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Guest Editorial

HDL-C AS A NEW PARADIGM IN ATHEROSCLEROTIC DISEASES

Atherosclerotic diseases are a leading cause of disability and death worldwide (1) and two thirds of these cases are associated with dyslipidemia. Even though low level of high density lipoprotein-C(HDL-C) (<40 mg/dl) is a potent and an independent risk factor of atherosclerosis, current National Cholesterol Education Program/Adult Treatment Panel (NCEP/ATP) guidelines mainly focus at reducing low density lipoprotein-C (LDL-C) levels with Statin therapy(2). Mega trials have conclusively shown that even after achieving the LDL-C goal in patients suffering from atherogenic dyslipidemia the coronary heart disease (CHD) risk decreases only by 25%–35% (3). Long term observational studies, such as the Framingham Heart Study in US, provided evidence about CHD relative risk which decreased by 2–3% with every 1 mg/dl increase in HDL-C while with every 1 mg/dl decrease in LDL-C the risk decreased by one percent (3). Therefore this evidence projects the importance of treating both lipid parameters effectively in order to decrease the CHD risk further.

Approximately 10% of the global population is affected by dyslipidaemia. In the developed countries (US, Europe and Japan) there are more than 240 million people with abnormal lipoprotein levels. Of these, more than 55 million have low levels of HDL-C and/or high triglyceride levels and could be candidates for HDL-C raising therapies. However, it should be noted that for low-risk patients the first step to controlling serum lipids levels might be a lifestyle change. Among other benefits, weight reduction, physical exercise and quitting tobacco smoking could increase HDL-C by 5–30 percent (4). Cardioprotective effect of HDL-C is mainly exerted by facilitating the reverse cholesterol transport and other adjuvant effects such as anti-inflammatory, antioxidant, anticoagulant and fibrinolysis. Recently published studies indicate a regulatory role for HDL-C in endothelial function. The binding of HDL-C to scavenger receptor class BI (SR-BI) leads to the activation of endothelial nitric oxide synthase (eNOS) and therefore enhances vasorelaxation(5).

HDL metabolism and reverse cholesterol transport (RCT) pathway

Atherogenic apoB containing lipoproteins (VLDL, LDL, IDL, apoA) deposit cholesterol into the arterial wall while on the other hand HDL mediates rapid egress of this deposited cholesterol by attachment to peripheral cells by SR-BI receptor and carries out efflux of cholesterol via ATP Binding Cassette

A-1 (ABCA-1) membrane transporter present on peripheral cells. The nascent HDL particles which facilitate this initial step are then acted upon by lecithin cholesterol acyl transferase (LCAT) enzyme found in plasma which esterifies the acquired free cholesterol and in the process transforms nascent HDL (pre-β1) into larger HDL-3 particles (3). These particles progressively increase to form spherical, more cardioprotective, mature HDL-2 Recently it has been discovered that ATP binding Cassette gradient 1(ABCG-1), an ABC transporter of unknown function, can promote cholesterol efflux from cells, including macrophages, to the major forms of plasma HDL that is, HDL-2 and HDL-3 (Fig. 1). In contrast to ABCA-1, ABCG-1 is a half transporter that might act as a homodimer. ABCG-1 is an liver X receptors (LXR) target gene and shows a particularly high expression level in macrophages (5).

HDL-2 are captured by the SR-BI receptor from plasma and depleted of their cholesterol content following which they are released back into the circulation (3). The excretion of hepatocyte cholesterol into bile is mediated by ABCG-5/ABCG-8, which are half-transporters that act as heterodimers. Therefore, at least three of the known mammalian members of the ABCG transporter family (there are six members in total) are LXR targets and have a role in reverse cholesterol transport(RCT) (5). Also another apoprotein associated with HDL in small quantities, i.e apoE was found to uniquely facilitate RCT by allowing cholesterol ester (CE)-rich core expansion in HDL (6).

Cholesteryl ester transfer protein (CETP) is a plasma glycoprotein which mediates the transfer of cholesterol esters from mature HDL particles to apoB containing lipoproteins in exchange for triglycerides from them (3). Approximately two thirds of cholesterol associated with HDL is removed by this pathway.

Currently the drugs used in the management of atherogenic dyslipidemia mainly focus at reducing LDL levels and elevate HDL levels only by a minimal to modest extent. Statins increase HDL by 5-15%, Bile acid sequestrants by 3–5%, Fibric acid derivatives by 10–20%, Ezetimibe 1–2% and Niacin increases it by a significant 15–35% but its associated toxicities limit its use (2).

Low HDL-**C** level in the presence of associated normal LDL-C level is also recognized as a potent risk factor but is currently managed by LDL lowering drugs as presently drug therapies which exclusively raise HDL-C levels have not been synthesized.

The idea that raising HDL levels could be of therapeutic benefit was first conceptualized in 1970 but until recently the receptors of HDL and reverse cholesterol pathway were not well characterized (3). Further, it has been difficult to study specific effects of HDL in isolation as current therapy affects multiple lipids. Hence much progress has only been made recently in developing drugs which target HDL (Fig. 1).

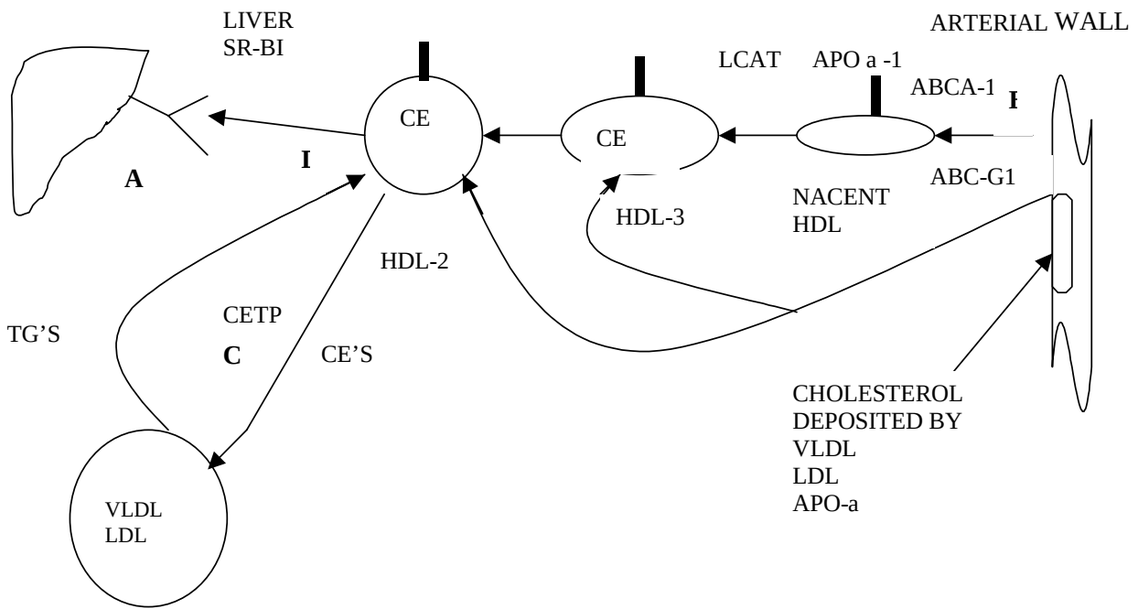

Fig. 1 : Intravascular HDL metabolism and potential targets for therapeutic normalization of abnormal metabolism and deficient biological activities of HDL.

Llipoproteins (VLDL,LDL,IDL,apoA) deposit cholesterol into the arterial wall and HDL mediates rapid egress of this deposited cholesterol by attachment to peripheral cells by SR-BI receptor and carries out efflux of cholesterol via ATP Binding Cassette A-1 (ABCA-1) membrane transporter present on peripheral cells. The nascent HDL particles which facilitate this initial step are then acted upon by LCAT enzyme found in plasma which transforms nascent HDL (pre-β1) into larger HDL_3 particles. These particles progressively increase to form cardioprotective, mature HDL-2 HDL-2 are captured by the SR-BI receptor from plasma and depleted of their cholesterol content following which they are released back into the circulation. CETP is a plasma glycoprotein which mediates the transfer of cholesterol esters from mature HDL particles to apoB containing lipoproteins in exchange for triglycerides from them. Potential targets for therapeutic normalization of abnormal metabolism and deficient biological activities of HDL include: (A) upregulation of apoAI synthesis in the liver (B) accelerated efflux of cholesterol and phospholipid from peripheral cells mediated by ABCA1 and/or ABCG1; (C) decreased activity of CETP resulting in the diminished heteroexchange of CE and triglyceride between HDL and triglyceride-rich lipoproteins with normalization of HDL-particle turnover; (D) inhibition of HDL2 holoparticle uptake by the liver mediated by hitherto unidentified receptor(s) for HDL holoparticles.

Emerging therapies to elevate HDL (Table I)

In a double blind, randomized control trial apoA-I Milano was given as 5 weekly injections of either 15 mg/kg or 45 mg/kg to 57 patients suffering from acute coronary syndrome. Results which were obtained were dramatic (7). A decrease in atheroma volume of up to 4.6% was noted in both treatment groups. In the treated groups, there were occasionally significant events, which may or may not have been drug-related, including gastrointestinal side effects, an episode of cholelithiasis, and one hypersensitivity reaction. The study was designed as a pilot study and compared 45 patients receiving the

TABLE I: Emerging therapies to elevate HDL.

	Drug	*Route of administration*	*Stage of development*
1	ApoA-1 Milano peptide	Injectable	III
2	ApoA-1 mimetic peptide(D4F)	Oral	Phase I
3	CETP Inhibitor	Oral/Injectable	Phase II/III
4	LCAT activators	Injectable	Phase I/II
5	PPAR alpha agonists	Oral	Phase I/II/III
6	LXR activators	Injectable	Preclinical
7	Rimonabant	Oral	III
8	EL/HL Inhibitors	Oral	Preclinical Phase I/II

apoA-I Milano infusions with 12 patients receiving placebo (5).

ApoA-I Mimetic Peptide (D4F) is a synthetic peptide containing 18 D-amino acids and therefore has the advantage of oral administration. It has an amphipathic helix with a hydrophobic face that binds lipids in a manner similar to apoA-I. When tested on atherosclerosis prone mouse models by adding D4F to drinking water and subsequently an increase in nascent HDL, decrease in macrophage accumulation and restored vascular reactivity was noted. Aortic atherosclerosis was reduced by 75 percent.

Cholesteryl ester transfer protein (CETP) inhibitors such as JTT705 are in Phase II clinical trial. The results of the dose finding trial done on 198 hyperlipidemic patients showed that the maximum dose of JTT705 (900mg/day) raised HDL by 34%, decreased LDL by 7% by inhibiting CETP upto 37 percent (8). Despite the potential benefit of such an antiatherogenic HDL profile in these subjects, there has been a conflict in the literature as to whether CETP deficiency is pro- or antiatherogenic in humans. Recently a CETP inhibitor Torcetrapib was pulled out of Phase III clinical trial after it was found cause to sudden death in few individuals even though it increased HDL levels significantly. Thus, it is unclear whether the CETP inhibitors currently in clinical trials would promote or prevent atherosclerosis and coronary heart disease.

LCAT gene defects are associated with low levels of HDL-C and an increased incidence of atherosclerotic diseases. LCAT activators enhance the activity of the LCAT enzyme therefore increase conversion of nascent HDL into larger, spherical, more cardioprotective HDL-2 particles. These LCAT activators are currently undergoing phase I/II trials.

The PPAR family of nuclear receptor transcription factors consists of the three members PPARα, PPARγ and PPARδ. While each of these nuclear receptors controls a distinct network of target genes a common feature of many of the PPAR-regulated genes is their involvement in fatty-acid metabolism.

Fibrates act as PPARα agonists; however, they are comparatively weak agonists. Therefore a number of potent and more selective PPARα agonists are currently under trial which promise to become useful drugs for managing metabolic syndrome and atherosclerosis. PPARα agonists induce ABCA-1 and SR-BI expression in macrophages, thereby enhancing the first steps of macrophage RCT. Expression of the major human HDL apolipoprotein genes apoA-I and apoA-II is activated in response to fibrate treatment in vitro and in humans via direct

transcriptional control by PPARα (9).

PPARγ is another member of the PPAR family of nuclear receptor transcription factors. It is most highly expressed in adipose tissue and has been shown to be essential for adipocyte differentiation and normal glucose metabolism. Glitazones act as high-affinity agonists by directly binding to PPARγ. It has been suggested that glitazones could act as potent inducers of ABCA-1 and promote macrophage cholesterol efflux; however, these findings have not been uniformly observed. The overall effect of glitazones on plasma HDL levels seems to be small, increasing HDL by an average of 3–5%, however, recently the insulin-sensitizing effects of PPARγ activators have been found be important in reversing pro-atherogenic changes in macrophage foam cells reflecting macrophage insulin resistance or in endothelial cells reversing impaired eNOS expression. Recent evidence indicates that *in vivo* PPARγ activators protect against atherosclerosis but do not up regulate ABCA-1. By contrast, they induced macrophage ABCG-1 and cholesterol efflux to HDL, indicating that this might be a key mechanism of protection by this class of drugs.

Another potentially important mechanism by which both PPARα and PPARγ agonists could attenuate the development of atherosclerosis is through an anti-inflammatory effect, which has been suggested for both these nuclear receptors (10). PPARα and PPARγ are expressed in cell types that are relevant to the regulation of the immune system and the pathogenesis of atherosclerosis, such as macrophages, monocytes, smooth-muscle cells and vascular endothelial cells. Since type 2 diabetes patients often suffer from dyslipidemia and other metabolic abnormalities, thus putting them at high risk for cardiovascular disease, with its dual (α/γ) PPAR activity, has the potential to be useful for the treatment of these patients. Several studies report that activation of both PPARα and PPARγ can inhibit NF-κB signalling and suppress the secretion of various pro-inflammatory cytokines. Other possible anti-inflammatory mechanisms that have been suggested for both receptors include inhibition of the vascular adhesion of monocytes by reduced chemokine-receptor and adhesion-molecule expression and inhibition of monocyte–macrophage migration into lesional areas. In the 12-week dose-ranging Study (GLAD) in Insulin Resistance (SIR), the dual PPARα/γ agonist **tesaglitazar** at 0.5mg and 1.0 mg doses reduced fasting insulin and plasma glucose levels and improved the atherogenic dyslipidemic profile in insulin-resistant, non-diabetic patients by increasing HDL. However, reductions in hematologic measures and reversible, dose-dependent increases in serum creatinine were seen in **tesaglitazar** treated patients (11). Ragaglitazar has been shown to increase HDL, lower triglycerides and increase insulin sensitivity in patients with type II diabetes and in animal models of diabetes.

The third member of the PPAR family of nuclear transcription factors, PPARδ has emerged as a powerful regulator of fatty acid catabolism and energy homeostasis and provides another potential drug target in the treatment of atherosclerosis. Little is known about the effect of PPARδ agonists in

humans, but the results obtained in some animal and cellular models hint at PPARδ as another promising drug target in the nuclear receptor superfamily.

Intense research has been devoted to developing activators of the LXR as drugs that can raise plasma HDL levels and inhibit the progression of atherosclerosis (5). LXRs are ligand-activated transcription factors that belong to the nuclear receptor superfamily. LXRs were first named orphan nuclear receptors as their endogenous activators were unknown when they were cloned.

Two isoforms, LXRα and LXRβ, have been identified and both have emerged as central regulators of genes that are involved in lipid metabolism. LXRα is primarily expressed in the liver, kidney, macrophages and intestine, whereas LXRâ is ubiquitously expressed. Oxysterols such as 22-OH cholesterol and 24-OH cholesterol have been identified as natural ligands for LXR, and several synthetic ligands have been well characterized. LXR target genes include the ABC transporters involved in cholesterol efflux (ABCA-1, ABCG-1 and ABCG-4), HDL-modifying enzymes (CETP and phospholipid transfer protein (PLTP)) and genes involved in cholesterol secretion into the bile (CYP7A, ABCG-5 and ABCG-8). Treatment with GW3965 (GlaxoSmithKline), a synthetic agonist of LXR, has been shown to inhibit the development of atherosclerosis in mice whereas macrophage-specific knockout of LXR by bone-marrow transplantation aggravates atherosclerosis (5). In the first RCT study in wild type mice, the experimental design involved treatment of animals with either vehicle or GW3965 (synthetic LXR agonist), an promotion of reverse cholesterol from macrophages to feces was seen. LXRs also regulate various enzymes involved in lipoprotein remodeling, including CETP. No adverse lipid related consequences were noted.

Endothelial lipase (EL) is a recently discovered member of the triglyceride lipase gene family (1, 2). Endothelial lipase is highly homologous to lipoprotein lipase (LPL) and hepatic lipase (HL), both of which are known to hydrolyze lipids within lipoproteins and thereby influence their metabolism. Endothelial lipase (EL) is believed to preferentially hydrolyse phospholipids from HDL particles while LPL and HL mainly act as triglyceride hydrolases. In preclinical studies, inhibition of endothelial lipase was found to raise HDL levels considerably. Adenovirus-mediated over expression of EL in LDL receptor-deficient mice reduced plasma concentrations of VLDL and LDL cholesterol by about 50%, whereas HDL levels were decreased to almost zero. By contrast, inhibition of EL activity by injection of a neutralizing polyclonal antibody resulted in a strong increase in plasma HDL levels in mice. When compared with a control antibody, the infusion of the inhibitory antibody resulted in a 25–60% increase in HDL cholesterol in three different mouse models (5)

Rimonabant is the first selective blocker of the cannabinoid receptor type1 (CB1) (12). In a one year long trial (RIO-LIPID S trial) which enrolled 1036 patients with mild to moderate obesity and untreated hyperlipidemia, where 20mg of Rimonabant

was given as a 5 mg daily dose or a 20 mg daily dose. At the end of treatment the patients on high dose rimonabant showed an increase in HDL levels by 23%, a 15% decrease in triglycerides also noted. In another trial with rimonabant (RIO-NA trial), 3045 obese patients enrolled and an increase in HDL levels of 24.5% was noted after one year treatment with 20mg of rimonabant compared with 3.8% in placebo group. Currently the drug is under review with FDA.

The HDL raising effect of nicotinic acid has been known for decades. Although the clinical efficacy of nicotinic acid is not proven, and the side effects will continue to limit its widespread use, the molecular mechanism of the nicotinic acid receptor might well be an attractive drug target for future drug discovery (6). In the treatment of dyslipidemia and coronary heart disease, niacin extended-release (ER) has also been introduced which uniquely targets the atherogenic lipid abnormalities of the metabolic syndrome. Niacin ER raises high-density lipoprotein cholesterol more effectively than other agents, while reducing triglyceride and lipoprotein (a) levels and increasing low-density lipoprotein particle size. It is formulated to minimize the toxicities and adverse effects associated with other niacin formulations, making niacin ER more tolerable for patients. Previous concerns of hyperglycemia have been addressed by numerous studies demonstrating that morbidity and mortality benefits outweigh potential increases in glucose levels. Niacin ER is an important lipid-lowering agent in preventing fatal and nonfatal coronary events and slowing the progression of atherosclerosis. Currently trials of Niacin(ER) and atorvastatin are being carried out.

There has been a general world-wide sharp increase in the prevalence of the metabolic syndrome and obesity in both adults and children and an associated increase in cardiovascular morbidity. This global epidemic affects not only the industrialized nations, but also the developing world. Low HDL levels are one of the hallmarks of the metabolic syndrome, and so the problem of low HDL as a risk factor is likely to increase in the future (5). The drugs aiming at raising HDL which are currently under trial have the potential to considerably lower incidence of coronary artery disease and other cardiac events in the next decade. Other targets which also need to explore include the ABCA1and ABCG1 transporters, SR-BI Receptors, Apo E etc. Thus these new drug targets in the management of atherosclerosis hold a great promise in future.

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