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Hypolipidemic Therapy and HDL

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ABSTRACT

Statins significantly reduce cardiovascular events by reducing low-density-lipoprotein cholesterol (LDL-C). However, despite achieving the LDL goals, a substantial residual risk remains. It appears that low levels of high-density lipoprotein cholesterol (HDL-C) constitute another independent risk factor for coronary artery disease (CAD). Therefore, raising HDL levels appears an attractive target to further reduce the cardiovascular risk. However, classical HDL-reducing drugs (nicotinic acid derivatives, fibrates) are not well tolerated and newer agents (torcetrapid), which seemed effective in doing that, had to be withdrawn due to increased mortality. Nevertheless, due to the importance of HDL in influencing the cardiovascular risk, efforts in developing safer drugs are continuing. Such pharmacological developments, as well as the nonpharmacological approaches to increase HDL, and other HDL-targeted therapies are discussed in this overview.

KEY WORDS: *cardiovascular disease; dylipidemia; HDL-cholesterol; hypolipidemic agents; nicotinic acid; CETP inhibitors; fibrates; statins*

INTRODUCTION

Cardiovascular disease remains the leading cause of morbidity and mortality in the developed countries. Over the last two decades we have witnessed an impressive reduction in cardiovascular risk mainly with the use of 3-hydroxy-3-methylglutaryl coenzyme A inhibitors (statins), which are directed to reduce low-density-lipoprotein cholesterol (LDL-C). The use of these drugs has resulted in a 21% reduction in the risk of major cardiovascular (CV) events, for every 39 mg/dl (1 mmol/l) decrease in LDL-C.[1] More aggressive LDL lowering using high-dose statins has led to even greater reductions in the risk of future CV events.[2,3] However, despite achieving the LDL goals recommended by ATP III guidelines,[4] a substantial residual risk remains, and it appears that an approximate 70% of coronary events have not been prevented in statin trials.[5,6] On the other hand, early epidemiological data showed that low levels of high-density lipoprotein cholesterol (HDL-C) constitute an independent risk factor for coronary artery disease (CAD).[7] Therefore, raising HDL levels appears an attractive target in the effort of further reducing cardiovascular risk.

ABBREVIATIONS
CAD = coronary artery disease
CETP = cholesteryl ester transfer protein
CV = cardiovascular
HDL = high-density lipoprotein
IVUS = intravascular ultrasound
LDL = low density lipoprotein
NO = nitric oxide
VLDL = very low density lipoprotein

EPIDEMIOLOGY

From the 1970s, the Framingham study showed that low HDL levels were associated with increased risk of cardiovascular events. More recent data confirm these observations, suggesting that a 2-3% increase in CV risk results from every 1mg/dl decrease in HDL-C.[8] The inverse relationship between HDL and CAD was also demonstrated in

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the PROCAM study, where patients with HDL-C <35 mg/dl had a 70% increased risk of developing CAD, compared to those with an HDL-C above this value.[9]

The prevalence of dyslipidemia varies with the population being studied and abnormalities in lipoprotein metabolism are more common in subjects with premature CAD than age-matched controls. There are several studies, suggesting that low levels of HDL are quite common in the general population, affecting 33% of men and 40% of women.[10] It should also be noted that low HDL is frequently a component of the metabolic syndrome, which affects 24% of adults older than 20 years in the US.[11,12] In these patients, as well as in those with type 2 diabetes mellitus, low HDL levels coexist with high triglycerides and small, dense LDL particles which consist the "lipid triad". It is therefore not surprising that the third report of the National Cholesterol Education Program (NCEP) ATP III guidelines, consider HDL levels below 40 mg/dl as an independent major risk factor for CAD, which should be addressed after the LDL and triglyceride goals are met.[4]

HDL METABOLISM AND PLEIOTROPIC EFFECTS

Although traditionally thought to protect against cardiovascular disease through reverse cholesterol transport, HDL acts through multiple pathways that are sometimes incompletely understood. In other words, HDL also exerts antiatherogenic effects that are non-cholesterol dependent.[13] Antioxidant, antiapoptotic, anti-inflammatory, antithrombotic and direct endothelial effects of HDL have all been investigated and possibly contribute to its antiatherogenic properties.

REVERSE CHOLESTEROL TRANSPORT

The metabolism of lipid molecules in the human body involves the transfer of cholesterol from the liver to the periphery, to be used for cell membrane integrity and steroid hormone synthesis, which is mediated by Apo- B containing lipoproteins (VLDL and LDL) and the transfer of cholesterol in the opposite direction i.e. from periphery to the liver, which is mediated by Apo A-I containing lipoproteins (HDL). The latter process is known as "reverse cholesterol transport".[14]

The major apolipoprotein of HDL is Apo A-I, comprising 70% of HDL protein. The liver and intestine synthesize Apo A-I which interacts with adenosine-triphosphate-binding cassette transporter A1 (ABCA1), located in the macrophages in order to achieve the transfer of free cholesterol from foam cells to the lipid-poor Apo A-I. Thus, nascent pre-b-HDL is created, which is converted to mature a-HDL (HDL2 and HDL3). HDL2 particles are smaller and denser, while HDL3 particles are larger and less dense. The interconversion between the two is achieved via the action of the enzymes, hepatic and endothelial lipase and lecithin-cholesterol acyltransferase (LCAT). Those mature HDL particles can accept more cholesterol from the macrophages via the ATP-binding cassette transporter G1 (ABCG1). The cholesterol obtained from the periphery can then be returned to the liver via two pathways. In the *direct pathway*, cholesteryl esters are taken by hepatocytes via the scavenger receptor type B1 (SRB1). In the *indirect pathway*, there is an exchange of cholesteryl esters for triglycerides between the mature HDL particles and VLDL and LDL, which is mediated by the enzyme cholesteryl ester transfer protein (CETP). HDL is enriched in triglycerides, while VLDL and LDL transfer their cholesteryl esters back to the liver via the LDL receptor. The latter mechanism may be responsible for 50% of cholesterol transfer back to the liver. The process of reverse cholesterol transport provides the most common explanation for the antiatherogenic effects of HDL, as cholesterol is taken from the periphery i.e. macrophages and foam cells and returned back to the liver to be excreted into the bile and gut. However, as already mentioned, HDL has other antiatherogenic properties that contribute to improve endothelial function and reduce the overall CV risk.

ENDOTHELIAL ACTIONS OF HDL

There are studies which suggest that HDL increases nitric oxide (NO) production from nitric oxide synthase (eNOS).[15,16] NO bioavailability is known to be impaired in the early stages of atheroma formation. In addition, therapies directed to increase HDL, like nicotinic acid[17] and infusion of Apo A-I,[18, 19] have been shown to improve endothelial function. There are also data which show that HDL-C improves the proliferation and migration of endothelial cells, thus promoting the integrity of the inner layer of blood vessels.

ANTIOXIDANT EFFECTS OF HDL

The oxidation of LDL is an important step in the formation of fatty streaks at the initial stages of atheroma development. HDL particles carry enzymes such as paraoxonase 1 and LCAT which, along with Apo A-I, exert antioxidant effects by inhibiting phospholipid oxidation that leads to the production of minimally modified LDL.[20,21] Interestingly, a recent study showed that low paraoxonase 1 activity was an independent predictor of coronary events.[22]

ANTIAPOPTOTIC EFFECTS OF HDL

Several particles such as oxidized LDL, TNFa and homocysteine contribute to the apoptotic cell death of endothelial cells, thus promoting disruption of the inner layer of blood vessels and inducing acute thrombotic events. There is evidence that HDL decreases endothelial cell apoptosis through several mechanisms, involving the TNFa pathway and the activation of protein kinase Akt.[23,24]

ANTITHROMBOTIC EFFECTS OF HDL

There is growing evidence that HDL particles have antithrombotic properties. The above mentioned increase in NO levels, along with increased prostacyclin release, as well as the decrease in the activity of adhesion molecules and the anti-apoptotic activity, act in concert to prevent thrombus formation.[25-27] In addition the sphingolipids, contained in the HDL particles, exert various antithrombotic effects. Finally, HDL also acts as an anticoagulant factor enhancing the inactivation of Factor Va by activated protein C and protein S.[28]

ANTIINFLAMMATORY EFFECTS OF HDL

Atheroma formation is definitely an inflammatory process. The expression of adhesion molecules such as vascular cell adhesion molecule (VCAM-1) and intercellular adhesion molecule (ICAM-1), leads to the attachment of monocytes to endothelial cells. The migration of the former to the sub-endothelial layer is stimulated by chemoattractants such as monocytes chemotactic protein-1 (MCP-1). HDL particles interrupt the TNFa stimulation of the adhesion molecules and also neutralize CRP's proinflammatory activity.[29,30]

NON-PHARMACOLOGICAL APPROACH TO INCREASE HDL LEVELS

AEROBIC EXERCISE

Aerobic exercise has been reported to increase HDL levels by 5-10%. Ideally, one should exercise briskly for 30 minutes, 5 times per week, and the strongest determinant of achieving optimal HDL levels is the total duration of exercise exceeding 120 minutes per week.[4,31] The patients most likely to benefit from exercise are males with low HDL levels, high triglycerides and abdominal obesity.[32]

SMOKING CESSATION

Smoking is known to reduce HDL levels although the mechanism remains elusive. Nevertheless, tobacco cessation is said to increase HDL levels by 5-10% and should be strongly encouraged via a multidisciplinary approach involving counseling and if necessary appropriate medications. Smoking also constitutes a source of oxidative stress that can impair the function of HDL.[4,33] One study showed that smoking cessation led to an increase in HDL by 4 mg/dl.[34]

WEIGHT REDUCTION

It is well known that obesity and in particular visceral obesity is associated with high levels of triglycerides, low levels of HDL and small, dense LDL particles. The patients who derive most benefit from weight loss in terms of HDL raising, are the overweight or obese ones. It has been reported that for every kilogram lost, an increase of 0.35 mg/dl is observed in HDL-C.[35] It is worth mentioning that according to ATP III guidelines, overweight and obese individuals should aim for a body mass index of less than 25, and the rate of weight reduction should be 2 kg per month.[4] In addition, weight loss also improves other cardiometabolic risk factors.

DIETARY MODIFICATIONS

Although the consumption of saturated and trans-fatty acids, increases HDL levels, it also increases LDL levels as well as the expression of adhesion molecules.[36] It appears that the consumption of n-3 polyunsaturated fatty acids increases HDL levels and provide cardiovascular benefits.[37] Moreover, a recent study showed that a low-fat, and rich in fibers diet, along with exercise is helpful in improving the quality of HDL particles, increasing their anti-inflammatory properties.[38] Therefore, patients should be advised to replace saturated and trans fatty acids with monounsaturated and n-3 polyunsaturated ones, consuming plant oils, nuts and fish.[4]

ALCOHOL CONSUMPTION

The beneficial effects of mild to moderate alcohol consumption on CV risk have been known for many years. It appears that 1-3 drinks per day offer some degree of protection, via various mechanisms, but they also increase HDL levels.[39,40] Of course every benefit will be lost if the alcohol consumption is not kept within the limits stated by the ATP III guidelines.[4] Moreover it appears that the benefits of alcohol are influenced by the patients' age and gender.

PHARMACOLOGICAL APPROACHES TO INCREASE HDL

NICOTINIC ACID (NIACIN)

Nicotinic acid is part of the Vitamin B complex and has been in use for the treatment of hyperlipidemia for the past 50 years. When tolerated, it is the most effective HDL-C increasing agent currently available. It has been shown to increase HDL-C by 15-35%, and a recent meataanalysis showed an average increase of 16%.[41] There are several mechanisms through which niacin increases HDL levels. It reduces VLDL synthesis by the liver and therefore attenuates the exchange of triglycerides for cholesterol mediated by CETP. This results in larger HDL particles, containing more cholesterol, which have delayed catabolism.[42] Secondly, nicotinic acid reduces the uptake of HDL by the liver.[43] Thirdly, it enhances reverse cholesterol transport via ABCA-1.[44] Finally, a recent study showed that niacin stimulates Apo A-I synthesis.[45] Other favorable effects of nicotinic acid on lipid metabolism include lowering of LDL and triglycerides as well as lipoprotein Lp(a) levels.[46] It appears that LDL particles are converted from small, dense and therefore more atherogenic to larger, buoyant and less harmful ones.

Many studies have proven the efficacy of nicotinic acid in

augmenting HDL-C with favorable clinical outcomes. The Coronary Drug Project, where niacin was used in patients with a history of myocardial infarction, demonstrated a reduction in CAD death, non-fatal myocardial infarction, cerebrovascular events and, although not shown at first, an 11% mortality reduction which became apparent 9 years after the termination of the trial.[47] Several studies have tested the efficacy of nicotinic acid combined with fibrates and bile acid sequestrants, all showing favorable outcomes on cardiovascular events.[48-50]

Niacin has also been combined with simvastatin in a study that tested their efficacy as well as the use of antioxidants in patients with clinical and angiographic evidence of CAD, normal LDL-C and low HDL-C.[51] The combination arm resulted in substantial decrease in LDL levels and a 26% increase in HDL-C. Angiographic regression was also observed in the simvastatin plus niacin arm and these patients were significantly less likely to have a first cardiovascular event (death, myocardial infarction, stroke, or revascularization). The study also showed that the use of antioxidants was not beneficial, which is also the conclusion of most other clinical trials. The efficacy of extended-release niacin on CAD patients with low HDL-C, already receiving a statin was tested in ARBITER-2 study, where the levels of HDL-C increased by 8 mg/dl on average and there was a trend towards decreased progression of carotid intima-media thickness at one year.[52]

Unfortunately the side effects of nicotinic acid, mainly facial flushing, pruritus and hepatotoxicity, have limited its use. Flushing is initially seen in 80% of patients and disappears over time. It is caused by stimulated biosynthesis of prostaglandin D2 which acts as a vasodilator. *Laropiprant*, a selective antagonist of the prostaglandin D2 receptor, was shown to reduce flushing, resulting in less patients discontinuing the medication.[53] Flushing is also reduced by the use of a longer-acting formulation of nicotinic acid instead of the immediate release one. The former has been shown to be equally effective in increasing HDL-C.[54] Interestingly, recent data show that flushing may be a sign of a good response to HDL levels, as patients in the ARBITER-2 study who reported flushing had a greater increase in HDL-C, compared to those without flushing.[55] The effects of combining simvastatin with prolonged-acting niconitic acid will be addressed in the AIM-HIGH study which enrolls patients with known cardiovascular disease with raised triglycerides and low HDL-C. Although there has been some concern about niacin disturbing glycemic control in diabetics with dyslipidemia, recent data have not shown any significant effect on glucose levels.[56,57]

STATINS

Statins are known to modestly increase HDL-C by 5-15% and this is more pronounced with rosuvastatin.[58] Statins increase hepatic Apo A-I synthesis, decrease levels of CETP and increase the activity of the antioxidant enzyme paraoxonase. Statins are known to have other beneficial effects besides LDL lowering. Nevertheless, patients in the heart Protection Study who had lower HDL-C levels, had a greater reduction in CV risk.[59] However, despite the great benefits derived from statin therapy, the only modest increase in HDL-C does not permit their use as monotherapy when the target is to increase HDL.

FIBRIC ACID DERIVATIVES (FIBRATES)

Fibrates act on peroxisome proliferator-activated receptor a (PPARa), a nuclear receptor which activates genes for Apo A-I and Apo A-II. With the increase in lipoprotein lipase activity, fibrates clear VLDL particles and lower triglycerides. As a result, they increase HDL-C by 10-20%, lower LDL-C by 10-15% and mostly reduce triglycerides by 40-50%. Both the Helsinki Heart Study [60] and the VA-HIT study,[61] treatment with gemfibrozil reduced cardiovascular events. In fact the VA-HIT trial was the first to demonstrate the beneficial effect of HDL-C raising on major coronary events, as a multivariate analysis correlated the CAD death and nonfatal MI with the levels of HDL-C independently of LDL and triglycerides.[62] One should bear in mind that the combination of statins with fibrates, and in particular gemfibrozil, should be closely monitored due to the increased risk of myopathy and *rhabdomyolysis*. The role of fibrates is mainly in the treatment of hypertriglyceridaemia and potentially in the treatment of high-risk patients with low HDL and high triglycerides.

NOVEL HDL-TARGETED THERAPIES

CETP INHIBITORS

The enzyme cholesterol ester transfer protein (CETP) holds a key role in reverse cholesterol transport, as already mentioned, since it exchanges cholesterol esters for triglycerides, permitting the uptake of cholesterol esters by the hepatocytes via the LDL receptor. Inhibition of CETP activity has resulted in increased levels of HDL and reduced levels of VLDL, LDL and triglycerides.[63] Torcetrapib, was a direct CETP inhibitor which was shown to significantly increase HDL.[64,65] Unfortunately, it was withdrawn from further development as the results of the ILLUMINATE trial,[66] where patients were treated with atorvastatin and torcetrapib or atorvastatin alone, showed a significant increase of cardiovascular events as well as all-cause mortality in the torcetrapib arm. This was despite a significant increase in HDL-C levels (72%) and an additional reduction in LDL-C. The disappointing results led to the early discontinuation of the trial. Three other trials with torcetrapib (ILLUSTRATE, RADIANCE 1 and 2), showed no decrease in coronary atheroma as evaluated by IVUS and no change in carotid intima-media thickness, despite the increase in HDL-C.[67-69]

In the ILLUMINATE trial, a significant increase in blood

pressure of 5.4 mmHg, was observed, compared to the placebo group. This effect was also noted in the other torcetrapib trials and it appears to be a molecule-specific effect rather than a result of CETP inhibition. This could be one of the reasons that led to the negative results of ILLUMINATE. Other potential explanations include the production of HDL particles that are defective in terms of reverse cholesterol transport, the production of pro-inflammatory HDL possibly through oxidation of phospholipids and finally possible interactions between atorvastatin and torcetrapib.[70] The above raise once more the issue of paying attention not only to the absolute HDL levels, but also to the quality of the HDL particles. For example, it is known that HDL2 particles, containing only Apo A-I, are mostly effective in the reverse cholesterol transport, while the HDL3 ones, containing both Apo A-I and Apo A-II, are less effective and indeed may increase visceral fat accumulation and impair VLDL metabolism.[71,72]

Newer CETP inhibitors include dalcetrapib and anacetrapib. *Dalcetrapib* has been investigated in animal and human studies and it seems to significantly raise HDL-C without significant side-effects,[73,74] both alone and in combination with statin therapy. Since the mechanism of action of dalcetrapib is different from that of torcetrapib, resulting in modulation rather than inhibition of CETP, the drug may prove valuable in the quest of raising HDL-C. *Anacetrapib* is another CETP inhibitor that has been tested in phase I and II clinical trials with encouraging results. The combination with atorvastatin resulted in significant LDL reduction and HDL increase, without any significant increase in blood pressure.[75] Obviously, further research is needed before any definite recommendations can be made regarding the use of CETP inhibitors in clinical practice.

THIAZOLIDINEDIONES (PPAR-γ AGONISTS)

They are part of the PPAR agonist family which includes fibrates (PPARa agonists). Their use is in type II diabetes mellitus and they have been shown to increase HDL levels by 5-15%.[76,77] However, in a recent meataanalysis, rosiglitazone has been associated with a 40% increase in the risk of myocardial infarction,[78] possibly due to an LDL-increasing effect and the precipitation of heart failure in predisposed patients. A dual PPARa and PPAR γ agonist (muraglitazar) has been developed but despite increasing HDL-C by 16%, it has been withdrawn as it increased mortality, nonfatal myocardial infarction, stroke, transient ischemic attacks and congestive heart failure.[79]

CANNABINOID-1 RECEPTOR BLOCKERS

Rimonabant was the first cannabinoid receptor blocker that was used for managing obesity. In the hope to improve cardiometabolic risk, it was tested in overweight and obese patients, as well as in patients with type II diabetes mellitus and in the dose of 20 mg/day, it resulted in significant weight loss, reduction in waist circumference, and improvements in several metabolic risk factors. Rimonabant was found to increase HDL-C more than expected from weight loss alone, implicating a direct action on HDL metabolism.[80,81] Unfortunately, it was also shown to have significant central nervous system toxicity, mainly anxiety and depression, which led to its withdrawal from further clinical trials.

APO A-I AND PHOSPHOLIPID THERAPIES

Apo A-I Milano is a naturally occurring mutant of Apo A-I discovered in a small village in Italy, Limone sul Garda. Although it is associated with very low HDL levels, it does not result in clinical atherosclerosis.[82] A recombinant form of Apo A-I Milano has been manufactured and tested in patients with acute coronary syndromes.[83] Patients received 5 weekly infusions of placebo or recombinant Apo A-I Milano at 15 mg/kg or 45 mg/kg and intravascular ultrasound was performed within 2 weeks following the acute coronary syndrome and repeated after 5 weekly treatments. The trial demonstrated a significant regression of coronary atherosclerosis as measured by IVUS.

Reconstituted human HDL (rHDL) was tested in the ERASE trial,[84] where patients were assigned to four weekly infusions of either placebo or two different doses of CSL-III, a reconstituted HDL consisting of Apo A-I from human plasma and soybean phosphatidylcholine. The results showed no significant reductions in percentage change in atheroma volume or nominal change in plaque volume, but there was a statistically significant improvement in the plaque characterization index on intravascular ultrasound, and coronary score on quantitative coronary angiography. The authors concluded that further research is warranted.

Apo A-I mimetics are peptides, smaller than the 243-aminoacid Apo A-I, which are developed to resemble the lipid-binding domain of Apo A-I. An orally active compound, D-4F was shown to reduce atherosclerosis in mice.[85] D-4F was recently tested in humans,[86] showing that it has a low bioavailability, which is improved under fasting conditions, and it was safe and well tolerated. It appears that the main mechanism of action of Apo A-I mimetic peptides is the remarkable binding affinity that oxidized lipids have for them, compared with Apo A-I.[87] D-4F has also been reported to induce pre-β HDL formation which is the form of HDL the most active in promoting cholesterol efflux from macrophages via ABCA1 transporter.[88] Although preliminary, the above results indicate that Apo A-I mimetic peptides are a promising therapeutic strategy.[87]

Besides Apo A-I, the HDL particle consists, to a great extent of phospholipids and these have recently been tested as therapeutic options. An oral synthetic phospholipid (DMPC) was tested in a murine apoE null model, showing an increase in HDL levels and function, thereby reducing atherosclerosis.[89] Phosphatidylinositol, another phospholipid, was tested in hu-

mans and also demonstrated an increase in HDL-C and Apo A-I.[90] A potential therapeutic target for the future is the inhibition of endolipase, which is an enzyme secreted by endothelial cells and hydrolyses HDL phospholipids, thereby reducing HDL levels. There is evidence that the levels of endolipase are inversely related to HDL levels.[91] Lastly, the two main transporters for cholesterol efflux from macrophages to HDL are ABCA1 and ABCG1. Both of them are regulated by the nuclear liver X receptor (LXR). Therefore the stimulation of LXR would appear promising as a means to augment reverse cholesterol transport. The available data are yet limiting but show a positive effect on atherosclerosis progression.[92,93]

CONCLUSIONS

Over the last few decades we have witnessed a dramatic decrease in cardiovascular risk mainly by targeting LDL cholesterol. The substantial residual risk that remains after statin treatment, represents a challenge and there are enough epidemiological as well as experimental and clinical data to show that it can be reduced by targeting low HDL cholesterol. The ATP III guidelines state that LDL is the primary target and HDL should be addressed after non-HDL goals are met, the preferred drugs being nicotinic acid and fibrates. Nevertheless, there are a lot of emerging therapies such as CETP inhibitors, rHDL and Apo A-I Milano as well as HDL-mimetic peptides that exhibit promising results and certainly constitute a hopeful prospective in reducing CV events in our patients.

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