THE INFLUENCE OF EXTRACELLULAR MATRIX COMPOSITION ON THE PATHOGENESIS OF CORONARY ATHEROSCLEROSIS

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Abstract - The modern concept of the development of atherosclerosis implies that the underlying pathogenesis of this disease is vascular remodeling as a response of the vessel wall to hypertension associated with hyperlipidemia and subsequent inflammation. However, even though this disease has been investigated for decades, both from a basic and clinical research aspect, there are still many doubts as to what the initial phase of the disease is. In contemporary literature there are an increasing number of papers that stress the importance of the extracellular matrix (ECM) of the blood vessels connective tissue, particularly proteoglycans, in the formation of early atherosclerotic lesions of human coronary arteries.

Key words: Atherosclerosis, extracellular matrix, coronary artery, immunohistochemistry, ultrastructure

INTRODUCTION

Connective tissue is found in all layers of the vascular wall in varying quantities: it is prominent below the basement membrane, forming subendothelial layer. A significant accumulation of connective tissue is also found in the adventitia (Stevens and Lowe, 1997). This tissue has the function of wall structural support; it represents the mechanical barrier to infectious agents, performs a defensive role through the activity of immunocompetent cells and macrophages, and finally, it is also the center of metabolic activity of the vascular wall (Fawcett, 1986). Connective tissue consists of the extracellular matrix (ECM) and connective cells. ECM of the vascular wall can be expressed in the form of a flexible, specialized thin layer, which represents the basement membrane and in the form of stromal (interstitial) ECM (Lačković, 1998).

The structure and function of the ECM

The ECM is resistant to compression and stretching. It has a very complex structure. It consists of a ground substance and the connective fibers that permeate it. The ground substance is an amorphous gelatinous substance which is made up of glycosaminoglycans, proteoglycans and adhesive, structural glycoproteins. These three families of macromolecules form different interactions between themselves, with fibers and connective tissue cells. They participate in important processes
such as cell adhesion, thrombosis and coagulation, calcification, organization and plaque rupture, cell migration and proliferation and LDL binding (Juliano and Haskill, 1993; Goldstein et al., 1979; Kaplan and Aviram, 2001). Glycosaminoglycans (GAG) (mucopolysaccharides, mucus) are hexosamine polysaccharide chains, covalently bound to different amounts of protein. These include chondroitin sulfate, dermatan sulfate and heparan sulfate. Usually they are divided into acidic and neutral (Jones, 2002; Katsuda and Kaji, 2003). Proteoglycans (PGs) are highly sulfated glycosaminoglycans with a centrally set protein core. They are synthesized by endothelial cells, fibroblasts, mast cells and vascular smooth muscle cells (VSMCs). The degree of polymerization of these large molecules determines the degree of viscosity of the ground substance (Jones, 2002; Merrilees et al., 2001). Endothelial cells predominantly synthesize biglycan and perlecan (Riessen et al., 1994; Schönherr et al., 1993), and VSMCs synthesize versican, biglycan and decorin (Edwards et al., 1994; Qwarnström et al., 1993). Adhesive glycoproteins are macromolecules that mediate in the linking of cells and ECM components and thanks to this, they are the most important integrative component of connective tissue. The most important adhesive glycoproteins of coronary arteries are fibronectin, laminin, entactin, thrombospondin, vitronectin and osteopontin (Wight, 1995).

The collagen and elastic fibers of the vascular connective tissue provide its elasticity, strength and flexibility. Collagen fibers also participate in the processes of differentiation, adhesion, proliferation and apoptosis (Plenz et al., 2003). They are synthesized by fibroblasts and other fixed connective tissue cells, and VSMCs of the synthetic phenotype (Katsuda et al., 1992). There are 13 types of collagen in the vascular wall whose amounts increase with the development of atherosclerotic plaque (Plenz et al., 2003). Elastic fibers are also synthesized by fibroblasts and VSMCs of a synthetic phenotype (Badesch et al., 1989; Ichiro et al., 1990). During synthesis, microfibrils are formed initially, after which there is a deposition of amorphous elastin within them (Vuković, 2003; Krettek et al., 2003). They are found in large amounts in atherosclerotic plaques, but their number decreases with the deposition of lipids, which, along with the deposition of the calcium around them, results in reduced plaque elasticity.

Elastic arteries have an extensive ECM with significant amounts of proteoglycans, elastin and collagen. As opposed to them, muscular arteries (for example coronary arteries) are more cellular, with an ECM which contains mostly collagen with a fewer number of elastic fibers organized in the form of internal and external elastic lamina and the rare, individual interlaminar elastic fibers (Ito et al., 1989; Mulvany, 1989; Tanasković and Andelković, 2009).

**ECM composition at the fatty streak stage (the early stage of coronary atherosclerosis)**

The composition of the ECM at the intima is very important in the initial development of atherosclerosis. Recent studies have shown that the PGs link to LDL with high affinity, which is a basic precondition for the accumulation of lipid droplets in the intima, i.e. the first step in the development of atherosclerosis (Katsuda and Kaji, 2003; Merrilees et al., 2001; Riessen et al., 1994).

A large amount of PGs in the subendothelium of coronary arteries in the fatty streak stage, which is much higher than in normal subendothelium and subendothelium of the initial phase, develops as a result of the synthetic activities of proliferating VSMCs. Although in the unmodified subendothelium of coronary arteries, as well as at the initial stage of atherosclerosis, there is a certain amount of acidic PGs, it has been proven that the PGs synthesized by proliferating cells contains 30% more acidic sulfated PGs, some of which are atypical of normal vascular tissue (Robbins et al., 1989). In the composition of newly synthesized PGs, heparan sulfate, versican, biglycan and keratan sulfate proteoglycan are predominantly represented (Wada et al., 2003; Robbins et al., 1989) (Figs. 1, 2).

The composition of PGs and their distribution in the subendothelium may have a crucial importance
Figure 1. Coronary atherosclerosis – the stage of the initial lesion. There are no visible morphological changes in the structure of the coronary artery wall. The presence of a small amount of highly sulfated glycosaminoglycans in the subendothelial layer is observed in c; histochemical staining: a - Weigert van Gieson; b - Periodic Acid-Schiff (PAS); c - alcian blue - PAS, pH 1.0; d - alcian blue - PAS, pH 2.5; e - Mallory's collagen stain; f - van Gieson's stain; original magnification × 16.
Fig. 2. Coronary atherosclerosis – the fatty streak stage. A largerge amount of highly sulfated glycosaminoglycans in the subendothelial layer is observed in d, compared to c; histochemical staining: a - Weigert van Gieson; b - Periodic Acid-Shiff (PAS); c - van Gieson’s stain; d - alcian blue - PAS, pH 1.0; e - alcian blue - PAS, pH 2.5; original magnification × 16; f - immunohistochemical staining of von Willebrand factor; original magnification × 32.
in the pathogenesis of atherosclerosis and can be the predisposing factor for the development of lesions within a certain type of artery. It is proven, namely, that in the coronary arteries, which are characterized by a high incidence of atherosclerosis during initial and early stage lesions, VSMCs predominantly synthesize versican and biglycan. Newly synthesized PGs are localized subendothelially in the form of focal accumulation. On the other hand, arteries such as internal thoracic artery and radial artery, that have been used as arterial grafts, because of their relative resistance to the development of atherosclerotic lesions, show a different PG composition. Within the internal thoracic artery, VSMCs predominantly synthesize decorin, biglycan to a smaller extent and similar amounts of versican as is the case with coronary arteries, while the radial artery has all three types of PGs evenly represented. In addition, with the internal thoracic and radial arteries, unlike the coronary arteries, PGs are diffusely distributed in the intima (Merrilees et al., 2001). Differences also exist in the distribution of PGs during the development of plaque. Versican dominates in the intima of the initial lesions, while biglycan (in the coronary arteries) and decorin (in the internal thoracic artery) are located within the composition of fatty streak and preatheroma (Riessen et al., 1994).

Versican, biglycan and decorin show high affinity for the binding of LDL. They promote macrophage activity and induce the deposition and retention of LDL, causing damage to endothelial cells and the proliferation and migration of VSMCs, which contributes to the development of atherosclerotic plaque. However, the fact that compared to coronary arteries arterial grafts develop less atherosclerotic lesions suggests that decorin in their intima shows weaker proatherogenic characteristics compared to the biglycan in the coronary arteries’ intima (Riessen et al., 1994; Labudović-Borović et al., 2010).

VSMCs synthesize PGs under the influence of growth factors and cytokines expressed in the vascular wall, especially platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β) and interleukin-1 (IL-1). It has been proven that PDGF and TGF-β promote an increase in the synthesis of versican and biglycan (Schönherr et al., 1993) while IL-1 which is synthesized by macrophages promotes decorin synthesis (Edwards et al., 1994) and inhibits versican synthesis in VSMCs (Qwarnström et al., 1993).

The fact that the presence of PGs in the subendothelial layer is an essential condition for the development of atherosclerotic lesions suggests that the initial phase of the lesion is actually the one that precedes the increased synthesis of PGs. That initial phase would, according to the theory of vascular wall remodeling, in fact be the compensatory dilation of the wall in conditions of hypertension (Lackovic 2010; Getachew et al., 2010; Nakashima et al., 2007, 2008). Compensatory dilatation of the
Fig. 4. Coronary atherosclerosis – complicated lesion. Intima is composed of thick collagen fibers between which there are lacunae with foam cells, remnants of cell necrosis or extracellular lipid deposits; histochemical staining: a - alcian blue - PAS, pH 1.0; b - alcian blue - PAS, pH 2.5; c - reticulin stain; d - Masson's trichrome stain; e - van Gieson's stain; f - orcein stain; original magnification ×8.
wall induces the synthesis of growth factors which causes the phenotypic modulation of VSMCs from the contractile to the synthetic phenotype, their migration to the subendothelium and the increase in the amount of PGs (Lackovic and Vukovic, 2006; Tanaskovic, 2010; Tanaskovic, 2010*). If hypertension is associated with the hypercholesterolemia in parallel with these processes, there is an accumulation of lipid droplets in the subendothelium, their binding to proteoglycans and subsequent chemical modification, which cause endothelial dysfunction and the expression of adhesion molecules, migration of monocytes and T cells into the vascular wall and foam cells formation (Glasser et al., 1996).

It has been shown that diabetes significantly accelerates the process of coronary atherosclerosis. It is, among other things, linked to an increase in the amounts of ECM components such as glycosaminoglycans (hyaluronic acid) and proteoglycans (biglycan) in intimal atherosclerotic lesions (McDonald et al., 2007). Moreover, it has been shown that increasing amounts of hyaluronic acid are associated with plaque erosion (Kolodgie et al., 2002). It was also shown that the amount of elastin decreases in the intima of the vascular wall in cases of diabetes. However, the exact mechanisms by which diabetes stimulates the accumulation of hyaluronic acid and biglycan and decreases the amount of elastin have not yet been exactly determined.

In addition to the ECM, the basement membrane (a specific form of ECM) and internal elastic lamina also have important roles in the pathogenesis of atherosclerosis. Preservation of the basement membrane (BM) continuity and the internal elastic lamina (IEL) is a “last defense” of the vascular wall before irreversible changes that affect the further development of lesions. A preserved BM has a protective characteristic because it slows down and hinders the migration of leukocytes to the vascular wall, but it cannot completely prevent this process because, under the influence of ox-LDL, the endothelium synthesizes the chemokines that promote the migration of leukocytes. In addition, lipids and endoglycosidase, products of adhesional monocytes and T lymphocytes, damage the BM and reduce the ability of endothelial cells to synthesize its components (Skalen et al., 1995; Neufeld and Schneeweiss, 1983; Guyton and Klemp, 1989). And above all, elastases or proteases secreted by macrophages can damage the ECM and cause the creation of defective elastic laminas (Assoian and Marcantonio, 1996). The loss of endothelial cells, BM damage and the presence of weak elastic lamina promote the development of atherosclerotic lesion (Ross, 1995).

The uniqueness of coronary arteries is that the IEL has already been weakened by the existence of duplication that occurs during normal elastogenesis. Immediately after birth, the fragmentation of the IEL occurs which is followed by the migration of VSMCs into the subendothelium. This leads to the accumulation of longitudinal beams of VSMCs and the creation of diffuse intimal thickening (DIT) (Vuković et al., 2010; Neufeld and Schneeweiss, 1983; Guyton and Klemp, 1989) (Fig. 3). Along with this process, elastogenesis continues in the coronary artery wall, which leads to the formation of yet another “incomplete” IEL (Eefting et al., 1997). The newly created lamina remains parallel to the “original” IEL, contains a smaller amount of elastin and is less functional than the lamina formed initially. Duplicated and incomplete IEL is a “weak point” of the coronary wall and in certain conditions (hypertension) contributes to vascular wall instability and represents another predisposition to atherosclerosis (Vukovic, 2003; Vukovic 2006).

Composition of the ECM in advanced stages of atherosclerosis

During the development of an atherosclerotic lesion, along with the active migration of VSMCs of the synthetic phenotype into the intima, there also occurs the deposition of intimal ECM enriched with collagen and elastin. At the preatheroma stage the fine network of collagen fibrils is evident around the VSMCs or basal lamina which thickens with the further aggregation of fibrils. At the fibroatheroma stage, and especially in organized fibrous plaque, the intima is composed of thick collagen fibers between
which there are lacunae with foam cells, remnants of cell necrosis or extracellular lipid deposits. In this way neointima develops from tissue of predominantly cellular composition to tissue in which the main component is ECM (Ross, 1995; Skalen et al., 1995; Libby and Clinton, 1993; Libby, 1996; Horkko et al., 1999; Assoian and Marcantonio, 1996) (Fig. 4). During the development of the plaque, the collagen content continues to grow so that in fibrous plaques collagen contributes to 60% of the total protein content. The most abundant collagen is type I (around 70%), associated with fibers of collagen type III, while the content of type V collagen increases with lesion progression (Katsuda and Kaji, 2003). It was determined by histochemical methods that collagen I predominantly expresses in the intima and outer media and in the adventitia of advanced lesions (Xu et al., 2002). On the other hand, the synthesis of collagen III increases in all parts of the wall, including the adventitia. Also, during the development of atherosclerotic plaque, the amount of collagen type IV increases, for the most part in the form of basal lamina. While at the early stages of atherosclerosis, medial VSMCs are surrounded by a relatively thin basal lamina, at the advanced stages, this layer thickens, reaching a thickness of up to ten times larger than the dimensions of the cells. The thickening of the basal lamina results in decreased proliferative activity, “isolation” of the cell by the disruption of intercellular junctions and represents the index of “aging” the cell that leads to cell death (Katsuda et al., 1992; Greilberger et al., 1998). Type VI collagen is also present in plaque. By bonding with collagen III and IV, collagen VI ensures the integrity of the plaque structure (Kielty et al., 1993). Type VIII collagen also contributes to plaque stability by forming a hexagonal connection with elastic fibers (Katsuda and Kaji, 2003).

Collagen synthesis in VSMC of a synthetic phenotype is influenced by cytokines and growth factors. TGF-β affects the increase in the synthesis of collagen type I, III, IV and V. In contrast, IL-1, TNF and INF-γ (interferon-γ) suppress the synthesis of collagen. PDGF affects the increase in the synthesis of collagen V and VIII, and inhibits the synthesis of collagen IV (Okada et al., 1993; Hiraga et al., 2000).

Besides the increase of the amount of collagen in early lesions, an increase in the synthesis of tropoelastin is present, organized around the lacunae of foam cells (Vukovic et al., 2006; Xu et al., 2002). Synthesized elastin initially expresses in the form of “spots” of high electron density that coalesce into larger masses of low electron density with a high degree of cross-references. However, in the later lesion phases, the volume of elastic fibers in the plaque reduces. Elastic fibers represent the places of elastin deposition of elastin and Ca ++ and are subject to the effects of elastin-degrading enzymes. They are synthesized by endothelial cells, but it is proven that the macrophages in plaque can synthesize tropoelastin (Krettek et al., 2003). TGFβ and IGF-1 promote elastin synthesis and EGF-1 inhibits it (Badesch et al., 1989; Ichiro et al., 1990).

Synthesized ECM in synthetically active VSMCs remains deposited around them. In this way, the isolation of intimal VSMCs is created, which contributes to the disintegration of cell junctions and further increase of collagen synthesis (Vukovic et al., 2006; Friedman, 1990; Frisch and Francis, 1994). The increased synthesis of collagen in lipid-rich lesions, especially at the edges of the plaque, can lead to the progressive multiplication of connective tissue and expansion of the atherosclerotic lesion (Assoian and Marcantonio, 1996; Xu et al., 2002; Friedman, 1990). In this way the fibrous plaque is created. In other words, the lesion progresses to a terminal stage (Vukovic et al., 2006; Tanaskovic et al., 2010). The mechanism of fibrous plaque emergence represents the creation of scar connective tissue with low metabolic activity (Andreeva et al., 1997; Xu et al., 2002; Frisch and Francis, 1994; Brodkey et al., 1995).

The development of lesions leads to a decrease of the amount of PGs in the composition of the ground substance and an increase in their polysaccharide components. In the later stages of atherosclerotic lesion, increased amounts of carboxylated mucins (glucuronic acid) are observed together with the
reduction of highly sulphated mucins (proteoglycans), which are a characteristic of the early stage of lesion. As the mucins are hexosamine polysaccharides covalently linked to different amounts of protein, the presence of carboxylated mucins indicates an increase in the polysaccharide components in the core protein, and, in other words, indicates the glycosylation to which the mucins are subjected to during the development of an atherosclerotic lesion (Totty, 2002).

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