STATINS, CHOLESTERYL ESTER TRANSFER PROTEIN INHIBITION, HIGH DENSITY LIPOPROTEIN PARTICLE METABOLISM AND HEART DISEASE RISK REDUCTION

Ernst J. Schaefer, M.D. and Bela F. Asztalos, Ph.D., Cardiovascular Research and Lipid Metabolism Laboratories, Tufts University, 711 Washington St., Boston, MA USA, Tel: (617) 556-3100, Fax: (617) 556-3103, E-mail: ernst.schaefer@tufts.edu

Abstract

Statins have been shown to decrease cholesteryl ester transfer protein activity (CETP) activity, and rearrange HDL particles. Cholesteryl ester transfer protein (CETP) inhibitors (JTT-705 and torcetrapib) are currently in clinical testing and significantly raise high density lipoprotein (HDL) cholesterol levels. Low HDL cholesterol is a significant independent predictor of coronary heart disease (CHD) while HDL raising has been associated with CHD risk reduction; however, there is debate about whether they will reduce atherosclerosis in humans. In rabbits torcetrapib markedly decreases clearance of HDL cholesteryl ester via an indirect pathway, but has no effect on total plasma cholesteryl ester clearance. In humans torcetrapib raises HDL apoA-I by modestly decreasing its fractional catabolic rate, while having a very profound effect on raising HDL cholesterol and large α-1 migrating HDL particles by more than 50%, with no effect on fecal cholesterol excretion. When JTT-705 at 600 mg/day was given to hypercholesterolemic patients already on pravastatin 40 mg/day, the combination was well tolerated and increases in HDL cholesterol of 28% were noted.

In our view CETP inhibitors in combination with statins will be profoundly beneficial in reducing human atherosclerosis, primarily because they normalize HDL particles and prevent the transfer of cholesteryl ester from HDL to atherogenic lipoproteins.

Introduction

Early research in the 1950s associated decreased HDL cholesterol with increased heart disease risk, which was later confirmed by prospective population studies. Low HDL cholesterol is as powerful a predictor for CHD as is elevated low density lipoprotein (LDL) cholesterol; however, in contrast to LDL cholesterol, it does not lose its power with age. Kindreds with various HDL deficiency states have premature CHD. Low HDL cholesterol is the most common lipid abnormality observed in families with premature CHD. Three familial forms of HDL deficiency are commonly observed in these families: combined hyperlipidemia (combined elevations of LDL and triglycerides), dyslipidemia (high triglycerides), and hypoalphalipoproteinemia (isolated low HDL). Support for the concept of HDL raising comes from the Lipid Research Clinics Trial with cholestyramine, the Helsinki Heart Study and the Veterans Affairs HDL Intervention Trial (VA-HIT) with gemfibrozil, the Scandinavian Simvastatin Survival Study and several angiographic trials using nicotinic acid along with other agents including the Familial Atherosclerosis Treatment Study (FATS) and the HDL Atherosclerosis Treatment Intervention Study (HATS). Surprisingly just five weekly infusions of apoA-I Milano/phospholipids (small discoidal preβ-1 HDL-like particle) provided more benefit on atheroma regression as assessed by intravascular ultrasound (IVUS) than 18 months of treatment with 80 mg/day of atorvastatin [1,2]. Most recently 24 months of therapy with 40 mg/day of rosuvastatin in 349 statin naïve
patients decreased LDL cholesterol by 53%, increased HDL cholesterol by 15%, and resulted in a very beneficial median 6.8% reduction in total atheroma volume [3]. Statins have been shown to decrease CETP activity, probably mainly by decreasing the concentration of triglyceride acceptor particles which have lost some triglyceride from their core [4].

The potential importance of CETP was recognized when it was noted that mice and rats lacked plasma CETP activity, had very high HDL, and were resistant to diet-induced atherosclerosis, while rabbits had very high CETP activity levels and developed marked elevations of apoB containing lipoproteins and significant atherosclerosis on diets rich in saturated fat and cholesterol. When Inazu and colleagues in 1990 reported very high HDL cholesterol, increased large HDL2, decreased CHD risk, and potentially enhanced longevity in Japanese kindreds associated with CETP deficiency, both Japan Tobacco (JTT-705) and Pfizer (torcetrapib) began development of CETP inhibitors [5]. Both products have been shown to decrease diet-induced atherosclerosis in rabbits. Moreover these agents have been shown to be very effective in raising HDL cholesterol in humans [6-8]. However some authorities have questioned their potential benefit. We have documented with Dr. Rothblat’s group that large HDL particles containing both apoA-I and apoA-II are efficient in interacting with the liver scavenger receptor-B1 (SR-B1) [9], and very large particles of this type accumulate in homozygotes with CETP deficiency [10]. We have documented in both the Framingham Offspring Study and the VA-HIT study that the B2/B2 Taq1B genotype (which is in very strong linkage disequilibrium with a promoter variant that decreases gene expression) which is seen in 20% of the normal population, is associated with decreased CETP activity, higher HDL cholesterol levels, and decreased risk of CHD [11,12]. In a healthy Ashkenazi Jewish population of mean age 98 years, almost one third (more than a three-fold increase over normal middle-aged subjects of the same ethnic background) were homozygotes for the I405V CETP variant, associated with decreased CETP activity and increased large HDL particles [13]. Such debates will only be settled by randomized, placebo-controlled clinical trials testing CETP inhibitors with statins versus statins alone, and these are underway.

The Function of HDL Particles and CHD Risk

HDL particles are found in plasma at density of 1.063-1.21 g/ml, and can vary considerably in size and composition, and are about 50% protein by weight, 30% phospholipids, 25% cholesterol (about 70% is esterified or has a fatty acid attached to it), and 5% triglyceride. Most HDL are spherical particles of α mobility on electrophoresis with protein, free cholesterol, and phospholipids on the surface, and cholesteryl ester and triglyceride in the core of the particle. The major proteins of HDL are apoA-I and apoA-II, and minor protein constituents include apolipoproteins A-IV, A-V, C-I, C-II, C-III, D, E, and serum amyloid A (SAA).

HDL in whole plasma or serum can be examined by non-denaturing two dimensional gel electrophoresis followed by immunoblotting with apoA-I antibody or other antibodies (see Figure 1). This technique separates HDL by size in the vertical dimension, and by charge in the horizontal dimension. The major HDL subspecies have α-mobility, ranging from large spherical α-1 HDL containing apoA-I without apoA-II, to intermediate-sized spherical α-2 and α-3 HDL containing both apoA-I and apoA-II, to small, discoidal α-4 HDL which contains apoA-I without apoA-II. All α HDL can contain the C apolipoproteins and SAA can be found on α-2 HDL. Adjacent to each of these α-mobility particles are pre α-mobility particles that all contain apoA-I without apoA-II. In addition there are small discoidal preβ-1 and large preβ-2 migrating HDL
which contain apoA-I without apoA-II (see Figure 1). In collaboration with Dr. George Rothblat’s group, we have recently documented that both preβ-1 and α-2 HDL levels are correlated with macrophage free cholesterol efflux via the ATP binding cassette protein A1 (ABC-A1) pathway, while the α-1 and α-2 HDL levels correlate with liver cell scavenger receptor B1 (SR-B1) mediated cholesterol efflux from liver cells [8]. Since this is a bi-directional process we hypothesize that these particles can also serve as very efficient cholesteryl ester donors.

Research in patients with rare inborn errors of HDL metabolism has given us a framework for HDL particle metabolism (see Figure 2). ApoA-I secretion (step 1) by the liver and intestine and combining with phospholipids is essential for the formation of small discoidal pre-beta 1 HDL. Efflux of cellular free cholesterol and phospholipids via ABCA1 (step 2) is essential for the conversion of preβ-1 HDL to small discoidal α-4 HDL. Esterification of free cholesterol (an HDL surface component) with the transfer of a fatty acid from the phospholipid lecithin or phosphatidylcholine to free cholesterol via lecithin:cholesterol acyl transferase (LCAT) (steps 3-5), and the movement of cholesteryl ester to the core of HDL is essential to form spherical HDL particles of α-3 and α-2 HDL. Coupled with this process is continued lipolysis of triglyceride via lipoprotein lipase (LPL) to form larger HDL particles (steps 3-6). The exchange of core cholesteryl ester for triglyceride via cholesteryl ester transfer protein (CETP) between α-2, α-1 and larger HDL and the apoB containing lipoprotein particles is essential for the formation of α-1 HDL and pre-α HDL particles containing apoA-I without apoA-II (step 7). Another option for cholesteryl ester on α-2, α-1 and larger HDL and a critical step in reverse cholesterol transport is the selective bi-directional transport of cholesteryl ester between HDL particles and the liver via SR-B1 (step 8) for ultimate excretion of cholesterol into the bile. Hepatic lipase (HL) is important for the lipolysis and removal of phospholipids from HDL (step 9) which results in the conversion of α-1 to α-2 HDL particles. The next two steps in HDL metabolism are the recycling of apolipoproteins, phospholipids, and free cholesterol from larger HDL particles to small α-4 HDL due to loss of HDL cholesteryl ester via SR-B1 (step 10) or to pre-β-1 HDL due to interaction of large HDL with endothelial lipase (EL) and secretory phospholipase A2 (sPLA2), which both hydrolyze phospholipid (step 11). Recycling of apolipoprotein A-I may occur many times. The final step in HDL particle metabolism is the cubulin-mediated clearance of preβ-1 HDL in the kidney (step 12), and excretion into urine.

In both the Framingham Offspring Study and the VA-HIT study we have clearly documented that low levels of α-1 and α-2 HDL apoA-I levels are much better at CHD risk prediction that is HDL cholesterol [14,15]. We have also collaborated with Dr. Greg Brown to examine samples from the HDL Atherosclerosis Treatment Study (HATS), and have documented that the substantial increases (mean 115%) in the large α-1 HDL particles with the niacin/simvastatin combination were correlated with less progression or more regression of coronary atherosclerosis in the study [16]. We have also examined the effects of statins on HDL particles, and found rosuvastatin to be the most effective statin not only in raising HDL cholesterol, but also α-1 HDL (Asztalos et al, unpublished observations), which may partly account for its significant benefit in promoting atheroma regression [3]. In our view this effect may relate to its longer plasma residence time than any other statin.

Effects of CETP Inhibition on HDL Particles and HDL Metabolism
In vitro studies have shown that torcetrapib binds to CETP with 1:1 stoichiometry and blocks both neutral lipid and phospholipid transfer from HDL to other lipoproteins by inducing a nonproductive complex between the transfer protein and HDL [17]. The effect of CETP inhibition with torcetrapib was studied in a rabbit model by Kee et al. [18]. In normal rabbits labeled HDL cholesteryl ester was removed by both a direct and an indirect pathway, as determined by multi-compartmental modeling. In the treated rabbits CETP activity was inhibited by 80-90% and the level of HDL cholesteryl ester was doubled due to lack of removal via the indirect pathway, but had no overall effect on total HDL cholesteryl ester clearance. These data indicate that the indirect pathway is due to transfer of cholesteryl ester to apoB-containing lipoproteins, and that overall net HDL cholesteryl ester removal from plasma is not compromised by CETP inhibition in a rabbit model [18].

We have examined the effects of torcetrapib at doses of 120 mg daily and the same dose twice daily alone or together with atorvastatin 20 mg per day versus placebo in 19 subjects with decreased HDL cholesterol levels [9,19]. Subjects were studied with a primed constant infusion of deuterated leucine in the fed state (small hourly frequent feeding). Stool collections were carried out to assess fecal sterol excretion. While there were very significant increases in HDL cholesterol of more than 50%, apoA-I plasma pool size increased by 8%, 16%, and 34% in the atorvastatin plus torcetrapib, torcetrapib 120 mg daily dose, and the torcetrapib twice daily dose groups, respectively, due to decreases in apoA-I fractional catabolism of 7%, 8%, and 21%, with no significant effect on apoA-I production or fecal cholesterol or bile acid excretion [19]. Moreover there were very significant increases in large α-1 HDL of 136% in the atorvastatin/torcetrapib group, 153% in the torcetrapib once daily, and 382% in the torcetrapib twice daily group, versus placebo [19]. In contrast to homozygous CETP deficiency these large α-1 HDL particles were totally normal, in that they contained apoA-I, but no apoA-II [19]. These data indicate to us that torcetrapib can more than normalize the HDL spectrum of particles, and coupled with our other data from Framingham, VA-HIT, and HATS, would suggest that this agent will have striking benefit in CHD risk reduction [19]. Moreover in these studies we have documented that torcetrapib lowers apoB-100 containing lipoproteins mainly by enhancing their fractional clearance and together with atorvastatin can also decrease LDL apoB-100 secretion (Millar et al, unpublished observations).

In a randomized, double-blinded, placebo-controlled trial in 155 patients with elevated LDL cholesterol already on treatment with pravastatin 40 mg per day, four weeks of therapy with the CETP inhibitor JTT-705 at 600 mg/day decreased CETP activity by 30% and LDL cholesterol by 5%, while increasing HDL cholesterol by 28%, HDL₂ cholesterol by 48%, and plasma apoA-I levels by 14% [20]. The drug was well tolerated and had no significant effects on plasma apoE levels. The authors hypothesize that this lack of an effect on apoE may constitute a beneficial difference for JTT-705 versus torcetrapib which increases apoE levels significantly, but in our view these differences may merely relate to more efficacious CETP inhibition and HDL raising induced by torcetrapib than by JTT-705 [6-8,19,20].

Conclusions

CETP inhibition is a promising new treatment strategy for raising HDL cholesterol and optimizing the entire spectrum of HDL particles. In our view the benefit of these agents is that they prevent the transfer of cholesteryl esters from HDL to atherogenic triglyceride-rich remnants of both intestinal and liver origin. CETP inhibitors would be predicted to be most
beneficial in combination with statins in patients with low HDL cholesterol who also have elevated triglycerides or combined hyperlipidemia. Statins potentiate the effects of CETP inhibitors by decreasing CETP activity due to reductions in the levels of triglyceride-rich lipoprotein acceptor particles. Clinical trials are currently underway to test the potential benefits and safety of CETP inhibitors, which can affect blood pressure levels.

References


Please address correspondence to:
Ernst J. Schaefer, M.D
Cardiovascular Research and Lipid Metabolism Laboratories
Tufts University
711 Washington St.
Boston, MA, U.S.A.
Tel: (617) 556 3100
Fax (617) 556 3103
Email: ernst.schaefer@tufts.edu
Figure legend:
Figure 1: ApoA-I-containing HDL subpopulation profiles of a control (a) and a CHD (b) subjects with much less less $\alpha$-1 HDL than the control. Panel (c) is a schematic representation of the individual HDL particles. Size is on the vertical axis going from large to small particles, and charge on the horizontal axis going from pre-$\beta$ to $\alpha$ to pre-$\alpha$ HDL particles. Lighter gray represents HDL particles containing apoA-I without apoA-II and darker gray represents HDL particles containing both apoA-I and apoA-II. All $\alpha$ HDL particles contain C apolipoproteins, while $\alpha$-2 HDL contains SAA. ApoA-IV and apoE are on their own separate HDL particles.

Figure 2: A conceptual model of HDL particle metabolism which has the following steps: 1) apoA-I secretion and combining with phospholipids to form pre-$\beta$-1 HDL, 2) the addition of cellular FC via ABCA1 to form $\alpha$-4 HDL, 3-5) cholesterol esterification via LCAT to form spherical $\alpha$-3 and $\alpha$-2 HDL, 3-6) lipolysis of triglyceride via LPL to form larger HDL particles with more CE core, 7) CETP mediated transfer of TG from TRL to HDL in exchange for CE to TRL to form $\alpha$-1 HDL, 8) net liver uptake of HDL CE via SR-B1, 9) conversion of $\alpha$-1 HDL to $\alpha$-2 HDL via the action of HL, 10) recycling of apolipoproteins, free cholesterol, and phospholipids to pre$\beta$-1 HDL with CE uptake via SR-B1, 11) recycling of apolipoprotein A-I to pre-$\beta$ HDL via the action of endothelial lipase and secretory phospholipase, which hydrolyze phospholipids, and 12) cubulin clearance of free apoA-I and pre$\beta$-1 HDL via the kidney.

Abbreviations: Free cholesterol (FC), cholesteryl ester (CE), Phospholipid (PL), triglyceride (TG), apolipoproteins (apos), high density lipoprotein (HDL), low density lipoprotein (LDL), triglyceride rich lipoprotein (TRL), ATP binding cassette protein A1 (ABCA1), scavenger receptor B1 (SR-B1), cholesteryl ester transfer protein (CETP), lecithin cholesterol acyl transferase (LCAT), lipoprotein lipase (LPL), hepatic lipase (HL).
Figure 2