular Patterns</td>
</tr>
<tr>
<td>EL</td>
<td>Endothelial Lipase</td>
</tr>
<tr>
<td>HDL-C</td>
<td>Cholesterol content of High Density Lipoprotein</td>
</tr>
<tr>
<td>HL</td>
<td>Hepatic Lipase</td>
</tr>
<tr>
<td>HMGCoA-R</td>
<td>Hydroxy Methyl Glutaryl CoA-Reductase</td>
</tr>
<tr>
<td>LDL</td>
<td>Cholesterol content of Low Density Lipoprotein</td>
</tr>
<tr>
<td>LDL-C</td>
<td>Cholesterol content of Low Density Lipoprotein</td>
</tr>
<tr>
<td>LDL-R</td>
<td>Receptors for LDL</td>
</tr>
<tr>
<td>LP -a-</td>
<td>Lipoprotein -a-</td>
</tr>
<tr>
<td>LPL</td>
<td>Lipoprotein Lipase</td>
</tr>
<tr>
<td>Lp-PLA2</td>
<td>Lipoprotein Phospho lipase A2</td>
</tr>
<tr>
<td>LRP-1</td>
<td>LDL receptor Related Protein-1</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>PAMP</td>
<td>Pathogens Associated Molecular Patterns</td>
</tr>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
</tr>
<tr>
<td>PLTP</td>
<td>Phospholipid Transfer Protein</td>
</tr>
<tr>
<td>RCT</td>
<td>Reverse Cholesterol Transport.</td>
</tr>
<tr>
<td>SR-B1</td>
<td>Scavenger Receptor B1</td>
</tr>
<tr>
<td>TG</td>
<td>Triglycerides</td>
</tr>
<tr>
<td>TRL</td>
<td>Triglyceride Rich Lipoproteins</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very Low Density Lipoprotein.</td>
</tr>
</tbody>
</table>

**CONFLICT OF INTEREST**

The authors confirm that this article content has no conflicts of interest.

**ACKNOWLEDGEMENT**

To Rodolfo E Guajardo I MD and Glanser Services SC for translation support.

**REFERENCES**


[14] Borggreve, S.E.; Hillege, H.L.; Wolfenbuttel, B.H.R.; de Jong, P.E.; Zuurman, M.W.; van der Steege, G.; van Tol, A.; Dullaart, R.P.F.; on behalf of the PREVEND Study Group. An increased coronary risk is paradoxically associated with common cholesteryl ester transfer protein gene variations that relate to higher high-

---


**Morales-Villegas et al.**


