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Matrix metalloproteases as a pharmacological target in cardiovascular diseases

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Abstract. – OBJECTIVE: Matrix metalloproteases (MMPs) are involved in the development and the progression of atherosclerosis and are related to an elevated risk of cardiovascular morbidity and mortality. An altered profile of MMPs and their tissue inhibitors (TIMPs) has been demonstrated in arterial hypertension, diabetes mellitus, obesity, metabolic syndrome and in cardiovascular disorders, such as coronary artery disease, atrial fibrillation, and heart failure.

MATERIALS AND METHODS: The aim of this review was to examine the literature data regarding the possible effects of some molecules, commonly employed in subjects with cardiovascular disease, on the expression and the activity of MMPs and TIMPs. A search was conducted with PubMed, Medline, using combinations of the terms "matrix metalloproteases," "TIMP," "activity regulation," "pharmacological therapy," "cardiovascular disease," "antihypertensives," "antidiabetic drugs," "statins" and "antiplatelet agents". Review articles were also searched.

RESULTS: Several drugs, and in particular diuretics, calcium-channel blocker, angiotensin receptor blockers, ACE inhibitors, statins, antidiabetic and antiplatelet agents, seem to influence, in different ways, the MMP pattern with beneficial effects on cardiovascular outcomes.

CONCLUSIONS: Keeping in mind these findings, the therapeutic strategy must be reconsidered in order to obtain a sensible MMP inhibition.

Key Words:
Matrix metalloproteases (MMPs), Tissue inhibitors (TIMPs), Cardiovascular diseases.

Introduction

Matrix metalloproteinases (MMPs) form a large family of zinc-dependent endopeptidases, which activity consists principally in extracellular matrix (ECM) protein degradation by cleavage of internal peptide bonds. They can be secreted by all the cells present into the vascular wall, even if macrophages seem to be determinant in atherosclerotic plaques[1]. Each MMP has a specific target substrate that defines its denomination, such as collagenase, gelatinase, stromelysin and matrilysin.

Collagenases, including MMP-1, -8, -13, and -18 cleave interstitial collagen I, II, and III and also other ECM and non-ECM molecules, such as bradykinin and angiotensin I. Fragments of collagen are then degraded by gelatinases[2]. Gelatinases A and B (MMP-2 and -9) can be secreted by endothelial cells, pericytes and podocytes, fibroblasts and myofibroblasts, monocyte derived macrophages and in particular by local tissue macrophages[3]. They are responsible for IV type collagen degradation, vasculature remodeling, angiogenesis, inflammation and atherosclerotic plaque rupture[4]. MMP-2 is constitutively expressed on cell surface, while MMP-9 is stored in secretory granules and it is inducible by exogenous stimuli, such as cytokines, growth factors or altered cell-matrix contacts[3,5]. Stromelysin-1 (MMP-3) and -2 (MMP-10) have similar substrate specificity but MMP-3 has higher proteolytic effects as compared to MMP-10. They degrade fibronectin, laminin, gelatins-I, III, IV and V, collagen fibres and proteoglycans[4]. Matrilysins, including matrilysin-1 (MMP-7) and -2 (MMP-26), may hydrolize fibronectin, gelatins and also may break human plasminogen producing a fragment that inhibit angiogenesis[5]. Membrane-Type MMPs (MT-MMPs), transmembrane or GPI (glycosylphosphatidylinositol)-anchored, can degrade type-I, -II, and III collagen and other components of ECM, and can activate pro-MMP to MMP[2]. In fact most MMPs are secreted as precursors (zymogens), which are activated in the extracellular space by several proteases, including plasmin and other MMPs[5].

The regulation of MMPs production and activity is highly complex. Radical oxygen species (ROS) growth factors, cytokines, and hormones can influence MMP transcription through the activation of the Mitogen-Activated Protein Kinase

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(MAPK), the inhibition of MAPK phosphatase, the inactivation of the histone deacetylase (involved in gene repression) or the recruitment of different chromatin remodeling factors[6].

Most of the MMPs are synthesized as precursors (pro-MMP) and must be activated to expose the catalytic domain with the Zn^{2+}-binding site. This activation can be effected by proteolytic modifications from several proteases, such as plasmin, thrombin, chimase, and membrane-type MMP (MT-MMPs)[5,7] and also by S-glutathiolation, S-nitrosylation and phosphorylation reactions[8,9]. MMPs can be activated also by heat treatment and low pH[2]. On the contrary, MMPs activity can be down regulated especially by the four tissue inhibitors of MMP (TIMPs): TIMP-1 inhibits MMP-1, MMP-3, MMP-7 and MMP-9, TIMP-2 inhibits especially MMP-2, TIMP-3 can inhibit MMP-2 and MMP-9, while TIMP-4 inhibits MT-1 MMP and MMP-2 activity[5]. TIMPs activities include regulation of cell proliferation, migration and invasion, anti-angiogenesis, and apoptosis[10]. Most of these activities arise from MMP inhibition, but TIMPs are also able to interact with some specific cell receptors, for example TIMP-3 binds vascular endothelial growth factor (VEGF) receptor on endothelial cells inhibiting angiogenesis[10].

An uncontrolled MMP activity causes tissue damage and functional alterations. MMPs can influence the development of several diseases through three different pathophysiological mechanisms: tissue destruction (cancer invasion, gastric ulcer, neuroinflammatory disorders, rheumatoid arthritis), fibrosis (liver cirrhosis, pulmonary fibrosis, atherosclerosis) and extracellular matrix degradation (alveolar wall destruction, dilative cardiomyopathy, aneurisms)[8]. As regards to cardiovascular diseases, MMPs contribute to the remodeling of basement membranes and the degradation of the components of the ECM, are involved in angiogenesis and in vascular smooth muscle cells contraction[2]. MMPs participate not only in the development of atherosclerosis, but also in its progression and in its complications: an increased MMP expression has been detected in atherosclerotic plaques and their activity may be responsible for plaque instability and rupture, and for an increased platelet aggregation[2].

An imbalance between MMPs and TIMPs is also associated with a higher risk of all-cause mortality and cardiovascular mortality[8]. In the last years we have examined the behaviour of the gelatinases (MMP-2 and MMP-9) and their tissue inhibitors (TIMP-1 and TIMP-2) in subjects with metabolic syndrome[11], in subjects with obstructive sleep apnea syndrome, which is associated with an elevated cardiovascular risk, and in patients with leg venous ulcers, before and after the compression therapy[12]. The aim of this research was to examine the literature data regarding the effects of some pharmacological molecules, commonly employed in cardiovascular disorders, on MMPs and TIMPs.

Molecules Able to Influence MMPs Activity

Since 1983 it is known that tetracyclines are potent MMP inhibitors chelating the catalytic Zn^{++} ion essential for MMP activity[13]. Doxycycline, in particular, at low doses inhibits MMP activity and also down-regulates the transcription of some MMPs mRNA and it is able to contrast the vascular remodeling[14]. In fact, doxycycline seems to improve the endothelium-dependent response to acetylcholine, to induce vasodilation and to prevent media calcification[14].

In the last decades, many researches have described how some drugs affecting the cardiovascular system may regulate MMP activities. These drugs include diuretics[15], calcium channel blockers[16,17], angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blockers, and statins[18] among others.

Diuretics

In animal model of renovascular hypertension, Ceron et al[15] have examined if the combination of hydrochlorothiazide and spironolactone, besides producing antihypertensive effects, is able to reduce MMP activity and vascular remodeling; they described that spironolactone produced weaker antihypertensive effects compared with hydrochlorothiazide or their combination, but the treatment with the two molecules or their combination induced antioxidant effects and attenuated MMP-2 up-regulation. They suggested that the antioxidant action of the diuretics was due to the direct inhibition of vascular NADPH oxidase activity[15].

In animal model of hypertensive heart failure, the treatment with eplerenone improved the cardiac remodeling decreasing the activity of MMP-2, MMP-7, MMP-12, and MMP-13 and regulating the expression of extracellular matrix proteins[19].

In subjects with chronic heart failure spironolactone enhanced cardiac function and signifi-

cantly decreased plasma levels of MMP-1 and MMP-9 and MMP-9/TIMP-1 ratio[20]; in this research the parameters of diastolic function and left ventricular end diastolic dimension were statistically correlated with MMP-9/TIMP-1 ratio[20].

ACE inhibitors and Angiotensin Receptor Blockers

In cultured smooth muscle cells and monocyte-derived macrophages the temocaprilat, which is an ACE inhibitor, and the olmesartan, which is an angiotensin receptor blocker, inhibited AGE-induced ROS generation and MMP-9 expression and activity[21].

The block of renin-angiotensin system probably attenuates the AGE/RAGE-activated signalling pathway and reduces the vascular involvement observed in diabetic subjects.

A 3 months period of anti-hypertensive treatment with candesartan or lisinopril normalizes MMP-9/TIMP-1 ratio promoting the reduction of plasma MMP-9 and the increase in TIMP-1 values[22]. Schieffer et al[23] have obtained the same result in subjects with coronary artery disease (CAD) treated with irbesartan or enalapril. On the contrary, Fontana et al[24] have observed no changes in gelatinases and their inhibitors activity after an 8 weeks therapy with enalapril in hypertensive subjects. In subjects with acute myocardial infarction (AMI) the effects of telmisartan or enalapril on gelatinases levels have been evaluated in the acute phase and after 6 months[25]. Both the molecules significantly reduced the activity of circulating MMP-2 and MMP-9 at days 7 and 14, but after 6 months MMP-2 activity increased[25]. MMP-9 plasma levels have been significantly decreased by a 12 months treatment with valsartan, trandolapril or their combination in AMI subjects and this effect was associated with a reduced left ventricular remodeling[26].

In hypertensives with high-grade internal carotid artery stenosis, 4 months of treatment with irbesartan reduced the expression of both the gelatinases in the plaque areas of intense macrophage infiltration, the inflammatory reaction and also the levels of oxidized LDLs increasing the plaque collagen content[27]. Then, the effect of irbesartan on plaque stabilization could be mediated by the inhibition of macrophages activity and the reduction of the oxidative stress. Other authors have demonstrated that the treatment with irbesartan reduced also the levels of collagenases (MMP-1 and MMP-8) in samples from high-grade carotid stenosis, but increased elastin degradation, suggesting that the MMPs are not involved in elastin degradation in the atheroma[28].

In addition, in spontaneously hypertensive rats, the renin inhibition with aliskiren reduced the myocardial expression of gelatinases and TIMP-1 after an ischemia/reperfusion injury attenuating the myocardial damage[29].

Statins

The treatment with atorvastatin decreases TIMP-1 concentration in healthy subjects with isolated hypercholesterolemia[30]. In hypercholesterolemic subjects diet plus atorvastatin for 2 months reduced plasma CRP levels and MMP-9 activity more than diet alone[31]. Another study[32] shows that pravastatin decreases MMP-9 concentration in men with mild hypercholesterolemia independently of changes in lipid levels, as a pleiotropic effect. Also simvastatin reduces MMP-9 levels and activity differently from fenofibrate, which shows no effect on MMPs[33]. In comparison with diet alone, a diet plus simvastatin decreases MMP-9 activity and MMP-9/TIMP-1 ratio in 14 weeks[34]. Simvastatin and rosuvastatin seem to have the same beneficial effect on MMP-9 and other inflammation markers[35], although rosuvastatin did not improve MMP-13 and TIMP-1 concentration in hypercholesterolemic subjects treated for 4 weeks[36]. In experimental models of LDL-receptor deficient rats, rosuvastatin inhibited the expression of MMP-2 and MMP-9 and decreased the atherosclerotic lesion area in aortic artery and aortic sinus, suggesting a pathogenetic role of the gelatinases in atheroma development[37]. In apolipoprotein E-deficient rats, simvastatin reduced MMP-9 levels and prevented ventricular hypertrophy and fibrosis, typical features of the Apo E -/- mice[38]; in addition, simvastatin reduced ROS production and increased nitric oxide levels[38].

Antiplatelet Agents

Platelets are a source of inflammatory mediators and cytokines and may accelerate the recruitment of inflammatory cells in atherosclerotic lesions, but the effects of antiplatelet agents on MMPs seem to be independent from platelet action.

In diabetic rats with induced coronary ischemia, a treatment with minocycline plus aspirin reduced MMP-2 and MMP-9 levels, infarct size and mortality more than the treatment with minocycline alone[39]. As it is known, hyper-

glycemia induces an overexpression of the gelatinases, which contribute to the vascular remodeling, to the cardiac damage, and the loss of contractile function. The inhibition of gelatinase activity probably improved the outcome of the acute coronary syndrome[39].

In a murine model of abdominal aortic aneurysm, clopidogrel inhibited macrophages infiltration, ROS production and gelatinases activity[40] while in cultures of human brain endothelial cells, dipyridamole attenuated the increase in MMP-9 levels induced by inflammatory and oxidative stimuli and the endothelial cytotoxicity[41].

Cilostazol is a specific cAMP phosphodiesterase type III inhibitor that reduces platelet aggregation and induces vasorelaxation. In culture of human monocytes cilostazol decreased the MMP-9 expression and activity and increased the TIMP-1 expression, affecting the invasive ability of monocytes upon differentiation toward macrophages[42]. The decreased invasive ability of these cells could influence the recruitment and infiltration of macrophages in the subendothelial space preventing the early stage of atherosclerosis. In a rat model of hemorrhagic stroke induced by tPA infusion after ischemia, the pretreatment with cilostazol reduced the cerebral hemorrhage in comparison with aspirin. This effect was probably due to an attenuated degradation of cerebrovasculature after the transient ischemia and the administration of tPA, mediated by the reduction in platelet endothelial cell adhesion molecule 1 (PECAM 1) and MMP-9 expression in the microvasculature of the ischemic brain[43].

Antidiabetic Agents

In streptozotocin-induced diabetic rats, the use of long-acting insulin for 20 weeks inhibited the aortic atherosclerosis process decreasing MMP-9 (but not TIMP-1) expression; this effect was associated with a reduced IL-6 expression by macrophages in atherosclerotic lesions [44]. In normoglycemic rats underwent a carotid balloon angioplasty, insulin reduced MMP-2 and MMP-9 expression and activity without affecting TIMPs[45].

Some studies[46,47] have indicated metformin as an agent able to influence cancer cell proliferation and migration and this effect has been correlated with the regulation of MMPs activity. In particular, in endothelial cells from human umbilical vein metformin decreased MMP-2 and MMP-9 expression probably acting on the AMP-activated protein kinase[46] while in fibrosarcoma cells metformin suppressed MMP-9 production inhibiting the transcription factor AP-1 (activator protein-1), independently of AMPK[47]. Similarly, other authors[48] observed that glibenclamide, in addition to cobalt chloride, attenuates MMP-9 expression in murine breast tumor cells. As it is known, MMP-2 and MMP-9 during tumour invasion are involved in cell migration by degrading extracellular matrix proteins[49,50].

In hypercholesterolemic mice with demonstrated carotid atherosclerosis pioglitazone reduced macrophage activity and MMP-9 production, inflammation, and also the plaque size[51]. MMP-9 mRNA expression by adipocytes and macrophages in the adipose tissue is elevated in obese and insulin resistant subjects and decreases after pioglitazone treatment[52]. In type 2 diabetic subjects treated with basal insulin and glargine, the addition of pioglitazone increased insulin sensitivity and reduced MMP-9 and hs-CRP levels, differently from metformin[53].

Conclusions

An impaired pattern of MMPs and TIMPs is associated with an elevated risk of atherosclerosis and cardiovascular disease and, in particular, an increase in MMP-9 and TIMP-1 plasma levels is related with higher all-cause mortality and cardiovascular mortality[8]. In literature there are evidences of an improvement in MMP/TIMP ratio with diet and exercise[54-57]. This review suggests that also a pharmacological therapy addressed to contrast the deregulated MMPs profile might attenuate the atherosclerotic process and might reduce the cardiovascular morbidity and mortality.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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