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REVIEW ARTICLE

Matrix metalloproteinases and cardiovascular diseases

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Abstract

Matrix metalloproteinases (MMPs) are extracellular enzymes that are important in many physiologic and pathologic processes. Their activity is regulated mainly by tissue inhibitors of metalloproteinases (TIMPs). MMPs expression is related with the classical cardiovascular risk factors as well as with inflammation. They play a central role in atherosclerosis, plaque formation, platelet aggregation, acute coronary syndrome, restenosis, aortic aneurysms and peripheral vascular disease. Many studies have shown that commonly prescribed antihypertensive medications, glitazones and statins may influence MMPs activity. The aim of the review is to present literature data on the role of MMPs and their inhibitors in cardiovascular disease. Hippokratia 2009; 13 (2): 76-82

Key words: metalloproteinases, extracellular matrix, atherosclerosis, acute coronary syndromes, aneurysms

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Extracellular matrix (ECM) plays a key role for the proper function of the different organs of the human body, including heart and vessels. Changes in the ECM have been implicated in the pathogenesis of several cardiovascular conditions including atherosclerosis, aneurysms, post-angioplasty restenosis and heart failure[1-3].

Matrix Metalloproteinases (MMPs) and their inhibitors (Tissue Inhibitors of Metalloproteinases, TIMPs) have a fundamental role in the remodeling of the ECM in both normal and pathological conditions. In addition, MMPs have an important role in cardiovascular diseases, including atherosclerosis[4], dilated cardiomyopathy[5] and myocardial repair following infarction[6].

In the present work we reviewed the existing literature data on the relationship between MMPs and their inhibitors with cardiovascular disease.

MMP structure and function

MMPs were discovered in 1962, in an effort to establish how the metamorphosing tadpole of a frog lost its tail[7]. MMPs are found in a variaty of living organisms, from the simplest bacteria up to the human[8]. For example it must be mentioned that metalloproteinase toxin-2 of Bacteroides fragilis shares 59% homology in the amino acid sequence with human interstitial MMPs[8].

MMPs not only take part in biological processes, such as ontogenesis (morphogenesis, angiogenesis, growth), and wound healing[9,10], but also during pathological remodeling like tumor growth[11]. Endothelial cells, smooth muscle cells and fibroblasts can produce MMPs[4]. Oxidative stress, which is involved in cardiovascular disease, can stimulate MMPs production and activation[12]. On the contrary, nitric oxide (NO) inhibits MMPs production by endothelial cells and smooth muscle cells[13,14].

MMPs can be divided into 6 groups: collagenases, stromelysins, matrilysins, gelatinases, membrane-type metalloproteinases and zinc- and calcium-dependent endopeptidases[8]. They are usually secreted from the cells as inactive proenzymes[8]. A proenzyme molecule is organized into the 3 basic structural domains: N-terminal propeptide, catalytic domain, and the C-terminal part of the molecule[8]. N-terminal propeptide consists of approximately 80–90 amino acids containing cysteine residue which interacts with catalytic zinc and this ensures the enzymatic latency of the proenzyme[8].

Regulation of MMPs activity is a complex process including three different levels of activation: a) Regulation of MMPs gene expression[15]. Gene expression can be inhibited by some factors like TGF-β, glucocorticoids and retinoic acid[16]. Genes for MMPs are expressed only if the tissue is remodelled under either physiological or pathological conditions[17]. Gene expression is influenced also by the ECM-cell and cell-cell interactions. As an example, we can mention glycoprotein EMMPRIN (extracellular matrix metalloprotinase inducer) that stimulates MMPs production and was first identified on the surface of human tumor cells[18]. b) Regulation of MMPs enzyme activity by cystein «switch» mechanism[19]. The mechanism by which MMPs are activated is mentioned above. However, it must be emphasized that there is a certain reserve of inactive MMPs bound to various components of ECM in the extracellular space. For example, MMP-2 binds to the ECM structures containing elastin, MMP-3 to basal membranes and occasionally to collagen fibrils and MMP-13 to proteoglycans, collagen and elastin[19]. c) Inhibition of MMPs by TIMPs. TIMPs are

proteins of size 21–30 kDa. Four homologous molecules termed TIMP-1, -2, -3, -4 have been described[20]. TIMPS are a family of specific inhibitors of MMPs which are essential for the regulation of normal connective tissue metabolism[21]. TIMP-1 is synthesized by most connective tissue cell types, including mesangial cells and macrophages[21]. TIMP-1 levels are increased in diabetic nephropathy[22] and polycystic kidney disease[23]. TIMP-2 has only 42% amino acid homology with TIMP-1 but a similar profile of MMP inhibitory activity. Whereas TIMP-1 is highly inducible by cytokines and growth factors, TIMP-2 expression closely matches the pattern of expression of MMP-2[24]. Expression of TIMP-1 and TIMP-2 is increased significantly in patients with glomerulosclerosis[25]. TIMP-3 shares only 37% sequence homology with TIMP-1 and is localized mainly to the ECM[26]. TIMP-4 is the main TIMP in the heart and has an important role in processes such as infarction, heart failure and cardiomyopathy[24]. Also, TIMPs exert a number of other biological effects in connective tissues, including growth factor activity, inhibition of apoptosis and inhibition of angiogenesis[27].

Extracellular matrix and atherosclerosis

It is known that the arterial wall consists of collagen types I and III, macrophages and smooth muscle cells. The evolution of the atherosclerotic plaque from the fatty streak to advanced plaque is associated with an increase in its content of collagen[28], in the number of smooth muscle cells[29], and in MMP-9 levels[4]. Inreased levels of MMP-9 are found more often in patients with unstable angina compared with those with stable angina[30]. Also, re-stenotic human coronary plaques demonstrate lower MMP-9 expression[29]. In patients with coronary artery disease higher MMP-9 levels are an independent risk factor of cardiovascular morality[31]. Increased TIMP-1 levels have been reported consistently in human atherosclerotic plaques, mainly in relation to areas of calcification[32]. Increased circulating TIMP-1 levels have also been related to stable coronary[33], carotid[34] and peripheral artery atherosclerosis[34].

Following an acute coronary syndrome, numerous MMPs are expressed and activated in the myocardium, with greater alterations in levels in the ischemic regions and less change in the areas remote from injury[36]. The same pattern is observed in the peripheral blood over several weeks after an acute coronary syndrome. Elevated levels of TIMP-1 have been reported[37], whereas reports of circulating MMP levels during and after acute coronary syndromes have been inconsistent[37-40]. In two studies, increased levels of MMPs were observed in the coronary, but not in the peripheral, circulation of patients with acute coronary syndromes[39,40]. Thrombolytic therapy is a powerful stimulus for MMP expression and collagen degradation[41]. In addition, coronary balloon angioplasty results in an immediate release of MMP-9 into the blood[42].

Extracellular matrix and cardioavascular risk factors

Many studies have shown that all major risk factors for atherosclerotic disease are associated with alterations in circulating levels of various ECM markers[43,44].

Age and gender

Older age and male sex have been related to higher plasma TIMP-1[43], but not MMP-9[45]. Also, hormone replacement therapy has been demonstrated to reduce plasma MMP-9 levels in postmenopausal women[46].

Dyslipidemia

Higher blood levels of some MMPs, especially MMP-9, have been observed with increasing total or low density lipoprotein cholesterol (LDL-C) levels[45,47]. In one study, the plasma TIMP-1 level was related to the total cholesterol/HDL-cholesterol ratio[51]. In experimental studies, oxidized LDL has been observed to increase and high density lipoprotein cholesterol (HDL-C) to decrease the production of MMP-1 and MMP-9[48,49]. In addition, oxidized LDL-C can also decrease TIMP-1 production[49].

Diabetes mellitus

Increased circulating levels of MMP-9 were observed in patients with diabetes[45,50], without any reports for the rest MMPs. However, we showed that plasma levels of MMP-2 and MMP-9 did not differ between diabetic and nondibetic subjects[51]. Only TIMP-1 levels were lower in diabetic subjects compared with nondiabetic subjects[51]. The above finding was in accordance with a study showing that TIMP-1 levels are decreased in diabetic subjects[45].

High glucose concentrations have been demonstrated to induce expression of MMP-1 and MMP-2 from endothelial cells and expression of MMP-9 from macrophages, with no effect on TIMP-1 expression[52]. In one recent study of patients with diabetes, intensified glycemic and cardiovascular risk management for one year decreased serum cholesterol and glycated hemoglobin levels, and had as a result the decreasement of TIMP-1 levels, without any significant change in circulating MMP-9 and TIMP-2[53].

Hypertension

Hypertension has been related with increased plasma levels of MMP-9[45] and TIMP-1[43]. In addition, increased levels of TIMP-1 in hypertensive persons has been reported in some[54,55], but not all[56], studies. Increased circulating MMP-9 levels have also been observed in subjects with systemic hypertension[57] or isolated systolic hypertension[58].

Obesity

Plasma TIMP-1[43], but not MMP-9[45], levels were positively related to body mass index (BMI) in one population-based study. One recent prospective study in obese

women showed that plasma MMP-9 levels decreased one year after gastric banding[59].

Smoking and alcohol

Large scale studies have shown that smokers had increased plasma levels of MMP-9 and TIMP-1[43,45]. In smaller studies, smokers have also been found to have higher plasma MMP-9 levels[60,61].

An inverse relation between usual alcohol intake and plasma TIMP-1 level was observed in one study[45]. Plasma MMP-9 levels have not been related to alcohol consumption[43]. Recent alcohol intake does not affect circulating levels of TIMP-1 and MMP-2[62].

Inflammation

Activation of neutrophils by inflammatory mediators causes release of MMP-8 and MMP-9 from their granules[63]. Experimental studies suggest that C-reactive protein (CRP) stimulates MMP-1 expression directly, but has no effect on TIMP-1 expression[64]. In small clinical samples, serum CRP levels have been related to circulating levels of MMP-9, but not to those of MMP-2 or MMP-3[65,66]. Interestingly, infecting macrophages or smooth muscle cells with Chlamydia pneumoniae induces MMPs production[67]. Recently, a strong association between the presence of Chlamydia pneumoniae antigen and production of MMP-9 in coronary atherosclerotic plaques has been demonstrated[68].

MMPs and cardiovascular disease

MMPs and atherosclerosis

MMPs influence the process of atherosclerotic lesion formation. One proposed mechanism of the pathogenetic role of MMPs includes the increased migration of vascular smooth muscle cells through the internal elastic lamina into the intimal space, where they proliferate and contribute to plaque formation[69]. In addition, MMPs activity may diminish plaque volume by degrading ECM in the intima[70]. In one study of TIMP-1-deficient mice (in which MMPs activity is increased), a reduced atherosclerotic plaque size was noted[71]. Hearts obtained within 24 h post-mortem from patients who died from causes other than coronary artery disease (CAD) showed increased expression of MMP-2 and MMP-9 in plaques of expansively remodeled versus constrictive remodeled segments of atherosclerotic coronary arteries[72]. Finally, one study compared plasma concentrations of MMPs between 53 male patients who had one or more significant stenosis (>50% of diameter) in the coronary arteries with 133 subjects free of cardiovascular diease[33]; plasma levels of MMP-9 were significantly higher in the patients with CAD, while plasma concentrations of MMP-2 and MMP-3 were significantly lower in patients with CAD[33].

MMPs and plaque rupture

There is a rapidly expanding body of evidence suggesting that acute coronary syndromes may be influenced by MMPs through degradation of the fibrous cap of vulnerable atherosclerotic lesions[73]. In one report, specimens from patients with unstable angina showed a 70% increase in intracellular MMP-9, indicating active synthesis, compared to specimens from patients with stable angina[30]. Similarly, plaques from patients undergoing carotid endarterectomy that were thought to be unstable (patients symptomatic within one month of surgery), showed a more intense stain for MMP-9 than plaques from patients with stable atherosclerotic disease[74]. Also, other studies reported similar findings for the other MMPs[75,76].

MMPs and platelet aggregation

The rupture of atheromatous plaques allows dissection of blood into the intima and subsequently the lipid-rich pool[77]. A sequence of events ensues, including platelet aggregation and thrombus formation, which can compromise arterial patency and result in acute coronary syndrome[77]. Some MMPs, like MMP-1 and MMP-2, have been demonstrated to be involved in platelet aggregation[78,79]. High concentrations of MMP-2 as well as MMP-9 have been shown to inhibit platelet aggregation[82]. Platelets have also been shown to have effects on MMPs secretion[82]. Thus, MMP-9 is synthesized by human monocytes when they are coordinately adherent to collagen and platelets[82].

MMPs and acute coronary syndromes

Kai et al measured MMP-2 and MMP-9 levels in 50 patients (22 with acute myocardial infarction, 11 with unstable angina, 17 with stable angina and 17 normal volunteers)[83]. MMP-2 levels were increased by 2-fold in the unstable angina and acute myocardial infarction groups versus the stable angina and controls and were sustained over the 7 day period[83].

Another study compared plasma levels of MMP-9 and TIMP-1 in patients with angiographically identified lesions in the left anterior descending artery verus normal subjects[39]. The study showed that during acute coronary syndromes plasma levels of MMP-9 and TIMP-1 were increased[39]. Another study evaluated the levels of MMP-1 and MMP-2 in subjects with acute myocardial infarction compared with healthy controls and found that only plasma MMP-2 levels, but not MMP-1, were increased[38].

Hirohata et al examined serum concentrations of MMP-1 and TIMP-1 in 13 consecutive patients after their first myocardial infarction who underwent successful reperfusion[37]. At the end of the study a significant increase of MMP-1 and TIMP-1 was observed, that peaked at 14th day[37]. Recently, Blankenberg et al reported a strong and independent association between plasma levels of MMP-9 and cardiovascular risk among 1,127 subjects with established CAD[31]. This association was independent of conventional cardiovascular risk factors, but attenuated after adjustment for CRP, IL-6, fibrinogen, and IL-18 levels[31].

MMPs and post-intervention restenosis of atherosclerotic lesions

Post-intervention resteuosis is a common adverse event after endoluminal treatment of atherosclerotic lesions. ECM remodeling by MMPs is involved in each of these processes. For example, balloon injury has been shown to increase MMP-2 and MMP-9 in carotid pig arteries[84]. Similar findings have been found in humans. Hojo et al found increased MMP-2 expression and activity in the coronary circulation following angioplasty and a significant positive correlation between MMP-2 levels post-angioplasty and the degree of angiographic restenosis[85]. Finally, studies using a MMPs inhibitor showed reduction of the intimal hyperplasia and collagen accumulation[86,87]. In addition, an experimental study showed that the use of doxycycline inhibits MMPs activation in the carotid arteries after angioplasty[88].

MMPs and aortic aneurysms

Histologic studies of aneurysmal aortas demonstrated specific changes in the extracellular matrix and the aortic wall, including decrease in elastin content and an increase in collagen synthesis[89]. Proteolysis of elastin also results in the release of elastin degradation product, release of MMP-1 and MMP-2, and smooth muscle cell proliferation[90]. Many studies in humans and animals reported increased total MMP-2 levels and MMP-2 activity in aneurysmal aortas compared with normal and atherosclerotic aortas[91,92]. The presence of MMP-9 in aortic tissue is associated with chronic inflammation[93].

Studies both in humans and in animals have shown a relationship between elevated aortic tissue MMP-9 levels and abdominal aortic aneurysms[94,95]. Petersen et al found that levels of MMP-9 were increased in ruptured aneurysms compared with intact large aneurysms[96]. Furthermore, it has been demonstrated that overexpression of TIMP-1 prevents both elastin depletion and aneurysm formation and rupture in a rat model of abdominal aortic aneurysms[97].

MMPs and peripheral vascular disease

After occlusion of a major artery, ischemic limbs revascularize via the distinct mechanisms of arteriogenesis and angiogenesis[98]. Many animal and human studies have shown that MMPs, especially MMP-2 and MMP-9, have both been associated with angiogenesis[99]. In an animal model of critical limb ischemia, after 28 days, the limb was revascularized, with perfusion reaching 50% to 80% of the nonischemic control limb[99]. The revascularization was related with the levels of MMP-2 and MMP-9[99]. In the contrary, MMP-9 knockout mice exhibit delayed and incomplete revascularization compared to wild-type mice[100]. The existing literature data on human are limited. However, a recent study demonstrated a linear correlation between plasma MMP-9 levels and the severity of ischemia in patients with varying degrees of peripheral arterial occlusive disease[35].

Patients with diabetes mellitus and peripheral arterial occlusive disease have a 5-fold increase in the rate of amputation caused by critical limb ischemia than do patients without diabetes[101]. An experimental model of limb ischemia showed diminished revascularization in diabetic compared to wild type aminals, a finding associated with increased expression of MMP-2 and markedly increased expression of MMP-12[102].

MMPs and cardiovascular medications

Several therapies commonly prescribed for patients with cardiovascular diseases may influence MMPs function. Nitroglycerin increases the expression and the activity of MMP-2, MMP-7 and MMP-9, and decreases TIMP-1 levels[103]. Similarly, heparin has been shown to induce MMP-1 and MMP-2 levels[104]. On the contrary, recent evidence has shown rosiglitazone decreases levels of MMP-9[105]. Calcium channel blockers like amlodipine and diltiazem increase the activity of MMP-1 and MMP-2 in cultured human vascular endothelial cells as well as TIMP-1 levels[106]. Decreased MMP-1 activity has been shown with angiotensin II[107]. Therefore, angiotensin converting enzyme (ACE) inhibitors may increase the activity of MMP-1[107]. Finally, losartan has been shown to increase MMP-2 activity in human vascular smooth cells[108].

Recent observations suggest that statins may exert their beneficial effects on the arterial wall in part by their effects on MMPs and TIMPs. Statins inhibit the secretion of MMPs from rabbit, human smooth muscle cells and macrophages[109,110] and increase plaque stability[111]. Statins suppress the development of experimental aneurysms in both normal and hypercholesterolemic mice, independently form the lipid-lowering treatment[112]. Simvastatin reduced serological markers of inflammation and plasma MMP-9 activity[113]. In addition, pravastatin decreased lipids, lipid oxidation, inflammation, MMP-2, and cell death and increased TIMP-1 and collagen content in human carotid plaques, confirming its plaque-stabilizing effect in humans[114].

Conclusion

In conclusion, MMPs and their inhibitors have a fundamental role in the process of atherosclerosis. MMPs are associated with the classical cardiovascular risk factors and are involved in the different stages of atherosclerosis. Although literature data suggest a role for MMPs activation in cardiovascular diseases, prospective data are needed to evaluate whether interventions aiming at modification of MMPs activity can reduce atherosclerosis. Finally, commonly prescribed medications affect plasma concentrations of MMPs and more research in humans in this field is warranted.

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