Chapter 18
Implications of Intracellular Proteolytic Activation of MMP-2 in the Heart

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Abstract Matrix metalloproteinases (MMPs) are a family of metalloproteases comprised of 25 related members, of which 24 are found in mammals. By cleaving their target proteins, MMPs play regulatory roles in signaling events, control the cellular environment, and modulate many bioactive molecules at the cell surface to influence cell behavior. However, MMPs are also localized inside the cell and can cleave intracellular substrates. In the heart, MMP-2 is widely expressed in nearly all cells and plays important roles in a variety of physiological and pathological processes, ranging from heart development to ischemia–reperfusion (I/R) injury that triggers an acute loss in heart contractile function. MMP-2 is abundantly expressed in cardiac myocytes and is directly activated by oxidative stress. This results in the S-glutathiolation of a critical cysteine in the prodomain which removes its coordination to the catalytic zinc and allows access of substrates to its catalytic domain, resulting in the proteolysis of specific sarcomeric and cytoskeletal intracellular proteins.

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MMP-2 activity is also regulated by its phosphorylation. Intracellular substrates of MMP-2 include troponin I, titin, myosin light chain 1, α-actinin, and glycogen synthase kinase-3β. The hydrolysis of specific sarcomeric and cytoskeletal proteins by MMP-2 contributes to contractile dysfunction after I/R injury pointing towards inhibition of MMP-2 as a possible therapy for the treatment of heart diseases associated with enhanced oxidative stress.

Keywords Matrix metalloproteinase-2 • Intracellular proteolysis • Oxidative stress • Ischemia–reperfusion • Doxycycline

1 Introduction

Proteolytic enzymes, proteases, proteinases, or peptidases are synonymous terms for enzymes that cleave other proteins or peptides. They are currently classified into seven classes according to MEROPS database: serine-, cysteine-, aspartic-, metallo-, threonine-, glutamic-peptidases, and asparagine peptide lyases [1].

The proteases are found in all organisms from viruses to humans, comprise more than 2 % of the human genome, and occur inside or outside of the cell. They control multiple biological processes including the cell cycle, autophagy, signaling, wound healing, inflammation, food digestion, protein and organelle turnover, immune response, infectious diseases, aging, blood pressure, cancer, and degenerative diseases among others [2, 3]. All these characteristics make proteolytic enzymes interesting targets for the development of prognostic biomarkers and drugs to be used in the treatment of many diseases. Some successful medications involving proteases include the captopril-like drugs, inhibitors of angiotensin-converting enzyme which are used in the treatment of hypertension and heart failure; the HIV-1 protease inhibitors present in anti-HIV cocktails; and human kallikrein-3, more popularly known as prostate-specific antigen, used as a diagnostic test for prostate cancer [4].

In the heart, proteases have been reported to participate in numerous processes either in physiological or pathological conditions [5–7]. We describe along this chapter the features of an important group of proteases in the heart, focusing on matrix metalloproteinases (MMPs), especially MMP-2.

2 Matrix Metalloproteinases

A family of structurally related zinc metalloproteases called matrix metalloproteinases (MMPs) is found in all vertebrates. The first report of MMP activity came in 1962 by Gross and Lapiere. They described a collagen-degrading activity in the culture medium of a tadpole undergoing morphogenesis which accounted for the hydrolysis of collagen in the tail as it is resorbed [8].

MMPs therefore play important roles in many physiological processes such as embryonic implantation, development, immune functions, and tissue remodeling but are also involved in pathological conditions including multiple aspects of cancer initiation and progression, inflammation, autoimmune diseases, osteoarthritis, vascular diseases, and neurodegenerative disorders [9–15]. Although MMPs have long been recognized for their ability to catalyze the hydrolysis of extracellular matrix (ECM) proteins, more recently attention has focused on MMPs activity to proteolyze non-extracellular matrix substrates both outside and inside cells [16–18].

There are currently 23 different endopeptidases identified as human MMPs [19]. There are some variations in their structure, but in general, MMPs are multidomain enzymes possessing several common structural characteristics. They have an N-terminal signal sequence which allows for subcellular targeting and extracellular export of the enzyme. However, the signal sequence of MMP-2 was recently found to be very inefficient in targeting the nascent protein to the endoplasmic reticulum for the secretory pathway, resulting in almost 50 % of MMP-2 in the cytosol. A splice variant of MMP-2 lacking the signal sequence was also found; therefore there are at least two intracellular MMP-2 moieties [20].

MMPs are synthesized in an inactive zymogen form with an autoinhibitory propeptide domain. The propeptide domain shields the neighboring Zn^{2+}-containing catalytic domain. The propeptide domain also contains a highly conserved PRCGVPD sequence, which is believed to assist in the binding of the cysteine thiol to Zn^{2+} in the catalytic domain, preventing access of substrate into the catalytic site. The PRCGVPD sequence thus plays an important role in the regulation of MMP activity [21, 22].

MMPs can be classified into five subgroups based on their primary structure, substrate specificity, and subcellular localization as collagenases (MMP-1, MMP-8, and MMP-13), gelatinases (MMP-2 and MMP-9), stromelysins (MMP-3, MMP-10, MMP-11, and MMP-12), matrilysins (MMP-7 and MMP-26), and membrane-type (MT)-MMPs (MT1-MMP through MT6-MMP) [23]. This original system of MMPs classification is now questioned due to the ability of several MMPs to biologically hydrolyze and target various non-matrix substrates both outside and inside the cell [17].

The activities of MMPs are regulated by diverse mechanisms at every step from their induction to their inhibition and degradation [9]. These include (a) gene transcription and translation; (b) posttranslational modifications such as glutathiolation and phosphorylation; (c) interaction with tissue inhibitors of metalloproteinases (TIMPs 1–4), which are their endogenous inhibitors; and (d) by compartmentalization (their intra-/extracellular localization) [24, 25].

2.1 MMPs in the Heart

Information about the role of MMPs in cardiac physiology is still scanty. In normal hearts, MMPs are present predominantly in their zymogen form (proMMPs) and are

often co-expressed in complex with their endogenous inhibitors, the tissue inhibitors of metalloproteinases (TIMPs) [26]. The presence of MMPs in the heart has been implicated in early heart development such as the role of MMP-2 in heart tube formation [27]. MMP-2 has also been shown to play important roles in angiogenesis [28] and heart valve development [29].

Although under pathological conditions the levels of MMPs 1, 2, 3, 7, 8, 9, 12, 13, and 14 have been reported to respond to cardiac tissue repair stimuli after chronic permanent coronary artery occlusion in humans and animal models, MMP-2 and MMP-9 are the most highly studied MMPs reported in heart and cardiovascular diseases including atherosclerosis, hypertension, heart failure, and ischemic heart diseases [30]. MMP-2 mRNA [29] and protein [31] are abundantly expressed in heart tissue [32], whereas MMP-9 is normally associated with activated inflammatory cells such as leukocytes and macrophages and is not present in cardiac myocytes, aortae, or vena cava from healthy humans and rats [33].

3 MMP-2 in the Heart

In the heart, MMP-2 is ubiquitously expressed in normal cardiac myocytes [31, 34] and fibroblasts [35], which implies that it has roles in normal heart physiology and which underlies several reports of its involvement in cardiac pathologies.

MMP-2 like other MMPs is synthesized as an inactive zymogen of 72 kDa. The autoinhibitory, hydrophobic propeptide domain PRCGVPD that shields the catalytic site of the enzyme is present at its N-terminus. The catalytic site contains a Zn^{2+} ion that is essential for its activity. Activation of MMP-2 can occur in the pericellular and extracellular compartments. The proteolytic cleavage of the propeptide domain present in 72 kDa MMP-2 occurs by MMP-14 in a multistep pathway that also involves tissue inhibitor of metalloproteinase 2 (TIMP)-2, resulting in enzymatically active 64 kDa MMP-2 in the extracellular compartment [36].

However, it is not necessary for 72 kDa MMP-2 to lose its propeptide domain in order to become an active protease. MMP-2 can also be activated upon exposure to reactive oxygen/nitrogen species such as peroxynitrite ($ONOO^-$), an important mediator of oxidative stress injury inside the cell via nonproteolytic modulation of the 72 kDa form via the S-glutathiolation of the cysteine residue in its inhibitory propeptide domain involved in its activation [37]. The cysteine moiety in the PRCGVPD propeptide sequence was found to be S-glutathiolated by low concentrations of $ONOO^-$ (1–10 μM), a modification that changes the conformation of the propeptide allowing contact of the substrate to the catalytic Zn^{2+} domain, thus resulting in its activation [37] (Fig. 18.1). On the other hand, it was also observed that higher concentrations of $ONOO^-$ (30–100 μM) led to MMP-2 deactivation [37, 38].

Phosphorylation is another significant regulator of MMP-2 activity. MMP-2 as expressed in the human fibrosarcoma (HT-1080) cell line is phosphorylated on S32, S160, S365, T250, Y271, and likely also on several other residues.

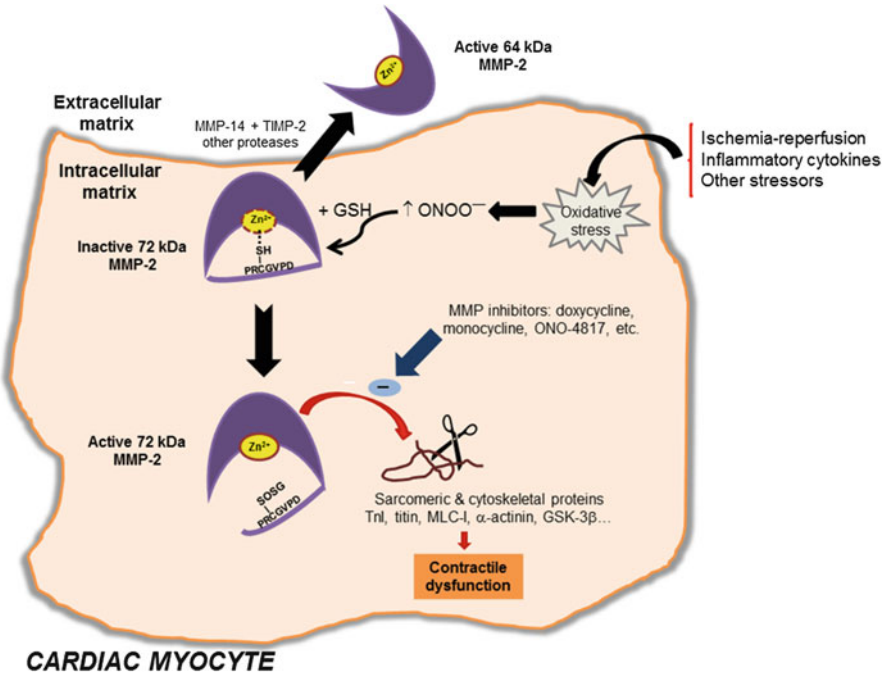

Fig. 18.1 Paradigm of MMP-2 in the cardiac myocyte. 72 kDa MMP-2 can be activated extracellularly by MMP-14 in the presence of TIMP-2 (or by other proteases) by the cleavage of its propeptide domain, yielding an active 64 kDa MMP-2 which acts on the extracellular matrix. I/R injury, proinflammatory cytokines, or other factors can cause an increased biosynthesis of peroxynitrite ($ONOO^-$) inside the cell that in the presence of cellular glutathione can result in the nonproteolytic activation of 72 kDa MMP-2 (without losing the propeptide domain). This activated 72 kDa MMP-2 can act intracellularly to hydrolyze susceptible sarcomeric and cytoskeletal proteins, causing myocardial contractile dysfunction. MMP-2 can be inhibited by sub-antimicrobial dosage of doxycycline, which improves contractile function

Phosphorylation of MMP-2 greatly reduces its enzymatic activity [39]. In silico analysis of the MMP-2 protein sequence shows that several kinases, including protein kinase C, protein kinase A, and glycogen synthase kinase-3, are potentially able to phosphorylate MMP-2 and consequently modulate its activity. However, the protein kinases and phosphatases responsible for MMP-2 phosphorylation in vivo are still unknown. In isolated rat hearts treated with okadaic acid at a low concentration that selectively inhibited protein phosphatase 2A but not protein phosphatase 1 activity, myocardial MMP-2 was kept in a more phosphorylated and less active state, which may account in part for the cardioprotective action observed with this inhibitor in hearts subjected to ischemia–reperfusion (I/R) injury [39]. More information about the phosphorylated and dephosphorylated forms of MMP-2 in the heart is needed; however, these data indicate that various posttranslational modifications in the cytosol can act to regulate intracellular MMP-2 as previously suggested [17, 40].

3.1 Extracellular MMP-2 in the Heart

The extracellular matrix actions of MMP-2 in the heart have been implicated to be crucial in embryonic heart development including angiogenesis, valve development, and heart tube formation [27–29]. In the latter, there seems to be a significant role of MMP-2 as demonstrated in chick embryos, where an MMP-2-neutralizing antibody or a selective MMP inhibitor were shown to inhibit MMP-2 activity and produce severe heart defects, including cardia bifida, abnormal left–right patterning, and a disruption in the looping direction, suggesting a key role of MMP-2 in cell migration and remodeling required for normal heart development [27]. Moreover, MMP-2 knockout mice survive at birth and are viable as adults; however, they display significantly retarded growth in comparison to the wild-type controls [41].

MMP-2 has also been implicated in various heart pathologies including postinfarct extracellular matrix remodeling [42]. In the myocardium of spontaneously hypertensive heart failure rats, there are reports of an increase in MMP-2 activity with age and cardiac hypertrophy [42, 43]. Increase of MMP-2 activity is also reported in parasitic myopathies caused by *Trypanosoma cruzi* (Chagasic cardiomyopathy) and coxsackievirus B3 (coxsackievirus B3 myocarditis) [44, 45].

3.2 Intracellular MMP-2 in the Heart

Ischemic heart disease is the most common cause of death in most western countries. There are many mechanisms that mediate I/R injury including an imbalance in energy substrate preference for ATP production and dysregulation of Na^+/H^+ as well as Na^+/Ca^{2+} exchangers [46]. Moreover, there is accumulation of intracellular Ca^{2+} due to impairment of sarcoplasmic reticulum Ca^{2+} uptake leading to activation of Ca^{2+}-dependent proteases and phospholipases [47, 48]. The sudden reintroduction of molecular oxygen during reperfusion reenergizes mitochondria and reactivates the electron transport chain, causing a rapid and large increase in the biosynthesis of reactive oxygen/nitrogen species including $ONOO^-$, the reaction product of nitric oxide and superoxide which activates MMP-2 [37, 49] (Fig. 18.1). Studies of the pathogenesis of myocardial I/R injury revealed a structural and functional remodeling of intracellular matrix components in the cardiac myocyte [50]. MMP-2 activation by $ONOO^-$ in particular has been shown to play an important role in pathogenic processes of the very early steps of myocardial I/R injury, also known as myocardial stunning [29] when myocardial muscle is still reversibly injured.

Besides the well-known activity of MMP-2 in extracellular matrix remodeling, studies from our group have shown that MMP-2 is localized to specific compartments inside the cell and also has very important intracellular roles [17]. MMP-2 is activated inside cardiac myocytes in injuries associated with increased oxidative stress including I/R injury [31] or exposure to proinflammatory cytokines [51], thus

cleaving susceptible intracellular proteins that are essential for myocyte contractile function, such as troponin I [34, 51], myosin light chain-1 [52], α-actinin [53], GSK-3β [54], and titin [55] (Fig. 18.1).

The cleavage of specific intracellular substrates by MMP-2, as a result of oxidative stress injury in the heart results in acute myocardial contractile dysfunction, demonstrates the importance of an intracellular fraction of MMP-2 that is not secreted from the cardiomyocyte. This results from two events: (1) a splice variant of MMP-2 lacking the signal sequence is expressed in both neonatal and adult human cardiomyocytes making it present exclusively in the cytosol, and (2) the signal sequence of canonical MMP-2 is inefficient in its ability to target newly synthesized MMP-2 to the endoplasmic reticulum and thereby does not restrict it to only the secretory pathway, resulting in a considerable portion of canonical MMP-2 which resides in the cytosol [17, 20, 40, 50].

In fact, there is evidence of diverse forms of intracellular MMPs. Lovett et al. showed a novel intracellular, N-terminal-truncated 65 kDa MMP-2 isoform which clearly distinguishes it from the 64 and 72 kDa MMP-2 forms. The 65 kDa MMP-2 isoform is not found under basal conditions. Redox stress induced by hypoxia or I/R injury activates a latent promoter in the first intron of the MMP-2 gene, generating a truncated mRNA transcript encoding the N-terminal-truncated 65 kDa MMP-2 isoform. This isoform lacks the secretory signal sequence and inhibitory prodomain and is then present in the cytosol and mitochondria as an active enzyme. The 65 kDa MMP-2 isoform hydrolyzes inhibitory IkB-α, thereby activating NFkβ/NFAT mitochondrial-nuclear stress signaling, inducing of an innate immunity transcriptome [56]. Other intracellular MMPs have been reported in different tissues: MMP-26 in breast carcinomas [57], MMP-10 in brain striatal cells [58], and MMP-3 in human liver [59]; however, whether more MMPs are also localized inside the cardiomyocyte, as MMP-2, is unknown.

3.3 Intracellular Targets of MMP-2 in the Heart

Troponin I (TnI)

TnI was the first intracellular MMP-2 substrate identified among the family of MMPs which shows that these proteases may have many yet undiscovered biological actions. TnI is a regulatory protein of actin–myosin interaction present in the thin myofilaments, known to be rapidly proteolyzed during acute I/R injury [34]. Our group showed using immunogold electron microscopy that MMP-2 was localized to the sarcomere in cardiac myocytes and confocal microscopy, and immunoprecipitation experiments revealed that MMP-2 also colocalized with TnI. TnI was highly susceptible to proteolysis by MMP-2 in vitro, and loss of TnI during I/R injury in isolated rat hearts was attenuated by MMP inhibitors, indicating that myocardial stunning injury is caused in part by MMP-2-mediated proteolysis of TnI.

Myosin Light Chain 1

The discovery of the localization of MMP-2 to the sarcomere led us to look for other intracellular MMP-2 substrates in hearts subjected to I/R injury. By using a pharmacoproteomics approach, myosin light chain 1 was identified as an intracellular MMP-2 target [52]. Myosin light chain 1 was reported to undergo proteolytic degradation in hearts subjected to I/R injury [60]. MMP-2 activity was found in preparations of thick myofilaments (which contain myosin light chain 1) prepared from rat hearts; immunogold microscopy localized MMP-2 to the sarcomere in a pattern consistent with the known distribution of myosin light chain 1, and purified myosin light chain 1 was susceptible to proteolysis by MMP-2 in vitro with a cleavage site near the C-terminus identified by mass spectrometric analysis [52].

Titin

Titin is another sarcomeric protein to which MMP-2 is colocalized. It is the largest known mammalian protein (3,000–4,000 kDa) and is found in both cardiac and skeletal striated muscles. It forms an intrasarcomeric elastic filament spanning nearly half the length of the sarcomere, from its N-terminus anchored at the Z-disc to its C-terminus in the M-line region. Titin also has elastic segments in the I band region that allow it to serve as a molecular spring. The titin molecule is the framework for the organized assembly of other myofilament proteins, thus helping to maintain the structural and functional stability of the myocyte [61]. Titin is also the molecular superstructure on which sarcomeric proteins are assembled during sarcomerogenesis in embryonic myocytes. In cardiac muscle, titin is of vast importance since it is a determinant of both diastolic and systolic function and the Frank–Starling mechanism of the heart [62]. We found that MMP-2 localizes to the Z-disc region of titin. Cleavage of titin in perfused rat hearts subjected to I/R injury, or in skinned cardiomyocytes incubated with MMP-2, was prevented with MMP inhibitors *o*-phenanthroline or ONO-4817. Hearts from MMP-2 knockout mice subjected to I/R in vivo did not show loss of titin content [55]. These data indicate that MMP-2 plays an important role in titin homeostasis, which directly affects the contractile function of the heart at the sarcomeric level.

Cytoskeletal Targets

Myocardial stunning injury in isolated guinea pig hearts is accompanied by the degradation of the cytoskeletal proteins desmin, spectrin, and α-actinin, although the protease(s) responsible was not identified [63]. α-Actinin is a cytoskeletal protein found at the Z line of the sarcomere. It connects actin filaments in adjacent sarcomeres and thus serves as a pivotal protein in transmitting the force generated

by the actin–myosin complex. Our group found that α-actinin and desmin (but not spectrin) are susceptible to cleavage by MMP-2 in vitro. Infusion of $ONOO^-$ into isolated, perfused rat hearts caused activation of MMP-2 with concomitant loss of myocardial α-actinin content, which was preventable by a selective MMP inhibitor, PD-166793 [53].

Nuclear Targets

The nuclear matrix has similarities with that of the extracellular matrix with a proteinacious structure that resembles the extracellular matrix in that it imparts structure and organization, as well as provides support for various processes [64], although the exact composition of the nuclear matrix is unknown. Proteolysis of nuclear matrix proteins, such as poly-ADP-ribose polymerase-1 (PARP-1) and X-ray cross-complementary factor 1 (XRCC1), is involved in important cellular processes such as apoptosis and regulation of the cell cycle [65, 66]. Kwan et al. discovered that MMP-2 activity and protein were found in nuclear extracts from human hearts where it co-immunoprecipitates with PARP-1, a chromatin-associated enzyme with multiple functions [67]. MMP-2 was able to efficiently cleave PARP-1 in vitro, and this may be a further means to regulate PARP-1 activity. Indeed, PARP-1 is only one of several putative nuclear targets of MMP-2 identified by unbiased, high-throughput degradomic approaches [18]. Thus MMP-2 is very likely to have several yet undiscovered biological functions in the nucleus.

Other Targets

MMP-2 is also suggested to be involved in myocardial apoptosis. Kandasamy et al. showed that glycogen synthase kinase beta (GSK-3β) is a target of MMP-2. GSK-3β is a serine/threonine kinase abundantly expressed in eukaryotes and is important in regulating glycogen metabolism and processes such as the cell cycle, apoptosis, and cell polarity [68]. GSK-3β is susceptible to proteolysis during oxidative stress and is therefore dysregulated by its increased kinase activity. Incubation assays of MMP-2 with GSK-3β resulted in the time- and concentration-dependent cleavage of GSK-3β, showing that GSK-3β may be a target of MMP-2 and that MMP-2 mediates its activity through cleaving the N-terminal of GSK-3β which contains the autoinhibitory phospho-serine 9 residue. The activity of GSK-3β was significantly enhanced upon incubation with MMP-2 and was prevented by MMP inhibitors GM-6001 or ONO-4817. H_2O_2 stimulated GSK-3β activity in cardiomyoblasts, and this was prevented with MMP inhibitors [54]. This may suggest that cleavage and activation of GSK-3β may be an additional means of the contribution of MMP-2 to oxidative stress-induced cardiac dysfunction. Inhibition of GSK-3β activity can also be cardioprotective by reducing apoptosis in the ischemic heart [54].

3.4 Intracellular MMP-2 vs. Calpain

Besides MMP-2, other proteases have been described to have intracellular roles in the heart, especially the calpain family of enzymes. Calpains are cysteine proteases activated by intracellular Ca^{2+}. Since Ca^{2+} overload is a significant determinant of cardiovascular malfunction, calpains are thought to contribute to heart disease. Calpains participate in a variety of cellular processes including cytoskeletal and sarcomeric remodeling, signal transduction, and cell death. Enhanced calpain activity by increased intracellular Ca^{2+} concentration following loss of Ca^{2+} homeostasis results in tissue damage as seen in myocardial infarct, stroke, and muscular dystrophy [69]. Increase in the intracellular concentration of Ca^{2+} can activate calpains which in turn may hydrolyze troponin I, α-fodrin, and ryanodine receptors and impair L-type Ca^{2+}-channel function [69, 70]. Two of the three ubiquitous forms of calpain are expressed in heart tissue: μ-calpain (calpain-1) requires micromolar Ca^{2+}, and m-calpain (calpain-2) requires millimolar Ca^{2+} for activation. Whether these are found directly in cardiac myocytes is unclear. Both calpains are heterodimeric proteins composed of a large 78–80 kDa subunit and a 29 kDa regulatory unit [71].

Calpain-1 has been implicated in the pathogenesis of myocardial stunning injury. Oxidative stress generated during I/R injury may result in the development of intracellular Ca^{2+} overload and activation of calpain activity in the heart [72]. Oxidative stress can also activate intracellular MMP-2 activity directly in the myocardium. Although the exact role of calpain in acute myocardial I/R is controversial [73], there are evidences of similar target proteins of MMP-2 and calpain such as troponin I, or calpain has been incorrectly identified as the protease responsible for some intracellular proteolytic activities. This can be inferred by the fact that calpain proteolysis of substrates in cardiac cells rests on the use of calpain inhibitors such as calpain inhibitor III (MDL-28170), ALLN, and PD-150606, which were found to also inhibit MMP-2 activity in vitro at commonly employed concentrations [74]. Thus, it would be interesting to investigate the suggested calpain substrates in the myocardium for their susceptibility to cleavage by MMP-2, and much more work is needed on the localization of calpains with their putative substrates.

4 MMP Inhibitors: Evolution and Clinical Application

The involvement of various MMPs in diverse human pathologies has attracted the attention of the pharmaceutical industry for the development of MMP inhibitors. About 60 MMP inhibitors have been pursued as clinical candidates since the first drug discovery program targeting this enzyme family began in the late 1970s. Major targeted indications included various cardiovascular diseases, cancer, arthritis, and chronic obstructive pulmonary disease [75]. Most pharmacological inhibitors of MMPs act by chelating the zinc ion in the enzyme's catalytic site. Such MMP inhibitors include batimastat, marimastat, GM-6001 (ilomastat/gelardin), o-phenanthroline, PD-166793, and ONO-4817. Although these compounds selectively inhibit MMP activity in comparison to other protease classes, they do not preferentially inhibit a

specific MMP [24]. Clinical trials in cancer with early MMPs inhibitors were unsuccessful due to the appearance of unanticipated side effects. However, these trials failed for several reasons including (1) limited target knowledge (the exact MMP to be inhibited was unknown during the development of the drugs), (2) lack of knowledge of intracellular MMP activity which has markedly broadened the biological roles of these proteases, (3) lack of understanding that MMPs can be activated by oxidative stress without requiring proteolytic removal of the propeptide domain, and (4) lack of understanding of MMP phosphorylation and whether drugs could be designed to target phosphorylated versus non-phosphorylated forms.

The role of MMPs has been since greatly expanded especially as a crucial pathological determinant in cardiovascular diseases associated with enhanced oxidative stress. The evidence that MMP-2 and other MMPs can be activated by prooxidant stress in the form of $ONOO^-$ suggests that they mediate some of the earliest detrimental actions of oxidative stress to the heart, particularly through the proteolysis of sarcomeric and cytoskeletal proteins [50]. Since there are currently no safe and therapeutically proven $ONOO^-$ blockers/scavengers, the ability to block the consequences of increased $ONOO^-$ stress using MMP inhibitors is a promising and novel alternative to treat cardiovascular diseases for which oxidative stress is a key component of the pathogenesis.

Tetracyclines are a group of broad-spectrum antibiotics commonly used to treat a variety of bacterial infections. Some members of the tetracycline class (doxycycline in particular) have been shown to have additional pharmacological actions, independent of their antibacterial effects, especially in their ability to inhibit MMP enzymatic activity, which for doxycycline occurs at sub-antimicrobial plasma concentration [76]. Of the tetracyclines, doxycycline, followed by minocycline, are the most potent MMP inhibitors. Doxycycline (in a sub-antimicrobial dose formulation) is the first and only clinically approved MMP inhibitor (by the US Food and Drug Administration and Health Canada) for the therapeutic treatment of periodontitis and rosacea.

An epidemiological study from the United Kingdom showed the possible clinical utility of tetracycline use for cardiovascular disease as it revealed a statistically significant reduction in the risk of first-time acute myocardial infarct seen only in patients who had taken tetracycline class, but not any other antibiotics of several different classes, comparing 3,315 such patients vs. 13,139 control subjects [77]. Thus, the cardiovascular benefits associated with tetracycline-class antibiotics may be connected to the inhibition of pathological MMP activity and suggest a possible therapeutic use. Furthermore, doxycycline was also able to prevent cardiac mechanical dysfunction triggered by endotoxic shock [78] and streptozotocin-induced diabetic cardiomyopathy in rats [79], models of heart failure known to involve increased biosynthesis of $ONOO^-$ [80]. Therefore, doxycycline is an attractive and possible alternative for the treatment of cardiovascular disease given its proven MMP inhibitor profile at sub-antimicrobial doses, low toxicity, excellent safety profile, and low cost. Although doxycycline is a possible candidate for therapy as an MMP inhibitor, the development of a drug which selectively blocks 72 kDa intracellular MMP-2 activated by $ONOO^-$ may become a next generation therapy for the treatment of many cardiovascular and other diseases.

5 Conclusions

Although originally thought to exclusively cleave proteins of the extracellular matrix and contribute to adaptive and maladaptive cardiovascular remodeling, MMP-2 is now also recognized as an important intracellular protease, and an increasing number of novel MMP-2 intracellular substrates and functions for it are being discovered. MMP-2 can be considered as an integral sarcomeric protein with roles in the proteolysis of susceptible sarcomeric and cytoskeletal proteins during injury to thus acutely diminish cardiac contractile function. Evidence suggests that MMP-2 may have biological actions in other subcellular compartments including caveolae, nuclei, and mitochondria. Although the role of MMP-2 in early myocardial I/R injury is becoming clear, more studies are needed to elucidate its role in myocardial cell death pathways and other cellular functions, both in physiological and pathological conditions. Finally, the development of specific intracellular MMP-2 inhibitors should be a possible therapeutic strategy to prevent or treat oxidative stress injury of the heart and other organs.

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