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Matrix Metalloproteinases: From Biology to Therapeutic Strategies in Cardiovascular Disease

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Research Interests:
1) Mechanisms of Myocardial Ischemia and Cardioprotection;
2) Physiological roles of mammalian Stress "Heat Shock" Proteins; and
3) Molecular Pathogenesis of familial αB-crystallin^{R120G} cardiomyopathy

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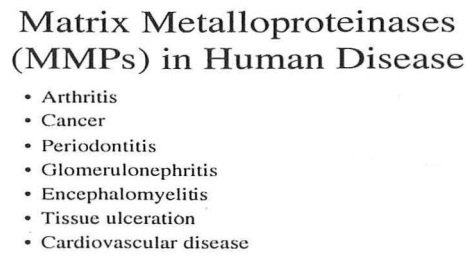
INTRODUCTION

Recent decades have witnessed substantial advances in our efforts to reduce morbidity and mortality from congenital and acquired forms of cardiovascular diseases (1). The spotlight for today's Grand Rounds, the connective tissue elements and matrix remodeling, draws attention to the biological roles and possible clinical implications of matrix metalloproteinases (MMPs) in certain cardiac and vascular diseases. MMPs have widespread clinical interests over a range of human pathologies from degenerative diseases such as rheumatoid arthritis, cancer metastasis, peridontitis, glomerulonephritis, and cardiovascular diseases (Fig. 1).



The conventional wisdom that the cardiomyocyte is a primary object of cardiac dysfunction is deeply ingrained but warrants reappraisal. New classes of pharmacological agents such as angiotensin converting enzyme (ACE) inhibitors, for example, have complemented the treatment of heart failure using inotropic agents (e.g. Digoxin). Such benefits have shifted our understanding about this complex syndrome away from the central dogma as primary abnormalities of cardiac contractility. Accumulating scientific evidence suggests that matrix metalloproteinases and endogenous inhibitors will be the likely targets of future therapeutic strategies for diverse classes of cardiovascular diseases ranging from acute ischemic syndromes, restenosis following angioplasty, coronary artery bypass grafting, and heart failure, to genetic disorders such as hypertrophic cardiomyopathy and Marfan's syndrome. Therefore, the primary goal of today's Grand Rounds is to demystify the extracellular matrix, which forms an indispensable part of the cardiac syncytium, and to provide a conceptual framework for considering potential therapies aimed at matrix remodeling in cardiovascular disease. Though distinct in clinical presentation and classification, the following two cases illustrate the spectrum of evidence in which matrix remodeling and MMP biology affect disease pathogenesis.

Cases:

Case 1. F. C. is a 62-year-old white woman presents with sudden death and cardiac enzymes consistent with inferior wall myocardial infarction. She was referred for cardiac catherization prior to electrophysiological study. Current medications: Metoprolol 50 mg BID; Aspirin 325 mg QD; Isordil 40 mg TID; Lovastatin; and Niacin. On physical Exam: BP 120/80, P 68, Weight 256

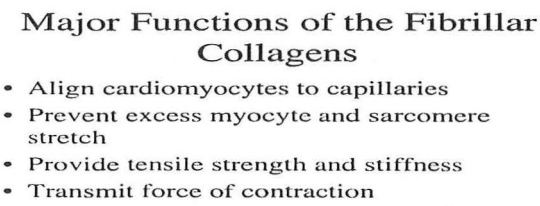
HEENT was unremarkable. Bibasilar crackles were audible. Cardiac exam showed normal S1 and S2, no murmurs, gallops, or rubs. Pertinent labs: Hct 30.5; Na 143, K+ 4.3, and Cr 0.7. The electrocardiogram showed normal sinus rhythm, right bundle branch block, and inferior Q waves. Coronary angiography revealed single vessel disease from an occluded RCA. The overall left ventricular function was mildly depressed (LVEF=0.41), and the ventriculogram showed a large inferior aneurysm without evidence of mitral regurgitation.

Case 2. C. G. is a 26-year-old Hispanic man with Marfan's syndrome was evaluated recently at an outside hospital for chest pain that radiated towards his back. In 1996, he had an aortic valve-conduit replacement (St. Jude's) for acute ascending aortic dissection. His medications on presentation included Coumadin and Lipitor. The chest radiograph showed mediastinal widening and chest and abdominal CT scan revealed a Debakey Type 1 aortic dissection extending to the iliac bifurcation. A large thrombus was present along the length of the abdominal aorta. His medical management included Metoprolol and temporary discontinuation of anticoagulation before he underwent surgical repair of the thoracic and descending aorta with a prosthetic graft.

THE EXTRACELLULAR MATRIX (ECM) OF THE CARDIOVASCULAR SYSTEM

The heart, a rhythmic muscle pump, is formed by a syncytium of heterogeneous cells that are integrated and interdependent with the 3-dimensional network of Type 1 fibrillar collagen, matrix proteases, integrin receptors, cytoskeletal myofibrils, and interstitial cells of the extracellular network **(2-4)**. Force-generating cardiomyocytes occupy seventy-five (75%) of the volume but account for only ~25-30% of the cells in the normal myocardium. The major cellular fraction (75%) consists of non-myocytes, predominantly fibroblasts, endothelial cells, vascular smooth muscle, and interstitial cells, while the remaining volume is interstitial matrix and connective tissue. Much of the elastic properties of the heart are derived from fibrillar collagen type I and III, which form a network of cables or struts which connect cardiomyocytes to each other, to capillaries, and to surrounding interstitial cells (Fig. 2) **(5)**.

Fig. 2.



Fibrillar collagen is resistant to proteolytic degradation and type I collagen has been proposed to possess the tensile strength of steel (6). Struts are thought to function as tethers and to possess recoil properties like springs. Through their elaborate connections and interdependence, the fibrillar network supports the alignment of cardiomyocytes, prevents excess cardiac stretch, and promotes efficient force-generation, all of which contribute to the intrinsic properties of myocardial stretch

and stiffness (7). Type IV collagen is attached to the basement membrane along with laminin, fibronectin, and heparan sulphate proteoglycan of the ECM, whereas type V collagen is diffusely distributed in the interstitium (8,9). Lastly, type VI collagen interconnects cardiomyocytes to the basement membrane, besides elastin, myofibrillar proteins, hetero-polysaccharides and glycoproteins found in the ECM (10).

Matrix Remodeling is Required for Developmental Morphogenesis, Wound Healing, and Tissue Repair. Neonatal cultured cardiomyocytes exhibit greater attachment for diverse classes of collagen I, II, III, IV, V, fibronectin, and components of the basement membrane that adult cardiomyocytes, which efficiently attach to type IV collagen and laminin, but only weakly to fibronectin **(8)**. During cardiac embrogenesis, cell proliferation, migration, and differentiation of myocardial cells is dependent on the active remodeling of connective tissue and trabecular network. Growth factors such as PDGF-A play important roles in such remodeling as shown by the deletion of PDGF-Aα receptor (Patch mutation) in mice, which exhibit severe cardiac and extracardiac abnormalities of morphogenesis caused by down-regulation of matrix metalloproteinases (MMP), MMP-2 and MT-MMP **(11)**. Unlike normal tissue, significant upregulation of collagenase expression and deposition in the interstitial space is a property of wound healing and tissue remodeling after acute myocardial infarction, all phases of human atherosclerosis **(12)**, and during heart failure from volume or pressure overload **(13,14)**. Recent advances in the cellular and molecular biology of tissue injury and repair indicate that a more thorough and critical evaluation on the dynamic processes that regulate collagen accumulation and degradation is warranted.

The Case for Matrix Remodeling by Metalloproteinases in the Pathogenesis of Cardiovascular Disease. Collagen is actively degraded by matrix metalloproteinases, reviewed in **(15)**. Collagenases have been identified by immunohistochemistry in the interstitial spaces surrounding cardiomyocytes, round myocyte bundles, pericardium and in close proximity to its substrate, collagen (reviewed in **(16)**. Small changes in collagen content forecast significant effects on myocardial phenotype ranging from increased systolic and diastolic function by collagen overexpression to ventricular dilatation or rupture due to errant collagen degradation. Three distinct mechanisms regulate collagenolytic activity in the heart. First, MMPs are secreted as inactive proenzymes, which provide an important mechanism for maintaining in their latency. Second, endogenous inhibitors of matrix metalloproteinases or TIMPs maintain inhibition of proteolytic activity. Lastly, there is tight control of gene expression at the transcription level of both MMPs and TIMPs **(17,18)**. Each of three pathways that maintain the normal collagen matrix activity in the myocardium will be discussed in greater detail below.

Matrix Metalloproteinases are Subdivided by Substrate Specificity. Matrix metalloproteinases (MMPs) have been classically divided into the interstitial collagenases, gelatinases, stromelysins, and a fourth unclassified subgroup ((**15**) & Table 1). The collagenase subgroup consists of interstitial collagenase (MMP-1), neutrophil collagenase (MMP-8), and collagenase 3 (MMP-13) **(19)**. The major distinguishing feature of these MMPs is their ability to degrade triple helical regions of interstitial collagens I, II, and III **(20,21)**. MMP-1 is synthesized in several different cell types including fibroblast, chondrocytes, epithelial cells and macrophages, whereas MMP-8 is found only in neutrophils **(22)**. Human MMP-1 and human MMP-8 share 57% identity in amino acid sequence **(22,23)**. MMP-13 is secreted as a 65,000 kD propeptide and is cleaved to an 55,000 kD protease. Type I collagen is the major matrix substrate of MMP-13, a functional homologue of

human MMP-1, which is not expressed in the rat. Interestingly, the best known substrate for MMP-1 is human alpha macroglobulin (α_2M) **(24)**, which exceeds human collagen type I by 150-fold as a substrate. Other substrates of these collagenases include casein, aggrecan, type VII, type X collagen and cartilage-linked protein **(25)**.

Table 1, (15)

Matrix metalloproteinases

Enzymes	MMP No.	M_r Precursor	M_r Active	Matrix Substrates
Collagenases				
Interstitial collagenase (EC 3.4.24.7)	MMP-1	52 000 56 000*	41 000 45 000*	Collagen I, II, III, VII and X, gelatins, entactin, aggrecan, cartilage link protein
Neutrophil collagenase (EC 3.4.24.34)	MMP-8	75 000*	65 000*	Collagens I, II, III, aggrecan, link protein
Collagenase 3	MMP-13	65 000	55 000	Collagen I
Gelatinases				
Gelatinase A (EC 3.4.24.24)	MMP-2	72 000	67 000	Gelatins, collagens I, IV, V, VII and XI, fibronectin, laminin, aggrecan, elastin, large tenascin-C
Gelatinase B (EC 3.4.24.35)	MMP-9	92 000*	84 000*	Gelatins, collagens III, IV, V, XIV, aggrecans, elastin, entactin
Stromelysins				
Stromelysin 1 (EC 3.4.24.17)	MMP-3	57 000 59 000*	45 000 28 000	Aggrecan, gelatins, fibronectin, laminin, collagen III, IV, IX and X, large tenascin-C
Stromelysin 2 (EC 3.4.24.22)	MMP-10	57 000	45 000 28 000	Aggrecan, fibronectin, laminin, collagen IV
Others				
Matrilysin (EC 3.4.24.23)	MMP-7	28 000	19 000	Aggrecan, fibronectin, laminin, gelatins, collagen IV, elastin, entactin, small tenascin-C
Stromelysin 3	MMP-11	55 000	45 000 28 000	Weak activity on fibronectin, laminin, collagen IV, aggrecan, gelatin
Metalloelastase	MMP-12	53 000	45 000 22 000	Elastin
Membrane-type MMP	MMP-14	66 000		Activate proMMP-2

* glycosylated

Gelatinases: Diversity among members of the MMPs family is derived from substitutions, deletions or additions of subdomains imposed on their basic structure. MMP-2 (gelatinase A) and MMP-9 (gelatinase B) are examples that have incorporated three repeats homologous to the type II module of fibronectin into their catalytic domains. In turn, these collagenases have been shown to selectively bind denatured collagen or gelatin **(26)**. MMP-2, a 72-kDa protein, is secreted from a variety of cells MMP-9 is found in neutrophils, macrophages, fibroblasts, chondrocytes, and T lymphocytes. The synthesis of MMP-9 is upregulated in response to cytokines, cell transformation, or infection. The unique type II domain of gelatinases, which consists of three repeats of eight amino acids, has at least three main functions. The fibronectin-like domain from MMP-2 appears to play a role in the hydrolysis of gelatin and cleavage of type IV collagen, and, therefore, is thought to

affect the interactions of the enzyme with the extracellular matrix **(26)**. In addition to digestion of gelatins, MMP-2 and MMP-9 bind and hydrolyze a variety of substrates such as elastin, aggregan and cartilage. While several of these substrates are not direct primary targets in the cardiovascular system, they do provide some insight into the broad functional and biochemical diversity of MMPs in disease pathogenesis.

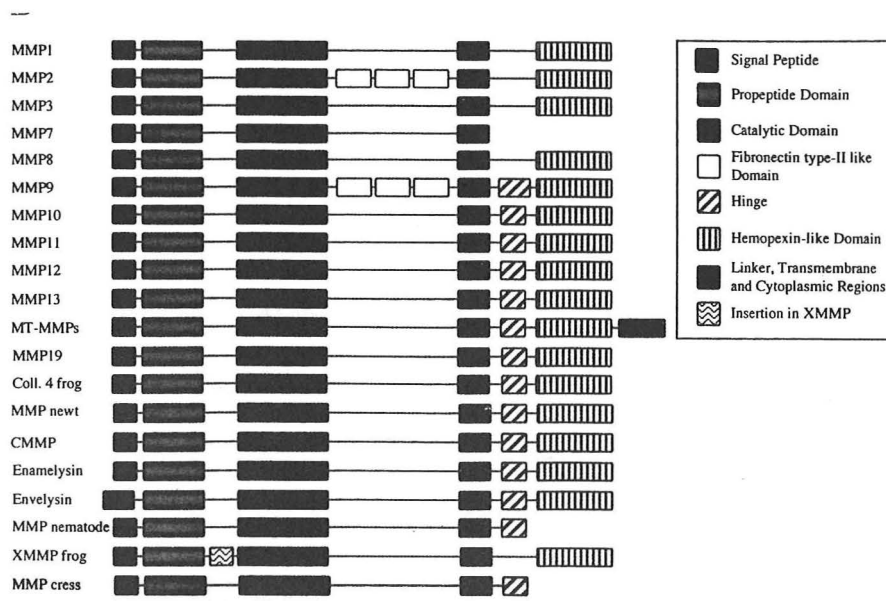
Stromelysins: MMP-3 (stromelysin-1) and MMP-10 (stromelysin-2) are the main enzymes in this subgroup that degrade gelatins, aggregan, fibronectin, laminin, and type IV collagen among others **(27)** & Table 1). Human MMP-3 and MMP-10 share 79% identity in amino acid sequence and 55% identity with other collagenases. None of these enzymes degrade triple helical collagens such as collagen type-I and -II. MMP-3 is produced in many connective tissue cells and is synthesized in response to treatments with cytokines, growth factors or phorbol esters.

Other Matrix Metalloproteinases. Because of significant structural and functional differences these members include matrilysin, which are MMP-7, MMP-11 or stromelysin 3, MMP-12, a macrophage metalloelastase, and a membrane-type matrix metalloproteinase (MT-MMP). Another member, MMP-13 (stromelysin-3), has significantly diverged from MMP-3 and MMP-10 and is therefore placed in the unclassified subgroup shown in Table 1.

Structural Properties of Matrix Metalloproteinases (MMPs). MMPs are related to the super family of zinc-peptidases, and to date, over 66 MMPs have been sequenced including 17 from humans (28,29). Analysis of multiple sequence alignments for 64 MMPs conducted by Massova and colleagues has identified 23 distinct subgroups (29). Indeed, MMPs are quite ancient in relation to bacterial sequence in *bacteroides spatulus* metalloproteinase toxin-2, which shares 59% sequence identity within a 27-amino acid stretch of human MMP-1 (30). Since the diversity of MMPs can be traced to the simplest bacteria, these results suggest that certain primordial MMPs may have evolved 3.5 billion years ago. Interestingly, most MMPs exhibit similar biological properties and substrate profiles in different organisms, and several closely related MMPs appear to have originated on chromosome 11 (29).

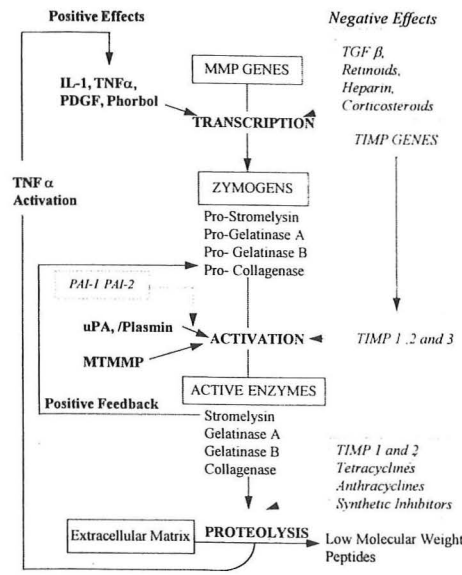
The basic organization for the MMP family consists of three highly conserved domains: an amino-terminal propeptide; a catalytic domain; and a hemopexin-like domain at the carboxy-terminal (29) & Fig. 3). The N-terminal propeptide contains at least 80-90 amino acids including a cystein residue and the highly conserved sequence (..PRCGXPD..). As all members of the MMP family are secreted as inactive zymogens, cleavage of the MMP propeptide by proteolysis is required for full activation. The central domain contains the active catalytic site and two zinc ions and at least one calcium ion. Although MMPs possess high affinity for both zinc and calcium ions, their precise biological roles remain to be elucidated. The C-terminal domain of MMPs is highly conserved and shows sequence hemology to the plasma protein, hemopexin. The hemopexin-like domain of MMPs plays a pivotal role in binding to substrates and interactions with the endogenous inhibitors, the tissue inhibitors of metalloproteinases (TIMPs) (17,18). While these three basic domains are common to all MMPs, other members of the family through addition or deletion have evolved into different subgroups that contribute to their structural and functional diversity and specificity (Fig. 3).

Fig. 3. (29).



Regulation of MMPs/TIMPs in the Cardiovascular System. At least three distinct pathways maintain inhibition of secreted extracellular matrix (ECM) components (Figure 4). Urokinase plasminogen activator (uPA) proteolytically cleaves precursor plasminogen into plasmin, a major activator of matrix metalloproteinases (31). In cultured human smooth muscle cells, potent inducers of MMP-2, TIMP-1 & 2 expression (32), include the growth factor, PBGF, and proinflammatory cytokines IL-1, and TNFα, which are highly expressed in activated macrophages of human atherosclerotic lesions (33,34). Cytokines may also modulate MMP/TIMP activities through plasmin activation and other cell mediated mechanisms. Several lines of evidence indicate the uPA-plasmin system regulates MMP-2 and MMP-9 expression. Both MMP-2 and MMP-9 are associated with the cell surface and binding of either uPA or plasminogen results in the selective activation of these gelatinases (35). In addition to uPA, plasminogen-activator inhibitors (PAI) and MMPs contain binding sites in their promoters for transcriptional activators and repressors. Ets-1, a transcription factor that regulates a number of ECM targets, has been implicated in cell migration and degradation of the extracellular matrix. In the chick heart, the Ets-1transactivator binds an upstream recognition motif, 5'-GGA (A/T)-3', or PEA3 domain (36). Ets-1 expression in the subepicardium and in trabecular sinusoids suggests that this regulator may play developmental roles in remodeling and formation of cardiac vessels (36).

Fig. 4, (36)



Role of the Urokinase-Plasminogen Pathway in Vessel Remodeling. From a historical perspective, cancer biologists were the first to focus on components of the ECM, especially in cancer metastases and angiogenesis **(37)**. Since MMPs are stored as latent proenzymes, plasmin-dependent pathways promote the cleavage of the stored zymogens, enabling their activation and subsequent degradation of ECM components. Specifically, one hypothesis is that tissue invasion by metastatic cells involves the roles of uPA, its specific receptor (uPA-R), its inhibitors PAI-1 and PAI-2 and other mediators in cell surface proteolysis **(37)**. Activation of uPA through binding of its receptor on the leading edge of a cell is thought to promote local entry and subsequent spreading of metastatic cells through proteolysis. Proof of principle was provided by several *in vitro* and *in vivo* studies although consistent results have proven elusive. Like the clotting cascade, plasmin cleaves and activates stromelysin, which has been shown to promote further activation of collagenases and MMP proenzymes through a positive feedback loop. Specifically, stromelysin activation increases collagenases activity by 5 to 8 fold through the specific cleavage of stromelysin at the carboxy terminal of collagenases **(38)**. The presence of $\alpha_5\beta_1$ integrin on the surface of keratinocytes and fibroblasts expressing collagenase will support the general notion such activation accompanies cell migration **(39,40)**.

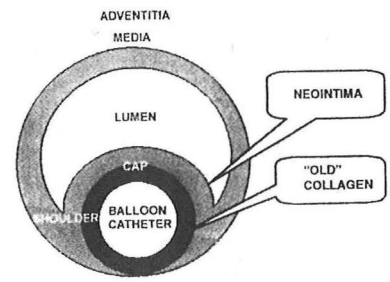
MATRIC METALLOPROTEINASES AND ATHEROGENESIS

MMPs/TIMPs in Atherosclerosis. Infiltration of circulating monocytes into the vascular endothelium is a well-recognized early event in the development of the atherosclerotic plaque **(41)**. Subsequent engorgement with resident macrophages containing lipids along with proliferation of smooth muscle cells are the hallmarks of advanced atherosclerotic lesions. The release and secretion of growth factors and cytokines such as TNFα, IL-1, and PDGF are proposed to stimulate

MMP synthesis in diseased lesions **(42,43)**. In experimental studies, gelatinase-A (MMP-2) is required for rat vascular smooth muscle cell (VSMC) migration and penetration of the basement membrane, which is predominantly composed of type IV collagen . Interestingly, VSMCs exhibit a proliferative phenotype capable of penetrating the basement membrane, suggesting that cell cycle control is a mechanism for homeostasis in the vessel wall by remodeling. In advanced lesions, circumferential stress is thought to be unevenly distributed across the fibrous cap, creating an imbalance in matrix formation and distribution **(43,44)**. In studies of human atherosclerotic plaques, Davies and colleagues have correlated the risk of thrombosis with the distribution of connective tissue in the shoulders of the plaque **(45)**. Other studies have shown that stromelysin mRNA transcripts are expressed in frozen sections of lipid-laden plaques. Because TNFα can induce the synthesis of stromelysin, it has been proposed that the distribution and activation of MMPs can effect mechanical stress and thereby contribute to plaque rupture and thrombosis. Besides these descriptive clinical studies, there are now increasing lines of evidence in experimental animal models to support a direct role of MMPs and TIMPs in atherosclerotic plaque initiation and stabilization, and in the progression of thrombotic disease **(46,47)**.

MMPs/TIMPs in Atherosclerosis: Effects of Collagen Turnover on Plaque Stabilization and Rupture. Several factors including foam cells, VSMCs, macrophages **(45)**, lipid deposits, and collagenous bone matrix proteins **(48,49)** accumulate in atherosclerotic plaques. Coronary thrombosis from abrupt rupture of a vulnerable plaque initiates a cascade of events in which MMPs have been implicated. The major source of MMPs may be macrophages that take up residence in lipid-laden plaques. In addition, macrophages are proposed to synthesize MMPs, which, in turn, degrade collagen and increase plaque vulnerability. Exposure of the coagulation system to components of the basement membrane can trigger thrombosis since collagen has a potent thrombogenic capacity **(40)**. To determine whether lipid accumulation directly affects collagen degradation, Rekhter and colleagues developed an ingenious system using an inflatable matrix-covered balloon for directly monitoring plaque formation and rupturing pressure **(50)** & Fig. 5). Plaque rupture upon balloon inflation, an index of plaque strength in rabbit atheroma, was 2.1 times lower in rabbits fed 0.5% cholesterol chow for 3 months compared with control animals fed a standard diet. Development of fibrous plaques was associated with foam cell, collagen loss, and collagen breakdown in cholesterol-fed animals, suggesting that hypercholesterolemia may contribute directly to plaque weakening **(50)**.

Fig. 5, (50).



MATRIX REMODELING AND ACUTE ISCHEMIC SYNDROMES

Immunohistochemical analysis and *in situ* hybridization have identified MMP-2, TIMP-1 and TIMP-2 expression in nondiseased and atherosclerotic arteries. As inactive MMPs require proteolytic cleavage for activation and/or derepression of TIMP inhibition, MMP expression *per se* does not reflect catalytic activity. Indeed, MMP-2 zymogen is secreted in 1:1 stoichiometry with its endogenous inhibitor, TIMP-2 (51,52), providing a mechanism for limiting the degradative capacity of MMPs in normal tissue and vessels. During tissue repair a subpopulation of fibroblasts undergo cellular transformation into cardiomyocyte-like phenotype, or myofibroblast (myoFb) in response to injury (53,54). Upregulation of Ang II, TGF-β_1, and endothelin receptors on myoFbs has been proposed to facilitate their response to regulatory signals (55). Because myoFbs express α-smooth muscle actin, these specialized cells exhibit contractile properties and effect fibrogenesis in the myocardium, cardiac valves, pericardium and vasculature during diverse pathophysiological conditions (56,57).

In human hearts harvested from transplant recipients, Henney and co-workers first demonstrated stromelysin 1 (MMP-3) and stromelysin 2 (MMP-10) transcripts in atherosclerotic plaques by *in situ* hybridization (48). Stromelysin transcripts were localized in the plaque, intima, and adventitia, but spared the media including cell types such as smooth muscle cells and macrophages. However, *in situ* zymography indicates MMP activity is a specific property of diseased arteries. For example, *in situ* gelatinolytic activity and ^{3}H-collagen IV degradation, have shown increased interstitial collagenase (MMP-1), 92 kD gelatinase (MMP-9), and stromelysins in human atheromatous plaques (12). In coronary atherectomy specimens, Brown and coworkers demonstrated similar expression of the 92 kD gelatinase-B (MMP-9), which is predominantly present in macrophages and smooth muscle cells, in 10 of 10 patients with unstable angina compared with 3 of 10 patients with stable ischemic syndrome (58).

MMPs and TIMPs Expression: Implications for Coronary Angioplasty and Restenosis. Percutaneous transluminal coronary angioplasty is a widely available therapeutic intervention for revascularization of acute and subacute ischemic syndromes **(59,60)**. The clinical indications for PTCA in the management of patients with ischemic syndromes have been reviewed recently at Grand Rounds. These remarks will not be comprehensive and are limited to recent advances implicating MMPs/TIMPs in the pathobiology of coronary restenosis, which, despite > 80% technical success rate of PTCA, remains a limitation in 25-50% of the patients within six months of the procedure. Strategies to prevent restenosis have remained elusive. Multiple factors have been implicated in recurrent restenosis resulting in arterial narrowing including the rapid growth of vascular smooth muscle cells and fibrocellular intimal hyperplasia **(61)**. In human samples after coronary atherectomy, macrophages were significantly increased in primary lesions implicating their etiologic role in MMP expression after PTCA **(62)**. During the tissue repair, markers of exaggerated wound healing are found during restenosis including intimal hyperplasia, increased collagen synthesis, and matrix deposits **(63,64)**. Recurrence of luminal obstruction is associated with > 80% increase in connective tissue, suggesting an imbalance that favors matrix synthesis over degradation**(65)**. Disruption of the basement membrane by balloon angioplasty stimulates the

proliferation and migration of smooth muscle cells and infiltration of inflammatory cells into the vessel wall(**66**).

In experimental studies, activation of MMP-2 in the neointima inhibits smooth muscle cell migration, which implicates MMPs in vascular remodeling. Likewise, balloon catheter de-endothelialization is associated with activation of MMP-2, which is temporally related to expression of membrane-type matrix metalloproteinase (MMP-14) (**67**). Further evidence of MMP-2/MT-MMP interactions comes from genetic analysis of platelet-derived growth factor receptor (PDGF) α mutant mice (**11**). PDGF α regulates MMP-2 expression, and null mice for the PDGF α receptor gene exhibit prenatal lethality caused by severe cardiac abnormalities attributed to connective tissue defects, along with marked reductions in expression of MMP-2 and its activator, MT-MMP (**11**). In animal models of carotid artery injury, gelatinase-A (MMP-2) expression is induced in 4-5 days after angioplasty; in contrast, gelatinase-B (MMP-9) expression is increased 1-2 days after injury (**61**). Likewise, the plasminogen activator system is activated after balloon or ischemic injury in both rodent and rabbit models (**68**), suggesting that its upregulation could directly trigger the cascade of MMP activation. Although no direct evidence exists currently that inhibition of MMPs can alter the recurrence of restenosis, this specific hypothesis remains an important one to test (**63**).

MATRIX REMODELING AND ACUTE MYOCARDIAL INFARCTION

Early events that re-establish timely reflow to the ischemic myocardium either through thrombolytics, direct angioplasty or from spontaneous clot lysis are essential for enhancing myocardial salvage and reducing morbidity and mortality. Nonetheless, recurrent ischemia either from rupture of an unstable plaque or congestive heart failure can complicate the clinical course of an acute myocardial infarction (AMI). During AMI, for example, matrix remodeling at the cellular level begins within minutes but could last for weeks to months before myocardial repair is complete. Thus, endogenous pathways that influence the synthesis and degradation of matrix components have clinical relevance in mitigating the effects of ischemic damage in the patients. In addition, other wound healing models will suggest that collagenolytic activity may contribute to the removal of damaged tissue and/or facilitate migration of interstitial cells during remodeling and tissue repair (69). One plausible hypothesis is that the release of cytokines, such as TNFα, TGFβ, and inflammatory cytokines, may contribute to the phenotype of cells expressing MMPs and TIMPs during repair of myocardial tissue (70).

Collagenolytic Activity in the Heart: The Effects of Myocardial Ischemia. In the isolated perfused heart, latent MMP proenzymes are activated by oxidized glutathione produced during ischemia-reperfusion. Within 40 minutes after coronary occlusion in the porcine heart, ultrastructural studies have demonstrated significant changes in the architecture and arrangement of collagen fibers. Disruption of collagen fibers, elastic fibers and microfilaments can be seen two hours after ischemia-reperfusion and the collagen network exhibits an irregular appearance with disruption of collagen banding pattern (**71**). Myocardial stunning, which results in severe myocardial dysfunction without ultrastructual signs of myocyte damage, is associated with disappearance of collagen weave and struts (**72**). These features become absent in the hypocontractile heart in which increased stiffness accompanies myocardial stunning (**73**). Likewise, total collagen content is reduced in the infarcted zone following coronary occlusion (1-3 hours) as the amount of insoluble collagen increases substantially. There are also corresponding

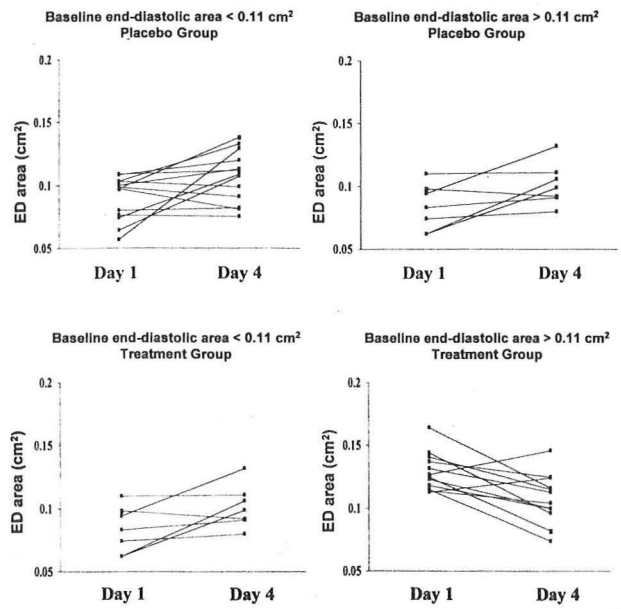
increases in the protease activities of collagenase, neutral proteinase and lysosomal serine proteases in the ischemic heart, suggesting that these enzymes may be activated and contribute to the overall increased proteolytic state *in vivo*. In addition, proteolytic activity also appears to be controlled regionally since increased collagenetic activity in rat ischemic myocardium is restricted to the infarct zone **(71,74)**.

Collagenetic Activity in the Heart: The Effects of Endogenous Inhibitors, TIMPs. Dual pathways that control the activation of MMPs and their targeted inhibition determine the net balance of collagenetic activity and breakdown in the extracellular matrix by TIMPs. Several lines of evidence support the general notion that MMP expression influences the morphological and ventricular chamber volume that accompanies acute myocardial infarction. Collagenolytic activity measured by zymography is increased in the postischemic heart. However, changes in MMP-1 activity, for example, are not associated with increased synthesis until seven days postinfarction. These data suggest that remodeling of the myocardium during early ischemia is related to activation of pre-existing MMPs in the extracellular matrix rather than new synthesis of these enzymes. TIMP-1 mRNA expression is accompanied with activation of MMPs within six hours after infarction (71). In addition, TIMP-1 transcription peaks after two days, which mirrors the onset of postischemic MMP activation. Thus, depletion of the pre-existing pool of MMPs during acute myocardial infarction is accompanied by subsequent increased synthesis presumably in a feedback mechanism to restore homeostasis (71). Measurements of chamber dynamics within two days of acute myocardial infarction, do not correlate with early changes in collagenolytic activity, suggesting that alterations in left ventricular geometry are delayed and occur several days postinfarction. These observations support the rationale that an MMP inhibitor may reduce late onset ventricular dysfunction in acute MI. As previously mentioned, MMP-1 and TIMP-1 are produced by fibroblasts and myofibroblasts (myoFb), which assume a myocyte phenotype with contractile activity, and whose proliferation and activation promote overexpression of MMPs and perhaps postischemic left ventricular dysfunction.

MMP Inhibition Attenuates Early Left Ventricular Enlargement After Experimental Myocardial Infarction. Activation of MMPs and subsequent disruption of collagen struts and fibers are proposed to play important roles in subsequent cell slippage, eccentric hypertrophy and changes in ventricular geometry. Other changes in tissue architecture, myocardial stiffness, wall thinning and dilatation may also contribute to acute cardiac decompensation, especially in patients with extensive myocardial infarction involving greater than 40% of the left ventricle. Until recently, however, support for the hypothesis that MMP inhibition can alter the morphological and ventricular dimensions after myocardial infarction was unavailable. In a randomized trial in which a broad spectrum MMP inhibitor (CP-471, 474) or placebo Rohde and colleagues showed there was significant attenuation of early left ventricular dilatation after experimental myocardial infarction in inhibitor-treated animals **(75)** (Fig. 6). In addition, there was no change in fractional shortening after infarction in animals receiving MMP inhibitor, while animals receiving placebo sustained a decrease in fractional shortening and larger increases in end-systolic and end-diastolic dimensions **(75).** Measurement of collagen indicated no differences in infarcted and noninfarcted regions in the treated and untreated groups of animals, suggesting that the effect of MMP inhibition is related to other effects besides collagen content. MMP inhibitor therapy had the most significant effect in the treated group whose baseline end-diastolic area was greater than 0.11 cm^2, suggesting that MMP inhibition would benefit post-MI patients at high risk for congestive heart failure **(76)**. These

results are reminiscent of the benefits from angiotensin-converting enzyme inhibitors on survival and left ventricular dilatation in experimental subjects and humans with modest to large infarctions **(77)**. In the future, it should prove feasible to determine whether specific or broad-spectrum MMP inhibition offers long-term benefits. In addition, separate analysis is needed to assess whether there are additional effects of MMP inhibition when administrated at the onset or immediately after coronary occlusion. Further studies are needed to clarify these potential beneficial effects in randomized trials in humans.

Fig. 6, (75).



Role of the Matrix Remodeling and Cardiac Rupture. Almost 15% of all in-hospital deaths with acute myocardial infarction receiving thrombolytic therapy are attributed to cardiac rupture **(78)**. Ventricular septal defect remains a dreaded complication of acute myocardial infarction with a reported incidence of 1-2% in the prethrombolytic era **(79,80)**. With typical onset 3-5 days post-MI, this complication in the first week after an infarction correlates angiographically with total occlusion of the infarct-related artery and minimal collateral vessels **(81,82)**. The mortality from VSD treated surgically is 45% and in patients treated medically is approximately 90% **(83,84)**. These poor outcomes are associated with the onset of cardiogenic shock and right ventricular dysfunction, typically with inferior-wall infarctions. While the foregoing figures have been well documented in the prethrombolytic era, Crenshaw and colleagues have recently evaluated the outcomes of patients in the Global Utilization of Streptokinase and TPA for Occluded Coronary Arteries (GUSTO-I) trial **(85,86)**. In GUSTO-1, 84 of 41,021 were diagnosed with a VSD, which is approximately 5-10 fold less than the previous era **(87)**. In addition, median time for the diagnosis of VSD was one day, and associated risk factors in patients included advanced age, female gender,

anterior infarction and no prior history of smoking. Because all patients selected for analysis received thrombolytic therapy less than six hours from the onset of chest pain, the lower incidence of VSD could be related to more aggressive therapy for acute thrombosis. However, the outcomes from the development of VSD are indistinguishable between the prethrombolytic and current area. The thrombolytic therapy is likely to reduce or prevent transmural necrosis, which may be a factor in cardiac rupture. However, it remains controversial whether the earlier onset of symptom with cardiac rupture is secondary to a "lytic" state associated with thrombolysis and adjuvant anticoagulation therapies.

In the Thrombolysis and Thrombin Inhibition in Myocardial Infarction 9 study, 65 of 3,759 (1.7%) patients with MI were reported with cardiac rupture in which the majority of events occurred less than 48 hours after initiation of thrombolytic treatment **(78)**. Risk factors in these patients include older age (greater than 70 years), female gender, prior angina or myocardial infarction and few collaterals angiographically. Like the GUSTO-I trial, the patient-related risk factors were similar in that cardiac rupture was independent of either the type of thrombolytic therapy or intensity of anticoagulation. Despite similarities in prognosis associated with cardiac rupture in the prethrombolytic and thrombolytic eras, much less is known about the etiology and mechanisms of this serious complication during acute myocardial infarction.

Following acute myocardial infarction, ventricular remodeling and scar tissue formation are well-recognized cellular processes that begin within minutes and extend for weeks and months. Because plasmin activates MMPs in the extracellular matrix, recent studies have examined changes in interstitial collagen, the substrates of activated MMPs caused by thrombolysis and plasmin activation. In patients receiving either streptokinase or tissue plasminogen activator, the amino terminal propeptide of type III collagen, a marker of plasmin degradation of ECM components, was increased 16 to 44%, suggesting that thrombolytic agents stimulated the breakdown of interstitial collagen **(88)**.

Disruption of u-PA Protects Against Cardiac Rupture After Experimental MI. Studies in transgenic models would support a direct role for specific extracellular matrix proteins in cardiac rupture. After acute myocardial infarction, the frequency of cardiac rupture was 33% in wild-type animals or mice lacking tissue type plasminogen activator, urokinase receptor, matrix metalloproteinase, stromelysin-1 or metalloelastase compared with non-infarcted sham-operated animals **(89)**. In contrast, no animals lacking the urokinase-type plasminogen activator, uPA, exhibited cardiac rupture, whereas disruption of gelatinase-B partly reduced cardiac rupture in null mice compared to controls, Fig. 7. At least two or more mechanisms may account for the phenotype abolishing cardiac rupture. First, the plasminogen/MMP pathway promotes the infiltration of inflammatory cells, especially neutrophils into the infarct zone. Accelerated degradation of collagen network may contribute to cardiac rupture **(78)**. Second, proteolysis by plasmin is important for mediating infarct healing through effects on cell migration and phagocytosis by infiltrating macrophages into the infarct region. However, mice lacking u-PA that exhibited impaired and delayed healing supports the important role of plasmin proteolysis in infarct healing and scar tissue formation. Neither u-PA nor plasmin can directly degrade interstitial collagen, although u-PA/plasmin appears to directly activate MMP-9, which, in part, may account for the lower frequency of cardiac rupture in MMP-9 deficient mice **(89),** Fig. 7.

Fig. 7, (89).

Disruption of uPA Protects Against Cardiac Rupture

Gene inactivation	Cardiac rupture (frequency; %)
wild-type	17/51 (33%)
t-PA$^{-/-}$	3/10 (30%)
u-PA$^{-/-}$	0/32 (0%)**
u-PAR$^{-/-}$	4/18 (22%)
MMP-3$^{-/-}$	3/9 (30%)
MMP-9$^{-/-}$	2/26 (7%)**
MMP-12$^{-/-}$	4/18 (22%)

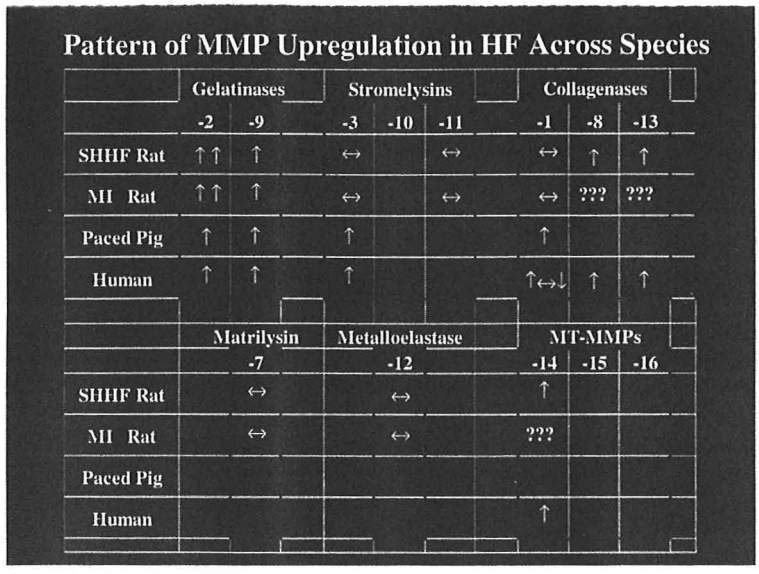
Other physiological functions for MMPs in tissue resorption. Besides evidence for direct roles in matrix degradation, recent studies in a model of disc herniation suggest MMPs help to stimulate release of bioactive substances that influence cell-cell communication and macrophage infiltration. Both MMP-3 (stromelysin-1, EC#3.4.24.17) and MMP-7 (matrilysin, EC#3.4.24.23) are expressed in human samples of herniated discs, suggesting that they may cleave cartilage proteoglycan aggrecan **(90,91)**. Using a recently developed organ culture system, Haro and colleagues demonstrated that macrophages secrete MMP-7, which stimulates the production of MMP-3 by chondrocytes **(92)**. However, the cytokine TNFα is required for efficient MMP-3 production, which mediates macrophage infiltration through a novel macrophage chemoattractant **(93)**. These results define another mechanism, for cell-cell interactions instead of catabolism, by which certain MMPs exert plieotropic functions during tissue repair.

MATRIX METALLOPROTEINASES IN HEART FAILURE

Inhibition of MMPs Can Retard the Development of Experimental Heart Failure. The transition from compensated to overt clinical signs of heart failure is associated with local changes in myocyte geometry, LV dysfunction, and remodeling of the extracellular matrix. To assess whether MMP expression plays a role during the early phases of congestive heart failure, Spinale and colleagues examined the zymographic activities of several MMPs and TIMPs following pacing-induced supraventricular tachycardia (SVT), which produced progressive CHF in pigs (94). Changes of LV dimension and myocyte length were present within 7 days of SVT and were exacerbated with prolonged stimulation. After 21 days of SVT, LV dimension had increased by 50%, LV fractional shortening fell by 56%, and myocyte velocity of shortening had declined 33% compared to normal controls (94). Even with pacing for 7 days, the collagen content of the LV myocardium declined >25%, during which MMP zymographic activity for the substrate, gelatin, was increased 80%. Specifically, there was a ~ 2-fold increase of interstitial collagenase (MMP-1),

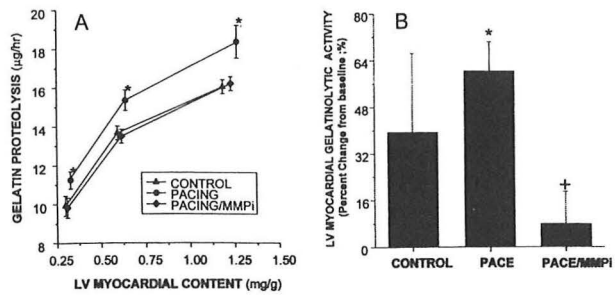
stromelysin (MMP-3), and the 72-kD gelatinase (MMP-2). Likewise, cardiomyopathic Syrian hamsters exhibit LV geometry and myocyte dysfunction. Taken together, these results demonstrate significant changes in several major components of the extracellular matrix during experimental CHF at the functional and cellular levels in diverse species (Fig. 8).

Fig. 8.



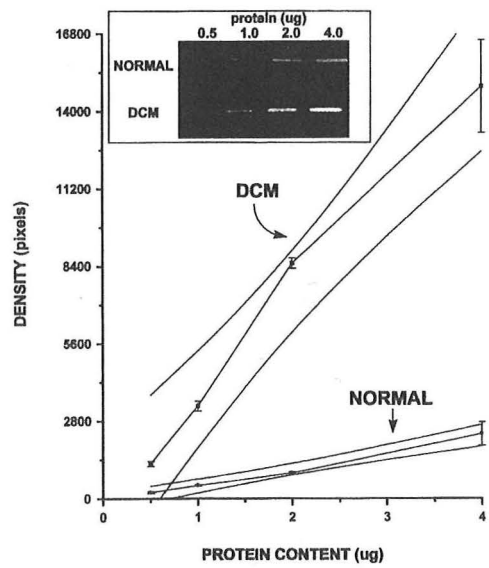
Remodeling is a dynamic process involving synthesis and degradation of both intracellular and extracellular components in living cells (6). Both human and experimental animal studies have demonstrated LV remodeling as a well-established hallmark preceding and during the development of LV dysfunction (95). Therefore, alterations of LV remodeling from either increased MMP expression and/or reduced endogenous tissue inhibitors of matrix metalloproteinases, TIMPs, have provided both challenges and opportunities for recent investigators. Experimental models of heart failure in pigs recapitulate the well-characterized clinical parameters such as contractile dysfunction and activation of the neurohumoral system, following chronic tachycardia by LV pacing (13,94). Treatment of CHF groups with the MMP inhibitor, PD 166793, reduced LV end-diastolic dimension, and pacing-induced LV peak wall stress, and increased LV endocardial shortening (25%), whereas LV midwall shortening was indistinguishable between treated and untreated groups (96). In addition, the expected increases in MMP activity were attenuated by MMPi treatment (Fig. 9). These studies demonstrate that MMPi can limit the development of LV dilation and wall stress, which are major beneficial effects in subclinical and overt heart failure.

Fig. 9,(96).



Matrix Metalloproteinases Activities in Patients with End-Stage Dilated Cardiomyopathy. Left ventricular remodeling has been shown to be an independent risk factor for morbidity and mortality in human heart failure **(7,77)**. Dilated cardiomyopathy (DCM) is accompanied by left ventricular dilation and remodeling, suggesting that alterations of the extracellular matrix may influence end-stage clinical manifestations. Disruption of the fibrillar collagen matrix may contribute to misalignment of adjacent cardiac myocytes and alterations in left ventricular geometry. Although collagen breakdown and MMP and TIMP activities are well-recognized features in various disease states, the major emphasis of recent studies in humans and experimental animal models has been to determine the specific abundance of components of the extracellular matrix in the left ventricular myocardium. In humans, Thomas and colleagues demonstrated that interstitial collagenous (MMP-1) was reduced whereas stromelysin (MMP-3) and the 92 kD gelatinase (MMP-9) were increased **(97)** (Fig. 10). The 72 kD gelatinase (MMP-2) remained unchanged in a survey of seven human idiopathic DCM hearts compared to eight normal controls. In addition, the levels of TIMP-1 and TIMP-2 were increased by 500% in patients with DCM. Likewise, Tyagi and colleagues demonstrated similar changes in collagen matrix of MMP-1, which is increased by 3 to 4 fold in DCM hearts **(98)**.

Fig. 10, (97).



Pharmacological Interventions: Effects of ACE Inhibition on MMP expression. Ultrastructural studies indicate distinct differences in the collagen matrix composition before and following chronic pacing with concurrent ACE inhibition or MMP (MMP-I) inhibition. Following three weeks of pacing, the collagen matrix, which in the normal myocardium exhibits a weave-like pattern and contains homogenous fibers wrapped around individual myocytes, is disrupted and becomes discontinuous between myocytes. This fibrillar pattern is substantially reduced during either ACE or MMP inhibition alone, whereas the collagen fibrillar network is significantly thickened. In the groups with combined ACE and MMP inhibition, there was disruption of the normal architecture in some regions whereas, in others, the collagen fiber weave is readily apparent, suggesting that these pharmacologic therapies can mitigate against and influence the deposition of the extracellular matrix in the interstitial space. MMP zymographic activity was increased in the rapid-pacing group compared with control animals, whereas the enzymatic activity was indistinguishable between the two groups with either ACE or MMP inhibition. The MMP inhibitor, PD166793, which has a chemical formulation (S)-2-(4 prime-bromo-biphenyl-4-sulfonylamino-3-methyl) butyric acid, has broad-spectrum MMP inhibitory activity, within 8-10 μM. Using purified human MMPs in *in vitro* assay systems, this MMP inhibitor is active against the gelatinases (MMP-2, 9), stromelysins (MMP-3, 7), and interstitial collagenase (MMP-1).

Evidence that combined ACE and MMP inhibition imparts beneficial effects on LV stiffness has several important implications. Myocardial wall stress is directly proportional to systolic pressure and the LV dimension and inversely proportional to wall thickness. In this experimental model of pacing-induced CHF, there is both an increase in LV chamber volume and a decrease in posterior wall thickness (99). MMP inhibition has no direct hemodynamic effects but appears to preserve LV wall thickness and reduce wall stress. In contrast, ACE inhibition reduces LV wall stress, maintains normal LV fillings, retards LV hypertrophy, and prevents the deleterious effects of LV remodeling (77). Because combined ACE and MMP inhibition exhibited substantial vasodilator effects, some authors have proposed that such effects are secondary to either activation or neurohumoral pathways or effects on vascular smooth muscle tone (100). Interestingly, MMP inhibition did not alter either systemic or pulmonary vascular resistance during pacing-induced CHF, suggesting that it would be appropriate in future studies to examine potential effects of MMP inhibition on the peripheral vasculature. Although there are limitations in the assessment of LV ejection performance, the benefits of either ACE or MMP inhibition during pacing-induced CHF may have direct influences on myocardial contractility.

Changes in myocardial collagen matrix can alter LV compliance. In pacing-induced CHF, myocardial stiffness is not significantly changed compared to controls, so it was surprising to observe that concomitant MMP inhibition increased myocardial stiffness. However, improvements in substrate specificity of MMP inhibition are needed since the timing of MMP inhibition may depend on the timing of the early or late phase in disease pathogenesis. For example, additional studies are needed to incorporate recent evidence that MMP-3 is increased, whereas MMP-1 is decreased in human cardiomyopathy. These results suggest that current strategies using nonselective and broad-spectrum MMP inhibition may need to be tailored as new insights about the respective alterations are obtained from clinical and experimental studies. One immediate future goal is to determine whether MMP inhibition influences TIMP expression *in vivo*.

MATRIX METALLOPROTEINASES AND MYOFIBRILLOPATHIES

MMPs and Marfan's Syndrome: Implications for Abdominal Aortic Aneurysm and Aortic Valves. Marfan's syndrome is an autosomal dominant disorder of connective tissue with variable penetrance of musculoskeletal abnormalities, ocular (i.e., ectopia lentis), and cardiovascular abnormalities (101,102). Cardiovascular complications from dilatation of the proximal aortic root and ascending aortic aneurysms, aortic dissections and rupture, and valvular insufficiency can reduce life expectancy (102,103). Prophylactic treatment of individuals with classical Marfan's with β-adrenergic receptor blockers (e.g. Atenolol, propranolol) reduces heart rate, decreases the rate of pressure change in the aorta and root dilatation, and improves survival by reducing the aforementioned secondary complications (104). In some cases approaching a critical root diameter of 55 mm, elective replacement of the aortic root with composite graft with or without valve-replacement is recommended to prevent premature death from aortic dissection and aneurysm (105,106). Uncertainty exists about the long-term durability of these surgical interventions, and the therapeutic response to beta-blockade is quite variable since in one trail some untreated patients exhibited less aortic distention than treated patients (104).

Some observers have attributed the well-recognized clinical variability of Marfan's syndrome to intragenic heterogeneity (107). The FBN1 gene consists of 65 exons, which is located on chromosome band 15q21.1, and encodes a large 350-kd protein whose 10-12 nm microfibrils are major extracellular components. Major constituents of the aorta include collagen type I, III, and elastin (Fig 11). Over 20 mutations (missense, nonsense, deletions) across most domains and evenly distributed over the 10-kb cDNA of the FBN1 have been described. Mutations of the fibrillin 2 gene cause a phenotypically related mirofibrillopathy, congenital contractural arachnodactyly. Although biochemical and genetic analyses for mutations of FBN1 have enabled prenatal diagnosis, the large number of mutations precludes a simple screening test (107). Fibrillin microfibrils are required for deposit of elastin, whose aggregation with fibrillin provides elasticity, which is defective in aortic vessels (107,108). Defects in the structural integrity of the vessel have raised the important possibility that matrix metalloproteinases could play a pathogenic role in the connective tissue abnormalities in Marfan's syndrome.

Fig. 11.

Connective Tissue	Known Constituents	Approximate Amounts, % dry wt	Characteristics or Functions
Aorta	Type I collagen	20-40	Fibril network
	Type III collagen	20-40	Thin fibrils
	Elastin, fibrillin	20-40	Provide elasticity
	Type IV collagen, laminin, nidogen	<5	Form basal lamina
	Types V and VI collagens	<2	Functions unclear
	Proteoglycans	<3	Provide resiliency

Recent immunohistological analysis of the aortic roots in 7 patients with Marfan's syndrome support the hypothesis that alterations of MMPs and endogenous inhibitors TIMPs may contribute to the cardiovascular and valvular lesions. In sections of thoracic aorta with Marfan's syndrome, extensive cystic medial necrosis (CMN), loss of elastic fibers, collagen, and smooth muscle cells primarily in central regions are present. Inflammatory cells are absent in CMN as are small elastic fibers that connect larger elastic lamellae (109). Normal aortic tissue expresses modest amounts of MMP-1, MMP-2, and MMP-9 in endothelial cells and macrophages, weak quantities in smooth muscle cells, and virtually none in elastic fibers. Expression of TIMP-1 and TIMP-2 is weak in corresponding cell types in normal aorta. In Marfan's syndrome, increased expression of MMP-2 and MMP-9 (gelatinase A and gelatinase B, respectively), which degrade basement membrane collagen type IV and partly digest elastin, was demonstrated in patients in abdominal aortic aneurysms compared to controls (109). The association of immunoreactive gelatinases, MMP2 and MMP-9, on damages elastin fibers parallels the similar observations in pulmonary emphysema, and relationships of MMPs and collagen type IV in alveolar damage and idiopathic pulmonary fibrosis (110). Affected aortic valves exhibited moderate immunoractivity of MMPs and weakly reactive TIMPs primarily in regions of myxoid changes containing fibroblasts/myofibroblasts. Increased expression of MMPs (MMP-1, MMP-2, MMP-3, and MMP-9), without corresponding elevation in TIMPs (TIMP-1 and TIMP-2), provides topographical evidence that abnormal remodeling may be a primary and not secondary role of this connective tissue disease.

The clinical decision to replace the prosthetic root without the valve may warrant reevaluation in light of underlying involvement of the cardiac valves. Alternatively, if MMP/TIMP imbalance plays an etiologic role in tissue destruction then future interventions may include specific forms of medical therapy targeted to the ECM in Marfan's syndrome (109). New insights into the functions of fibrillin-1, and the roles of MMP/TMP expression in tissue homeostasis, could forge new opportunities using small animals models of the human disease to tackle the mechanisms of disease pathogenesis and potential therapeutic strategies targeted to the extracellular matrix for Marfan's syndrome (111), Fig. 12.

Fig. 12.

Matrix Metalloproteinases and Inhibitors in Marfan's Syndrome

A Hypotheses

- MMP expression is activated in the aorta and cardiac values;
- TIMP expression is down-regulated; and
- MMP/TIMP imbalance is an etiologic factor in tissue destruction and is a potential target for future medical therapy

MATRIX METALLOPROTEINASES:THERAPEUTIC IMPLICATIONS AND CRITIQUE

Therapeutics Strategies for MMPs/TIMPs: Role of Angiotensin-Converting Enzyme. The benefits of angiotensin-converting enzyme inhibitors on clinical outcomes in individuals with congestive heart failure are well recognized in animal models and patients. Tissue remodeling has major functional consequences in patients with progressive congestive heart failure. Angiotensin-converting enzyme inhibitors are the best known therapeutic agents, which favorably impact LV remodeling in heart failure (55). However, the precise mechanisms are still poorly understood. One mechanism of ACE therapy is proposed to attenuate LV remodeling during the early and late phases of ventricular repair after injury (77). By reducing LV afterload, a major determinant of LV wall stress, ACE inhibition is thought to reduce myocardial oxygen consumption and improve ventricular efficiency. A central question, however, is whether LV remodeling and ventricular dilation is primary or secondary consequences in disease pathogenesis. Alteration of the collagen matrix composition or structure could affect myocyte alignment along with LV geometry (7). Since alterations of MMPs/TIMPs levels have been implicated in LV remodeling and heart failure (112), these mediators logically have been the targets of recent investigations (14,96,113,114), (Fig. 13 & 14).

Spinale and colleagues examined the effects of ACE or MMP inhibitor therapy in pacing-induced CHF in pigs (100). After 21 days, MMP inhibitor therapy reduces LV dilation that accompanies chronic pacing but, correspondingly, augments myocardial stiffness. ACE inhibition expectedly has similar effects on LV dilation but does not affect myocardial or chamber stiffness. In contrast, combined ACE and MMP inhibition improved LV pump function without impairment of myocardial stiffness seen with MMP inhibition alone. These results indicate that ACE inhibition

modulates MMP activity through a pathway that affects LV chamber size and mechanics (100). Thus, the beneficial outcome associated with ACE inhibition may involve a mechanism mediated by MMP activity.

Fig. 13.

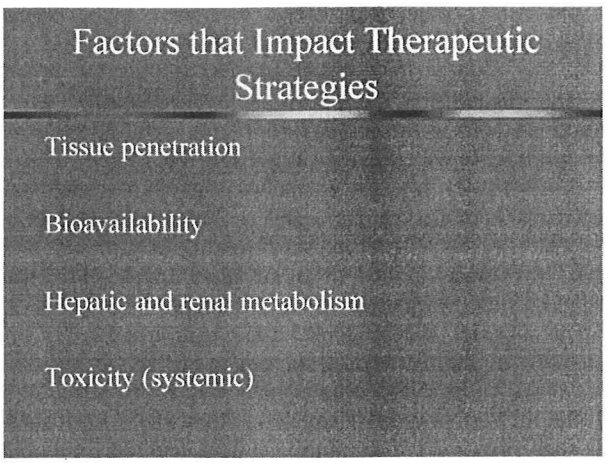
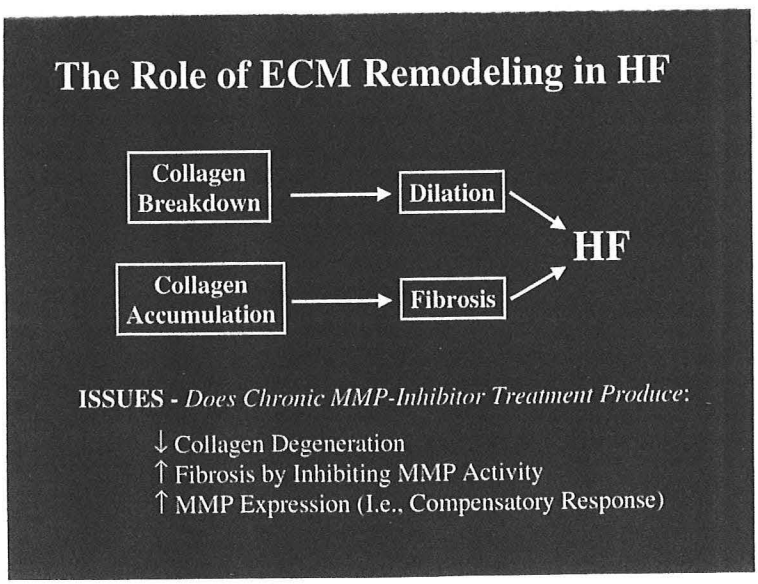


Fig. 14



Summary and conclusion. The cases selected for today's Grand Rounds pose unique diagnostic and therapeutic challenges for the clinician but, perhaps, aptly illustrate fundamental similarities affected by the cellular and biochemical pathways involving matrix metalloproteinases. The activities of MMPs and TIMPs participate a well-orchestrated contest to main tissue integrity. Overexpression of MMPs tilts the balance in favor of irreversible tissue destruction of joints, for example, in rheumatic disease, and efforts to curtail errant ways are been waged (115). Thrombolytic therapy and PTCA represent effective strategies for restoring antegrade flow in occluded vessels, but multiple factors preclude the majority of patients with acute myocardial infarction from receiving either of these treatments. Tissue healing and remodeling is independent of these interventions making the biology of matrix metalloproteinases universally applicable. Although the current state of existing knowledge from experimental models and patients is descriptive, such information lays the foundation for ongoing and future research aimed at the fundamental mechanisms in disease pathogenesis. Basic lessons from biochemistry, enzymology, crystal structure, mechanisms of gene expression will likely impact the development of therapies involving matrix proteinases and their inhibitors. In addition, formidable challenges face investigators using existing pharmacotherapeutics ranging from bioavailability to tissue penetration to toxicity in animal models. For congenital diseases such as Marfan's syndrome, which primarily affects the connective tissue, future therapies may be targeted to the underlying pathobiology involving matrix metalloproteinases. Strategies aimed at correction of the genetic defect may be complemented with those to prevent or ameliorate fundamental imbalances in matrix turnover and deposition. The challenges and opportunities are ahead and remain sharply defined.

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