Mini-review

The role of matrix metalloproteinases in heart disease

Jack P.M. Cleutjens *

Cardiovascular Research Institute Maastricht (CARIM), Department of Pathology, University of Limburg, PO Box 616, 6200 MD Maastricht, Netherlands

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1. Introduction

The heart can be subdivided into cellular and extracellular compartments. Approximately one third of cells from this cellular compartment are cardiomyocytes whereas two thirds of the cells are non-cardiomyocytes. Because of their dimensions, cardiomyocytes occupy two thirds of the total myocardial volume. Non-myocytes, predominantly fibroblasts, endothelial cells and to a lesser extent mast cells, macrophages and smooth muscle cells in the media of the myocardial vasculature, together with the extracellular matrix and tissue fluid, fill the remaining one third of cardiac tissue. The extracellular matrix compartment consists of a structural network of interstitial type I and III fibrillar collagens, which have a high rigidity and are extremely resistant to proteolytic digestion [1]. Besides the fibrillar collagen network, the extracellular compartment consists of basement membranes, which in the heart surround individual cardiomyocytes and smooth muscle cells, and separate endothelial cells, even in capillaries, from the media or surrounding connective tissue. Basement membranes contain a structural backbone of type IV collagen to which other components like laminins, fibronectin, heparan sulphate proteoglycan, and other extracellular matrix components can attach [2]. Type V collagen has been localized in basement membranes and interspersed in the interstitium [3]. Type VI collagen was found predominantly between cardiomyocytes linking basement membranes of the individual myocytes to the surrounding extracellular matrix and interstitial cells. The cardiac extracellular matrix also contains hetero-polysaccharides (glycosaminoglycans), glycoproteins (heparan sulphate and chondroitin sulphate proteoglycans), microfibrillar proteins (fibrillin and fibulin) and elastin.

The fibrillar collagenous network of the myocardium forms a continuity which consists of struts, connecting cardiomyocytes to other cardiomyocytes, cardiomyocytes to capillaries, cardiomyocytes to large collagen fibres and fine connections between large collagen fibres. These struts function as tethers and springs between myocytes. Furthermore, the collagenous weave, surrounding groups of myocytes, is associated with cardiac elasticity. Coiled perimysial fibres form a fibrillar network around blood vessels and a complex network in the subendocardium and subepicardium [4]. Functions of this fibrillar collagenous network are: alignment of cardiomyocytes and cardiomyocytes to capillaries; prevention of excess myocyte and sarcomere stretch; transmission of myocyte-generated force to the ventricular chamber; and provision of tensile strength and stiffness to the tissue [4,5]. Only a small increase in the amount of collagen results in increased systolic and diastolic stiffness, whereas decreased levels of collagen, either due to decreased collagen synthesis or increased collagen degradation, can lead to myocardial dilatation or even rupture. Disruption of the fibrillar collagen network, which can occur with increased collagenolytic activity, can have multiple adverse consequences on the architecture and function of the myocardium [6]. MacKenna et al. [7] perfused rat hearts in vitro with bacterial collagenase for 60 min and observed decreased collagen content, damage to the endomyosal struts and increased ventricular volume.
and sarcomere lengths without altering ventricular compliance. These findings suggest that fibrillar collagen may be more important for maintaining tissue architecture by prevention of overstretching sarcomeres than by contributing to passive ventricular stiffness.

The enzymes involved in degradation of extracellular matrix components can be divided into four different classes: matrix metalloproteinases (MMPs) and serine proteinases (plasmin, neutrophil elastase, cathepsin G) which react at neutral or slightly alkaline pH and cysteine (cathepsins B, L and S) and aspartic proteinases (cathepsin D) which have optimal activity at acidic pH [8].

Degradation of extracellular matrix is a normal process which is associated with morphogenesis, growth, angiogenesis, development and wound healing under normal and pathological conditions [8]. This review will focus on fibrillar collagen degradation given the structural role of interstitial fibrillar collagens in governing tissue architecture and function.

2. Collagen degradation

Fibrillar collagens (type I and III collagen) are extremely resistant to cleavage by most proteinases. Their collagen fibrils are tightly apposed, highly cross-linked and resistant to most extracellular matrix degrading enzymes [1,8]. Covalently bound carbohydrate and fibril interaction with glycoproteins of the surrounding extracellular matrix components make it difficult to degrade interstitial collagens. The enzymes capable of degrading native fibrillar collagen helices are interstitial collagenases also called collagenase (MMP-1) and neutrophil collagenase (MMP-8). MMP-1 and MMP-8 cleave interstitial collagens at unique Gly-Leu or Gly-Ile sites in the native triple helix at 3/4 from the N-terminal end, generating 3/4 (TC^A) and 1/4 (TC^B) collagen fragments [8,9]. Due to thermal degradation and loss of stability, these fragments unfold their triple helix conformation. The so formed single α-chains (gelatins) can be further degraded into amino acids and oligopeptides by a number of less specific proteinases such as gelatinases (72 kDa gelatinase A (MMP-2) and 92 kDa gelatinase B (MMP-9)), stromelysins and serine proteinases such as elastase and cathepsin G (Table 1 and Fig. 1) [9].

Another pathway of externalized collagen fibre degradation is endocytosis (phagocytosis) followed by intracellular degradation of larger collagen fragments by lysosomal enzymes at acidic pH. Predominantly cysteine proteinases and not matrix metalloproteinases produced by connective tissue cells (fibroblasts) and inflammatory cells (macrophages) could be involved in the intracellular degradation pathway [10]. Whether this intracellular degradation pathway plays a major role in degradation of mature collagen in the heart is still unknown.

Besides intracellular degradation of mature collagen fibres, also intracellular degradation of procollagen molecules can occur. After transcription of procollagen mRNA and translation into procollagen α-chains, these chains undergo hydroxylation and other modifications. Finally these α-chains are processed into triple helical procollagen and move from the endoplasmic reticulum to the Golgi apparatus. From there these procollagen triple helices are packed into vesicles and externalized by exocytosis. According to Bienkowski [11] intracellular breakdown

| Table 1  |
|----------|---------|-----------------|
| **Matrix metalloproteinases** | **Enzyme** | **Substrate** |
| Collagenases | MMP-1 | Interstitial collagenase (EC 3.4.24.7) | Type I, II, III, VII, X collagens, gelatins, proteoglycans, entactin |
| | MMP-8 | Neutrophil collagenase (EC 3.4.24.34) | Type I, II, III collagens |
| Gelatinases | MMP-2 | Gelatinase A, 72 kDa type IV Collagenase (EC 3.4.24.24) | Gelatins, type IV, V, VII, XI collagens, fibronectin, laminin, elastin, proteoglycans |
| | MMP-9 | Gelatinase B, 92 kDa type IV Collagenase (EC 3.4.24.35) | Gelatins, type IV, V collagens, elastin, entactin, proteoglycans |
| | MMP-3 | Stromelysin 1 (EC 3.4.24.17) | Gelatins, type III, IV, IX, X collagens, collagen telopeptides, proteoglycans, fibronectin, laminin |
| | MMP-10 | Stromelysin 2 (EC 3.4.24.22) | Type IV collagen, proteoglycans, laminin, fibronectin |
| | MMP-12 | Membrane-type MMP | Type IV collagen, gelatin, proMMP-2 |
| Others | MMP-11 | Stromelysin 3 | Unknown |
| | MMP-7 | Matrilysin, PUMP-1 (EC 3.4.24.33) | Proteoglycans, fibronectin, gelatins, type 1V collagen, elastin, entactin |
| | MMP-12 | Metalloelastase (EC 3.4.24.65) | Elastin |
| | MT-MMP | Membrane-type MMP | |
of high molecular pro-α-chains and procollagen can be divided into two pathways: basal degradation and enhanced degradation. Basal degradation is characterized by random procollagen degradation located in either smooth endoplasmic reticulum or Golgi complex. Approx. 15% of the total newly synthesized procollagen is degraded by this pathway. The basal degradation does not involve lysosomal proteases and is not inhibited by colchicine. Enhanced degradation or intracellular degradation by lysosomal activity (Fig. 1) is located in the Golgi complex and recognizes and sorts procollagen abnormalities [3]. Enhanced degradation is mediated by lysosomal proteases in lysosomes and can be blocked by colchicine. Up to 80% of the newly synthesized collagen can be very rapidly degraded intracellularly even before the collagen fibrils are externalized [12]. Although the regulation of this degradative pathway is unclear it may prevent secretion of defective molecules or play a role in the regulation of collagen deposition and fine tuning of an orderly and balanced collagen network [1, 3].

Matrix metalloproteinases are metal-binding proteases, secreted as a latent proenzyme, which require extracellular activation. All MMPs have a Zn^{2+} binding site (HEXGH) as in other zinc-containing proteases. Furthermore the MMPs require Ca^{2+} for stability and exhibit a preferred cleavage specificity for the N-terminal side of hydrophobic residues [8, 9, 13]. Activation of the latent proenzyme (zymogen) occurs by cleavage of a region of approx. 10–80 amino acids from the N terminus. The exact mechanism for activation of latent MMPs is not completely understood. MMP-1 and stromelysin can be activated in vivo by plasmin or urokinase-type plasminogen activator (uPA) [9, 13]. Stromelysin (MMP-3) and other peptidases are also able to activate latent collagenase. Mercurial compounds, plasmin and trypsin can be used in vitro to activate the latent proenzyme [9, 13]. In the activation process a relatively inaccessible sulfhydryl group is involved. The cysteine residue to which the sulfhydryl group is coupled is modified, exposed or released by proteolysis and replaced by water. This results in concomitant exposure of the active site, followed by an autolytic cleavage of the cysteine-containing fragment [9].

Collagenases present in an inactive form can be either inactive proenzymes or an inactivated complex of active collagenase with its inhibitor (tissue inhibitor of matrix metalloproteinases) which cannot be dissociated by gel-filtration or ion-exchange chromatography. Whether in the latter case MMP can still be dissociated from its inhibitor and retain its activated state again is still unknown.

Just recently Sato et al. [14] described the membrane-type matrix metalloproteinase (MT-MMP), which is a cell surface-associated MMP. This MT-MMP has a potential transmembrane (TM) domain at the C terminus which is expressed on the surface of invasive tumour cells. Expression of MT-MMP resulted in specific activation of 72 kDa progelatinase A (MMP-2). Whether this or similar mem-

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**Fig. 1.** Collagen turnover pathways. Schematic representation of interstitial collagen synthesis and degradation pathways. MMP-1 = interstitial collagenase; MMP-2 = 72 kDa gelatinase; MMP-8 = neutrophil collagenase; MMP-9 = 92 kDa gelatinase; MMP-3 = stromelysin 1. (A) Lysosomal intracellular degradation of newly synthesized procollagen fibres. (B) Non-lysosomal intracellular degradation of newly synthesized procollagen fibres. (C) Intracellular degradation of phagocytosed mature collagen fragments.
brane-type MMPs occur in the myocardium is still under investigation.

Most of the matrix metalloproteinases can be inhibited by a member of the family of tissue inhibitors of metalloproteinases (TIMP-1, TIMP-2 and TIMP-3) [8]. TIMPs bind to the active site of the metalloproteinases by blocking access to substrate. This is a very tight non-covalent binding with a very high $K_d$ ($10^{-9}$–$10^{-10}$). TIMP binds to metalloproteinases in a 1:1 molar ratio [8]. TIMP-1 is able to bind less tightly to latent proMMP-9 and TIMP-2 to proMMP-2, suggesting another possibility to regulate MMP activity.

Therefore, small concentration changes of either components can result in marked changes in collagenolytic activity. TIMP-1 forms a complex preferentially with MMP-9 (92 kDa type IV procollagenase), whereas TIMP-2 selectively complexes MMP-2 (72 kDa type IV procollagenase). TIMP-3, also called ChIMP-3, was first described in chicken and localized in the "insoluble" extracellular matrix [15]. Mouse TIMP-3 is found in the developing mouse embryo, where it was observed in the myocardium. TIMP-3 has a possible role in the embryonic extracellular matrix remodelling and cardiac development [16]. Whether TIMP-3 is also expressed in adult animals or with cardiovascular diseases is not yet known. $\alpha_2$-Macroglobulin, $\alpha_1$-macroglobulin, $\alpha_1$-inhibitor-3 and other serum components can act as potent inhibitors of collagenase [9]. These macroglobulins serve as substrate for collagenase. After cleavage these macroglobulins bind to collagenase and inhibit its matrix degrading capacity [9,15].

Increased collagenolysis is due to activation of the latent pool of collagenases stored in the extracellular compartment. Collagenase activation has been demonstrated in the remodelling process found during wound healing in various tissues. Furthermore, collagenolytic activity can be increased by a decrease in the concentration of inhibitor. Decreased TIMP levels were found in aortic aneurysms suggesting increased collagenolytic activity was due to an imbalance in the collagenase inhibitor levels resulting in extracellular matrix degradation and subsequent aneurysms [17]. Decreased TIMP can lead to an increase in collagenase activity in metastatic cells. This enables tumour cells to penetrate their extracellular matrix rich environment and migrate to neighbouring tissue [18]. Khokha et al. [19] demonstrated that an upregulation of TIMP suppresses metastatic capacity of cells. A fine tuned balance between collagenase and its inhibitors is necessary for normal tissue remodelling and function [1,8]. Small changes in this balance can have dramatic effects and form a potential danger for normal tissue function.

Besides the above described mechanisms to regulate collagenolytic activity by activation of latent proenzymes and inhibition of proteolytic activity, gene regulation at the transcriptional level has to be taken into account [20]. Cytokines and growth factors have been shown to induce or stimulate MMP synthesis. 12-O-Tetradecanoylphorbol-13-acetate (TPA), IL-1, TNF-α, PDGF can stimulate, whereas corticosteroids, retinoic acid, heparin, and IL-4 inhibit MMP gene regulation. Not all MMPs react to the same stimuli. TGF-β stimulates MMP-2 and MMP-9 but inhibits MMP-1 and MMP-3 synthesis. Promoter regions of MMPs contain a tumour promoter-responsive element (TRE) which binds the transcription factor AP-1, a c-fos/c-jun heterodimer. The MMP expression is greatly modulated by cytokines and growth factors described above and involves gene products of the Fos and Jun families of oncogenes. A more detailed description of cytokine regulation of MMP gene expression can be found in the reviews of Mauviel [21] and Ries and Petrides [22]. Also TIMP-1 expression can be induced by hormones and cytokines, whereas TIMP-2 expression is largely constitutive, following the pattern of MMP-2 expression. Promoter sequences between TIMP-1 and TIMP-2 are different but consistent with the differential regulation of these TIMP genes, suggesting a molecular mechanism for coregulation of MMPs and TIMPs [20].

3. Collagenolytic activity in the heart

Until now most research concerning MMPs and TIMPs has been conducted in tumour biology, atherosclerosis and restenosis. There are almost no data on the role of MMPs and TIMPs in cardiac research or disease. Montfort and Pérez-Tamayo [23] from Mexico City were the first to demonstrate by immunohistochemistry that collagenase was present in myocardium in the interstitium between cardiomyocytes, round myocyte bundles and in the pericardium. Collagenase was found in the neighbourhood of its substrate, fibrillar collagen. Antibodies generated so far do not discriminate between active or latent collagenase. Moreover, in normal rat heart collagenolytic enzymes were demonstrated to be predominantly present as latent proenzymes [24]. Activation of these latent proenzymes in isolated perfused hearts was demonstrated by disulphide reagents, such as oxidized glutathione, which is also produced in ischaemic hearts [25,26]. Collagenolytic capacity is present in normal myocardium, which can be activated during oxidative stress or other activation pathways such as activation by plasmin and other metalloproteases.

Structural changes in architecture and arrangement of collagen fibrils could be observed by scanning transmission electron microscopy in a porcine model as soon as 40 min after coronary occlusion. The collagen network becomes irregular in arrangement and by 2 h after occlusion the collagen banding pattern disappeared and collagen fibrils, elastic fibres and microfilaments were disrupted, which could be related to cell injury [27]. In a dog model of myocardial stunning, where no ultrastructural signs of cardiomyocyte necrosis occur, the collagen weave surrounding myocytes disappeared completely and collagen
struts became sparse or completely absent, leading to enhanced compliance and less effective contraction of the stunned myocardium [28]. Increased collagenolytic activity was due to activation of the latent pool, whereas total collagenolytic capacity was not changed [29].

In a rat model of coronary ligation, 1, 2 and 3 h after ligation total collagen content was decreased in the infarct zone due to an increased breakdown of insoluble collagen. Also collagenase, neutral proteinase and lysosomal serine protease activities were increased two- to threefold, suggesting a role for these enzymes in collagen degradation after myocardial infarction [30]. In a rat myocardial infarction model a transient increase in collagenolytic activity was found only in the infarcted left ventricle and not in non-infarcted tissue or sham-operated controls [31]. In this study no increase in MMP-1 activity could be determined before day 2 after infarction. Collagenolytic activity (MMP-1) in the infarcted left ventricle was increased from day 2 until day 7 following coronary ligation and declined thereafter. This suggests that MMP-1 is not the enzyme which breaks down the collagenous struts and fibres early after ischaemia. Other collagenolytic enzymes such as serine proteases (e.g. plasmin, cathepsin G) or lysosomal cysteine proteinases (e.g. cathepsin B) could be involved instead. Besides activation of MMP-1, MMP-2 and MMP-9 were also found to be activated post infarction. These gelatinases can degrade the one- and three-quarter fragments of fibrillar collagen generated after cleavage with MMP-1 [8,13], which could account for the concomitant increase of collagenolytic activity of these MMPs. Besides, using gelatins as a substrate these MMPs could also break down basement membrane type IV collagen from necrotic cardiomyocytes. In contrast to the activation of latent collagenases from day 2 onwards, MMP-1 mRNA expression was detected only at day 7 following coronary ligation and only in the infarcted region of the left ventricle [31]. Post-translational activation of stored extracellular latent collagenases seems to play a more important role in the regulation of myocardial collagenase activity following infarction than the synthesis of MMP-1 mRNA. After depletion of the latent pool of collagenase through activation and utilization, MMP-1 mRNA is synthesized to replenish the latent procollagenase pool. The heart may therefore recognize and regulate its own store of collagenase through signals that remain to be elucidated.

The effect of very early changes in collagenase activity after cardiac ischaemia [28–30] are not supported by measurements of whole chamber dynamics. LV chamber stiffness remained within the range of normal values during the early phase (≤2 days after MI) in all rats with infaracts but decreased significantly during the late phase (from day 7 on) in rats with moderate and large infarcts in association with the extent of ventricular enlargement [32]. These findings match very well with the time course of collagenolytic activity after MI described by Cleutjens et al. [31] and suggest a correlation between increased collagenolytic activity and ventricular enlargement and function.

TIMP-1 mRNA expression accompanied the activation of MMP-1, MMP-2 and MMP-9 [31]. Six hours after infarction TIMP-1 mRNA was found to be increased in infarcted tissue reaching a peak on day 2. This peak overlapped the pattern of MMP activation, which began to rise 2 days after infarction. Although this is only circumstantial evidence, these findings suggest a direct regulation of TIMP-1 transcription by activation of latent collagenases, similar to the increase of TIMP-2 expression when MMP-2 activity is increased [33]. TIMP-1 and MMP-1 mRNA synthesizing cells were only found in the infarcted left ventricle in the area surrounding the necrosis. These cells were phenotyped using in situ hybridization and immunohistochemistry on parallel tissue sections. MMP-1 and TIMP-1 mRNA producing cells were not cardiomyocytes, macrophages or endothelial cells but stained with vimentin and some of them with smooth muscle actin, suggesting that these cells are fibroblasts or myofibroblasts [31].

A net balance in collagen degradation is therefore established by the equilibrium that exists between collagenase production and activation on the one hand, and TIMP production on the other. Following infarction, the heart appears to regulate this balance and therefore the extent of collagen degradation.

The result of increased loss of collagenous struts and tethers due to activation of collagenases can be cell slippage, cellular elongation (eccentric hypertrophy), and cell dropout (apoptosis). These changes can lead to loss of structural support, distortion of tissue architecture, reduction in stiffness, wall thinning, dilatation and even rupture of the myocardium. Although many authors speculate about the role of collagen degradation in the onset of cardiac dilatation, inefficient left ventricular performance and congestive heart failure, there are no studies yet to prove this hypothesis. These events are mostly secondary to myocardial infarction. Activation of MMPs could also be the cause of acute coronary ischaemia. This process is usually characterized by rupture of an atherosclerotic plaque, which leads to intracoronary thrombosis and subsequent myocyte necrosis. Brown et al. [34] described that active synthesis of 92 kDa gelatinase, observed by intracellular immunostaining of this enzyme, by macrophages and smooth muscle cells in atherosclerotic lesions could play a role in the development of acute coronary ischaemia and was associated with unstable angina.

The detrimental effect of MMPs could be circumvented by controlling collagen degradation. There are two major pathways that will lead to decreased collagen degradation. The first is to inhibit MMP synthesis and activation and the second pathway is to inhibit the already activated MMPs. Metal chelators (EDTA, 1,10-phenanthroline) or compounds with reactive sulphhydryl groups (cysteine, diithiothreitol and D-penicillamine) can reduce the col-
lagenolytic activity directly. Inhibitors of metalloproteinases such as isolated or recombinant TIMP or antibiotics (tetracyclines, minocycline) [35] can be used to regulate collagenolytic activity by inhibiting activated collagenases and/or forming a complex with proteinases. Just recently the first clinical trials using synthetic MMP inhibitors (such as Galardin and Batimastat) have been completed [35]. Although these studies are conducted in treatment of corneal ulcers and inhibition of pleural effusion in lung cancer, it is feasible that such treatment can be used in the future for other clinical applications, such as cardiovascular diseases. More research is needed to determine the specificity, safety, toxicity, bioavailability and efficacy of these drugs.

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References

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