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RESEARCH ARTICLE

CHOLESTEROL HOMEOSTASIS: LIPOPROTEINS IN ACTION

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ABSTRACT

Apart from its functions like maintenance of cell membrane fluidity, vitamin D biosynthesis on skin cells, steroid hormones synthesis and neurotransmission in cells of the brain, cholesterol increase may have important impact in normal homeostasis. Atherosclerosis, the resultant effect in hypercholesterolemia is the leading cause of deaths in developed countries. Undoubtedly, the metabolism of lipoproteins serve as an important mode of cholesterol distribution and this review gives an overview and insights related to this metabolism of lipoproteins.

INTRODUCTION

Cholesterol being an important molecule has been subject of research for the past two centuries. Heart attacks, resultant of hypercholesterolemia is the leading cause of deaths when compared to deaths caused by all types of cancer [1]. All the eukaryotic cells require cholesterol for various processes like maintenance of membrane fluidity, vitamin D biosynthesis on skin cells, steroid hormones synthesis in steroidogenic tissues etc., Inbuilt regulation exists within the cells for the synthesis of cholesterol, utilisation and excretion and to maintain the homeostasis. Excess within the cells are put in the circulation that deposits in the arteries leading to formation of atherosclerotic plaque formation and subsequently coronary artery disease. Clinically, measurement of circulating cholesterol and related lipids provides a tool for prediction of risk of heart diseases. The relationship between the rise in circulating concentration of serum cholesterol and heart diseases has been reported earlier [2]. Circulation of cholesterol, a lipophilic molecule in the aqueous blood is extremely complex involving many carrier proteins termed as lipoproteins. The heterogeneous lipoproteins differ in size, shape, composition and function and correlate well with the heart diseases resultant of increased cholesterol in the circulation. High density lipoprotein (HDL) cholesterol delivers cholesterol from tissues to the liver back for channelling it for excretion in various forms such as bile acids etc., This has been described as good cholesterol in ordinary terms. Low density lipoprotein (LDL) cholesterol accumulates the cholesterol within the cells and is the source of cholesterol in cells other than the endogenous synthesis. Very low density lipoproteins (VLDL), chylomicrons, intermediate density lipoproteins (IDL) are other classes of lipoproteins that have their identical roles in the control of homeostasis of cholesterol metabolism.

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High density lipoprotein and its role in transport of cholesterol

HDL-c is one of the five major groups of lipoproteins (LDL, VLDL, IDL, chylomicrons and HDL) which enable lipids like triglycerides and cholesterol to travel in the aqueous blood environment and in healthy individuals about 30% of the cholesterol in the blood is carried by HDL. HDL-c is the smallest of all the lipoproteins and it is high dense because of large protein content when compared to others. The measurement of circulating concentrations of HDL-c serves as an index for the assessment of risk factors related to heart attacks and inversely related with coronary artery disease [2, 3]. The major pathway of HDL-c metabolism is through reverse cholesterol transport (RCT). The major components of HDL are apolipoproteins (Apo) A1 that covers the HDL particle in an anti-parallel, double belt structure [4]. Ten transmembrane amphipathic helices are the secondary structure of ApoA1 [5]. The APoA1 is recognised by some proteins involved in the RCT for various processes. The ApoA1 serves as a molecule for the recognition by the lecithin cholesterol acyl transferase (LCAT), ATP binding cassette

protein (ABC) A1 and the scavenger receptor B1 (SR-B1). ApoA1 does not constitute only the structural part of HDL, but serves as a recognition molecule for RCT, so deficiency of ApoA1 do not form HDL normally and 70-80% reductions in both plasma cholesterol and HDL was observed in mice [6]. Studies in mice and human suggested that the ApoA1 deficiency causes profound effects on pathology of atherosclerosis [7], but some mutations in ApoA1 (apoA1-milano) R173C has been implicated in risk free health despite having decreased HDL-c and hyper triglyceridemia [8]. The potential athero protective effects of ApoA1 mutations are not very well understood. Although previous studies has confirmed that there is more cholesterol efflux capabilities in this mutation, recent studies argued that efflux rates were not significantly different between wild and mutant ApoA1 in the cell lines study [9]. The first step in the RCT is the efflux of the cholesterol mediated largely by the ABCA1 gene product. ABCA1 promotes the cholesterol efflux by catalyzing the transfer of cholesterol and phospholipids from cells in peripheral tissues to the HDL [10]. Cholesterol induced differentiation of macrophages in the blood vessel into foam cells is the prerequisite for atherogenesis [11].

The discoidal HDL binds to the ABCA1 on the cell surface, internalized and within endosomes gets loaded with lipid pools and again released into the circulation as Pre-β2-HDL [12, 13]. The importance of this protein ABCA1 as revealed by disease phenotype such as Tangier's disease when there are inactivating mutations, clinically characterised by orange tonsils, peripheral neuropathy and predisposition to coronary artery disease [11,14]. The discoidal Pre-β2-HDL is converted to spherical α-HDL via LCAT mediated conversion of cholesterol into cholesteryl esters, ApoA1 serves as a cofactor for this conversion that makes HDL a preferential substrate for LCAT action [15, 16]. This conversion of cholesterol into cholesteryl esters serves dual purposes, cholesterol esters being more hydrophobic than the free cholesterol occupies in the centre and maintains a concentration gradient favoring addition of free cholesterol further to the HDL to carry [17]. The interchange of lipoproteins of different classes is modulated by the activities of lipases present in the circulation. Lipases are water soluble enzymes that hydrolyze ester bonds of water insoluble substrates such as triglycerides, phospholipids and cholesteryl esters.

Endothelial lipase, hepatic lipase and lipoprotein lipases remodel circulating lipoproteins and effectively maintain the homeostasis of lipid metabolism. Endothelial lipases hydrolyse phospholipids of VLDL, chylomicron and HDL [18]. The modulation of phospholipids in the HDL by endothelial lipases is critical for determining the mode of cholesterol efflux either by SR-B1or ABCA1 [19]. The hepatic lipase plays a major role in the hydrolysis of VDL and IDL triglyceride [20]. Lipoprotein lipase is involved in the hydrolysis of triglyceride rich lipoprotein particles in muscle, adipose and macrophages and the hydrolysis generates free fatty acids and glycerol for energy metabolism [21]. Apart from these hydrolytic enzymes there are two important lipid transfer proteins, cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP). CETP catalyses the exchange of cholesteryl esters inside HDL for triglycerides of LDL and VLDL [22]. PLTP regulates the size and composition of HDL both by lipid exchange and particle remodeling. In mice (which naturally lack CETP) the expression of the human ortholog has been found to be both pro-atherogenic [23] and antiatherogenic [24]. Humans carrying CETP mutations has markedly increased HDL and linked to the increased longevity [25, 26] and many studies have shown clearly the role of CETP and its relationship with circulating HDL levels. Inactivating mutations in PLTP in mice has decreasing concentrations of HDL in the circulation. Transgenic mice with increased over expression of PLTP have severely reduced HDL and inhibited RCT though there is an increased level of Pre-β-HDL [27, 28]. While these studies have undoubtedly addressed the roles of both the proteins that keep in check of the circulating levels of HDL, they also generated many unanswered questions to provide a complete balance in the maintenance of HDL concentration. The cholesterol efflux mediated by SR-B1 is the primary event for HDL mediated metabolism of cholesterol in the liver. It serves both for the efflux of cholesterol from the cell surface as well as the delivery for excretion of cholesterol in the liver cells. The importance of SR-B1 in the cholesterol metabolism is understood in animal studies where in mice lacking or inactivated SR-B1 show a two fold increase in the plasma cholesterol levels [29]. Many studies strongly suggest that SRB1 is required for the maintenance of normal cholesterol levels. There is a selective uptake of cholesteryl esters from the HDL into the liver cells via SR-B1 which is distinct and different from ABCA1 and LDL receptor mediated processes [30]. This clearance of cholesterol esters circulates back into the HDL and once again the cycle of RCT begins. The presence of ApoA1 in the HDL is the cause for the binding preference to the SR-B1.

Low Density lipoproteins and cholesterol distribution

Cells that require cholesterol from outside sources apart from endogenous synthesis are delivered by LDL and through metabolism of LDL-c. The quantity of circulating LDL is a well known risk factor for heart disease and many lipid lowering therapies targets LDL-c primarily [31]. Genetic defects of LDL-c lead to familial hypercholesterolemia that affect the population ~1:500 and is the consequence of varied mutations in the LDL-receptor, ApoB and other genes [32]. The major source of the cholesterol is from the diet and from the diet lipids are digested starting from the oral cavity to the duodenum sub-layer of the intestine. This crude emulsions consists of free cholesterol, triglycerides, free fatty acids and phospholipids. Secretions from the liver (bile acids) are essential for the absorption of the cholesterol. Bile salts emulsify triglycerides and cholesteryl esters and are hydrolysed by the pancreatic lipase and carboxyl ester lipase respectively. The brush border membranes and intestinal enterocytes in the jejunum are the regions where passive absorption of cholesterol takes place. Garcio-Calvo and colleagues discovered that Niemann-pickC1-like (NPC1L1) protein is a putative target of the drug that is used for the treatment of hyper lipidemia and identified this protein as a key molecule that enables cholesterol absorption. Mutations in the above protein are associated with the decreased absorption of the cholesterol and circulating levels of LDL-c [33]. The other proteins that contribute to the absorption of cholesterol are ABCG5/G8 genes which negatively regulate the absorption, the mutations of which causes increased absorption of cholesterol (especially plant sterols) and causes premature atherosclerosis [34]. The packaging of cholesterol takes place

inside enterocytes and as chylomicron put into the circulation. Cholesteryl transferase protein esterifies large amounts of free cholesterol [35] and mono and di-acyl glycerol acyl transferases synthesize triglycerides from free fatty acids [36]. The incorporation of ApoB-48 protein with the cholesteryl esters, phospholipids and triglycerides by the microsomal triglyceride transfer protein (MTP) causes the complete assembly for transportation [37]. Mutations in the above protein cause erroneous assembly or no assembly with poor formation of both chylomicrons and VLDL [38]. The nascent chylomicrons are transported to the golgi apparatus, where several additional triglycerides are recruited and transported via vesicular structures to clathrin-coated pits and exocytosed [39]. The entire digestion and packaging of dietary lipids will take about one hour, half life of the lipids in chylomicrons is just 4.5min [40]. Addition of ApoC2, Apo E from HDL, a process that yields mature chylomicrons will take place in the blood stream. ApoC2 in the chylomicron activates the LPL, which catalyses the hydrolysis of triglycerides in chylomicrons which distributes the fatty acids to tissues [41]. LPL deficiency causes hypertriglyceridemia, decreased HDL and LDL, and accumulation of chylomicrons in the plasma. The chylomicron remnants formed from chylomicrons after the action of LPL causes its clearance in hepatic cells via ApoE interaction. The other lipoprotein VLDL is assembled starting from the rough endoplasmic reticulum of the hepatocytes, followed by processing in the golgi apparatus (VLDL-VLD2) to form the mature VLDL.

As chylomicrons, VLDLs exchange ApoC2 and ApoE, with HDL in circulation and distribute free fatty acids to muscle and adipose tissues expressing LPL. When VLDL loses the triglycerides they become IDLs, which are either removed by the liver or further acted upon by the lipase to develop into LDL. LDL-c is taken up by the cells through LDLr which interacts mainly with ApoB100 on LDL. The extracellular portion of LDLr consists of three protein modules including a domain with seven contiguous cysteine rich repeats (referred to a LDLR type A, or LA domains) [42]. The receptor-ligand complex is endocytosed into the cells that occurs at clathrin coated pits. The acidic conditions within the cells catalyse and dissociate LDL from the LDLr. LDL particles are degraded into lipid components and amino acids by enzymes of the vesicle. Lysosomal acid lipase hydrolyses the cholesteryl esters into free cholesterol which can be incorporated into the cell membranes and transformation into other products like bile acids or steroids depending upon the cell context. The denovo synthesis of cholesterol is metabolically regulated and expression levels of HMGcoA reductase as well as LDLr are negatively controlled by intra cellular cholesterol. Sterol regulatory elements are present in the promoter regions of LDLr and HMGcoA reductase genes. Transcriptional factors like SRE binding proteins (SREBPs) are required for transcription of these genes containing SRE.

Conclusion

Cholesterol is obtained by the cells for its use for various physiological functions through de novo synthesis, or receptor mediated endocytosis of exogenous and endogenous cholesterol packaged in LDL. Cholesterol is transported in the circulation as triglyceride rich particles and hydrolyzed by LPL to release fatty acids for the usage. Now, cholesteryl-ester rich lipoproteins interact with the LDLr and selectively taken into the cells as a source for cholesterol. HDL-c synthesized in the liver has a critical task of eliminating the cholesterol from LDL and peripheral circulation in the process of RCT. The advents of new technologies in the scientific research assure that these metabolisms of lipoproteins continue to be investigated until a preventive medicine for hyperlipidemia becomes in reality.

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