Targeting Matrix Metalloproteinases in Atherosclerosis and Cardiovascular Dysfunction

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Matrix metalloproteinases (MMPs) are the primary mediators of extracellular remodeling and their properties are useful in diagnostic evaluation and treatment. They are zinc-dependent proteases. MMPs have been involved in the mechanisms of atherosclerosis in various arterial areas, ischemic heart disease and myocardial infarction, atrial fibrillation and aortic aneurysms. Recently, MMP9 has been implicated in dyslipidemia and cholesterol synthesis by the liver. Increased MMP expression and activity has been associated with neointimal arterial lesions and migration of smooth muscle cells after arterial balloon dilation, while MMP inhibition decreases smooth muscle cell migration in vivo and in vitro.

Key words: matrix metalloproteinases, atherosclerosis, ischemic heart disease

Matrix metalloproteinases (MMPs) are the main mediators of extracellular remodeling. Due to these properties attempts are being made to use them both in the diagnosis and treatment of cardiovascular diseases. Tissue remodeling and inflammation play an essential role in the onset and progression of atherosclerosis, ischemic heart disease and heart failure [1]. Myocardial extracellular matrix is not a static structure but it contributes to myocardial adaptation to stress and pathological remodeling [2]. Left ventricular hypertrophy alters the geometry of this ventricle. Myocardial extracellular matrix consists of a fibrillar collagen network, a basement membrane, proteoglycans and glycosaminoglycans [3]. Collagen types I and III ensure the integrity of myocytes and are essential for maintaining the structure and arrangement of myofibrils. Recently, MMP9 has been implicated in dyslipidemia and hepatic cholesterol synthesis [4]. Chronic inflammation plays a very important role in the pathogenesis of atherosclerosis, frequently complicated by myocardial infarction or stroke. Moreover, vascular smooth muscle cells and extracellular matrix proteins, especially collagen types I and III, and proteoglycans participate in atherosclerotic plaque formation [5].

MMPs are zinc-dependent proteases responsible for the degradation of extracellular matrix proteins. MMP8, also known as collagenase 2, is involved in the atherogenic process. Left ventricular remodeling after myocardial infarction or viral infection is also mediated by MMPs which act on collagen and elastin.

The extracellular matrix and the cardiovascular system

Recent progresses have highlighted the roles of extracellular matrix [1, 6, 7]:

-structural integrity of the heart and blood vessels;
-framework for cell anchoring;
-mediates cell adhesion and communication;
-integrates mechanical forces at cellular level;
-mediates diastolic stiffness;
-promotes cell survival or apoptosis;
-reservoir for growth factors and cytokines;
-have a role in remodeling and inflammation.

Although MMPs play an important role in physiological remodeling, they are also involved in many pathological processes: tumor angiogenesis, metastasis, rheumatoid arthritis, neointimal vascular hyperplasia, atheroma plaque rupture.

Liu P. et al. described the members of the MMP family and their substrate for action.

MMPs classification and their action on substrate [1]:

Collagenases:
-MMP-1 (interstitial collagenase) - acts on collagen I, II, III, VII, gelatin, MMP2, MMP9;
-MMP8 (neutrophil collagenase) - acts on collagen I, II, III, V, VII, X, gelatin;
-MMP13 (collagenase-3) - acts on collagen I, II, III, IV, gelatin, fibronectin, laminin;

Gelatinases:
-MMP2 (gelatinase-A) - acts on gelatin, collagen I, IV, V, VII, X, XI, fibronectin, laminin, elastin;
-MMP9 (gelatinase-B) - acts on gelatin, collagen III, IV, V, VII, X, elastin, vitronectin;

Stromelysins:
-MMP3 (stromelysin-1) - acts on collagen III, IV, V, IX, X, gelatin, fibronectin, laminin, tenascin, MMP 1, 7, 8, 9, 13;
-MMP10 (stromelysin-2) - acts on collagen III, IV, V, IX, gelatin, laminin, casein, fibronectin, MMP1, 8;
-MMP11 (stromelysin-3) - acts on collagen IV, gelatin, fibronectin, laminin;

Membrane type MMPs:
-MMP14 (MT-1) - acts on collagen I, II, III, gelatin, fibronectin, laminin, vitronectin, proteoglycans, activates pro-MMP2 and pro-MMP13;
-MMP15 (MT-2) - activates pro-MMP2;
-MMP16 (MT-3) - activates pro-MMP2;
-MMP17 (MT-4) - activates pro-MMP2;
-MMP24 (MT-5) - activates pro-MMP2;
-MMP25 (MT-6) - gelatinolytic activity.

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MMPs and vascular remodeling in atherosclerosis

Atheroma plaque formation involves structural alterations leading to the accumulation of cells, extracellular matrix and lipids in the arterial intima. Increased expression of MMPs and their activation results in the accumulation of smooth muscle cells in the atherosclerotic lesions [8, 9]. Increased MMP expression and activity has been associated with neointimal arterial lesions and migration of smooth muscle cells after arterial balloon dilation, while MMP inhibition decreases smooth muscle cell migration *in vivo* and *in vitro*.

There are still many unknowns about leukocyte infiltration in the intima; MMPs could also facilitate this step. MMP2 facilitates the interaction between T cells and extracellular matrix.

Atheroma plaque initiation is determined by vascular leukocytes and invasion of smooth muscle cells into the subendothelial space. In the same way a neointimal hyperplasia is also formed after angioplasty and stenting. Plasminogen activator 53 and gelatinases are elevated after vascular injury.

Studies have shown an increased direct interaction between monocyte cells and a paraformaldehyde fixed layer and elevated MMPs in activated macrophages [10].

MMPs activity is also influenced by smoking. Smoking determines through MMPs emphysematous, as well as atherosclerotic lesions [11]. The epidemiology of cardiovascular diseases that have smoking as a risk factor is also related to MMP activation. Excessive extracellular matrix destruction is a major factor for the dilation and formation of aortic aneurysm. It has also been shown that MMP9 was elevated in intracerebral aneurysms [12]. The secretion of MMP1 and MMP3 by macrophages stimulated *in vitro* or *in vivo* depends on NF-B activation. NF-B is required for the up-regulation of MMP1, MMP3, and MMP9 in vascular smooth muscle cells. Nitric oxide inhibits MMPs activation.

TNFα and ILα induce MMP expression in endothelial cells. Smoking increases monocyte adhesion to endothelial cells.

There are few studies assessing the direct effect of smoking on MMPs. Exposure of endothelial cells to cigarette smoke induces expression of MMP1, MMP8 and MMP9. Specimens obtained from smokers after carotid endarterectomy revealed higher MMP12 concentrations [13].

MMP2 level was elevated in patients with unstable angina or myocardial infarction, compared to controls. MMP9 was correlated with the degree of severity of coronary artery disease; the highest levels were found in patients with damage to 2 or 3 coronary arteries. It was concluded that during acute coronary syndromes MMP9 level is significantly increased [14]. A first practical application of these studies is the use of some MMPs as markers of atheroma plaque vulnerability. MMP9 was correlated with smoking and diabetes mellitus [15].

The vulnerable atheroma plaque is characterized by increased infiltration with inflammatory cells (monocytes/ macrophages, T cells, neutrophils). MMP9 has collagen degradation effects that will mediate left ventricular dilatation.

Atherosclerosis affects the entire artery tree. It becomes symptomatic when the atherosclerotic plaques reach a certain size. Positron emission tomography is a modern method that can highlight areas of decreased blood flow.

MMPs in other cardiovascular disease and possible implications on the treatment

MMP molecules have been linked to the risk of atrial fibrillation. Population studies have shown that body mass index is a very important predictor of development of atrial fibrillation. Based on these findings, epicardial fat was also studied and it was correlated with the risk of atrial fibrillation [16]. MMPs and several interleukins have been implicated in the pathogenesis of atrial fibrillation.

The mRNA expression of MMPs is influenced by a variety of chemical agents, neurohormones, corticosteroids, and cytokines. Some studies have shown that varieties of MMPs are activated in ischemic heart disease but are not expressed in cardiomyopathies of other etiologies. For example, in myocardial infarction, MMP expression is time-dependent and also depends on a given location of ischemic and necrotic areas [17].

The polymorphism of genes encoding MMPs is involved in many disorders. The genes encoding MMP3 and MMP9 have been more extensively studied. The 5A allele was associated with elevated MMP3 promoter and protein levels. In contrast, 6A allele has a lower MMP3 promoter activity. An increase in MMP3 level occurs in patients homozygous for the 5A allele, while in patients homozygous for the 6A allele MMP3 level decreases. Elevated MMP9 has been associated with decreased cardiovascular survival.

MMPs have also been implicated in the pathogenesis of obstructive hypertrophic cardiomyopathy and heart failure. They are zinc-dependent endopeptidases, having collagenase and gelatinase activity. The main effect is degradation of the extracellular protein matrix. MMPs play an important role in the development of fibrosis and in the remodeling that occurs after myocardial infarction, in dilated or hypertrophic obstructive cardiomyopathy. MMPs are inhibited by specific inhibitors called TIMPs.

In obstructive hypertrophic cardiomyopathy, MMP activity is modulated by the renin- angiotensin-aldosterone system, oxidative stress, endothelin 1, and TNF-á. Due to the imbalance between MMPs and TIMPs, fibrosis develops excessively in obstructive hypertrophic cardiomyopathy [18-20].

The role of TIMPs is to prevent the exaggerated MMP-mediated degradation. TIMP1 and TIMP2 have been shown to inhibit angiogenesis and TIMP3 is involved in apoptosis. Experimental studies have shown that inhibition of some of the MMPs has been effective in blocking cellular multiplication and inhibiting metastasis. MMP inhibition after myocardial infarction could prevent the development of heart failure [21].

In myocardial infarction it was also noted the increase in MMP2 levels, in direct relation to cardiac dysfunction, destruction of Troponin I and myosin light chain. From this point of view, doxycycline, which is known to have inhibitory effects on MMPs, has been indicated [22]. Doxycycline could interfere by direct inhibition of MMPs but also by treatment of *Chlamydia pneumoniae* infection.

Chronic heart failure continues to generate cardio-vascular morbidity and mortality despite the use of important therapeutic classes such as angiotensin converting enzyme inhibitors or beta-blockers. Currently, the main causes of heart failure are arterial hypertension and chronic ischemic heart disease. Clinical and experimental studies have demonstrated that the progression to heart failure is determined by an initially adaptive remodeling which subsequently complicates with fibrosis, ventricular dilatation and risk of arrhythmia [23].

The severe prognosis of patients with heart failure has led to researches focused on new biomarkers that are active in this condition. Particular attention has been given

to MMP family, enzymes capable of degrading the cardiac extracellular matrix. MMPs are essential in maintaining the balance between extracellular matrix formation and degradation, cavitary remodeling depending on it [24]. Inhibition of MMP actions has been shown to reduce pathological ventricular remodeling.

Kameda et al. raised the assumption of a link between oxidative stress, MMP activation, and left ventricular dilatation. The authors reported a significant positive correlation between left ventricular diastolic volume, MMP2 and MMP9 activity, and pericardial levels of 8-iso-prostaglandin F2á, an oxidative stress marker.

MMPs have also been implicated in various aspects of vascular pathology. MMP collagenases degrade collagen within atherosclerotic lesions and may lead to atheroma plaque rupture. MMP2 and MMP9 gelatinases degrade the collagen fragments and may promote complication of the atheroma plaque [25- 27].

Formation of thoracic and abdominal aneurysms is characterized by elastin degradation, collagen synthesis and accumulation of inflammatory cells. At the level of aortic aneurysms there are high concentrations of MMP2, MMP-9 and MMP-12. Experimental studies have demonstrated the elastolytic role of MMPs in aortic aneurysm formation. Increased collagen content causes increased arterial stiffness. However, MMP2 does not appear to be a convenient target in the prevention and therapy of aortic aneurysms [28, 29].

Atherosclerotic coronary artery disease was associated with activation of MMP8 and MMP9. MMP activation was associated with high cardiovascular risk in general.

Conclusions

MMPs are a large family of enzymes involved in cardiac and vascular processes, which could be influenced by specific mediators with the goal of treating some cardiovascular disorders.

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