Role of lipid and lipoprotein metabolizing enzymes in the development of atherosclerosis

Ramaswamy Subramanian, Manisha Ramaswamy & Kishor M. Wasan*

Division of Pharmaceutics and Biopharmaceutics, Faculty of Pharmaceutical Sciences, University of British Columbia, 2146 East Mall, Vancouver B.C., Canada Y6T 1Z3

Cardiovascular disease is the primary cause of mortality in developed and developing nations. With an increase in the aging population, there is a surge in the incidence of atherosclerotic cardiovascular diseases. One of the most common and lethal manifestations of atherosclerosis is coronary heart disease, accounting for 50% of the atherosclerotic cardiovascular diseases in men and women younger than 75 years. Peripheral arterial diseases, manifested mainly as intermittent claudication, constitute approximately 10% of the atherosclerotic cardiovascular events. According to the American Heart Association 2001 Heart and Stroke Statistical Update, atherosclerosis accounts for 75% of all deaths due to cardiovascular diseases. Therefore, atherosclerosis continues to remain the primary cause of health concern for the population at large. The aim of this review is to discuss the role of enzymes that are involved in the metabolism of lipids and lipoproteins in the development of atherosclerosis.

Overview
Cardiovascular disease is the primary cause of mortality in developed and developing nations. With an increase in the aging population, there is a surge in the incidence of atherosclerotic cardiovascular diseases. In the U.S., 44% of the nation’s mortality has been attributed to cardiovascular disease. According to an estimate in 1997, the economic cost of atherosclerotic cardiovascular disease in the U.S. amounted to $259 billion. One of the most common and lethal manifestations of atherosclerosis is coronary heart disease, accounting for 50% of the atherosclerotic cardiovascular diseases in men and women younger than 75 years. Approximately 10% of the atherosclerotic cardiovascular events are constituted by peripheral arterial diseases, manifested mainly as intermittent claudication. According to the American Heart Association 2001 Heart and Stroke Statistical Update, atherosclerosis accounts for 75% of all deaths due to cardiovascular diseases. Thus, atherosclerosis continues to remain the primary cause of health concern for the population at large. The aim of this review is to discuss the role of enzymes that are involved in the metabolism of lipids and lipoproteins in the development of atherosclerosis.

Atherosclerosis
Atherosclerosis is a complex multifactorial inflammatory disease, characterized by the presence of lesions due to the accumulation of lipids in the walls of large and medium-sized arteries. The earliest event is the formation of atherosclerotic lesion or “fatty streak” due to endothelial dysfunction, characterized by inflammatory cellular infiltration, mainly of monocyte-derived macrophages and T-lymphocytes. Fatty streaks are usually present in humans in several major vessels, namely aorta, coronary artery and cerebral artery during different stages of life. The vessel endothelium develops pro-coagulant instead of anti-coagulant properties as a result of injury resulting in the production of cytokines, chemokines and growth factors. If continued unabated, the inflammatory response stimulates the migration and proliferation of smooth muscle cells to form an intermediate lesion. This leads to a gradual thickening of the arterial walls, which is compensated by dilatation of the vessel wall or “remodeling”. In later stages, continuous migration and activation of monocyte-derived macrophages and T-lymphocytes causes release of chemokines, cyto-

Abbreviations: LDL—low-density lipoprotein, HDL—high-density lipoprotein, VLDL—very low-density lipoprotein, IDL—intermediate-density lipoprotein, LCAT—lecithin cholesterol acyltransferase, CETP—cholesteryl ester transfer protein, PLTP—phospholipid transfer protein, LPL—lipoprotein lipase, HL—hepatic lipase, apo—apolipoprotein, HDL-C—HDL-cholesterol, TC—total cholesterol, SR-B1—scavenger receptor B1.
kines and growth factors, which leads to further damage of the vessel wall and develops focal necrosis (Fig 1). The clinical manifestation of atherosclerotic plaque formation is acute vascular occlusion due to the formation of a thrombus or clot, which can lead to ischemia of vital organs, such as heart causing myocardial infarction, brain resulting in strokes and lower extremities causing peripheral artery disease.56.

Risk factors for atherosclerosis

Several risk factors have been identified in the development of atherosclerosis, some of which are causal and independent, while others have not been completely established and are classified as conditional or permissive factors. The major causal factors include cigarette smoking, high blood pressure, elevated low-density lipoprotein cholesterol, reduced serum high-density lipoproteins, diabetes and advancing age.6. Some genetic determinants of atherosclerotic cardiovascular diseases include, elevated levels of homocysteine and elevated levels of haemostatic factors, such as fibrinogen and plasminogen activator inhibitor type 1.7-8. Studies have suggested that low level of antioxidants is also a potential causative factor for the development of atherosclerosis.9. In humans, specifically in men, abdominal obesity due to

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Fig. 1 — Schematic representation of initiation and progression of atherosclerotic lesion formation (A) Fatty-streak formation; (B) Formation of advanced lesion and (C) formation of unstable fibrous plaque (Adapted with permission of Massachusetts Medical Society, from N. Engl. J. Med., 340 (1999), 115-26). Copyright (1999), Massachusetts Medical Society)
accumulation of intra-abdominal fat has been suggested to be a major predisposing factor in the development of atherosclerosis. Other predisposing factors include lack of physical activity, family history of premature coronary artery disease and ethnic origin of the subject. It is beyond the scope of this review to discuss all the factors responsible for causing atherosclerosis. Further discussion will, therefore, focus on the involvement of lipids and lipoproteins in the development of atherosclerotic cardiovascular diseases.

The risk factor that remains at the top of all causal factors for the development of coronary heart disease in humans is hypercholesterolemia. Several medical recommendations have been made to counteract this condition, including consumption of food low in saturated fats, regular physical exercise and refraining from smoking. Individuals with elevated plasma low density lipoprotein (LDL) levels appear to be at a greater risk for developing coronary heart disease, secondary to atherosclerosis. Specifically, very high total and LDL cholesterol has been linked to the development of coronary artery disease. Several clinical studies have suggested that lowering LDL cholesterol significantly reduces the risk of developing coronary events. However, observations from other studies suggest that ~80% of the patients who develop coronary artery disease have the same total cholesterol as individuals who do not suffer from coronary artery disease. In addition, a large number of patients treated with cholesterol lowering medications continue to suffer from clinical events related to coronary artery disease. Results from epidemiological studies have suggested an inverse relationship between plasma high density lipoprotein (HDL) levels and the incidence of atherosclerosis. In fact, a 1% increase in the concentration of HDL has been proposed to cause a 3% reduction in the risk of developing atherosclerosis.

Lipoproteins and lipoprotein subtypes

Lipoproteins are macromolecular aggregates of lipids and proteins that are responsible for the transport of water insoluble nutrients through the vascular and extravascular fluids from their sites of synthesis or absorption to the peripheral tissues for storage or catabolism in the production of energy. Structurally, a spherical lipoprotein particle is made up of a lipid core, consisting mainly of cholesterol and triglycerides surrounded by a surface monolayer of amphipathic lipids, namely, phospholipids and unesterified cholesterol and specific proteins called apolipoproteins. Based on their densities, lipoproteins are classified into five types: triglyceride-rich chylomicrons (density <0.95) and very low-density lipoproteins (VLDL, density 0.95-1.006), intermediate density lipoproteins (IDL, density 1.006-1.019), low-density lipoproteins (LDL, density 1.019-1.063) and high-density lipoproteins (HDL, density 1.063-1.21). In terms of size, chylomicrons are the largest of all the lipoproteins with a diameter of 100-1000 nm, LDL is the second smallest (18-25 nm), whereas HDL is the smallest lipoprotein with a mean diameter of 7-12 nm. In humans, four subspecies of LDL, namely LDL I-IV can be found. LDL I is the largest and least dense, whereas LDL IV is the smallest and most dense LDL subspecies. In healthy humans, the large, buoyant LDL I is the predominant LDL subtype and the dense LDL IV is only present in small amounts. HDLs originating from both hepatic and intestinal sources are small and poor in cholesterol. However, the nascent, hepatic HDL is disk-shaped, whereas the intestinal HDL is spherical and varies in its protein composition. Based on analytical ultracentrifugation and gradient gel electrophoresis, HDL from both the hepatic and intestinal sources can be classified as HDL. The cholesterol ester content in HDL3 is increased following its interaction with the enzyme, lecithin cholesterol acyl transferase (LCAT). Subsequently, the particle becomes larger and less dense to form HDL2. The denser, cholesterol-poor HDL2 has a density of 1.125-1.21 g/ml, whereas, the less dense, cholesterol-rich HDL2 has a density of 1.062-1.125 g/ml.

In addition to their role in transporting water-insoluble nutrients, lipoproteins are also involved in other biological processes, namely, coagulation of blood, tissue repair and immune reactions. Studies from our laboratory over the past several years have
demonstrated that various hydrophobic drugs, such as cyclosporine A, amphotericin B, amnycin, nystatin, and clozapine associate with human plasma lipoproteins, which results in their altered pharmacological activity. For example, the antiproliferative effects of the widely studied immunosuppressant, cyclosporine A, are enhanced when the drug is associated specifically to LDL, but not VLDL or HDL. The clinical implication of such association is increased nephrotoxicity of cyclosporine A, as observed in kidney and heart transplant patients with elevated total plasma cholesterol levels.

Lipid and lipoprotein metabolism

The metabolism of lipids ingested in the form of dietary fats is initiated in the intestine, where triacylglycerols are hydrolyzed in the lumen to form monoa-cyl glycerols and fatty acids, which are transported across the intestinal membrane by fatty acyl binding protein. In the enterocyte, triacylglycerols are resynthesized and packed with the apolipoprotein, apoB-48 and other lipids to form chylomicrons. Once released into the systemic circulation, chylomicrons acquire cholesterol, apoE and apoCs from HDL. ApoE and apoCs are required for metabolizing chylomicrons.

The peripheral circulation, apoCII located on the surface of chylomicrons activates the enzyme, lipoprotein lipase (LPL), which hydrolyzes triacylglycerols to form fatty acids and releases apoE and apoC back into the systemic circulation. The endogenously synthesized triacylglycerols are transported from the liver to extrahepatic tissues by VLDL, which is assembled and secreted in the liver. The nascent VLDL, composed of VLDL, triacylglycerols, small amount of cholesterol ester and apoB-100, is transformed into mature VLDL when it acquires cholesterol esters as well as apoCII, apoCIII and apoE from HDL. The mature VLDL interacts with LPL resulting in the hydrolysis of triacylglycerols accompanied by the release of phospholipids, apoCs and apoEs from the surface coat. The resulting particle is called VLDL remnant or IDL, which is rich in cholesteryl ester acquired from the hydrolysis of triglycerides and from HDL. IDL is then taken up by the liver or converted to LDL upon interaction with hepatic lipase. Both chylomicrons and VLDL have similar particle sizes and share some properties of catabolism. Thus, both are rapidly cleared from the plasma, with an average half life of 15-30 min. The lipoprotein particles are not cleared by the liver unless subjected to lipolytic modifications by LPL and hepatic lipase.

The smallest lipoprotein, HDL is primarily involved in the removal of cholesterol from the peripheral tissues and its transport to the liver by a process called “reverse cholesterol transport”. The apo AI-containing precursor of HDL, called nascent HDL acquires cellular cholesterol and phospholipid through a transport process mediated by the ATP-binding cassette transport protein, ABCA1. Within the discoidal HDL particle, unesterified cholesterol is esterified to cholesteryl ester by LCAT. HDL cholesteryl ester can either be taken up specifically by the liver via the action of scavenger receptors B1 (SR-B1) or transferred to apoB-containing lipoproteins in exchange for triglyceride by the enzyme, cholesteryl ester transfer protein (CETP). The process of reverse cholesterol transport is depicted in Fig 3. The enzyme, hepatic lipase (HL) hydrolyses triglycerides and phospholipids from HDL to generate smaller lipid-free HDL particles. Another triglyceride lipase, namely endothelial lipase may also promote catabolism of HDL by hydrolyzing the phospholipids.

The following section will discuss the various lipid and lipoprotein metabolizing enzymes and their role in atherosclerosis.

Lipid and lipoprotein metabolizing enzymes

I. Lipoprotein lipase (LPL)

A key enzyme responsible for the metabolism of lipoproteins is LPL, which is anchored to the luminal surface of the vascular endothelium by highly charged heparan sulphate proteoglycans. The primary function of LPL is to hydrolyze core triglycerides in triglyceride-rich lipoproteins and convert them into remnant particles, such as chylomicrons, IDL and LDL. In addition, LPL also stimulates the hepatic removal of lipolyzed lipoproteins and transfers the sur-
face components of triglyceride-rich lipoproteins to HDL. LPL is synthesized predominantly in the skeletal muscle, cardiac muscle and adipose tissue, and to a lesser extent in the kidneys, adrenal glands, brain and macrophages. When present in its unglycosylated form, LPL is inactive, and in order to attain maximum activity the enzyme must exist in the form of a dimer. In order to be functionally active, LPL requires the presence of apolipoproteins, specifically apoCII as an essential cofactor. LPL belongs to the triacylglycerol gene family, which also includes hepatic lipase (HL), pancreatic lipase and endothelial lipase. Structurally, LPL consists of a larger N-terminal domain, containing 312 amino acids (1-312) and a smaller C-terminal domain made up of 105 amino acids (313-418). It also contains heparin-binding sites in the N- and C-terminal domains, apoCII-binding site in the N-terminal domain and another binding site for LDL receptor-related protein in the C-terminal region. The catalytic site of LPL is located in the N-terminal domain and is covered with a lipid binding lid. The catalytic function of LPL is enabled by the interaction of lipoprotein substrates with the C-terminal domain, which leads to a conformational change in the enzyme and results in the opening of the lipid-binding lid.

As research has been intensified to identify the putative factors causing atherosclerosis, novel roles for LPL have emerged. More than two decades ago, Zilversmit proposed that the local action of LPL on VLDL and chylomicrons in the vascular endothelium produces high concentrations of cholesterol that would be taken up into the arterial wall leading to the deposition of cholesterol at these sites. This led to the suggestion that LPL plays a role in the development of atherosclerosis. Subsequent studies have proposed that the macrophage-derived LPL has proatherogenic function. Thus, Renier et al. demonstrated that macrophages derived from inbred mice that are susceptible to atherosclerosis have a 2-3-fold elevation in LPL mass, activity and mRNA compared to the atherosclerosis-resistant mice, which lead to the suggestion by the authors that LPL may be pro-atherogenic in this animal model. Additional evidence for the atherogenicity of macrophage-derived LPL comes from studies where macrophage-derived foam cells were identified as the main source of the enzyme in atherosclerotic lesions. In a more recent study, the extent of formation of atherosclerotic lesions in transgenic apoE-knockout mice that specifically express macrophage-derived human LPL was found to be significantly greater than the control human LPL-null, apoE knockout animals, further supporting the notion that macrophage-derived LPL is pro-atherogenic. It has been suggested that the atherogenicity or otherwise of LPL is dependent on its site of expression. Evidence from a recent study on LPL transgenic mice deficient in apoE suggests that the atherogenic potential of LPL or lack of it depends on the balance between two different sources of the enzyme, namely, vessel wall and plasma LPL. The observations from this study indicate that elevated plasma LPL in the absence of any increases in macrophage-derived LPL decreases the susceptibility of the animals to develop atherosclerosis. Thus, the pro- or anti-atherogenic effects of LPL depend on the pool of enzyme that is altered. Contrary to the observations in rodents, rabbits overexpressing human LPL and fed with a diet containing 0.3% cholesterol were found to have no atherosclerotic lesions compared to the control animals, suggesting that LPL exerts anti-atherogenic function in this animal model. The species-dependent effects of LPL on atherogenesis could be due to lack of CETP in rodents, which may result in the increased formation and accumulation of pro-atherogenic particles in the vessel wall due to lack of catabolism of HDL cholesterol. However, this hypothesis may not be entirely true since observations from a recent study in our laboratory have demonstrated that extravascular administration of a surfactant commonly used as a pharmaceutical excipient, Poloxamer 407 (P-407) to rats causes marked elevation in the plasma activity and protein concentration of CETP in addition to marked hyperlipidemia (unpublished observations). In earlier studies it was shown that, rodents treated with P-407 show progressive development of atherosclerotic lesions. Thus, either additional mechanisms of atherogenesis must exist in P-407-treated rodents or the enhanced CETP activity must generate more pro-atherogenic particles in these animals. Despite several years of research and innumerable studies, the evidence for pro- or anti-atherogenic potential of this LPL in atherosclerosis is controversial and the precise role of this enzyme in this multifactorial disease state remains to be established.

II. Hepatic lipase (HL)

Hepatic lipase is an enzyme involved in the hydrolysis of triglycerides and probably phospholipids in VLDL remnants, which leads to a more efficient uptake of these particles and generation of LDL.
is also involved in reverse cholesterol transport. In this process it mediates the conversion of triglyceride-rich HDL2 to triglyceride-poor HDL3, a step that releases cholesteryl esters, phospholipids, fatty acids and glycerol, which are taken up by the liver. Several in vitro studies have demonstrated that HL catalyzes the hydrolysis of diglycerides, triglycerides, and phospholipids in native lipoproteins. HL is a glycoprotein of approximately 65 KDa, which is synthesized primarily in the hepatocytes of the liver. The enzyme is anchored to the vascular endothelium of the liver and on the surface of hepatocytes by means of heparan sulfate proteoglycans. By facilitating the process of reverse cholesterol transport, hepatic lipase accelerates the transfer of cholesteryl esters to stereidogenic tissues, such as the ovaries and adrenal glands. In addition, HL also plays an important role in lipoprotein metabolism as shown in animals, where deficiency of the enzyme was induced by injecting anti-HL antibodies and also by heat inactivation.

Studies in humans have demonstrated an inverse correlation between plasma HDL levels, particularly HDL2 subfraction, and post-heparin plasma HL activity, which suggests that HL plays a significant role in the catabolism of HDL. The combined action of CETP-mediated transfer of cholesteryl ester and HL-mediated hydrolysis of triglycerides and phospholipids depletes the core of large HDL and help to form smaller HDL, as well as lipid-free apoA-I and preβ-HDL. Independent of its lipolytic activity, HL appears to serve as a cofactor in the selective uptake of HDL lipids, which is mediated by scavenger receptor-B1 and by an additional potential HDL binding site in hepatocytes.

In humans, deficiency in HL activity is characterized by hypercholesterolemia, hypertriglyceridemia, accumulation of β-VLDL remnants, LDL and HDL. Similarly, mice with targeted disruption of HL gene have elevated plasma HDL cholesterol and phospholipids, but normal plasma triglyceride levels. The clinical implication of HL deficiency in humans is increased susceptibility to premature coronary artery disease despite elevated levels of plasma HDL-cholesterol. In fact, in a recent clinical study on patients undergoing elective coronary angiography, a significant inverse correlation was obtained between plasma HL activity and the risk of developing coronary artery disease. HL activity was found to be lower in patients with coronary artery disease than control subjects. Evidence for the potential anti-atherogenic effects of HL have also been obtained in animals, such as mice and rabbits, where transgenic overexpression of HL leads to a reduction in plasma HDL-cholesterol concentration, but does not cause atherosclerosis. Extravascular administration of certain chemicals, such as P-407 causes significant inhibition of plasma HL activity in rodents, which may be responsible for the development of atherosclerosis in this animal model. (also Subramanian et al., unpublished observations). Recent evidence suggests that high HL activity is anti-atherogenic in familial hypercholesterolemia but pro-atherogenic in hypertriglyceridemia, whereas in normolipidemia, HL appears to have little effect on the risk of developing coronary artery disease.

III. Lecithin cholesterol acyl transferase (LCAT)

Cholesterol from the peripheral tissues is transported to the liver for elimination in the bile by reverse cholesterol transport, a process initiated by the enzyme LCAT, which converts cholesterol to cholesteryl esters on the surface of lipoproteins, predominantly HDL. Plasma LCAT is a 63 KDa protein containing 416 amino acids and four N-linked glycosylation sites. The enzyme is secreted by the hepatocytes in the liver and released into the plasma. Due to its involvement in reverse cholesterol transport, LCAT performs many important functions in the interconversion of lipids and lipoproteins, such as:

a. Stabilizing the shape and size of lipoproteins by maintaining the balance between unesterified and esterified cholesterol.

b. Creating the gradient necessary for the transfer of unesterified cholesterol from the tissues to the plasma and also transferring cholesteryl esters to the tissues. The esters undergo hydrolysis in the tissues to release free cholesterol, which can be utilized for important physiological processes.

LCAT reaction is initiated by activation of the phospholipid-cholesterol bilayer by specific domains on apolipoprotein A1 (apo A1) followed by hydrolysis of lecithin, mostly in the sn-2 position to release a fatty acyl group due to LCAT phospholipase A2-like activity. Finally, the acyl group is transferred to the acceptor, 3β-hydroxy group of cholesterol. Studies by Adimoolam and Jonas have suggested that LCAT contains a surface region of 25 amino acids (C50-74) that is involved in its binding to lipoproteins.

The esterification of cholesterol on the surface of lipoproteins by LCAT leads to the remodeling of the lipoprotein, predominantly HDL, and results in the formation of larger HDL particles that are known to...
offer protection against coronary artery disease. However, there is still widespread controversy surrounding the anti-atherogenic potential of LCAT in humans. While some studies suggest that patients with coronary artery disease typically demonstrate high cholesterol esterification rates and high CETP activity, others indicate that individuals with partial or complete LCAT deficiency are not at added risk for the development of coronary artery disease. Further, the notion that formation of large HDL is favored by high LCAT and LPL activities has been challenged by some investigators who have demonstrated that the lowest level of large HDL particles (HDL-
 2b) are found in those individuals who are at the highest risk of developing coronary artery disease. However, there is some degree of consensus regarding the anti-atherogenic potential of LCAT in experimental animals. Thus, overexpression of LCAT has been shown to protect rabbits against diet-induced atherosclerosis. Likewise, deficiency of LCAT has been demonstrated to cause hyperlipidemia and promote atherosclerosis in transgenic mice lacking LDL receptors and apoE. Downregulation of LCAT in certain pathophysiological conditions, such as chronic renal failure promotes hyperlipidemia, which may lead to the development of atherosclerotic cardiovascular disease. In other conditions, such as diabetes mellitus, the observations on plasma LCAT activity have been very inconsistent depending on the methods used for measurement of the enzyme activity. Where radioactive cholesterol was used as the exogenous substrate, LCAT activity was reported to be either diminished in type 1 and 2 diabetes, not altered, or elevated in patients with type 2 diabetes. In those studies where endogenous radioactive cholesterol was used as the substrate, LCAT activity was demonstrated to be either decreased in type 1 diabetes, not altered or increased. As a result, the significance of alterations in in vitro plasma LCAT activity on atherogenesis in diabetic patients is still not clear.

It is now becoming increasingly evident that prolonged exposure to certain chemicals causes hyperlipidemia and alterations in plasma LCAT activity. In this context, a recent study from our laboratory has demonstrated that, extravascular administration of P-407 to rats causes significant hyperlipidemia, characterized by dramatic increases in total plasma cholesterol and triglycerides. In addition, the plasma LCAT activity, measured as the rate of esterification of endogenous cholesterol was also elevated significantly in the P-407-treated animals (unpublished observations). In earlier studies it was shown that in mice, P-407-induced hyperlipidemia was also accompanied by progressive development of atherosclerotic lesions. If LCAT is considered to be anti-atherogenic in animals, the observations in the P-407-treated rodent model appear to contradict the findings from rabbits and transgenic mice discussed earlier in this section. The observations would tend to support either additional pro-atherogenic mechanisms in our rodent model or suggest an inability of the P-407-treated rodent to completely and efficiently catabolize/eliminate the increased plasma cholesterol stores. In addition to chemicals, other factors, such as smoking has also been demonstrated to significantly inhibit plasma LCAT activity in humans. The authors conclude that inhibition of LCAT, among other enzymes may result in the generation of atherogenic lipoprotein phenotype seen in smokers.

IV. Cholesteryl ester transfer protein (CETP)

The cholesteryl esters formed from LCAT reaction are redistributed between plasma lipoproteins along with triglycerides, and to lesser extent phospholipids by CETP, a 74 KDa hydrophobic glycoprotein containing 476 amino acids. Due to its involvement in reverse cholesterol transport, CETP causes equilibration of lipids between the different lipoprotein fractions. The overall effect of CETP activity is a net mass transfer of cholesteryl esters from HDL to triglyceride-rich lipoproteins and LDL and of triglyceride from triglyceride-rich lipoproteins to LDL and HDL. CETP-mediated transfer of cholesteryl esters from HDL results in the "remodeling" of HDL particles, characterized by a decrease in its cholesterol content, apoA-I content and particle size.

CETP along with another transfer protein, phospholipids transfer protein (PLTP) belong to the lipid transfer/lipoplyasacharide-binding protein (LBP) gene family, which also includes LBP and bacterial/permeability-increasing protein (BPI). All four proteins can bind lipopolysaccharides, phospholipids and a variety of other lipids. CETP is secreted by a number of cells, including, monocyte-derived macrophages, B-lymphocytes, hepatocytes, adipocytes, Caco-2 cells and HepG2 cells and once released, it circulates in the plasma, bound mainly to HDL. The expression of CETP is species-dependent, with undetectable levels in rodents, moderate levels in humans and high levels in rabbits. A strong correlation appears to exist between plasma HDL cholesterol and CETP activity, at least in experimental animals.
Thus, mice, rats and dogs have very low to undetectable CETP activity but high HDL-cholesterol to total cholesterol (HDL-C/TC) ratio, whereas, hamsters, rabbits and monkeys have low HDL-C/TC ratio and high CETP activity.\textsuperscript{106}

Based on CETP activity, animals can be classified as "resistant" or "susceptible" to atherosclerosis. The former group includes cat, dog, mouse and rat, whereas, the latter group includes chicken, pig, rabbit and man.\textsuperscript{107} However, studies in mice have demonstrated that atherogenesis can be induced in this "resistant" animal model by prolonged exposure to certain surfactants, such as P-407\textsuperscript{106,109}. Recent observations from our laboratory suggest that the atherogenic potential of P-407 in rodents may be due to its ability to stimulate the activity of various lipid metabolizing enzymes, including CETP (unpublished observations). Although studies have indicated that CETP may be both pro- and anti-atherogenic, the precise role of this enzyme in atherosclerosis is not clear. In cholesterol-fed mice, overexpression of simian CETP has been shown to aggravate atherosclerosis, compared to wild type mice, suggesting a pro-atherogenic role of CETP in this multifactorial disease state.\textsuperscript{108} In addition, rabbits injected with a vaccine that produces anti-CETP antibodies demonstrate reduced susceptibility to develop atherosclerosis, providing further evidence for a pro-atherogenic function of CETP.\textsuperscript{109}

In a recent study on rabbits, Okamoto et al. used a newly developed inhibitor of CETP, JTT-705 and demonstrated that, in vivo administration of this agent causes marked reduction in diet-induced hyperlipidemia and atherogenic lesions while increasing plasma HDL-C levels.\textsuperscript{110}

Although there is some degree of consensus between animals with regards to the pro-atherogenic role of CETP, studies in humans have been controversial and less conclusive. In CETP-deficient humans, absence of CETP protein has been identified as the major risk factor for the development of coronary artery disease, when HDL levels are comparable between the subjects.\textsuperscript{111} This is in contrast to another recent study in humans, where immunoreactive CETP was detected in foam cells from aortic and coronary atherosclerotic lesions, which were largely derived from macrophages. In addition, transfection of human CETP cDNA into COS-7 cells resulted in increased efflux of cholesterol from the cells, a phenomenon not observed in macrophages deficient in CETP.\textsuperscript{112} The authors suggest that expression of CETP in macrophages from human atherosclerotic lesions may have a potential anti-atherogenic function. It appears that in humans, the atherogenicity or lack of atherogenicity resulting from CETP deficiency depends on the magnitude of the associated elevation in HDL cholesterol. Thus, subjects with HDL cholesterol > 1.55 mmol/L have been suggested to be at reduced risk of developing atherosclerotic cardiovascular diseases, whereas, in subjects with a CETP gene mutation accompanied by a modest elevation in HDL cholesterol, the incidence of coronary heart disease appears to be higher.\textsuperscript{111,113} Modulation of CETP \textit{in vivo} appears to be a potential therapeutic intervention that could provide beneficial effects in the treatment of atherosclerosis. This is exemplified in a recent study, where a novel inhibitor of CETP, JTT-705 was shown to cause dose-dependent inhibition of plasma CETP activity and increase in HDL cholesterol without affecting plasma triglycerides, PLTP and LCAT activity in a randomized phase II clinical study in humans. While the pharmacodynamic effects of JTT-705 were not determined in this study, it certainly appears that inhibiting plasma CETP activity could have anti-atherogenic effects in humans.

Overall summary

As the world battles with growing incidence of atherosclerotic cardiovascular diseases, there is an increasing need to unravel the mechanisms underlying this potentially life threatening disorder. While some of the causal factors for atherosclerosis are natural, such as advancing age and genetic susceptibility, others, such as smoking and sedentary lifestyle can be controlled by the concerned individual. Regardless of the individual factors, hyperlipidemia, characterized by hypercholesterolemia has been recognized as the underlying denominator in the development of atherosclerotic cardiovascular disease. This review has been written with the intention of drawing the attention of the scientific community to the fact that, in addition to the traditionally recognized factors, alterations in the activities of lipid and lipoprotein metabolizing enzymes can have a profound influence on the development of atherosclerosis.

From the above discussion, it is evident that alterations in the activities and expression of LPL, HL, LCAT and CETP can be both pro- and anti-atherogenic. Despite innumerable studies in various species, there is still a lack of consensus regarding the beneficial or detrimental effects of these enzymes on atherosclerosis. In addition, observations in animal models do not always appear to correlate with the ob-
servations in humans. Finally, studies in transgenic animals suggest that the effect of modulation of lipid metabolizing enzymes is species-dependent. From the therapeutic standpoint, interesting observations from a recent study in humans indicate that modulation of the activity of at least one of these enzymes, CETP using synthetic inhibitors can offer protection against atherosclerotic cardiovascular diseases. Whether modulation of LPL, HIL and LCAT can also provide similar benefits against atherosclerosis in humans is at present a matter of conjecture. However, the realization that development of atherosclerotic cardiovascular disease can be controlled at the level of enzymes that regulate cholesterol and triglyceride metabolism opens the pandora's box for the design of new therapeutic modulators aimed at preventing the initiation and/or progression of atherosclerosis.

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