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Review

The response-to-retention hypothesis: From theory to the potential therapeutic approaches

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ABSTRACT

The key initiating process in atherogenesis is the subendothelial retention of apolipoprotein B-containing lipoproteins. Retained lipoproteins are chemically and enzymatically modified, inducing a chronic inflammatory response. Therapeutic approaches for atherosclerosis treatment have been focused on the control of risk factors, such as hypercholesterolemia, hypertension, and diabetes mellitus. Nonetheless, the efficacy of these strategies is limited and unfortunately the health problem persists. On the other hand, development of therapies targeting the interactions between low-density lipoproteins and components of the extracellular matrix has been poorly addressed. In this work, we review the response-to-retention hypothesis and the recent data on therapeutic approaches targeting the retentive process of proatherogenic low-density lipoproteins.

1. Introduction

Cardiovascular diseases (CVD) are the main cause of death in western countries [1]. The major underlying disease is atherosclerosis, which manifests as a complex process, leading to the often fatal clinical event [2].

The epidemiological association between plasmatic low-density lipoproteins (LDL) and coronary heart disease has been well-established [3]. Subsequently, studies of patients with familial hypercholesterolemia demonstrated that increased LDL levels, without other cardiovascular risk factor, could cause accelerated atherosclerosis [4]. Entrapment of LDL, by proteoglycans (PGs) in the intima layer appears, has been considered as an initial step which triggers inflammation and atherosclerosis development [5,6]. This process constitutes the basis of the "response-to-retention" hypothesis of atherosclerosis, suggested by Williams and Tabas in 1990s decade of the past century.

Despite the cholesterol-lowering effect and anti-inflammatory properties of statins and other lipid-lowering drugs, atherosclerosis remains progressing in a significant proportion of patients [7]. On the other hand, antioxidants have failed in the primary and secondary prevention of cardiovascular diseases [8]. Thus, there is a widespread agreement on the need of novel therapies directed to avoid the retention of LDL in the artery wall, which would complement current therapies.

The aim of the present review was to discuss the importance of the retentive processes of LDL in the arterial wall on atherosclerosis progression as well as the potential therapeutic approaches focused on this target.

2. Arterial wall proteoglycans

PGs are macromolecules composed of a protein core and linear long-chain carbohydrates, called GAGs. GAGs consist of repeating disaccharide units bearing negatively charged sulfate and carboxyl groups. GAGs are covalently bound to the protein core by tetrasaccharide linkage [GlcA-Gal-Gal-Xyl]. Different types of GAGs have been identified: chondroitin sulfate (CS), dermatan sulfate (DS), heparan sulfate (HS), heparin, keratin sulfate, and hyaluronan [9,10].

GAGs play different role in physiology, including the stabilization of the fibrillar extracellular matrix (ECM), control of hydration and cell cycle, among others [11]. Within the ECM, PGs are distributed in two different locations. PGs of pericellular environment are attached to the cell plasma membrane, whereas those secreted by cells are part of the extracellular space and usually do not physically connect with cells. The extracellular PGs are versican, decorin, and biglycan. Perlecan, syndecan and glypican represent the pericellular subclasses [9].

Vascular smooth muscle cells (VSMC), endothelial cells, and macrophages synthetize arterial wall PGs. The composition of PGs

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synthesized by vascular cells varies with the physiological state of the cells. Numerous factors, including extracellular matrix components, transforming growth factor (TGF), platelet-derived growth factor (PDGF), oxidized LDL (oxLDL), and non-esterified fatty acids modulate PGs synthesis [12]. In particular, ox-LDL increases the size of GAGs chain length having a greater affinity for native LDL [13]. Altered patterns of PGs synthesis characterized much pathological states, including atherosclerosis [12].

3. Molecular interactions leading to low-density lipoprotein retention

Lipoprotein entry within the intima layer depend on plasma levels, lipoproteins size, charge and composition [14]. The small, dense LDL subclass is associated with increased lipoprotein binding to arterial PG *in vitro*. Furthermore, the conversion of apoB lipoproteins into a small, dense form by *in vitro* phospholipase A2 treatment increases their affinity to PGs [15,16]. Other properties of LDL also affect the capacity of lipoproteins to entry within subendothelium and to interact with ECM molecules. For example, the high content of cholesterol increases the LDL affinity for arterial wall PGs, by mean of conformational changes in one of the PG-binding sites of apoB-100 [17]. Furthermore, the content of sphingomyelin (SM) in VLDL/LDL surface can promote the retention of these proatherogenic lipoproteins [18]. Up to 18% of total plasma phospholipid exists as SM [19], being SM content of atherosclerotic lesions higher than that of normal arterial tissue [20]. Plasma SM levels in apolipoprotein E-deficient mice (apoE$^{-/-}$) are 4-fold higher than those in wild-type mice [21]. In humans, plasma SM levels have been identified as an independent risk factor for coronary heart disease [22,23], having a prognostic value in patients with acute coronary syndrome [23].

Studies in mice expressing apoB-100 with mutations in its CS-binding region established how lipoproteins-CS interact [24,25]. These studies also demonstrated that mice expressing the PG binding-defective LDL had less atherosclerotic lesions due to a decreased interaction of mutated LDL with arterial wall PGs. LDL retention within ECM is mediated by direct binding of positively charged domains on apoB to negatively charged groups of retentive molecules, including sulfated groups of GAG [26]. Studies of Borén et al. [24] have clarified the required molecular determinants of apoB lipoproteins to interact with PGs. PG-binding site of apoB100, identified as site B (residues 3359 to 3369), possess positively charged arginine and lysine residues which are critical for binding to CS/DS PGs. In later stages of atherogenesis, LDL retention can be enhanced by GAGs chains elongation, increasing cholesterol oxidation and oxLDL internalization by intimal macrophages [25].

4. Proteoglycans in early atherosclerosis

Retention of LDL in the intimal layer favors chemical modifications, such as reactive oxygen species-mediated oxidation, lipase modification, or glycation, leading to aggregation and posterior cellular uptake leading to foam cells formation [27]. Aggregated and fused lipoprotein particles bind to PGs more tightly than do non-modified lipoproteins [28]. Modified LDL, including oxLDL, triggers the inflammatory reactions, including leucocytes recruitment, proatherogenic nuclear factors activation, proinflammatory cytokine expression, reactive oxygen species generation, contributing altogether to plaque growth and instability [29,30].

PGs that contain CS side chains seem to play a key role in proatherogenic lipoprotein retention, mainly in early stages of atherogenesis [31]. Biglycan and versican have been identified as the most important of the CS-containing PGs in apoB lipoprotein retention within human arteries [2]. In our species, atherosclerosis-prone areas of the arterial tree show high content of CS-containing PG, such as lumican [32]. Furthermore, in larger stages, these arterial sites are characterized by CS-PGs accumulation, occurring side chain elongation, and increasing of sugar sulfation degree [33].

On the other hand, rats VSMC, which overexpress human biglycan, produce ECM with high affinity to apoB lipoproteins [34]. Studies of Nakashima et al. revealed that biglycan distribution in thickened regions of human intima co-localized with lipids in the early phase of type I atherosclerotic lesions, suggesting that biglycan plays a key role in initial deposition of lipids in the arterial wall. Indeed, co-localization of biglycan and apoB/apoE apolipoproteins, are also found in early and advanced human and murine atherosclerotic lesions [2,28]. Recently, it was demonstrated that increased vascular biglycan expression predisposes to increased lipid retention and atherosclerosis development in LDL receptor-deficient mice (LDLr$^{-/-}$) expressing the human biglycan transgene [35]. These findings altogether suggest that molecular interactions between extracellular PGs and lipoproteins predispose and aggravate atherosclerosis development.

5. Accessory molecules: promoters of lipoproteins retention in advanced lesions

In later stages of atherosclerosis, endothelial cells and macrophages secrete "accessory molecules", such as sphingomyelinase (SMase) and lipoprotein lipase (LpL), which contribute to lipoprotein fusion and aggregation [6,37,38].

Much *in vitro* studies implicate the secretory form of acid SMase (S-SMase) in proatherogenic modifications of LDL particles. The synthesis of S-SMase is stimulated by proinflammatory cytokines and reactive oxygen species [38,39]. This enzyme mediates the hydrolysis of SM to ceramide on the surface of atherogenic apoB-lipoproteins [37]. The increase of ceramide content on lipoproteins promotes their aggregation [36], which in turn promotes the retention by PGs, affecting the efflux of large PGs-LDL aggregates from lesions, or stimulating macrophages uptake and foam cells formation.

The role of S-SMase in atherogenesis *per se* has been demonstrated by several human and animal studies. S-SMase is present in human and murine atherosclerotic lesions [40], and aggregated lipoproteins extracted from human atheroma are enriched in ceramide, suggesting the hydrolysis by SMase [36]. Studies of Devlin et al. [41], showed that acid SMase deficiency in two murine models of hypercholesterolemia abrogated atherosclerotic lesion development and decreases the arterial trapping of proatherogenic lipoproteins.

On the other hand, LpL has been found on the endothelium surface and within the intima. This enzyme binds GAGs, decorin-coated collagen and LDL, suggesting that LpL bounded to PGs and collagens within the intima leads to retention of LDL by acting as a molecular bridge [42]. Gustafsson et al. suggested that retention of LDL is initiated by direct LDL-PGs binding, but later shifts to indirect binding with bridging molecules, such as LpL [43]. These evidences involve the bridging molecules in lipoprotein retention and atherogenesis. In addition, S-SMase and LpL act in a synergistic form to increase the retentive processes of LDL [36,38].

5.1. Targeting proteoglycans-lipoproteins binding to prevent atherosclerosis

Despite the anti-hypercholesterolemic and anti-inflammatory effect of statins, atherosclerosis still remains as a leading cause of