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Review

Lipoproteins as modulators of atherothrombosis: From endothelial function to primary and secondary coagulation

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ABSTRACT

Atherothrombosis is a complication of atherosclerosis that causes acute cardiovascular events such as myocardial infarction and stroke. Circulating lipid levels are highly correlated with atherosclerotic plaque development. In addition, experimental evidence suggests that lipids also directly influence thrombosis and influence the risk and the outcome of acute cardiovascular events. Plasma lipoproteins influence three aspects important to atherothrombosis: endothelial function, platelet aggregation (primary coagulation) and secondary coagulation. Overall, VLDL, LDL and oxLDL promote thrombus formation, whereas HDL shows antithrombotic actions. In this review we will address the current knowledge about modulation of atherothrombosis by lipoproteins, summarizing findings from *in vitro* and *in vivo* animal studies, as well as from observational and interventional studies in humans. We will conclude with future perspectives for lipid modulation in the prevention of atherothrombosis.

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1. Introduction

1.1. Pathophysiology of atherosclerosis and atherothrombosis

Atherosclerosis is a lipid-driven progressive inflammatory disease, characterized by the accumulation of lipids and fibrous elements in medium and large sized arteries [1]. Atherosclerosis develops largely asymptomatic over a lifetime. However, as lesion development progresses, atherosclerosis can become complicated by atherothrombosis. This can be caused by either plaque rupture or superficial erosion of the plaque [2]. Upon rupture or erosion, subendothelial collagen and thrombogenic plaque material, such as macrophage tissue factor (TF), are exposed to the arterial circulation. This leads to thrombus formation on top of the ruptured or eroded plaque [3]. Within 1 min after rupture, platelets adhere and aggregate on collagenous plaque components. After 3 min, the thrombus is characterized by thrombin and fibrin formation, and by the activation of coagulation, a process entirely triggered by plaque-derived TF [2]. This thrombus formation can lead to rapid occlusion of the vessel, and cause myocardial infarction, ischemic stroke and sudden death. This deadly nature of atherothrombosis has made it a critical target for investigation.

1.2. Plaque rupture versus plaque erosion

Autopsy studies done several decades ago showed that plaque rupture most commonly led to fatal coronary atherothrombosis [4,5], whereas a minority of the fatal events was caused by superficial erosion of the plaque. These studies also demonstrated that plaques prone to rupture, so-called vulnerable plaques, are characterized by a thin fibrous cap, and a large lipid core with a relative abundance of inflammatory leukocytes [5]. Although the concept of the vulnerable plaque has been largely accepted and widely used in research, lately it has been the subject of debate. Questions have been raised about the dominant mechanisms implicated in atherothrombosis. Recent evidence suggests that plaques with thin fibrous caps and large lipid pools seldomly rupture and cause clinical events [6,7]. Often multiple presumed vulnerable plaques reside in coronary and other arteries. However, these do not inevitably rupture.

As opposed to lesions associated with plaque rupture, vulnerable plaques underlying areas of superficial erosion do not have thin fibrous caps. Furthermore, they harbor fewer inflammatory cells and lack large lipid pools [8]. Interestingly, from studying specimens from the Athero-express biobank we know that there has been a shift in human atherosclerotic plaque morphology over approximately the last 12 years. Plaques obtained from more recent patients with symptomatic carotid artery disease show significantly more fibrous, non-inflammatory characteristics. Moreover, this trend is also visible in asymptomatic patients [9–11]. This shift is possibly due to altered disease demographics and changes in risk factor profiles, such as (passive) smoking and lipid lowering treatment [9]. Lipid lowering reinforces the fibrous cap, decreases the lipid pool and reduces inflammation in both animals and humans [10–12]. Possibly, this shift in plaque characteristics could lead to a subsequent shift in plaque rupture *versus* erosion occurrence [13,14]. The consequences of this possible shift are under investigation.

1.3. Lipoproteins

Lipoproteins are macromolecular complexes of lipids and proteins that are essential for the transport of cholesterol, triglycerides and fat-soluble vitamins in the blood. Based on their relative densities, five major classes of lipoproteins can be distinguished, being chylomicrons (CM), very-low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL).

CM, VLDL, IDL and LDL serve to deliver dietary and hepatic triglycerides and cholesterol to peripheral tissues. In humans, the main structural apolipoprotein (apo) on CM is the apoB48 molecule, while VLDL, IDL and LDL are identified by an apoB100 protein. Moreover, a specific subtype of LDL can be distinguished, lipoprotein (a) (Lp(a)), an LDL-like particle with an apolipoprotein (apo(a)) moiety attached to it.

Native HDL is primarily formed by the liver and the intestine and serves as a cholesterol acceptor from peripheral tissues. In that way HDL mediates reverse cholesterol transport from the periphery to the liver, where it can be excreted *via* bile or repackaged as VLDL for delivery to tissues or used for the generation of native HDL particles. HDL is heterogeneous in terms of its density, size, shape, surface charge and composition [15].

Based on shape, HDL can be divided in spherical and non-spherical particles, which are often referred to as pre-β HDL based on their surface charge. Pre-β HDL can be divided in lipid-poor apoA-I molecules, single apoA-I molecules complexed with a small number of phospholipids, or discoidal particles which contain two or three apoA-I molecules complexed with multiple phospholipid molecules and a small amount of unesterified cholesterol. Upon esterification of free cholesterol to cholesterol esters by the enzyme lecithin cholesterol acyltransferase (LCAT), discoidal HDL can mature into spherical HDL particles. Spherical HDL particles can be divided into two major subclasses based on density: small dense HDL_3, and larger, less dense HDL_2. HDL particles can contain over 80 different proteins, more than 200 lipid species, and several microRNAs. Among these proteins, apoA-I is the most abundant on HDL particles, followed by apoA-II. A minor subpopulation of HDL carries apoE as their main apolipoprotein [16].

High levels of cholesterol are strongly correlated with the incidence of cardiovascular disease. In healthy individuals, cholesterol levels are below 5 mmol/L. A rise of 2 mmol/L cholesterol increases the risk of death by cardiovascular disease by 50% [17]. This is most likely attributable to LDL, the main carrier of cholesterol in human plasma. In contrast, large population studies have consistently shown that low HDL cholesterol, as well as apoA-I levels are independent, inverse predictors of cardiovascular disease risk [18–22]. There is also ample experimental evidence for a causative role for LDL in the development of atherosclerosis. At places in the arterial tree with turbulent blood flow, LDL can accumulate in the arterial intima, where it is prone to oxidative modification. Oxidized LDL (oxLDL) is taken up by macrophages, which, upon excess cholesterol loading, become foam cells. Macrophage foam cell formation in the arterial intima is the start of an atherosclerotic plaque [23]. Epidemiological studies have shown that low HDL is associated with an increased risk for CVD as a result of atherosclerotic plaque development. Although a causal role for HDL is still under debate, many studies have shown protective effects of HDL on the artery wall. [24]. An important mechanism by which HDL is protective lies in their function as cholesterol acceptor. Macrophages are able to efflux excess cholesterol by transporting this to HDL particles *via* ATP binding cassette (ABC) transporters, which reduces foam cell formation.

Due to these pivotal roles of LDL and HDL in the initiation and progression of atherosclerotic lesions, it is reasoned that this is their main role in the pathogenesis of cardiovascular disease. However, lipoproteins are being more and more recognized as multi-purpose players in cardiovascular disease. VLDL, LDL and HDL all carry a variety of proteins, aside their lipid constituents and apolipoproteins that influence their