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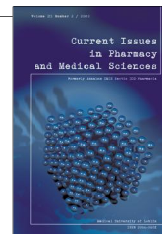

The role of glycosaminoglycans and proteoglycans in atherosclerosis

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ABSTRACT

Numerous studies point out to the role of glycosaminoglycans (GAG) and proteoglycans (PG) in the pathogenesis of atherosclerosis. Both GAG and PG take part in the formation of *tunica intima* and *tunica media*, contribute to the neointima formation, and can be found in the glycocalix layer. These molecules influence many properties of blood vessels, such as permeability, viscoelasticity, hemostasis, lipid metabolism, and extracellular matrix organization. The PG from atherosclerotic arteries had higher binding affinity for LDL, which suggests that alterations in the vascular PG contents predispose to atherosclerosis development. Atherogenic LP retained in the intima in association with GAG/PG, undergo several modifications, such as the oxidation or aggregation. LDL exposed to CS PG is more susceptible to such modification, which favours uptake by the macrophages. Moreover, oxy-LDL stimulated SMC to synthesize longer chains GAG and enhanced LDL binding properties. For this reason GAG structure and metabolism are the potential targets of the therapeutic investigation.

Keywords: atherosclerosis, glycosaminoglycans, proteoglycans, lipoproteins, heparan sulfate.

INTRODUCTION

Atherosclerosis is a complex process, one of the most interesting medical problems over the last 50 years. This pathological disorder involved in the macrovascular disease, leads to severe clinical cardiovascular complication – the myocardial infarction, when it occurs in the coronary arteries, or stroke, when it occurs in the cerebral arteries. Apart from the well-known, classical risk factors, used in the assessment of the atherosclerosis risk degree, the new factors that could be useful in the early diagnosis and prevention are still being looked for. Numerous studies point out to the role of glycosaminoglycans (GAG) and proteoglycans (PG) in the pathogenesis of atherosclerosis. Both GAG and PG, take part in the formation of *tunica intima* and *tunica media*, contribute to the neointima formation, and can be found in the glycocalix layer [18]. These molecules influence many properties of blood vessels, such as permeability, viscoelasticity, hemostasis, lipid metabolism, and extracellular matrix organization [16]. It has been proved that the intimal thickenings are the precursors of the more advanced atherosclerotic lesions, and that intimal GAG/PG play important roles in atherosclerosis, especially by lipoprotein (LP) binding and interaction. It is likely that the discovery of the precise mechanisms of the regulations of the synthesis and metabolism for the individual GAG/PG in the vascular wall will enable to compile a pharmacological prevention strategy, which could not only prevent the development of the lesions, but also seize the progression.

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GLYCOSAMINOGLYCANS AND PROTEOGLYCANS – STRUCTURE AND BIOLOGICAL SIGNIFICANCE

Glycosaminoglycans (GAG) chains are unbranched, linear, high-molecular weight polisaccharides. They consist of the repeating disaccharide units containing an amino sugar with *N*-acetylgalactosamine (GalNAc) or *N*-acetylglucosamine (GlcNAc), and a uronic acid, such as glucuronate or iduronate. Next modification - sulfation and carboxylation, gives them a highly negative charge [4,7]. Basing on the disaccharide components, GAG are divided into six types, including keratan sulfate (KS), chondroitin sulfate (CS), dermatan sulfate (DS), heparan sulfate (HS), heparin (HE) and hialuronic acid (HA). In contrast to the others, HA is a much longer disaccharide polymer, of the order of 1000 kDa, but is not sulfated (still, it obtains its negative charge from the carboxyl groups), and is not covalently bonded to a core protein [18]. During the biosynthesis, GAG chains are linked to the core proteins in the Golgi apparatus forming PG. Thus, PG contains highly negatively charged GAG, which are attached to the core protein. The diversity of these compounds is related to the variable expression of the genes coding for the core proteins, and the resulting alteration in the composition and sequence of the aminoacids, as well as the differences between the length, kind, level of modification and location of GAG chains [4].

PG can be divided into three categories, according to their cellular location, i.e.: extracellular matrix PG (aggrecan,

perlecan, versican), cell surface PG (syndecan, glipican, decorin) and intracellular PG (sergycin). Cell surface PG act as the receptors because they can bind several ligands: growth factors, chemokines, enzymes, ECM components, platelet secretory products, anticoagulants, and this way modulate their concentration, distribution, and biological activity [6]. Furthermore, they interact with various molecules of the ECM, leading to the rearrangement of cell cytoskeleton, and transmitting signals promoting cell adhesion and migration [13]. In each model for the processing of ligands bound to PG, the nature of the cell surface PG is a key determinant of the following cellular catabolism [6].

Because of the fact that these compounds are so widespread, they assist the proper functioning of almost all the systems and organs, and their effects have both the structural and modular character. They take part in the transfer of information, support, and regulation. The highly ionic charge of GAG chains is critical in determining the water content or turgor of ECM and influences tissue viscoelastic behavior. Binding of water by agrecan provides cartilage with the ability to resist compression. These molecules help define functional compartments and act as filtration barriers. GAG/PG have essential roles in urinary, circulatory, nervous, muscular, skeletal and immune systems, as well as embryological development and wound repair [4, 16]. The best known HS ligand interaction, important for the coagulation and inflammation, is the binding of antithrombin III to HS. Some GAGs contribute to the negative charge in the glomerular basement membrane (GMB), and changes in their sulfation and concentration may be associated with the development of nephropathy [12]. In respiratory system PG are important components of the alveolar and pulmonary capillaries. In reproductive system, they take part in the ovarian function and the placenta development. The HS degradation products, obtained by the action of human heparanase, are directly involved in the tumor progression and metastasis [13]. In turn, sulphated GAG, e.g. HS, were indentified as constituents of PrP^{Sc} plaques, facilitating their conversion in the brains of Creutzfeldt-Jakob disease [7].

VASCULAR GLYCOSAMINOGLYCANS AND PROTEOGLYCANS

The luminal surfaces of the blood vessels are covered by a gel-like, endothelial glycocalyx layer, which behave as an adhesion and transport barrier to water and larger solutes [18]. Additionally, this barrier plays some important roles in the mechanotransduction of shear stress. The essential components of the glycocalyx are GAG, especially HS, accounting for more than 50% of the total GAG pool, with the rest comprising CS and HA. The transmembrane syndecans that associate with the cytoskeleton and the membrane-bound glypicans are the major PG found on endothelial cells. It has been suggested that glypican core protein of the glycocalyx mediate the NO response to shear stress, whereas syndecan core proteins mediate the remodeling response for a longer period of time [18].

Arterial intima is the innermost layer of the artery, well-organized structure composed of the endothelial cells, subendothelial tissue, and elastic lamina. The thin subendothelial tissue consists mainly of ECM with smooth muscle cells (SMC). GAG and PG are the main components of the ECM and form a tight and negatively charged network [2].

There are over 20 different PG identified in the vascular wall, produced by vascular SMC and EC. The main PG in the vascular wall are: CSPG – versican, CSPG/DSPG – biglican and decorin, HSPG – perlecan, CSPG – mimecan [1]. The most frequent extracellular PG is versican, the subsequent are biglican and decorin. In atherosclerotic damages, the thickening of the intima occurs together with the enrichment with versican and biglican, whilst the intima enriched with decorin is characteristic for the atherosclerosis-resistant arteries [1]. In addition, the expression of decorin is induced during formation of neovessels both *in vitro* and *in vivo* [16].

Versican interacts with HA to create viscoelastic matrices that are required for arterial SMC proliferation and migration. Versican is present both in the early stage and in the advanced atheroslerosis, and is localized in the inner layer of the DIT (diffuse intimal thickening) of the arteries. Despite its having the affinity for binding LDL, and a larger number of binding sites to LDL than biglican, versican is not often colocalized with LP and is absent in the lipid core of the lesions. Interestingly, its presence is noticed in the thrombus, suggesting a possible role in thrombosis: platelets bind well to aorta versican [14].

Biglican is present in the intima of normal human vessels. During DIT in coronary arteries, immunohistochemical study indicates biglican in outer layer, in the same localization as LP. It is thought that such a distribution of biglican has a decisive role in the initial deposition of LP. Moreover, the colocalization of biglican with apoB and apoE has been found in both early and the advanced alterations in the vessels. It means that this PG binds not only LDL, but also the HDL particles containing apoE [14].

Decorin is the small leucine-rich PG, and is present in the intima of normal vessels like biglycan, but in the lower levels. It is presumed that decorin plays a role in atherogenesis by linking LP to collagen fibres and apoB. Mechanical stress causes a decrease in the expression of decorin, which could lead to the disorganization and loosening of the collagen in the ECM.

Perlecan is a HSPG and consists of a core protein with 3 binding sites for HS side chain in domain I, and an additional site in domain V. It is the major extracellular HSPG in blood vessel located in both the intima and media of vascular wall [16]. In the animal experiments, its role in atheroscerosis is not consistent in the different species. In mice, it is visible in both the early and the advanced stadia, while in monkeys only in the advanced stadium. In addition, it colocalizes with apoA1, which suggests that perlecan acts as a retaining factor for HDL in athorosclerotic lesion [14].

Leta et al. [11] compared the GAG content of human venous and arterial walls. They reported that the most abundant GAG in the human veins is DS, whereas C4/6S is preponderant in the arteries. The concentrations of C4/6S and HS are ~4.8 and ~2.5 – fold higher in arteries than in veins, whereas DS contents are similar in the two types of blood vessels [11].

According to Tovar et al. [19], aortic content of total GAG does increase during the first 40 years of life, especially CS, mainly C-6-S, whereas DS remains constant. Changes in the content of hyaluronic acid and HS are less apparent. Interestingly, it has been shown that the age-related changes in GAG composition of the arterial wall do not contribute to the increased deposition of plasma LDL, because in healthy arterial human wall, together with the increase in age, concentration of GAG with low affinity for LDL also increases. It is proposed that the atherosclerosis augmenting with age may be a reflection of the longer period for lipid accumulation to occur, and not alterations in GAG composition in older arteries that promote a raised binding of LDL with age [19].

THE "RESPONSE TO RETENTION" HYPOTHESIS

The response to retention hypothesis was written as first by Williams and Tabas in 1995 [17].

That hypothesis assumes that the underlying mechanism initiating the development of the atherosclerotic plaque was the retention of atherogenic, apoB-containing LP in the subendothelial matrix by their interaction with ECM molecules, particularly PG. This interaction may be direct, or mediated via bridging molecules, such as lipoprotein lipase (LPL), or secretory phospholipase A2 (sPLA2) [17]. This process is an initial event in atherogenesis and probably begins with predisposing stimuli, e.g. cytokines and mechanical strain, that enhance synthesis of PG. Furthermore, the local production of PG is characterized by the high binding affinity for LP [14]. The response to retention hypothesis is not contradictory to the chronic inflammation hypothesis, because retained and modified LP can stimulate the recruitment of macrophages and T-cells [14].

GAG obtained from aortas give three peaks, indentified as hyaluronic acid, HS and a mixture of DS together with C4/6S. Arterial hyaluronic acid and HS do not bind LDL, whereas DS and C4/6S form both soluble and insoluble complexes with this LP [19]. Subsequent research shows that high-molecular weight chains of both DS and CS can interact with LDL, but their degree of polymerization and their molecular weight have significance and ultimately describe the level of affinity for LDL. For this reason CS, and especially DS, have especially high affinity for plasma LDL [19].

The reaction between LP and PG is ionic in nature and occurs between the negatively charged sulfate and carboxyl groups on the GAG side chains of PG, and the positively charged arginine and lysine residues of apolipoproteins (apoB100) [10, 14]. The complex LP-PG exhibits increased susceptibility to modifications, such as oxidation and aggregation, and leads to uptake by macrophages to form the foam cells. Furthermore, modificed LP enhance the production of PG with a high affinity for LP [10, 14].

LP bind to PG through ionic interactions, and the factors that increase PG binding affinity for LP are length or sulfation of the GAG chain. In addition, species of PG differ by binding affinity for LP, yet *in vivo* binding site availability seem to influence binding more than the affinity. This confirms colocalization of apoB and biglycan in human atherosclerosis, as well as biglycan and perlecan in mice, even though it is versican that has more LDL binding sites than biglycan. It is probable that several other compounds can act as bridging molecules between GAG and LP, e.g. LPL, apoE, SMase and sPLA2. LPL – enzyme responsible for TG hydrolysis of chylomicrons, VLDL and IDL, is bound to PG and collagens in arterial intima, leading to retention of LDL by acting as a molecular bridge [17].

THE ROLE OF GLYCOSAMINOGLYCANS AND PROTEOGLYCANS IN ATHEROSCLEROSIS

Subendothelial retention of atherogenic LP is an initialising event in atherogenesis. Many studies indicate that the LP-binding PG contribute to retaining atherogenic LP in the intima, especially the CS/DS proteoglycans (CS/DSPG), such as versican, biglycan and decorin. The sulfation pattern of CS is a key player in protein interactions, causing atherosclerosis by high affinity for Lp(a) and LDL [8]. In particular, biglycan has been proposed to be an essential PG in both human and murine atherosclerosis [17], in turn Pillarisetti notes that perlecan is a key antiatherogenic molecule [15]. Atherogenic levels of LDL, oxyLDL decrease not only perlecan core protein synthesis but also enhance HS degradation by stimulating endothelial secretion of heparanase. HS has been further implicated in presentation and stabilization of lipoprotein lipase and hepatic lipase on cell surfaces, and in the transport of lipoprotein lipase from extravascular cells to the luminal surface of the endothelia [9].

In turn Tran-Lundmark et al. [20] investigated the role of perlecan HS chains in vascular disease using mice expressing HS-deficient perlecan. The mutation leads to a significant depletion of the arterial HS content and promotes increased SMC proliferation and formation of the intimal hyperplasia. They concluded that the HS chains of perlecan promote atherosclerosis in mice, most likely through increased retention of lipoproteins. Because of the difference in PG expressed in mice and humans, it is difficult to determine a role of perlecan in human disease [20].

It has been proved that GAG length is a main determinant of PG binding affinity, with longer chains showing higher-affinity binding. Molecules known to enlarge GAG on PG include TGF-β1, platelet-derived growth factor (PDGF), oxyLDL, free fatty acids and angiotensin II. GAG chains on PG are also elongated in proliferating cells [1]. The next factor crucial for LDL binding is sulfation of GAG chain. It has

also been suggested that the degree of sulfation, rather than the position of the sulfate groups on GAG, determines LDL binding [1].

Also the modifications in LDL particles, such as lipolysis, increase its binding affinity for PG. The reason is that they induce changes in the surface monolayer of LDL that may modify the conformation of apo-B100, which then exposes a new PG-binding site [2, 10]. Two PG binding sites have been described in apo-B. Site A is only exposed after degradation of phospholipids, by sPLA2, whereas site B is permanently exposed [2]. Site A becomes functional in sPLA2-modified LDL and acts cooperatively with site B resulting in increased PG-binding activity. The increased binding for PG of cholesterol-enriched LDL is solely dependent on site B. This mechanism is likely to be mediated by a conformational change of site B, and contrasts with sPLA2-modified LDL, independent of site A [5].

Lähdesmäki et al. [10] indicated that in acidic pH of ECM increases phospholipolysis by sPLA2, and the PG binding of the TG-rich LP, and so may increase the progression of the atherosclerotic lesions. The potent binding of LDL, VLDL and IDL to PG at acidic pH is likely to prolong their residence time in the ECM, which again can lead to the prolonged exposure to sPLA2 [10]. In turn, GAG-LDL complexes are more easily internalized by macrophages than LDL alone, thereby enhancing the formation of foam cells. Also GAG induced structural alterations in LDL molecules may potentiate their atherogenic effects [19].

Other components of LDL, such as apo-E or apo-CIII can also mediate binding of LP to PG, by bridging (apoE), or by facilitating PG interaction (apoCIII) [2]. Study of apo-E and apo-CIII contents in LP indicated that particles with low affinity to PG had high contents of these apolipoproteins. It is visible on the example of VLDL that apo-E and apo-CIII rich, large VLDL have lower affinity to PG than smaller VLDL [10].

Modified, electronegative LDL(-) are increased in subjects with high a cardiovascular risk. This particles have a higher density, smaller size and could bind to PG, owing to their increases apoE and apoCIII content compared to LDL(+). Furthermore, the presence of LDL (-) increased binding affinity for arterial PG compared to LDL (+) [2]. The study preformed with anti-apoB-100 mAbs showed that the major difference of apo B100 immunoreactivity between native LDL and LDL(-), affecting both the amino- and carboxyl-terminal ends, suggests that both terminal extremes are more accessible in LDL(-) than in native LDL. This property would favor LDL(-) in aggregation and retention in the superficial PG-rich layer of the arterial intima [3].

Because the main characteristic of LDL(-) is its increased electronegative charge, it was expected to have a reduced interaction with the negatively charged sulfate and carboxyl groups on PG. According to Bancells et al. [2] the additional interactions appear, therefore electronegatively charged particles are not a limiting factor of all LDL (-) particle interaction with PG, apparently because it can be counteracted by other particle properties that favor the PG interaction, as apo E and apo CIII. Apo E has two binding sites for heparin but does not play a major direct role in the increased binding of LDL(-) to arterial PG. In turn, the content of apo CIII in LDL particles is very low and these results indicate that its role is negligible in the binding of LDL to PG. What is important, it has been found that lipolysis by SMase or sPLA2 promotes aggregation of LDL and increases their binding affinity to PG. PLC-like activity hydrolyzes sphingomyelin, phosphatidylcholine in LDL, yielding phosphorylocholine, which is released. The increase in these hydrophobic molecules causes aggregation, because the ceramide-, mono- and diacylogliceol-, enriched domains act as nonpolar targets on the particle surface and initially lead to particle aggregation [2].

The GAG from different localizations vary in composition and in binding affinity for plasma LDL. It is presumed that the distribution of GAG in the wall may be modified, which suggests that GAG composition could be one of the several factors that determine the susceptibility of a given artery to atherosclerosis. In vitro investigation shown that several stimuli related to the known cardiac risk factors alter GAG synthesis. These include shear stress, angiotensin II, hypoxia, certain cytokines and native or modified LP [19].

One of the mediators taking part in atherogenesis are growth factors. These factors can modulate synthesis of GAG by inducing phenotypic change in the arterial SMC and by increasing the synthesis of PG, with greater affinity for plasma LDL [19]. For example, elevated level of TGF-β, commonly seen in diabetes, can alter vascular PG synthesis and increase PG binding affinity for LDL [17]. It has been proved that PG produced by TGF-β -treated cultured SMC show longer GAG chains and higher binding affinity to LDL than GAG produced by control SMCs. Although the examinations on the mice models suggest that TGF-β promotes plaque stabilization by collagen synthesis stimulation, inhibition T-limthocyte activation, and foam cells formation, whereas the other examinations relating to the human models show that TGF-β acts pro-atherogenically. Research on the expression of TGF-β and its receptors in human atherosclerotic lesions, suggested that TGF-β is active in lipid-rich lesion and contributes to the pathogenesis of atherosclerosis [15].

Among the GAG examined in the context of atherosclerosis, the most inconvenient one is perlecan. Depending on the cell types and the environment, it can have different, sometimes contradictory biological effects. Seger et al. [16] called this phenomenon “perlecan paradox”, describing it on the example of the influence of perlecan on the two different types of vessels-building cells: SMC and EC. In SMC, perlecan has been shown to be a potent inhibitor of adhesion, migration and proliferation, and may represent a novel strategy to inhibit intimal hyperplasia after arterial injury. However, the mechanism by which perlecan inhibits SMC has not been fully explored. In contrast to its inhibitory effect

on SMC, perlecan appears to be a potent pro-angiogenic agent. There are several possible explanations of this duality of function depending on cell type. These differences may be due to the HS side chains that directly affect cell signaling, or through interactions with heparin-binding growth factors, particularly FGF-2 and its receptor. For example, some genes may be induced by heparin in SMC but not in EC. Differences in the ECM of SMC and EC also profoundly affect cellular behavior. These opposing effects merit further studies. The diverse biological effects of perlecan are ideal for the prevention of in-stent restenosis. Perlecan inhibits SMC proliferation and arterial thrombosis while enhancing EC proliferation, which may promote rapid healing after stenting [16].

CONCLUSION

The complete mechanisms responsible for the development or even initiation of atherosclerosis are still unknown; hence, the research on the role of the particular GAG and PG in these processes is an interesting area of a future work. Certainly, one of the causes is the lack of good models for the study of atherosclerosis. The morphological profiles of atherosclerosis are different between the human and laboratory animal, and it may be partly misleading to extrapolate the results received from the animal models to the human ones.

It is likely that the discovery of the precise mechanisms of the synthesis and metabolism regulations of the individual GAG/PG in the vascular wall will enable to compile a pharmacological prevention strategy, which could suppress any lesions and slow disease progression. The therapeutic potential is that shorter GAG chains and modified sulfation may lead to the lower-affinity binding of LP, and thus reduced retention of LP in the vessel wall, and less atherosclerosis [1, 17].

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