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Review

Matrix Metalloproteinases and Coronary Artery Diseases

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Summary: Matrix metalloproteinases (MMPs) play an important role in cardiovascular remodeling by degrading the extracellular matrix. Enhanced MMP expression has been detected in the atherosclerotic plaque, and activation of MMPs appears to be involved in the vulnerability of the plaque. Circulating MMP levels are elevated in patients with acute myocardial infarction and unstable angina. Increased MMP expression is also observed after coronary angioplasty, which is related to late loss index after the procedure. These observations suggest that MMP expression may be not only related to instability of the plaque, but also to the formation of restenotic lesions. The development of therapeutic drugs targeted specifically against MMPs may be useful in the prevention of atherosclerotic lesion development, plaque rupture, and restenosis.

Key words: atherosclerosis, acute coronary syndrome, restenosis, plaque rupture

Introduction

In human atherosclerosis, unstable atherosclerotic plaque is an important event that triggers acute coronary syndrome. Plaque rupture frequently correlates with loss of the extracellular matrix (ECM) at certain locations, often in the shoulder areas of the plaque. Focal destruction of the ECM renders the plaque less resistant to mechanical stresses imposed during systole and therefore vulnerable to rupture. Recent findings have revealed enhanced expression of matrix metalloproteinases (MMPs) in the vulnerable region of plaques and this contributes to the weakening of plaque caps by degrading the ECM. This review aims to highlight the involvement of MMPs in coronary artery diseases and describes the potential use of MMP inhibitors in their treatment.

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Matrix Metalloproteinases and Their Inhibitors

Matrix metalloproteinases are a family of zinc-containing endoproteinases that share structural domains but differ in substrate specificity, cellular sources, and inducibility. The list of MMPs has grown rapidly in the past several years, and by now > 20 mammalian members have been cloned and identified. All MMPs share the following functional features: (1) they degrade ECM components, (2) they are secreted in a latent proform and require activation for proteolytic activity, (3) they contain Zn^{2+} at their active site, (4) they need calcium for stability, (5) they function at neutral pH, and (6) they are inhibited by specific tissue inhibitors of metalloproteinases (TIMPs).[1] The members of the MMP family can degrade all of the components of the blood vessel wall and therefore play a major role in both physiologic and pathologic events that involve the degradation of ECM components.

Based on their substrate specificity and primary structure, the MMP family can be subdivided into four groups (Table I).[2, 3] The first group, the collagenases, includes MMP-1 (interstitial collagenase), MMP-8 (neutrophil collagenase), and MMP-13 (collagenase-3), which can all cleave fibrillar collagens (type I, II, and III). Group 2, the gelatinases (MMP-2 and MMP-9), is well-known for its ability to degrade gelatins, which are fragments of collagens degraded by collagenases. Gelatinases are also capable of cleaving interstitial collagens. Group 3 is comprised of the stromelysins (MMP-3, -10, and -11), so named because they are active against a broad spectrum of ECM components, including proteoglycans, laminins, fibronectin, elastin, and some types of collagen. Group 4 contains the membrane-type MMPs (MT-MMPs), which degrade several ECM components and are also able to activate other MMPs.

Fully activated MMPs can be inhibited by interaction with naturally occurring, specific inhibitors, the TIMPs. At present, the TIMP family consists of four structurally related members, TIMP-1, -2, -3, and -4.[3, 4] The TIMPs bind noncovalently to

TABLE I Matrix metalloproteinases (MMP) family members and their substrates

Subgroup	MMP number	Substrate
Collagenases	1	Collagens (I, II, III, VII, X), gelatin, proteoglycans
	8	Collagens (I, II, III), gelatin, proteoglycans
	13	Collagens (I, II, III), gelatin, fibronectin, laminins
Gelatinases	2	Gelatin, collagens (I, IV, V, VII, X)
	9	Gelatin, collagens (IV, V, VII, X)
Stromelysins	3	Collagens (III, IV, V, IX), fibronectin, laminins, elastin, gelatin, proteoglycans
	10	Collagens, (III, IV, V, IX), gelatin, proteoglycans
	11	Collagen IV, fibronectin, laminins, gelatin, proteoglycans
Membrane-type MMPs	14	Collagens (I, II, III), fibronectin, laminins, activates proMMP-2 and -13
	15	Fibronectin, laminins, activates proMMP-2
	16	Collagens (III, IV), fibronectin, gelatin, activates proMMP-2
	17	Collagens (III, IV), gelatin
	24	Activates proMMP-2
	25	Gelatin

active MMPs in a 1:1 molar ratio. Inhibition is accompanied by their ability to interact with the zinc-binding site within the catalytic domain of active MMPs. There is a certain degree of specificity in the activity of different TIMPs toward distinct members of the MMP family. Whereas TIMP-1 potentially inhibits the activity of most MMPs, with the exception of MMP-2, TIMP-2 is a potent inhibitor of most MMPs, except MMP-9; TIMP-3 has been shown to bind MMP-1, -2, -3, -9, and -13; TIMP-4 inhibits MMP-1, -3, -7, and -9.[1]

Regulation of Matrix Metalloproteinases

Regulation of MMPs occurs at three levels: induction of expression, activation of the latent forms, and regulation by TIMPs. Induction of MMPs at the transcriptional level is mediated by a variety of inflammatory cytokines and growth factors, such as interleukin-1 (IL-1), IL-6, tumor necrosis factor-α (TNF-α), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and basic fibroblast growth factor (bFGF).[3,4] There are significant differences between the regulation of MMP-2 and the remainder of the MMP family. The latter are inducible by cytokines and growth factors, but MMP-2 has a more constant pattern of expression. In contrast, other cytokines such as IL-4, interferon-γ (IFN-γ), and IL-10 inhibit the synthesis of MMP-1, MMP-3, and MMP-9.[5–7]

Direct cell-cell contact may be an additional factor in the regulation of MMP expression in the atherosclerotic plaque. It has been suggested that activated T-cells play a pivotal role in the induction of MMP-1, MMP-3, MMP-9, and MMP-11 expression in macrophages and vascular smooth muscle cells (VSMCs).[8,9] Lee *et al.*[10] reported that interactions between monocytes and VSMCs stimulate MMP-1 and MMP-3 secretion in VSMCs. We also reported increased MMP-1mRNA and protein expression in human monocytes and vascular endothelial cells through their direct contact.[11]

Although transcriptional regulation is essential for MMP production, all MMPs are expressed as inactive zymogens, and matrix degradation requires the latent enzymes to be activated by proteinases such as plasmin, trypsin, chymase, elastase, or kallikrein. Among them, plasmin is a potent activator of most MMPs.[12]

On the other hand, the activity of MMPs is controlled by TIMPs. Although TIMP expression appears to be less affected by cytokines and growth factors, TIMP-1 is induced by IL-10 and TIMP-3 is induced by PDGF and transforming growth factor-β (TGF-β).[5,7]

Reactive oxygen species (ROS) have also been shown to modulate vascular MMP activity potently.[13] In an experimental hypercholesterolemic rabbit model, treatment with an ROS scavenger, *N*-acetyl-cysteine, markedly decreased the expression and activation of the macrophage-derived MMP-9.[14] Reactive oxygen species can trigger activation of MMP precursors, which may be related to the mechanism by which *N*-acetyl-cysteine decreases MMP-9 activation. Oxidized low-density lipoprotein (LDL) may also play an important role in the regulation of MMPs in atherosclerosis, because it upregulates MMP activity, by inducing MMP-9 expression while reducing TIMP-1 expression in macrophages.[15]

Atherosclerosis

It is widely accepted that atherosclerosis is initiated by chemical and/or mechanical injury of the endothelium, followed by transendothelial infiltration of circulating monocytes into the intima where they become activated and elaborate a variety of cytokines and growth factors.[16] In response to these stimuli, VSMCs migrate from the media to the intima and undergo proliferation. Matrix degradation is a prerequisite for both the recruitment of monocytes and migration of VSMCs, because in such maneuvers the cells have to transverse the extracellular barriers, including the basement membranes (consisting of collagen type IV and laminin) underlying the endothelium and surrounding each smooth muscle, as well as a dense mesh of interstitial collagen. In recent studies using knockout mice, electrical injury of femoral arteries in mice,

which stimulates intimal thickening, caused enhanced expression of MMP-2 and MMP-9.[17] In TIMP-1-deficient mice intimal thickening was significantly higher compared with that in wild-type controls.[18] Together, these observations support a role for MMP involvement in intimal thickening, particularly in migration of VSMCs.

Previous studies demonstrated that lipid-laden macrophages from human atherosclerotic plaque elaborate MMP-1 and MMP-3,[19] and culture of macrophages with fibrous caps of human atherosclerotic plaque induces MMP-dependent collagen breakdown.[20] Henney *et al.*[21] detected the presence of MMP-3 transcripts in coronary atherosclerotic lesions, which were colocalized with large clusters of lipid-laden macrophages in the shoulder areas of the plaque. Galis *et al.*[22, 23] reported that atherosclerotic plaque and lesion-free arteries had different patterns of MMP expression; MMP-2 together with TIMP-1and TIMP-2 were expressed by VSMCs in all layers of nonatherosclerotic arteries, whereas MMP-1, MMP-3, and MMP-9 were localized to macrophages, VSMCs, and the endothelium in the fibrous cap and shoulders of the lesions. Other researchers have also detected the expression of several other MMPs including MMP-1, MMP-2, MMP-7, MMP-9, and MMP-12 in the shoulder areas of plaque.[24–29]

Acute Coronary Syndrome

Acute coronary ischemia is usually initiated by rupture of the atherosclerotic plaque, leading to intracoronary thrombosis and occlusion. Collagen content contributes critically to the plaque stability. Cheung *et al.*[30] reported that the rat myocardium subjected to ischemia-reperfusion injury released MMP-2. Brown *et al.*[31] reported that MMP-9 was commonly expressed in coronary atherectomy specimens from patients with recent plaque rupture.

Recently, Kai *et al.*[32] reported that circulating MMP-2 and MMP-9 levels on admission were elevated in patients with acute myocardial infarction (AMI) and unstable angina. Inokubo *et al.*[33] also reported that plasma levels of MMP-9 were significantly increased in the coronary circulation in patients with AMI and unstable angina compared with those in control subjects, suggesting a process of active plaque rupture in acute coronary syndrome. Hirohata *et al.*[34] and we[35] also observed increased plasma MMP-1 and MMP-2 levels, respectively, in patients with AMI. In our study, plasma levels of TIMP-2 did not change during the course of AMI. Increased MMP expression may modulate vascular and ventricular remodeling in acute coronary syndrome.

Restenosis

The role of MMPs in iatrogenic postprocedural vasculopathy has also attracted a great deal of interest. The development of percutaneous coronary intervention (PCI) has provided a powerful means for treating ischemic heart disease. However, 25–40% of patients have a recurrence of their symptoms within 6 months because of restenosis at the original site. This is due to a combination of events, that is, the migration and rapid growth of medial VSMCs, producing a characteristic lesion of fibrocellular intimal hyperplasia.

In vitro models have been used to demonstrate the induction of collagenase and stromelysin gene expression in response to mechanical injury in VSMCs.[36] In animal models, it has been reported that balloon injury upregulates local gelatinase expression in damaged arteries.[37, 38] Degradation of the extracellular matrix in plaques dilated by PCI may facilitate contact of tissue factors in the vascular wall with circulating blood and activation of the extrinsic coagulation pathway. Sawicki *et al.*[39] also reported an MMP-2-mediated pathway of platelet aggregation. Bendeck *et al.*[37] found a correlation between MMP-2 activity and the degree of VSMC migration in balloon-injured rat carotid arteries. Southgate *et al.*[38] found that upregulation of MMP-2 activity paralleled the time course of VSMC migration in pig carotid arteries after PCI. It is interesting to note that Cheng *et al.*[40] demonstrated that human TIMP-2 gene transfer inhibited VSMC migration and delayed neointimal development in balloon-injured rat carotid arteries.

We previously investigated changes in MMP-2 levels in the coronary circulation after PCI in patients with angina pectoris.[41] Blood samples were drawn from the coronary sinus before and after PIC. Plasma MMP-2 levels in the coronary sinus increased significantly 4 h after PCI, whereas TIMP-2 did not show significant changes. A positive correlation was observed between MMP-2 level 4 h after PCI and late loss index 6 months after PCI. These in vitro and in vivo findings suggested that increased levels of MMPs in dilated coronary arteries lead to vascular remodeling and late restenosis by promoting migration of VSMCs and formation of thrombus.

Therapeutic Implications

Statins: Hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) have been widely used for treatment of hyperlipidemia. In atherosclerotic lesions, collagen is the major component of extracellular matrix, comprising up to 40% of the total protein. The accumulation of collagen is influenced by its de novo synthesis and deposition and by degradation of existing collagens by MMPs.

Previously, Bellosta *et al.*[42] reported that lipophilic statins such as fluvastatin and simvastatin reduced MMP-9 secretion by mouse and human macrophages in culture. We found that clinical concentrations of fluvastatin decreased MMP-1 expression in cultured human endothelial cells.[43] Fukumoto *et al.*[44] administered pravastatin, fluvastatin, or placebo to Watanabe heritable hyperlipidemic rabbits for 52 weeks and found that MMP-1, MMP-3, and MMP-9 expression by macrophages in the intima were lower in both the pravastatin and fluvastatin groups than in the placebo group. Statins may directly inhibit MMP expression and achieve plaque stabilization through effects that are independent of their cholesterol-lowering properties.[45]

Calcium-channel blockers: Mason *et al.*[46] reported membrane antioxidant effects of the calcium-channel blocker amlodipine. The chemical structure of amlodipine contributes to distinct biophysical membrane interactions that lead to the po-

tent lipid antioxidant effect, independent of calcium-channel modulation. We investigated the effects of amlodipine and nifedipine on the expression of MMP-1 in cultured human endothelial cells.[47] Amlodipine, but not nifedipine, significantly decreased MMP-1 levels in endothelial cells. The mechanism by which amlodipine decreases MMP-1 levels may be related to its membrane antioxidant activity. Like amlodipine, lacidipine is a highly lipophilic compound and shows prolonged binding to lipid membranes.[48] Recently, Bellosta *et al.*[49] reported that lacidipine decreased the secretion of MMP-9 by human macrophages, while nifedipine showed no effect. Together, these studies suggested that, by intercalating into hydrophobic compartments of the cell at high concentrations, lipophilic calcium-channel blockers may effectively inhibit the propagation of unstable radicals and contribute to stabilization of the plaque by interfering with MMP secretion. Indeed, the recent Prospective Randomized Evaluation of the Vascular Effects of Norvasc Trial (PREVENT) demonstrated that patients with documented coronary artery disease treated with amlodipine experienced marked reductions in the rate of unstable angina and coronary revascularization compared with patients receiving placebo.[50] Also in Coronary Angioplasty Amlodipine Restenosis Study (CAPARES), amlodipine significantly reduced the frequency of repeat PCI and clinical events after PCI.[51]

Gene therapy: In many recent studies, gene therapy has been used to examine the role of MMPs in atherosclerosis and whether TIMPs are good candidates for treatment of atherosclerosis. Using VSMCs transfected with TIMP-1[52] and adenoviral delivery of TIMP-1,[53] it was shown that overexpression of TIMP-1 reduced intimal thickening by 40 and 30%, respectively. Adenoviral delivery of TIMP-1 into apoE-deficient mice fed a lipid-rich diet, reduced lesion area by approximately 30%.[54] Histologic and immunohistochemical analysis revealed increases in collagen, elastin, and VSMC α-actin content and a marked reduction in macrophages.

Autologous saphenous vein coronary artery bypass graft surgery is complicated by late graft failure due to neointima formation and subsequent atherosclerosis. George *et al.*[55] performed adenovirus-mediated overexpression of TIMP-3 in pig saphenous veins before interposition grafting into carotid arteries in vivo to assess neointimal formation. Neointimal formation was reduced by 58% in 28-day vein grafts. They also used a highly reproducible organ culture model of neointimal formation in human saphenous vein to investigate the effects of adenovirus-mediated gene transfer of TIMP-1.[56] Overexpression of TIMP-1 significantly inhibited neointimal formation by 54% after 14 days. These observations confirmed the importance of MMPs in neointimal formation and highlighted the potential for application of TIMP gene therapy.

Conclusions

Matrix metalloproteinases play a crucial role in initiating acute coronary syndrome by degrading ECM components, which leads to vulnerability of the plaque as well as formation of atherosclerotic and restenotic lesions. Although the use of MMP inhibitors may have unforeseen adverse effects if used in the wrong setting, development of therapeutic drugs specifically targeted against MMPs may be useful in the prevention of atherosclerotic lesion development and cardiac events.

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