

New Approaches to Atherosclerotic Cardiovascular Disease. The Potentials of Torcetrapib

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Abstract: Atherosclerosis is the leading cause of death and disability in the developed world. Although the low-density lipoprotein (LDL) cholesterol lowering drugs reduce the mortality and morbidity associated with coronary artery disease, considerable mortality and morbidity remains. Reverse cholesterol transport mediated by high-density lipoproteins (HDL) may provide an independent pathway for lipid removal from atheroma. The current NCEP ATPIII include HDL-cholesterol 1.6 mmol/l as a negative risk factor. Torcetrapib is an inhibitor of cholesteryl ester-transfer protein (CETP) that increases high-density lipoprotein (HDL) cholesterol levels. The drug increases HDL-cholesterol and apolipoprotein A-I levels and decreases LDL-cholesterol and apolipoprotein B levels. The effect is showed in monotherapy and when administered in combination with statins. In addition, torcetrapib did not significantly change the serum levels of cholesterol and triglycerides. The raising HDL-cholesterol with torcetrapib could be a new approach to atherosclerotic cardiovascular disease although new trials based on hard clinical end points are necessary.

Keywords: Atherosclerosis, HDL-cholesterol, LDL-cholesterol, statins, torcetrapib, CETP.

INTRODUCTION

Atherosclerosis is an important cause of death in developed countries with high morbidity associated, therefore, it leads to a high sanitary cost. Cardiovascular diseases cause 38 % of death in North America and are the main death cause in European men under 65 years, being the second cause in women [1]. Important assays have shown a beneficial effect of statins due to their action lowering cholesterol levels and LDL-cholesterol, together with other pleiotropic mechanisms not related with dyslipemia. Despite this information and the use of aggressive therapies, a high morbimortality with vascular origin persists. It is necessary to find new therapeutic targets complementing the existing ones to solve this sanitary problem.

The aim of this revision is to comment the data published in the literature that reflect the utility of increasing HDL-cholesterol in order to reduce cardiovascular morbimortality, antiatherogenic mechanisms of HDL-cholesterol and torcetrapib characteristics, with special reference to its action mechanism through CETP (Cholesterol Ester Transfer Protein) inhibition and the preliminary results of the use of the drug in humans.

HDL-CHOLESTEROL. PROTECTING EFFECT

The cholesterol-HDL particle is a protein complex constituted by different apolipoproteins, including the most abundant apoA-I, apoA-II, present in 2/3 of the HDL particles and associated enzymes such as paroxonase, platelet-activated factor acetylhydrolase (PAF-AH), lecithin: cholesterolacyltransferase (LCAT) and plasma glutathione selenoperoxidase (GPX3) [2 - 6]. The outer layer is amphipathic and contains free cholesterol, apolipoproteins

and phospholipids. The inner layer is hydrophobic and contains cholesteryl esters. ApoA-I plays an important protector role facing atherosclerosis as cholesterol acceptor whereas the role of apoA-II has not been clearly established.

The protector role of HDL-cholesterol has been demonstrated in several studies both demographic and related with clinical assays. In one case-control study [7] performed with 6859 men and women, pertaining to different ethnic groups, the mean of HDL-cholesterol levels was lower in subjects with coronary disease than in healthy controls, remaining the significance after adjusting by levels of LDL-cholesterol and triglycerides. In Framingham Study [8], the fact that lower levels of HDL-cholesterol were the main risk factor of lipidic origin [9] was observed. After 12 years of monitoring, the analysis was repeated and the same fact was maintained. In four American prospective studies (Framingham Heart Study -FHS-, Lipid Research Clinics Prevalence Mortality Follow-up Study - LRCF-, Coronary Primary Prevention Trial -CPPT-, and Multiple Risk Factor Intervention Trial - MRFIT) the increase of 1 mg/dl of HDL-cholesterol leads to a significant fall of coronary disease ranging between 2 % and 4.7 % in the different studies [10]. The Tromso Heart Study [11] valued/estimated/assessed in a prospective way/manner in young males, aging 20-49, the relation between HDL-cholesterol and coronary risk, and an inverse relation between both parameters. In old males, with a 21-year monitoring, it was observed that low levels of HDL-cholesterol increased coronary mortality [12]. The effect persisted despite adjusting other risk factors and was particularly intense in diabetic individuals.

In the German PROCAM study, the relative risk of coronary disease was six fold higher in patients with HDL-cholesterol under 35 mg/dl [13]. In two prospective studies performed with gemfibrozil, the Helsinki Heart Study [14], in primary prevention and the Va-Hit Study [15] in secondary prevention, the decreasing of cardiovascular

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events was associated to the increase of HDL-cholesterol in the treated group. The Bezafibrate Coronary Atherosclerosis Intervention Trial (BECAIT) assessed the protector effect of bezafibrate on the progression of focused atherosclerosis angiographically mediated in patients with recent heart attack [16]. The treated group showed an increase of HDL-cholesterol of 90 % with a reduction of the disease progression. The HDL Intervention Trial (HIT) had the aim of evaluating the benefit obtained through the increase of HDL-cholesterol in patients with normal values of triglycerides and LDL-cholesterol [17]. A reduction in the heart attack incidence, death with cardiovascular origin and icus in the treated group with gemfibrozil was observed.

These facts were also revealed in trials that evaluate the effect of statins on the reduction of coronary events. Five of six published studies showed an inverse relationship between HDL-cholesterol levels and coronary risk [18]. In some of those studies, specifically in LIPID and 4S [19, 20], it was revealed the fact that part of the beneficial effect of statins was due to the increase of HDL or apoA-I, the main apolipoprotein of this particle. Recently, EPIC study showed that lower levels of HDL-cholesterol were a risk factor considering the recurrence of cardiovascular events [21].

All these data, corroborated by the presence of HDL-cholesterol lowered in metabolic syndrome [22], suggest a possible protector effect of HDL-cholesterol, being necessary to increase its levels in order to lower the adverse events of the coronary disease. Statins, in primary and secondary prevention, have successfully and remarkably decreased the coronary events, even though a noticeable morbimortality persists, in spite of achieving very low levels of LDL-cholesterol [23]. Considering the above information, an effective therapeutic option would be the increase of HDL-cholesterol values.

ANTIATHEROGENIC MECHANISMS OF HDL-CHOLESTEROL

There are various epidemiologic studies that reveal the existence of a protecting role of HDL-cholesterol in cardiovascular diseases. However, the involved mechanisms have not been clearly established, even though various mechanisms have been described [24]:

- Efflux of cholesterol from macrophages located in vascular walls
- Inhibition of LDL-cholesterol oxidation
- Inhibition of inflammation
- Stabilization of endothelial tissue through nitric oxide (NO)

1. Efflux of Cholesterol from Cholesterol Loaded Macrophages

Atherosclerosis is an inflammatory chronic disease characterized by macrophages and T lymphocytes accumulation in arteries accompanied by an increase of plasmatic concentration of inflammatory markers [25, 26]. Monocytes reach the arterial wall, where they become macrophages expressing scavenger receptor with the ability to uptake LDL-cholesterol originating foam cells. These

activated macrophages start up the inflammatory mechanisms that perpetuate the inflammatory process comprising the fatty grooves [27].

HDL-cholesterol take part easing the outflow of cholesterol excess from peripheral tissues (macrophages in this case) being led afterwards to the liver and removed by bile excretion. This process is named reverse cholesterol transport [28]. The efflux of cholesterol from macrophages occurs by means of three different mechanisms. The ATP-binding cassette (ABC) transporter superfamily are membrane proteins which employ ATP as an energy source and are used for passing of diverse elements through them. Macrophages are rich in ABCA1 whose expression is controlled by the intracellular cholesterol levels [29]. It is sterified to oxysterol, which is attached to a transcription factor of LXR, which forms a dimeric structure with the X-retinoid receptor. The formed complex stimulates the expression of the carrier gene. The lack of function of this carrier, due to diverse mutations, causes Tangier Disease, characterized by absence of HDL-cholesterol and reduction of LDL-cholesterol without a remarkable increase of atherosclerosis risk [30]. This system contributes to the efflux of cholesterol from the macrophage located in the vascular wall but has low influence on plasmatic levels of HDL [31]. However, it has an important role as antiatherogenic agent. There are other independent mechanisms different from the ABCA1 system that contribute to plasmatic levels of HDL (ABCG1 and ABCG4) and help its incorporation to major HDL-2 and HDL-3 particles which determine plasmatic levels [32].

Other mechanisms can take part, as the aqueous diffusion to phospholipid-containing acceptor [33] and the SR-B1 system that promotes a bidirectional flow of cholesterol between cells and HDL, assisting the efflux when the HDL are rich in phospholipids and poor in cholesterol [34].

Cholesterol proceeding from peripheral tissues is captured by Nascent-HDL particles (rich in triglycerides and poor in cholesterol) synthesized in the liver and small bowel or by hydrolysis of rich particles in triglycerides with later shape modifications into spherical particles named HDL-2 and HDL-3. These particles suffer a esterification process by lecithin-cholesterol acyltransferase (LCAT) creating cholesterol esters which are transferred to the liver following three routes. Cholesteryl-ester transfer protein (CETP), which transfers cholesterol from HDL to rich in triglycerides lipoproteins, which are subsequently catabolized following the route of the LDL receptor; scavenger receptor B1 (SR-B1) who mediates the selective uptake of cholesterol esters by the liver; endocytosis of HDL holoparticles through a catabolic hepatic receptor [35].

2. Inhibition LDL Oxidation

The LDL-cholesterol particles penetrate inside the macrophages located in the blood vessel walls wherein they oxidize themselves in order to produce their proatherogenic effect. This oxidation occurs when the lipidic hydroperoxides produced by the metabolic route of lipoxygenase and mieloperoxidase achieve a determined threshold [27]. The oxidized LDL penetrate inside the vessel walls and bind to it through the same VCAM-1 and E-

selectin. They are captured by two scavenger receptors (CD-36 and SR-B1) located in the macrophage and then their activation occurs. Such activation determines the synthesis of adhesion and chemotactic molecules. The inhibition of the oxidation performed by the HDL-cholesterol is carried out by apoA-I which remove hydroperoxides and by diverse enzymes (paraoxonases, platelet-activated factor acetylhydrolase -PAF-AH-, LCAT, plasma glutathione selenoperoxidase) which destroy the lipidic hydroperoxides and hydrolyze the oxidized phospholipides [36].

3. Inhibition of Inflammation

An early event in atherogenesis is the adhesion of monocytes to the endothelium through adhesion molecules (VCAM-1, ICAM-1, E-selectine), which are rapidly synthesized in response to cytokines and C-reactive Protein [37,38]. Native and reconstituted HDL-cholesterol are capable to inhibit the expression of these adhesion molecules. Also are capable of inhibiting the expression of MCP-1. The mechanism responsible for this facts is the blockade of an endothelial enzyme, sphingosine kinase, acting in the pathway of NF- κ B, used by TNF- to stimulate the expression of adhesion molecules [27,39]

4. Stabilization of endothelial tissue through nitric oxide (NO)

Vascular endothelial injuries perform a key role in the pathogenesis of atherosclerosis. The integrity of endothelia maintains a series of activities that generate an atheroprotector effect being the NO a key piece in the maintenance of this function. The HDL-cholesterol particles take part inhibiting the effects of oxidized LDL-cholesterol and lead to vasodilatation through the release of NO after attaching to SR-B1. The NO activation is mediated by NOS (endothelial nitric oxide synthetase) [40].

TORCETRAPIB

Low HDL-cholesterol levels constitute a lipid abnormality frequently observed in patients with known coronary disease. Several alternative therapies have been searched in order to increase the level of HDL-cholesterol and decrease the vascular risk. Cholestyramine increases HDL-cholesterol level from 3 to 5%, statins from 5 to 15% and fibrates from 10 to 15%. The magnitude of such effects is not very considerable, especially when compared with LDL-cholesterol reduction, which ranges from 15 to 30% for cholestyramine, 30 to 50% for statins and 10 to 15% for fibrates [41]. Apart from the above mentioned, clinical information regarding three types of drugs are also available: agonists of nuclear receptors (PPAR α , PPAR γ , PPAR δ), CETP inhibitors and apolipoproteins infusion or HDL synthetic particles, which could act as cholesterol acceptors [42]. Many therapeutic alternatives have been designed to inhibit CETP: immunological modulators of protein activity [43,44], JTT705, drug which binds to protein through disulfide bridges [45] and torcetrapib [46-51], the drug which was the object of our analysis.

Torcetrapib is a molecule, whose chemical formula is 4-[3,5-Bis-trifluoromethylbenzyl-methoxycarbonyl-amino]-2-ethyl-6-trifluoromehtyl-3,4-dihydro-2H-quinoline-1-carboxylic acid ethyl ester. It acts by means of the cholesterol

ester transfer protein (CETP) inhibition. This mechanism allows for HDL-cholesterol increasing and reduces LDL-cholesterol and apolipoprotein B. Low levels of HDL-cholesterol constitute the lipid abnormality most frequently seen in patients with known coronary disease, having the currently available drugs a moderate effect on this fact. Statins increase them in only a 5-10%, while fibrates and niacines rarely achieve more than a 25%. This fact justifies the necessity of introducing new drugs like the drug commented in the revision. Next, we are going to analyse the effect of CETP, target protein of torcetrapib, on vascular risk, and the studies carried out with this drug.

1. Cholesteryl Ester Transfer Protein (CETP)

The CETP is a glycoprotein physically associated with HDL particles. It helps transporting cholesterol ester from HDL to lipoproteins that contain apolipoprotein B (Very low density lipoprotein - VLDL -, VLDL remnants, Intermediate density lipoprotein - IDL - and low density lipoprotein - LDL -). This process is followed by the transference of triglycerides in the opposite direction [52].

CETP concentration ranges from 1 to 3 μ g/ml plasma in standard subjects and could duplicate or triplicate in subjects suffering hypercholesterolemia, especially if triglyceride levels are high. As we have mentioned before, the disclosure of a CETP genetic deficit on human beings was associated with high levels of HDL, which have a possible protector effect on atherosclerosis. Therefore, the possibility of using inhibitors of this substance as a therapeutic option has brought up. Theoretically, CETP inhibition could present a proatherogenic effect when reducing the metabolic pathway of reverse cholesterol. However, in population small studies this has not been seen, and no relation between mass and CETP activity and cardiovascular diseases [53,54] has been observed. Chinese population that presents myocardial infarction or ictus showed CETP levels and activities superior to those of the controls [55].

Recently, Boekholdt *et al.* [56] analysed the relation between CETP plasmatic levels and posterior risk for coronary disease in a prospective study of case-control. 735 cases of 1400 control were included and a positive lineal correlation with cholesterol-LDL, as well as a negative lineal correlation with cholesterol-HDL were observed. After adjusting through non lipidic risk factors, the study aimed the enhancement of the vascular risk regarding the highest quintile of CETP, and particularly for those subjects who presented high triglyceride levels. The benefit of low levels of this protein is related with the increase of HDL-cholesterol. This fact was observed in the present study and also in a meta-analysis carried out by the same authors. Such information justifies its use as a therapeutic target.

2. Effect of Torcetrapib on cholesterol metabolism. Studies on human

By the time the present review was carried out, few clinical studies on this molecule were published. A summary of them is displayed on (Table 1). The first one is a clinical assay with healthy volunteers [57]. Forty subjects were involved in the study. Five groups of 8 subjects were created and each was randomised to placebo (n=2) or torcetrapib (n=6) at 10, 30, 60, and 120 mg daily and 120 mg twice a

Table 1. Summary of Results in Clinical Trials with Torcetrapib

Study type	Number of subjects	Dose/day	Weeks of treatment	HDL-C increase	LDL-C decrease
Healthy Volunteers, different doses [57]	6 per dose (total=40)	10 to 120 mg and 120 mg b.i.d	2	16 to 91%	21 to 42%
Single-blind, placebo-controlled [58]	19 (part associated with atorvastatin* n=9)	120 mg and 120 mg b.i.d (n=6)	4 - 8	46 to 106% (61% in association with atorvastatin)	NS (17% in association with atorvastatin)
Multi-center, double-blind, placebo-controlled, different doses [62]	~30 per dose, total=236 (part associated with atorvastatin*)	10 to 90 mg	8	9 to 54.5% (up to 40.2% in association with atorvastatin)	16.5% (up to 18.9 % in association with atorvastatin)

Between brackets bibliographic reference.

NS= There wasn't statistically significant differences regarding to placebo.

*Dose of atorvastatin was 20 mg/day in all the studies where it was employed.

day during 14 days. Plasma CETP activity, plasma CEPT concentration, plasma lipids and apolipoproteins were determined. Torcetrapib with different doses was accepted by all patients. Mean inhibition of CEPT activity was 12 ± 17 , 35 ± 17 , 53 ± 8 and 80 ± 6 for the 30, 60, 120 mg daily and 120 mg twice-daily groups respectively.

Regarding the concentration of CEPT plasma a doses and time dependent increase was observed. The HDL-C was significantly increased to all doses in regard to placebo, from 16% to 91%, without substantial changes in TPC. The lack of effect on TPC is due to a decrease of non HDL-C, with LDL-C decreased 21% with 120 mg daily and 42% with 120 mg twice a day dosing. At the highest dose, apoA-I and apoE increased, respectively, by 27% and 66%, whereas apoB decreased by 26%. Cholesteryl ester content decreased and triglyceride increased in the non HDL plasma fraction, with contrasting occurring in HDL.

Another study was single blind, placebo-controlled in subjects who presented HDL-C below 40 mg per deciliter [58]. A total of 19 were enrolled: 9 were also treated with 20 mg of atorvastatin [59,60]. The study consisted in 17 men and 2 women. All participants received placebo during 4 weeks. Afterwards they received 120 mg per day of torcetrapib during 4 weeks. A subgroup (n=6) that did not take atorvastatin received 120 mg of torcetrapib twice per day during the next 4 weeks.

TPC, TG, LDL-C, HDL-C and subclasses were determined, unesterified and esterified cholesterol, phospholipids, apolipoproteins and CEPT activity. During the clinical assay, minor and moderate side effects were noticed, therefore the treatment was not interrupted. The side effects perceived were headache, asthenia, dispepsia, sweating, ache, simplex herpes, zoster herpes, amnesia or abnormal thinking. CETP activity was less than 28 ± 16 and 65 ± 16 percent regarding the placebo for those who had received 120 mg per day and 120 mg twice per day, respectively. The treatment with 120 mg per day of torcetrapib increased the plasmatic concentration of HDL-C

in 46% and 106% when they received 120 mg of the drug, twice per day, without substantial changes in the TPC.

The torcetrapib alone did not modify, with statistic significance, the LDL-C. It was only observed a 17% decrease when associated to atorvastatin. The apolipoproteins A-I and A-II increased about 12% to 36% with the minimum and maximum torcetrapib dose employed during the study, whereas the apolipoprotein B decreased about 10% to 17%.

The triglycerides decreased 26% only when CEPT inhibitor was employed on a 120 mg doses twice per day. Another study carried out with the same patients analyzed the effect of torcetrapib on apolipoprotein A-I (apoA-I) containing HDL subspecies, apoA-I turnover and markers of reverse cholesterol transport.

With regard to the placebo, the use of torcetrapib on 120 mg dose per day increased the amount of apoA-I in 1-migrating HDL in the atorvastatin (136 %, $p < 0.001$) and nonatorvastatin (153 %, $p < 0.01$) cohorts, whereas an increase of 382 % ($p < 0.01$) was observed in the 120 mg twice daily group. HDL apoA-I pool increased in the nonatorvastatin cohort (16 ± 7 %, $p < 0.0001$) y 34 ± 8 %, $p > 0.0001$). These changes were attributable to reductions in HDL apoA-I fractional catabolic rates. In addition, torcetrapib did not significantly change serum markers of cholesterol or bile acid synthesis or fecal sterol excretion [61].

The last study published in the literature, until the present review was written as an abstract, regarding multicentric clinical studies (n=162 y n=174, respectively), double-blind. Such studies include men who present HDL-C lower than 44 mg/dl and women who present HDL-C less than 54 mg/dl [62]. One of them used several doses of torcetrapib alone (10, 30, 60 and 90 mg) or placebo and the others used atorvastatin 20 mg doses per day. The assay carried out with the CEPT inhibitor has shown a decrease of HDL-C of about 9% to 54.5% employing 30 mg up to the maximum doses.

LDL-C decreased 16.5% only at the 90 mg dose. The assay associated with atorvastatin decreased the HDL-C about 8.3% to 40.2% by employing 30 mg of torcetrapib up to the maximum dose. The LDL-C decreased 18.9% at 90 mg dose of torcetrapib. Both studies did not show dose dependent increase on the side effects, however small increases in the systolic and diastolic blood pressures were observed.

There are not many clinical studies carried out with torcetrapib and they were performed during a short period of time. Such studies have shown that the levels of HDL-C increase, however no conclusions on morbidity, mortality or security on long term basis can be extracted.

CURRENT & FUTURE DEVELOPMENT

The epidemiologic studies mentioned with the therapeutic studies carried out with gemfibrozil have shown the relation between low levels of HDL-cholesterol and cardiovascular morbimortality, and also the benefit obtained from the increase of HDL-cholesterol. The use of an inhibitor of the CETP, torcetrapib, in order to increase the HDL-cholesterol could be a good therapeutic alternative. Indeed, nowadays there is not much information regarding the efficiency and security of the drug. The published studies have a small sample size subrogated markers (HDL-cholesterol, apoA-I, LDL-cholesterol, triglycerides) have been used in order to assess the effect. Prospective studies are necessary, with more patients and aimed to reach several aspects associated with cardiovascular morbimortality (death caused by cardiovascular problems, heart attack and coronary stenosis).

The effects of this drug on total cholesterol and cholesterol-LDL are minor; therefore, it will be mostly used with statins, especially for secondary prevention and it is also likely to be used on primary prevention in metabolic syndrome.

An interesting feature fact are the anti-inflammatory and antioxidant effects of torcetrapib resulted from the increase of HDL-cholesterol. The association to statins could produce a synergic effect with them and also improve the pleiotropic action of the statins. It could allow its use on other chronic diseases, such as osteoporosis, whose epidemiology is similar to that of atherosclerosis, case in which statins could be useful.

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