Markers of post myocardial infarction remodeling

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1. Introduction

In spite of improved and intensified post- myocardial infarction (MI) therapy (angiotensin-converting enzyme inhibitors, b-blockers, statins, acetylsalicylic acid, angiotensin receptor blockers, clopidogrel, and aldosterone antagonists) and the demonstrated benefits of reperfusion strategy (lysis or intervention), adverse left ventricular (LV) remodeling that progresses to dysfunction remains a significant complication following MI [1]. Furthermore, post-MI survivors who develop heart failure on therapy have a 10-fold greater risk of dying [1]. Therefore, the search for circulating markers of adverse LV remodeling after MI is of crucial importance, as a simple plasma or serum marker that can identify those at risk of adverse remodeling would benefit patients in need of intensive therapy, allow triage and reduce costs [1].

2. Pathophysiology of post MI left ventricular remodeling

Immediately after coronary artery occlusion, irreversible cell necrosis may occur within minutes. Factors affecting the amount of necrosis include the patency of the infarct-related artery, the presence or absence of a preconditioning stimulus, and the amount of the collateral blood flow [2]. Myocardial infarction is followed by a complex and interrelated sequence of events termed post-infarction left ventricular remodeling which describes the compensatory responses of the cardiovascular

system when faced with an acute loss of myocardial contractile function [2,3]. Myocyte necrosis and the resultant increase in load trigger a cascade of biochemical inter- and intra-cellular signaling processes that initiates and subsequently modulates reparative changes which include dilatation, hypertrophy, and the formation of discrete collagen scar (Table 1) [4]. Size, location and trasmurality of the infracted tissue are also major determinants of post-MI left ventricular remodeling [4].

Table 1. Components of post-infarction ventricular remodeling

Infarct expansion
Neurohormonal activation
Myocardial hypertrophy
Global ventricular dilatation
Scar formation

Adapted from Yousef ZR, Redwood SR, Marber MS. Post-infarction left ventricular remodeling: where are the theories and trials leading us? Heart 2000;83:76-80

Emphasis merits the fact that LV remodeling after MI is a complex, dynamic and most important a time- and space- dependent process that progresses in parallel with healing over weeks or months [4,5]. Post-infarction remodeling has been arbitrarily divided into an early phase (within 72 hours) and a late phase (beyond 72 hours). Furthermore, post-infarction remodeling has been described as two distinct processes, a process affecting the infarct and peri-infarct zone (IZ) and a process affecting the non-infarct zone (NIZ) [4,5]. The early phase involves the expansion of the infarct zone, which may result in early ventricular rupture or aneurysm formation [4]. Late remodeling involves the left ventricle globally and is associated with dilatation, distortion of ventricular shape and mural hypertrophy [4]. The failure to

normalize increased wall stresses results in progressive dilatation, recruitment of border zone myocardium into scar and deterioration of contractile function [4].

3. Cardiac extracellular matrix

The myocardium consists of three integrated components: myocytes, extracellular matrix and the capillary microcirculation. The cardiomyocyte is terminally differentiated and develops tension by shortening [4]. Extracellular matrix (ECM) provides a stress-tolerant, elastic scaffold that couples myocytes and maintains the spatial relations between myofilaments and the capillary microcirculation [4]. The ECM couples adjacent myocytes by intercellular struts that align myofilaments to optimize force development, distribute force evenly to ventricular walls and prevent sarcomeric deformation [4]. Furthermore, this framework preserves myocardial architecture, provides an environment for cells to migrate, grow and differentiate and also stores growth factors, hormones and cytokines thus establishing between cells communication [4].

Cardiac ECM is composed of matrix proteins (mainly collagen and elastin as well as more specialized proteins like fibrillin, fibronectin), membrane proteins, proteoglycans and bioactive signaling molecules [6]. Of the many collagen types, the major fibrillar collagens are types I and III, which constitute the bulk of cardiac ECM [4]. 85% of cardiac collagen is type I, which is associated with thick fibers that confer tensile strength and resistance to stretch and deformation, whereas 10% is type III, which is associated with thin fibers that confer resilience [4]. While the cardiac myocyte occupies the majority of myocardium volume, the cardiac fibroblast is the most abundant cell type in the myocardium [6]. Cardiac fibroblasts are the main

regulators of ECM levels through at least three mechanisms i) by regulating the synthesis and deposition of matrix molecules [6]; ii) by mediating matrix degredation and turnover by production and release matrix degrading enzymes (matrix metalloproteinases – MMPs) and their inhibitors (TIMPs) [6]; and iii) by maintaining mechanical tension on the collagen network [6]. Of interest, specific and distinct ECM changes have been documented in the initiation, progression and maintenance of adverse LV remodeling after myocardial infarction [7].

4. Matrix metalloproteinases and their inhibitors

Matrix metalloproteinases (MMPs) are a family of zinc-dependent proteases that are involved in the turnover of ECM [6]. The MMP family comprising more than 25 individual members can be divided into five classes based on their substrate specificity (Table 2) [6,8,9].

Table 2. Matrix metalloproteinases identified post-myocardial infarction

Class	Number	Size	Producing cell
Collagenases			
Interstitial collagenase	MMP-1	52/57	Fibroblast
Collagenase-2	MMP-8	75	Neutrophils
Collagenase-3	MMP-13	54	Undefined
Gelatinases			
Gelatinase-A	MMP-2	72	Macrophages, fibroblasts and myocytes

Gelatinase-B	MMP-9	92	Neutrophils, macrophages and myocytes
Stromelysins			
Stromelysin-1	MMP-3	52/58	Myocytes
Matrilysin	MMP-7	28	Undefined
Elastases			
Elastin	MMP-12	53	Undefined
Membrane-type			
MT1-MMP	MMP-14	66	Fibroblasts, vascular smooth muscle cells and myocytes

Adapted from Vanhoutte D, Schellings M, Pinto Y, Heymans S. Relevance of matrix metalloproteinases and their inhibitors after myocardial infarction: A temporal and spatial window. Cardiovasc Res 2006;69:604-613

MMPs are capable of degrading all components of ECM and therefore play a key role in ECM metabolism [6,8]. All cell types found in the myocardium either under basal conditions (myocytes, fibroblasts, endothelial cells) or in response to a stimulus i.e. inflammatory, ischemic insult (neutrophils, macrophages) are capable of expressing one or more of MMP species [6,8]. Furthermore, a large reservoir of recruitable MMPs exists within the cardiac matrix, which upon activation can result in a rapid surge of ECM proteolytic activity [6].

The first four groups (Table 2) of MMPs represent the secreted MMPs and are produced as a latent proform. Pro-MMPs bind to specific ECM proteins and remain enzymatic inactive until the propeptide domain is cleaved through a cysteine switch

mechanism [8,10]. Pro-MMPs are activated by serine proteases, trypsin, chymotrypsin, and plasmin [8,10]. In addition, several MMPs are substrate for other pro-MMPs, leading to autocatalytic activation of pro-MMPs [8]. The last group of MMPs (MT-MMPs) is activated once positioned in the cell membrane [8].

MMPs are regulated at multiple levels, including transcription, translation, secretion, and activation [6]. MMP activity is non-specifically blocked in the plasma by a2 macroglobulin and heparin [6]. MMPs are also specifically blocked in the tissue by tissue inhibitors of metalloproteinases (TIMPs) [6]. The TIMP family is low molecular weight proteins currently composed of four members (Table 3).

Table 3. Tissue inhibitors of matrix metalloproteinases

Class	Molecular weight	Expression	Location
TIMP-1	28	Inducible	Secreted
TIMP-2	21	Mostly constitutive	Secreted
TIMP-3	24	Inducible	Matrix associated
TIMP-4	22	Constitutive	Secreted

Adapted from Lindsey ML. MMP induction and inhibition in myocardial infarction Heart Fail Rev 2004;9:7-19

The fourth member (TIMP-4) has high constitutive expression in the myocardium [6]. All TIMPs inhibit all MMPs with different specificities. TIMPs bind to the active site of activated MMPs in a 1:1 molar ratio and form nonconvalent bonds. The binding of a TIMP to an MMP blocks the active site and prevents substrate access [6]. TIMPs can also bind to latent MMPs at the amino terminus to prevent autoactivation [6].

5. Extracellular matrix remodeling after myocardial infarction

Cumulative evidence suggest that the extracellular matrix plays a critical role in both early and late LV remodeling after myocardial infarction [1] and also involves both

the infracted and the non-infarcted myocardium [1]. A unique temporal and spatial profile of MMPs and TIMPs on the remodeling process has been determined through the use of several animal studies as well as clinical studies in humans.

5.1. Animal studies

5.1.1. Early wound healing phase (0-7 days) post MI

MMP-9 expression is present in its active form within 24 hours following myocardial infarction in mouse, and it is mainly expressed by infiltrating neutrophils and macrophages of the infarct area [8]. On day 3, MMP-9 activity further increases in non-infarcted, border and infracted myocardium of mice [8]. Neutrophils are thought to be the predominant source of MMP-9 during this early inflammatory phase [8]. After reaching its peak activity, MMP-9 gradually decreases, whereas MMP-2 activity starts to increase rapidly at day 4, reaching a maximum by 7 days, remaining elevated afterwards [8]. During this phase, activated macrophages, fibroblasts as well as myocytes appear to be the major source of MMP-2 [8]. MMP-3 expression is also up-regulated shortly after MI, however not unitl 48 hours [8]. In rabbits MMP-3 expression reaches its maximum at 4 days post-MI and remains upregulated throughout a 14 days period after the initial ischemic insult [8]. In rats, MMP-1 activity increases on day 3, reaches a peak on day 7 and then declines to its normal levels [8]. Fibroblasts are the major source of MMP-1 [8]. During this early phase, despite their possible beneficial effects in post-MI healing, activation of MMPs may also promote detrimental actions, such as infarct expansion, thinning of the infarct-related myocardium and cardiac rupture [8].

A loss of TIMP-mediated control has been reported in LV remodeling following MI [8]. In rats and mice, although an increase in TIMP-1 mRNA is observed within 3 days following MI, no increase in their protein level is documented [8]. Furthermore, in rabbits protein expression of TIMP-1 is reduced in the infarcted tissue [8]. Like TIMP-1, TIMP-2 protein levels do not change during this early phase [8]. Finally, TIMP-4 protein levels drop substantially during 1 week post-MI where after it restores to its basal levels [8].

All together these studies suggest a shift of the MMP/TIMP balance towards increased proteolytic activity and ECM degredation within the first few days following MI [9].

5.1.2 Granulation and early remodeling phase: 7-21 days post MI

After reaching their peak levels within the first 7 days post-MI both MMP-9 and MMP-2 activity decreases, but still remain significantly elevated between 7-14 days post-MI compared with baseline, both in mouse and rat [8]. Proliferative myofibroblasts may be the source of the increased gelatinase's levels during this time window [8]. Furthermore, MMP-3 and MMP-13 activity remains elevated during this time-window post-MI [8]. Finally, MMP-8 protein levels start to elevate at 2 weeks post-MI and neutrophils seem to be responsible for the aforementioned rise of MMP-8 levels [8]. Following MI in rats TIMP-1 and TIMP-2 mRNA stays markedly elevated from day 14 to day 21 at the site of MI but not in the non-infarcted myocardium [8]. Similarly, TIMP-1 and -2 protein levels increase from 2 weeks following MI and then decrease gradually.

5.1.3. Late remodeling phase: >21 days post MI

In a sheep model of post-MI, MMP-1 and MMP-9 levels are significantly reduced within the border and MI regions at 8 weeks post-MI, whereas MMP-2 levels increases substantially within the border and MI regions [8]. In contrast to the acute MI setting, a different set of MMPs emerges at 8 weeks after MI [8]. MMP-8, -13 and MT1-MMP are increased within border and MI regions while MMP-3 and -7 are reduced [8]. TIMP abundance decreases significantly in the border and MI region 8 weeks post-MI [8].

5.2 Clinical studies

In the clinical setting, increased plasma levels of MMP-9 after MI is a fairly consistent finding [1]. Following reperfusion therapy, serum levels of MMP-1 and TIMP-1 correlated with echocardiographic indices [6]. In specific, MMP-1 levels increased from day 4 to day 14 and then started to decline, while TIMP-1 levels were below control levels for the first day, increased through the second week and then declined [6]. These suggest that while both MMP-1 and TIMP-1 levels fall initially, TIMP-1 is upregulated before MMP-1 [6]. In patients with ischemic etiology heart failure increased levels of MMP-8, MMP-9, TIMP-1 and TIMP-2 have been documented. MMP-2 levels have been inversely correlated with LV volumes and with serum NT-pro BNP levels [11,12]. A recent study, showed an early and robust increase in MMP-9, MMP-8 as well as a reduction in MMP-2 levels after MI [13]. Furthermore in the same study an increase in TIMP-1 and TIMP-2 levels in the later post-MI periods and a uniform fall in TIMP-4 levels was also noted[13].

6. Specific aspects of circulating MMPs profile after myocardial infarction

Although many studies have demonstrated that plasma / serum levels of MMPs and TIMPs yield a unique temporal signature after MI, there are inherent limitations that should be recognized. Circulating profile of MMPs after a myocardial infarction depends on MI location. Squire et al, found that after MI MMP-9 levels did not differ between anterior and inferior MI whereas MMP-2 levels were higher in anterior MI and TIMP-1 levels were higher in inferior MI [11]. Furthermore, extent of myocardial infarction is also a major determinant of serum / plasma levels of MMPs [1]. A potential cofounder in acute MI studies is that increased MMP levels have also been correlated to plaque rupture reflecting atherosclerosis-related inflammation and progression [1]. Plasma profiling MMPs and TIMPs are at best only surrogate markers for local myocardial levels and cannot provide information on regional disturbution and proteolytic activity [13]. However, clinical and experimental data demonstrate that the major source of the changes in MMPs and TIMPs observed in the plasma after MI is the myocardium [13].

7. Conclusions

Cumulative evidence suggests that a distinct MMPs/TIMPs profile with respect to their temporal and spatial expression/activity window exists after myocardial infarction [8]. Furthermore, circulating MMPs/TIMPs levels are correlated to the degree of adverse left ventricular remodeling and to the associated prognosis [6]. Future, larger studies that examine in specific the temporal and spatial profile of MMPs and TIMPs in the post MI healing phases are warranted.

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