Matrix metalloproteinases in cardiovascular disease

Peter Liu MD, Mei Sun MD PhD, Sawsan Sader PhD

P Liu, M Sun, S Sader. Matrix metalloproteinases in cardiovascular disease. *Can J Cardiol* 2006;22(Suppl B):25B-30B

Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes that are regulated by inflammatory signals to mediate changes in extracellular matrix. Members of the MMP family share sequence homology, act on interstitial protein substrates, acutely participate in inflammatory processes and chronically mediate tissue remodelling. MMPs are important in vascular remodelling, not only in the overall vasculature architecture but also, more importantly, in the advancing atherosclerotic plaque. MMP activation modifies the architecture of the plaque and may directly participate in the process of plaque rupture. MMPs also participate in cardiac remodelling following myocardial infarction and development of dilated cardiomyopathy. Soluble MMPs are now potential biomarkers in delineating cardiovascular risk for plaque rupture and coronary risk. They also constitute innovative direct or indirect targets to modify cardiovascular tissue remodelling in atherosclerosis and heart failure.

Key Words: Atherosclerosis; Cardiovascular; Heart failure; Matrix metalloproteinases

MATRIX METALLOPROTEINASES IN CARDIOVASCULAR DISEASE

As the burden and spectrum of cardiovascular disease change from acute catastrophic events such as myocardial infarction and sudden death to chronic diseases such as vascular atherosclerosis and heart failure, tissue remodelling and inflammation take front and central roles. Tissue remodelling is a two-pronged process: cellular component remodelling and extracellular matrix remodelling. Extracellular matrix is now viewed as an equal partner in organ function and remodelling (Table 1), and matrix metalloproteinases (MMPs) are the major mediators of extracellular matrix remodelling. The recent research on the role of MMPs has promoted them to a prominent role in understanding the processes of remodelling, and potentially acting as diagnostic and therapeutic tools.

What are MMPs and why they are relevant in cardiovascular disease?

MMPs are a family of latent zinc- and calcium-dependent enzymes, which, when activated, are responsible for the breakdown of the extracellular matrix in many diseases (1). MMPs play a central role in organ development and subsequent tissue remodelling in inflammation and injury. The action of MMPs is increasingly appreciated in understanding the formation of

Rôle des métalloprotéines matricielles dans la maladie cardiovasculaire

Les métalloprotéines matricielles (MPM) forment une famille d'enzymes protéolytiques dont la régulation est assurée par des signaux inflammatoires qui amènent des changements au niveau de la matrice extracellulaire. Les membres de la famille des MPM partagent une homologie séquentielle, agissent sur les substrats des protéines interstitielles, participent intensément aux processus inflammatoires et influent à plus long terme sur le remodelage tissulaire. Les MPM sont importantes dans le remodelage des vaisseaux, car elles affectent non seulement leur architecture globale, mais également et surtout, la progression de la plaque d'athérome. L'activation des MPM modifie l'architecture de la plaque et peut participer directement à sa rupture. Les MPM sont en outre impliquées dans le remodelage cardiaque après un infarctus du myocarde et dans le développement de la myocardiopathie dilatée. Les MPM solubles sont désormais des biomarqueurs potentiels qui permettent d'identifier le risque cardiovasculaire associé à la rupture de la plaque et le risque coronarien. Elles constituent en outre de nouvelles cibles directes ou indirectes pour modifier le remodelage du tissu cardiovasculaire en présence d'athérosclérose et d'insuffisance cardiaque.

the atherosclerotic plaque or the development of cardiomyopathies. The rupture of the atherosclerotic plaque is mediated through the action of MMPs on the local fibrous cap, the consequence of which may turn a chronic disease into acute myocardial infarction or sudden death. The progress of ventricular remodelling after a myocardial infarction or viral injury is also mediated through MMPs' breakdown of the collagen and elastin framework, leading to ventricular hypertrophy and dilation. The consequence of this could be severe congestive heart failure accompanied by repeated hospitalization, or death.

TABLE 1
Role of extracellular matrix in the cardiovascular system

Biological roles of the extracellular matrix

Structural integrity of the heart and blood vessels
Framework for cell anchoring and function
Mediates cell adhesion, cell phenotype and cell-cell communication
Integrates mechanical force and lines of force transmission
Mediates diastolic stiffness
Promotes cell survival or apoptosis
Reservoir for growth factors and cytokines
Turns over and remodels in inflammation, injury or growth/involution

Heart & Stroke/Richard Lewar Centre of Excellence, and the Department of Physiology, University of Toronto; and Division of Cardiology, Toronto General Hospital, University Health Network, Toronto, Ontario

Correspondence: Dr Peter Liu, Heart & Stroke/Richard Lewar Centre of Excellence, NCSB 11-1266, Toronto General Hospital, 200 Elizabeth Street, Toronto, Ontario M5G 2C4, Canada. Telephone 416-340-3035, fax 416-340-4753, e-mail peter.liu@utoronto.ca

Figure 1 A Three-dimensional rendering of a member of the family of matrix metalloproteinases (MMPs). The catalytic domain contains the active enzymatic site that will bind collagen or gelatin. However, in the proenzyme form, the active site is shielded by a propeptide that is cleavable by free radicals, cytokines or other MMPs to activate the enzyme. The enzyme is stabilized and anchored in some cases through the hemopexin-like and linker domains (Reproduced from reference 2). B Structural relationships of the various identified family members of the MMPs. All the family members share the signalling peptide, propeptide, catalytic and hemopexin-like domains. MMP-2 and MMP-9 also share a fibronectin linker (Reproduced from reference 2). CMMP MMPs from chicken embryo fibroblasts; MT Membrane-type; XMMP MMPs from the frog *Xenopus laevis* embryos

There have been exciting recent advances in understanding the mechanisms of the biology of MMPs, their contributions to pathology, the potential of these molecules to act as diagnostic or prognostic tools, and their use as direct or indirect targets for therapy.

The MMP family members

MMPs are a class of metal-linked enzymes whose protease activity is zinc-dependent, and they require calcium for biological action and stability. MMPs are secreted by smooth muscle cells, endothelial cells, monocytes, macrophages and inflammatory T-cells, initially in a proenzyme form (zymogens). Their latency is maintained by a propeptide domain that is linked by a cysteine bond, and hydrolysis of the zinc-cysteine frees the propeptide and uncovers the enzymatic site on the parent MMP molecule (2).

All MMP family members share a common structural framework (Figure 1A). The three-dimensional structure consists of a signal peptide, followed by a propeptide domain. The latter is used to shield the aperture in the catalytic domain, which contains the active site for digestion of substrates such as collagen or elastin. In MMP-2 and MMP-9, there is also a fibronectin-like domain within the catalytic site (2). Finally, there is a hemopexin-like domain, which may be attached also to a transmembrane linker for MMP members that have a transmembrane localization.

From a phylogenetic point of view, most of the MMPs share basic structural homologies (Figure 1B). This is particularly themed based on their function. For example, MMP-2 and MMP-9, which are collagenases sharing common substrates such as collagen IV, have very close structural homologies. Similarly, the membrane-type (MT)-MMPs, which are membrane-bound forms of MMPs, important in the processing of signalling peptides such as cytokines, also share a significant amount of the structural homology.

MMPs are extremely potent protein degradation and modifying enzymes; thus, their biological actions are very tightly controlled. Table 2 lists the common types of MMPs, their

designations and common substrates. One can appreciate that there is a significant overlap of their activities and preferred substrates, providing some degree of redundancy and tissue localization. The initial levels of the proenzyme or zymogen of MMPs are tightly regulated through transcriptional mechanisms, with potent regulation by the pro-inflammatory cytokines. Cytokine signals such as tumour necrosis factor- α (TNF- α), interleukins and special extracellular MMP inducers are very effective in regulating the production of proenzymes by smooth muscle cells, myocytes or fibroblasts, and transporting them into the extracellular matrix (3). Other growth factors, such as platelet-derived growth factor and CD40 ligands, can also effectively control the transcriptional mechanisms of MMP family members (Figure 2).

Once produced, the MMPs reside in a proenzyme form in the ground substance of the extracellular space. However, when they encounter additional activation signals such as oxygen free radicals, ischemic triggers such thrombin or chymase, or angiotensin-converting enzyme (ACE) from mast cells, then the propeptide is released, uncovering the active site of the enzyme (4). This process can also be catalyzed by MT-MMPs or other proteolytic agents, such as plasminogen activators (urokinase-type plasminogen activator) or plasmin. The latter are activated through inflammation and coagulation cascades. Inhibition of either MMPs, plasminogen activators or cytokines can all reduce cardiac rupture following myocardial infarction as a result of MMP effects on matrix disruption (5,6).

However, to provide further checks and balances in the system, additional strategies to regulate MMP function are also present in nature. There are naturally occurring inhibitors of MMPs (tissue inhibitors of metalloproteinases [TIMPs]), which can partially or completely neutralize MMP function in activated states. There are currently four known members in the TIMP family – TIMP1 to TIMP4. Each TIMP member has its specific ranges of interactions with the various members of the MMP family. Furthermore, some of the TIMPs (such as TIMP3) are also MT-MMPs, and have an important function

TABLE 2
Matrix metalloproteinases (MMPs) members and their common substrates

Enzyme name	MMP classification	Common substrates
Collagenases		
Interstitial collagenase	MMP-1	Collagens I, II, III, VII, X, gelatin, MMP-2, -9
Neutrophil collagenase	MMP-8	Collagens I, II, III, V, VII, X, gelatin
Collagenase 3	MMP-13	Collagens I, II, III, IV, gelatin, fibronectin, laminin
Gelatinases		
Gelatinase A	MMP-2	Gelatin, collagens I, IV, V, VII, X, XI, fibronectin, laminin, elastin
Gelatinase B	MMP-9	Gelatin, collagens III, IV, V, VII, X, elastin, vitronectin
Stromelysins		
Stromelysin-1	MMP-3	Collagens III, IV, V, IX, X, gelatin, fibronectin, laminin, tenascin, MMP-1, -7, -8, -9, -13
Stromelysin-2	MMP-10	Collagens III, IV, V, IX, gelatin, laminin, casein, fibronectin, MMP-1, -8
Stromelysin-3	MMP-11	Collagens IV, gelatin, fibronectin, laminin
MT-MMPs		
MT-1	MMP-14	Collagens I, II, III, gelatin, fibronectin, laminin, vitronectin, proteoglycans, activate pro-MMP-2 and pro-MMP-13
MT-2	MMP-15	Activate pro-MMP-2
MT-3	MMP-16	Activate pro-MMP-2
MT-4	MMP-17	Activate pro-MMP-2
MT-5	MMP-24	Activate pro-MMP-2
MT-6	MMP-25	Gelatinolytic activity

MT Membrane-type

in regulating TNF- α and other cytokine signal peptides. There are also artificial MMP inhibitors, the most well known of which is the tetracycline family of antibiotics. There are now also a number of synthetic MMP inhibitors, which may have a variable range of function in blocking only selected members of the MMP family or blocking all of the MMPs.

More recently, there have been intriguing experimental results to suggest that MMPs may not only reside in the extracellular matrix, but may also be present intracellularly. The intracellular MMPs may perform important functions such as protein processing and digestion of the small structural proteins, and may also perform regulatory functions in processing signalling peptides. It has been suggested that MMPs may be important in the processing of components in the actin-myosin interface. This may lead to the transient cardiac dysfunction or myocardial stunning following ischemia-reperfusion (7).

MMPs in atherosclerosis and plaque rupture

The active atherosclerotic plaque consists of a lipid core, an external fibrous cap and a mixture of inflammatory cells, such as lipid-laden macrophages, in between. The fibrous cap is generally made up of extracellular matrix laid down by the smooth muscle cells, even though the matrix itself is acellular in nature. The components include collagen (providing strength) and elastin (providing flexibility), and when the plaque grows or recedes, the fibrous cap is resorbed (destruction) and

Figure 2) Mechanisms of regulation of matrix metalloproteinases (MMPs). The presence of proenzyme is regulated through transcriptional activation by inflammatory cytokines. The proenzyme can be converted into an activated enzyme by a number of environmental influences. Finally, there are both natural and artificial inhibitors to modulate their potent biological action by providing a variety of checks and balances. ACE Angiotensin-converting enzyme; EMMPRIN Extracellular MMP inducers; IL Interleukin; MT Membrane-type; OFR Oxygen free radicals; TIMP Tissue inhibitors of metalloproteinases; TNF Tumour necrosis factor; uPA Urokinase-type plasminogen activator; Zn Zinc

Figure 3) Actively remodelling atherosclerotic plaque that can lead to plaque rupture. In a remodelling plaque, there is a balance between matrix synthesis and matrix degradation – the latter is mediated by matrix metalloproteinases (MMPs), particularly MMP-9 and MMP-3. The MMP activation and, hence, matrix degradation is enhanced by the presence of cytokines such as tumour necrosis factor (TNF), interferons (IFNs) elaborated by macrophages, T lymphocytes and activated smooth muscle cells (SMCs). This is stimulated by the presence of oxidized low density lipoprotein (LDL) and C-reactive protein (CRP). In the presence of infection, inflammation or excessive mechanical stress, the MMP activity can further hasten the disintegration of the fibrous cap, leading to plaque rupture and its consequences

redeposited (construction) – the familiar ‘remodelling’ process (Figure 3). Under stable conditions, the destructive and constructive processes are balanced, and the plaque protects the lumen of the vessel from exposure to the underlying thrombogenic material. However, under proinflammatory conditions, the remodelling process can be shifted in favour of resorption of the matrix, leading to a potentially dangerous weakness in the cap and resulting in plaque rupture.

There is now good documentation of the increased presence of the MMPs in the active atherosclerotic plaques. Examination of either animal models of atherosclerosis or human coronary specimens from atherectomy or autopsy sources demonstrates the colocalization of MMP-9 and

Figure 4) Postinfarction ventricular rupture is partially dependent on the presence of tumour necrosis factor (TNF) activation of matrix metalloproteinase (MMP)-2 and MMP-9 in the myocardium. In this mouse model (6), the wild-type (WT) animals suffer significant mortality from rupture postinfarction due to very high levels of MMP-9. However, in the TNF knockout animals, in whom the cytokine levels were much lower, there was a reduction in MMP-9 and a minimal amount of rupture. B Infarct border; Is Infarct region; N Noninfarct region; S Sham

MMP-3 in the advancing edges of the atherosclerotic plaque (8). The MMPs found are often associated with the presence of inflammatory cells, such as macrophages or T lymphocytes (9). The presence of the MMPs is also associated with sites of potential weakness in the plaque where ruptures tend to occur or where extensive remodelling is taking place (10).

Interestingly, the natural inhibitors of MMP, the TIMPs, are also found in the atherosclerotic plaque (9). However, at sites where increased TIMPs are present, there is often associated vascular calcification (8). This may mean that vascular calcification is nature's way of stabilizing the remodelling process, and may be a consequence of TIMP action over that of MMPs. Overall, when there is imbalance in the MMP and TIMP activity ratio in favour of MMP activation, there is increased vascular remodelling and increased atherosclerotic plaque formation (10).

Activation of the MMPs locally, whether through activation of inflammatory signals or free radicals, leads to the degradation of collagen and elastin into peptide fragments, weakens its structure, and permits disruptions such as plaque rupture. The presence of inflammatory cells, such as activated macrophages and T cells in the advancing zone of the atherosclerotic plaque, helps to accelerate this process (10). Recently, studies using transgenic knockout mice deficient in MMP-9, when crossed with the atherogenic apolipoprotein E knockout mice, despite cholesterol feeding showed much less atherosclerotic burden (11). This was not replicated by MMP-12 knockout animals. This suggests strongly that MMPs such as MMP-9 plays a major contributory role in plaque growth and remodelling in the presence of increased cholesterol.

Interestingly, Lee et al have shown recently (12) that MMPs may also have feedback on the inflammatory process as well. For example, inhibition of the elastase system actually

decreases the degree of inflammatory cell infiltrates. The activation of MMPs can also lead to local growth factor production. The alteration of the matrix, together with the mitogenic signals, promotes smooth muscle cell migration into the intima and transformation of these cells into macrophage-like structures. Therefore, the activation of MMPs sets up a vicious cycle of recruitment of additional inflammation, the increased transformation of cells contributing to the atherosclerotic plaque growth, as well as the promotion of the potential rupture of newly formed plaques (12). The resultant forces of remodelling, and the increased inflammatory changes, further enhance the production and activation of local MMPs.

It is interesting, then, to note that most of the risk factors contribute ultimately to the activation of MMPs. Smoking, diabetes, homocysteine and increased lipids all contribute to the oxidative stress in the intima and media of blood vessels, leading to activation of MMPs. In addition, the presence of lipids (especially oxidized low density lipoprotein) stimulates the cytokine release from activated macrophages and T cells, leading to increased expression and activation of MMPs. Finally, the circadian variation in blood pressure surges also creates a mechanical stimulus to enhance an already weakened fibrous cap by other risk factors. Of course, small ruptures that occurred earlier lead to local clot formation, and release more tissue-like plasminogen activator, urokinase-type plasminogen activator and plasmin, which in turn will also enhance MMP activity.

MMPs in heart failure

The activity of MMPs has been shown to be increased in the progression of heart failure. The level of MMPs, and their induction and activation systems are increased in pathological specimens of human heart failure (3). Furthermore, in the setting of myocardial infarction, MMPs are increased very significantly within hours of the infarction, following the local activation of cytokines and infiltration of inflammatory cells (13). However, as the healing process continues, the MMP concentrations decrease, but then are followed by a second wave of activation, which is associated with more ventricular dilation and progression toward heart failure (6).

A significant component of regulation of MMP production following myocardial infarction is induced by the local inflammatory cytokines. In an animal model of myocardial infarction (14), the authors demonstrated that an excess of TNF in the myocardium is directly related to the increased production of local MMP-9 and MMP-2, which is associated with changes in integrin isoform transition. This can lead to such aggressive collagen dissolution that acute myocardial rupture may result (Figure 4). However, if this process continues without rupture, the heart becomes significantly dilated, and this is associated with decreased function and poor survival (6). However, if the gene for TNF is removed through genetic manipulation in the host animal, there is a significant reduction in the levels of the inflammatory cytokines, which is associated with the reduction in MMP activation. This results in a dramatic reduction in the incidence of rupture, and a reduction in the subsequent heart size and development of heart failure.

Similar strategies have been used by other investigators to demonstrate this cytokine-MMP remodelling linkage. Bradham et al (15), from Dr Spinale's group, demonstrated that in the classic canine pacing induced heart failure model, the administration of a TNF- α blocker reduced MMP-9 and

Figure 5) In a model of viral myocarditis, the affected cardiac tissue often undergoes remodelling and necrosis, with a significant amount of inflammatory infiltration (**Left**) (12). However, in the same model, when exposed to an elastase inhibitor, not only were the tissue necrosis and remodelling attenuated, but there was also a surprising degree of reduction of inflammatory cell infiltration (**Right**). This suggests that there is a vicious cycle set up in which inflammation begets matrix metalloproteinase activation, which begets more inflammation

MMP-13 activation, and attenuated an associated increase in end-diastolic volume.

The results of evaluating the role of MMP inhibitors in modifying cardiac remodelling following myocardial infarction have been somewhat variable. This is likely due to the fact that there is a biphasic response of MMP activation following infarction. Therefore, the administration of the appropriate inhibitors of MMPs in the early phase can reduce the incidence of rupture that is the result of the excess of inflammatory signals. However, to produce the appropriate cardiac remodelling more chronically, the inhibitors should be administered later in the process of infarction, targeting the second wave of MMP activation. Unfortunately, excessive or prolonged administration of MMP inhibitors may result in excessive scar formation. This may convert systolic heart failure into diastolic heart failure, with consequences of excessive fibrosis and paradoxical collagen deposition.

MMPs as diagnostic or prognostic markers

Additional research into the field has shown that MMPs may be released into the blood in sufficient quantities to act as diagnostic or prognostic markers. Recently, Blankenburg et al (16) conducted a study in patients with existing coronary artery disease. They found that soluble MMP-9 measured in the blood provided the most efficient prognostic information beyond that offered by traditional prognostic markers such as cholesterol or other inflammatory markers. In their population, they found that in those with the lowest levels of soluble MMP-9, there was less than a 2.5% event rate during follow-up, in contrast with those individuals with the highest quartile of MMP-9 levels, in whom the event rate approached 16%. There was also synergism with inflammatory markers such as interleukin-18, exceeding the value of the common prognostic markers such as troponin or C-reactive protein.

Similarly, we and others (5,6) have found that the soluble MMPs in the blood can be important in patients with heart failure. Several studies have now shown that the elevated levels of MMP-9 in the blood may predict those with cardiac dilation. This suggests that the active MMP activities in the heart that lead to collagen digestion and cardiac dilation can actually

be quantitated in the blood, thereby reflecting the degree of active remodelling.

A new role for MMPs may reside in stem cell mobilization following cardiac injury. In the process of the investigation of the cell mobilization following injury, in the very active field of cell-based therapy following myocardial infarction, investigators have found that MMP-9, for example, is present in the bone marrow and is responsible for the release of mononuclear cells into the circulation. It appears that following ischemic injury, there can be local production of inflammatory cytokines, such as TNF, platelet-derived growth factor or vascular endothelial growth factor. These factors enter the circulation and, ultimately, reach other sites such as the bone marrow. This can then lead to local increases in MMP-9 in the bone marrow, which then leads to the release of mononuclear cells, including the stem cells, from the bone marrow stroma. These mononuclear cells then migrate through the vasculature into areas of ischemia. Of course, many of these mononuclear cells develop into inflammatory cells such as macrophages or lymphocytes. However, a number of them also contain primitive markers of stem cells, which may help in the local injury environment to transdifferentiate into local structures, such as the microvasculature, to assist in the wound healing process.

NEW THERAPEUTIC OPPORTUNITIES

From the foregoing discussion, it can be noted that risk factors lead to inflammatory cell activation which in turn triggers MMP activation. This then weakens the fibrous cap, leading to plaque rupture. Therefore, this is consonant with the concept that aggressive reversal of risk factors in patients who are at risk for coronary events decreases the inflammatory process and, hence, stabilizes the plaque.

The authors have shown previously, in collaboration with Drs Rabinovitch and Husain, that elastase inhibition, in the setting of viral myocarditis, cannot only decrease the development of cardiomyopathy, but also decrease inflammation (Figure 5) (12,17). Similarly, in the setting of myocardial infarction, inhibition of elastases or overexpression of an endogenous inhibitor, elafin, can also attenuate the cardiac dilation, dysfunction and inflammation (18).

In the setting of pure volume overload through the creation of arteriovenous fistula, Chancey et al (19) have confirmed that the administration of an MMP inhibitor can effectively decrease cardiac dilation, reduce wall stress and left ventricular hypertrophy, and preserve ventricular function.

This has raised the intriguing possibility that MMP inhibitors may modify the development of adverse cardiac remodelling and heart failure postmyocardial infarction. This has indeed been borne out with basic proof of concept studies (20), particularly with the inhibition of MMP-9, one of the major gelatinases involved in postinfarct remodelling (21,22). Most of these studies demonstrate a reduction in ventricular size and improvement in ventricular function with administration of the MMP inhibitors (23-25). Unfortunately, a number of leading candidates have been withdrawn from active development for this indication because of fibromyalgia side effects in earlier trials attempting to decrease metastasis in cancer.

On the other hand, many of the currently protective treatment strategies may already partially attenuate MMP activation as part of its mode of action. For example, decreasing the

cytokine and inflammatory response can limit the bioactivity of MMP, and may form part of the benefit of treatments such as acetylsalicylic acid or statins. There is also suggestion that ACE may assist in activating MMPs, hence the benefit of ACE inhibition (4). The effectiveness of heparin and antibiotics may also help to decrease inflammation and MMP activation, thus stabilizing the plaque. In the future, other strategies to decrease inflammation (such as TNF inhibitors and peroxisome proliferator-activated receptor activators), to decrease oxidation (such as antioxidants) and, finally, to direct inhibitors of MMP may all represent additional intriguing approaches to tackle the problem of plaque rupture and ventricular remodelling.

ACKNOWLEDGEMENTS: Supported in part by grants from the Heart and Stroke Foundation (HSF) of Ontario, and the Canadian Institutes of Health Research (CIHR) and CIHR Group Program in Heart Failure (CHF-CORE), CIHR Canadian Heart Failure Interdisciplinary Health Research Network (CHFNET), and CIHR Strategic Program in Training for Cardiovascular Excellence (TAC-TICS) Partnership Programs of the HSF and CIHR.

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