Journal of Cardiovascular Pharmacology and Therapeutics
2016, Vol. 21(4) 368-371

DOI: 10.1177/1074248415615237
cpt.sagepub.com
SAGE

Hypothesis: Metalloproteinase Inhibitors Decrease Risks of Cardiovascular Disease

Michelle Lizotte-Waniewski, PhD[1], Keith Brew, PhD[2], and Charles H. Hennekens, MD, DrPH[1]

Abstract

The hypothesis that matrix metalloproteinase (MMP) inhibitors reduce risks of cardiovascular disease in humans is plausible, unproven, and difficult to test, due, in part, to differences in specificity and route of administration. Endogenous tissue inhibitors of metalloproteinases (TIMPs) are tight-binding, protein inhibitors that function *in vivo* and can be engineered to enhance specificity for desired targets. Nonetheless, TIMPs have been difficult to test, in part, because their secondary functions, including cell growth promotion and angiogenesis, raise concerns about side effects and they cannot be delivered orally. In contrast, doxycycline and other chemically modified tetracyclines are broad-spectrum, reversible MMP inhibitors with lower affinity but can be taken orally and have US Food and Drug Administration approval. The completed phase 2 randomized trials in humans of MMP inhibitors have methodologic limitations but generally show no significant benefits with adverse effects. At present, the principal research challenge is to achieve a better understanding of the complexities of biological functions of MMPs and subsequently to conduct large-scale phase 3 trials.

Keywords

cardiovascular disease, acute myocardial infarction, heart failure, molecular biology

Introduction

The transitory proteolytic breakdown of specific protein components of extracellular matrices (ECMs) is a necessary component of many normal physiological processes, including developmental tissue remodeling, wound healing, and embryo implantation.[1] Conversely, anomalous proteolysis is a pathologic feature of cardiovascular and other inflammatory diseases.[2] Members of the large matrix metalloproteinase (MMP) family play major roles in degradation of all matrix proteins. Thus, the use of exogenous inhibitors of specific MMPs has the potential to treat or prevent diseases involving pathologic ECM breakdown.[2]

Numerous synthetic MMP inhibitors have been tested in randomized trials, mainly in cancer, and their results have been disappointing. The findings of no significant evidence for benefit have been attributed to deficiencies in specificity, kinetics, and limitations in trial design.[3]

In animal, but not human, models of myocardial infarction (MI), inhibition of MMPs by synthetic inhibitors reduces adverse remodeling of the left ventricle (LV).[4] Further, endogenous tissue inhibitors (TIMPs) that regulate the activities of MMPs *in vivo*, but have been generally dismissed as leads for developing clinical treatments, can be exogenously introduced to reduce adverse post-MI LV remodeling and inflammatory processes.[4,5] In addition, tetracycline, which was initially discovered as an antibacterial agent and used to develop a family of antibiotics and derivatives, also inhibits MMPs. In rodent hearts, doxycycline has a cardioprotective effect during reperfusion mediated by MMP-2 inhibition.[6] In one small phase 2 randomized trial in humans, doxycycline reduces the adverse LV remodeling, infarct size, severity of MI, and LV dysfunction.[7] Doxycycline is approved by the US Food and Drug Administration for treating tissue degradation by MMPs in periodontitis.[8,9] Thus, synthetic MMP inhibitors, TIMPs, and chemically modified tetracyclines (CMTs) are promising but unproven agents to decrease risks of cardiovascular diseases.[10]

Basic Research

Matrix Metalloproteinases

The 23 MMPs encoded by the human genome are classified as collagenases, gelatinases, stromelysins, and membrane-bound MMPs, based on their substrates and structures.[1] Unregulated

[1] Department of Integrated Medical Science, Charles E. Schmidt College of Medicine, Florida Atlantic University, Boca Raton, FL, USA
[2] Department of Basic Science, Charles E. Schmidt College of Medicine, Florida Atlantic University, Boca Raton, FL, USA

Manuscript submitted: June 29, 2015; **accepted:** September 13, 2015.

Corresponding Author:
Charles H. Hennekens, Department of Integrated Medical Science, Charles E. Schmidt College of Medicine, Florida Atlantic University, 777 Glades Road, Boca Raton, FL 33431, USA.
Email: chenneke@health.fau.edu

MMP proteolysis is associated with increased risks of cardiovascular diseases. Specifically, MMPs are prominent players in matrix destruction post-MI, with gelatinases A and B (MMP-2 and MMP-9) playing important roles during the early post-MI period.[2,11] Matrix metalloproteinase 2 is expressed in the heart, but several other MMPs, including MMP-9, are not. These enzymes are produced by inflammatory cells, particularly neutrophils, which migrate into the cardiac matrix post-MI. The ECM that is degraded and remodeled post-MI is composed of type I, III, and IV collagens, fibronectins, laminins, and matricellular proteins including osteonectin, periostin (osteoblast-specific factor 2), and thrombospondin 1.[12] The cardiac ECM is not merely structural but interacts with and influences myocytes and fibroblasts through cell surface integrins.[12] In addition to the breakdown of ECM and other extracellular proteins by secreted MMPs, nonsecreted forms of MMP-2 can target intracellular proteins including sarcomere proteins such as troponin I, myosin light chain 1, α-actinin, and titin causing cellular damage and death.[13]

Like other proteases that do not function in digestive processes, active forms of MMPs normally have restricted lifetimes *in vivo* and their production is firmly regulated at the levels of synthesis, activation, and through inhibition. They are synthesized as inactive precursors, or pro-MMPs, which are activated through proteolytic removal of N-terminal pro-domains; this process can be catalyzed by other proteases, or autolytically, or promoted by chemical modification (oxidation).

Tissue Inhibitors of Metalloproteinases

Tissue inhibitors of metalloproteinases 1 through 4 are tight-binding, endogenous inhibitors of the MMPs, whereas circulatory MMPs are inhibited by the serum protein, α_2-macroglobulin. Like many proteins, TIMPs have an array of additional "moonlighting" functions. For example, TIMP-2 also mediates the activation of pro-MMP-2 in the pericellular environment. Tissue inhibitors of metalloproteinase 2 binds to the noncatalytic hemopexin domain of pro-MMP-2 and forms a membrane-anchored complex by also binding to the active site of a molecule of membrane-type MMP-14. The pro-MMP-2 component of this complex is then activated by proteolytic cleavage of the pro-domain by a second MMP-14 molecule. Tissue inhibitors of metalloproteinase 4 also binds to pro-MMP-2 through the hemopexin domain, in competition with TIMP-2, but it forms a complex that cannot be activated. Since TIMP-4 is the most abundant TIMP in the heart, pro-MMP-2 activation is normally regulated.[13] Post-MI, however, reperfusion generates reactive oxygen species that activate pro-MMP-2 and others by oxidizing a cysteine in the pro-domain.[14] This process triggers an upsurge in proteolytic activity arising from endogenous cells in the myocardium, together with the inflammatory cells that invade post-MI.[2,15]

Tissue inhibitors of metalloproteinases are also potential therapeutic agents for MMP-related pathologies.[13] As endogenous proteins, TIMPs are verified as *in vivo* MMP inhibitors. Although they are generally irreversible inhibitors of a broad spectrum of MMPs, they can be engineered to narrow their range of targets.[13] Tissue inhibitors of metalloproteinase 3 inhibits the largest array of MMPs including several proinflammatory molecules such as tumor necrosis factor α (TNF-α)-converting enzyme or A Disintegrin And Metalloproteinase 17 (ADAM-17) that releases a soluble, active form of the inflammatory cytokine, TNF-α , from a membrane bound precursor. TIMP-3 also inhibits the aggrecanases, A Disintegrin And Metalloproteinase with thrombospondin type 1 motifs 4 and 5 (ADAM-TS4 and ADAM-TS5), that initiate the degradation of the proteoglycan, aggrecan, in cartilage.[13] Little free TIMP-3 is present in tissues since it binds tightly to sulfated oligosaccharides in the ECM and is also endocytosed by cells after it has been secreted.[16,17] Hyaluronic acid hydrogel was used to encapsulate recombinant human TIMP-3, to which it binds through electrostatic interactions, allowing slow release *in vivo*. In pigs, MI was induced by coronary ligation, and the hydrogel/TIMP-3, hydrogel alone, or saline was injected into the MI region in subgroups. During 14 days of observation, both infarct size and LV dilatation were reduced in the TIMP-3-treated pigs that also showed lower levels of inflammatory cytokines and increased smooth muscle actin content relative to the controls.[5] The continuous delivery of TIMP-3 obstructed post-MI remodeling in the animals treated with TIMP-3. Although TIMP-3 was effective at mitigating adverse MI effects in pigs, the fact that TIMP-4 is expressed in a narrow range of organs, and is the predominant TIMP in the heart, directs attention to this inhibitor for the treatment of cardiac disease. In addition, overexpression of human TIMP-4 from an adenoviral construct, or through a cardiac-specific overexpression transgenic construct in post-MI mice, improved LV dilatation as well as ejection fraction and increased fibrillar collagen expression in the MI region, suggesting that exogenous TIMP-4 promotes matrix stabilization.[18]

Clinical Research With MMP Inhibitors

In the human heart, exogenous MMP inhibitors have the potential to minimize harmful effects of LV remodeling post-MI. Nonetheless, some MMPs have crucial functions that must be maintained. Thus, any clinical applicability of MMP inhibitors must include selective inhibition of desired targets or localized delivery to a tissue. Multiple synthetic MMP inhibitors have been developed, including some that appeared to be specific *in vitro* and had efficacy in animal models of various human diseases. When these were tested in randomized trials in humans, none had efficacy and many caused serious adverse events including anemia, musculoskeletal, and liver abnormalities.[19] This may reflect their nonspecific binding to other MMPs and proteins in humans. Specifically 2 types of inhibitors have been tested in humans, each in a phase 2 randomized trial of small sample size of LV remodeling post-MI. Each of these is useful to formulate but not to test the hypothesis of clinical benefits in cardiovascular disease.[20] These inhibitors include PG116800, a hydroxamate inhibitor, and doxycycline (Table 1).[4,21,22] The trial of PG116800 was reported to show no significant evidence of benefit, but the point estimate is

Table 1. Randomized Trials in Humans of MMP Inhibitors and LV Remodeling.

MMP Inhibitor	Molecule Type	Sample Size	Primary Outcome
PG-116800	Hydroxamate	253	PG-116800 did not reduce LV remodeling or improve clinical outcomes following myocardial infarction
Doxycycline	Modified tetracycline	110	Doxycycline reduced adverse LV remodeling, infarct size, severity of myocardial infarction, and LV dysfunction

Abbreviations: LV, left ventricle; MMP, matrix metalloproteinase.

compatible with no effect as well as small to moderate benefit or harm. In addition, there may have been inadequate dosing.[4] In contrast, doxycycline significantly reduced the adverse LV remodeling, infarct size, severity of MI, and LV dysfunction. These discrepancies in findings may be real but also may have been due, at least in part, to the size of the samples as well as the lower affinity of doxycycline for MMPs in comparison with most synthetic MMP inhibitors and TIMPs (K_i in the 10^{-5} mol/L range vs $\leq 10^{-9}$ mol/L).[21,22]

Research Challenges in Hypothesis Testing

Matrix metalloproteinase inhibitors are a plausible but unproven means to treat various clinical manifestations of cardiovascular diseases. Although TIMPs are tight-binding, essentially irreversible, inhibitors of MMPs that function *in vivo* and can be engineered to enhance their specificity for desired targets, they are difficult to test clinically because their secondary functions on cell growth and differentiation, cell migration, and angiogenesis raise concerns about serious adverse effects. Tissue inhibitors of metalloproteinases cannot be delivered orally but, when embedded in a matrix, can be delivered locally and released slowly. In addition, doxycycline and other CMTs are broad-spectrum MMP inhibitors that bind reversibly and with lower affinity. They can be taken orally and may be more clinically useful than high-affinity inhibitors that completely block enzyme action. One of the research challenges in testing the hypothesis is the need for rigorous testing and exploration of less conventional approaches. Support for this approach derives from the previously discussed promising results using a modified form of TIMP-3 in an animal model and other clinical studies with the relatively weak inhibitor, doxycycline. The latter may imply that partial, relatively nonspecific inhibition of MMPs may yield more favorable results.[7,23]

Conclusions

The completed randomized trials of MMP inhibitors showing no significant benefits have clear limitations in their design features that can be addressed. A greater and more fundamental research challenge is to better understand the biological functions and complexities of MMPs and their natural inhibitors. Such basic research findings may form the basis to conduct large-scale phase 3 randomized trials in humans. At present, the potential clinical and public health impact of MMP inhibition in the treatment of cardiovascular diseases is enormous. Thus, the adequate testing of this important and timely hypothesis will be extremely difficult but requires methodological rigor to be able to conclude whether there are clinical benefits or, to paraphrase Huxley, it is a beautiful hypothesis that is slain by ugly facts.[24]

Authors' Note

M. Lizotte-Waniewski contributed to conception and design; acquisition, analysis, and interpretation; drafted the manuscript; critically revised the manuscript; gave final approval; and agreed to be accountable for all aspects of work ensuring integrity and accuracy. K. Brew contributed to conception and design; acquisition, analysis, and interpretation; critically revised the manuscript; gave final approval; and agreed to be accountable for all aspects of work ensuring integrity and accuracy. C. H. Hennekens contributed to conception and design; acquisition, analysis, and interpretation; critically revised the manuscript; gave final approval; and agreed to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of Conflicting Interests

Dr. Lizotte-Waniewski, Professor Brew and Professor Hennekens reported that they are funded by the Charles E. Schmidt College of Medicine at Florida Atlantic University. Professor Hennekens reported that he serves as an independent scientist in an advisory role to investigators and sponsors as: Chair or Member of Data and Safety Monitoring Boards for Amgen, AstraZeneca, Bayer, Bristol Myers-Squibb, British Heart Foundation, Cadila, Canadian Institutes of Health Research, DalCor, Genzyme, Lilly, Regeneron, Sanofi, Sunovion and the Wellcome Foundation; to Aralez/Pozen, the United States (U.S.) Food and Drug Administration, UpToDate, and legal counsel for Pfizer and Takeda; receives royalties for authorship or editorship of 3 textbooks and as coinventor on patents for inflammatory markers and CV disease that are held by Brigham and Women's Hospital; has an investment management relationship with the West-Bacon Group within SunTrust Investment Services, which has discretionary investment authority and does not own any common or preferred stock in any pharmaceutical or medical device company.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

References

1. Mandhare MN, Patil PH, Jagdale DM, Kadam VJ. Targeting matrix metalloproteinases: an important strategy in cancer therapeutics. *Int J Pharm Sci Rev Res*. 2012;12(2):27-39.
2. Vanhoutte D, Schellings M, Pinto Y, Heymans S. Relevance of matrix metalloproteinases and their inhibitors after myocardial

infarction: a temporal and spatial window. *Cardiovas Res*. 2006; 69(3):604-613.
3. Vandenbroucke RE, Libert C. Is there new hope for therapeutic matrix metalloproteinase inhibition? *Nat Res Drug Discov*. 2014;13(12):904-927.
4. Spinale FG, Villarreal F. Targeting matrix metalloproteinases in heart disease: lessons from endogenous inhibitors. *Biochem Pharmacol*. 2014;90(1):7-15.
5. Eckhouse SR, Purcell BP, McGarvey JR, et al. Local hydrogel release of recombinant TIMP-3 attenuates adverse left ventricular remodeling after experimental myocardial infarction. *Sci Transl Med*. 2014;6(223):1-10.
6. Cheung P-Y, Sawicki G, Wozniak M, Wang W, Radomski MW, Schulz R. Matrix metalloproteinase-2 contributes to ischemia-reperfusion injury in the heart. *Circulation*. 2000;101(15): 1833-1839.
7. Cerisano G, Buonamici P, Valenti R, et al. Effects of a timely therapy with doxycycline on the left ventricular remodeling according to the pre-procedural TIMI flow grade in patients with ST-elevation acute myocardial infarction. *Basic Res Cardiol*. 2014;109(4):412.
8. Golub LM, Ramamurthy NS, McNamara TF, Greenwald RA, Rifkin BR. Tetracyclines inhibit connective tissue breakdown: new therapeutic implications for an old family of drugs. *Crit Rev Oral Biol Med*. 1991;2(3):297-321.
9. Peterson JT. Matrix metalloproteinase inhibitor development and the remodeling of drug discovery. *Heart Fail Rev*. 2004;9(1): 63-79.
10. Hennekens CH, DeMets D. Statistical association and causation: contributions of different types of evidence. *JAMA*. 2011;305(11): 1134-1135.
11. Patterson NL, Iyer RP, delCastro Bras LE, et al. Using proteomics to uncover extracellular matrix interactions during cardiac remodeling. *Proteom Clin Appl*. 2013;7(7-8):516-527.
12. Bowers SLK, Banerjee I, Baudino TA. The extracellular matrix: at the center of it all. *J Mol Cell Cardiol*. 2010; 48(3):474-482.
13. Brew K, Nagase H. The tissue inhibitors of metalloproteinases (TIMPs): an ancient family with structural and functional diversity. *Biochim Biophys Acta*. 2010;1803(1):55-71.
14. DeCoux A, Lindsey ML, Villareal F, Garcia RA, Schulz R. Myocardial matrix metalloproteinase-2: inside out and upside down. *J Mol Cell Cardiol*. 2014;77:64-72.
15. Cadete VJJ, Arcand SA, Chaharyn BM, et al. Matrix metalloproteinase-2 is activated during ischemia/reperfusion in a model of myocardial infarction. *Can J Cardiol*. 2013;29(11): 1495-1503.
16. Yu WH, Yu S, Meng Q, Brew K, Woessner JF. TIMP-3 binds to sulfated glycosaminoglycans of the extracellular matrix. *J Biol Chem*. 2000;275(40):31226-31232.
17. Troeberg L, Lazenbatt C, Anower-E-Khuda MF, et al. Sulfated glycosaminoglycans control the extracellular trafficking and the activity of the metalloproteinase inhibitor TIMP-3. *Chem Biol*. 2014;21(10):1300-1309.
18. Zavadzkas JA, Stroud RE, Bouges S, et al. Targeted overexpression of tissue inhibitor of matrix metalloproteinase-4 modifies post-myocardial infarction remodeling in mice. *Circ Res*. 2014; 114(9):1435-1445.
19. Milner JM, Cawston TE. Matrix metalloproteinase knockout studies and the potential use of matrix metalloproteinase inhibitors in the rheumatic diseases. *Curr Drug Targets Inflamm Allergy*. 2005;4(3):363-375
20. Hennekens CH, DeMets D. The need for large-scale randomized evidence without undue emphasis on small trials, meta-analyses, or subgroup analyses. *JAMA*. 2009;302(21):2361-2362.
21. Hudson MP, Armstrong PW, Ruzyllo W, et al. Effects of selective matrix metalloproteinase inhibitor (PG-116800) to prevent ventricular remodeling after myocardial infarction. *J Am Coll Cardiol*. 2006;48(1):15-20.
22. Cerisano G, Buonamici P, Valenti R, et al. Early short-term doxycycline therapy in patients with acute myocardial infarction and left ventricular dysfunction to prevent the ominous progression to adverse remodeling: the TIPTOP trial. *Eu Heart J*. 2014;35(3): 184-191.
23. Djafarzadeh R, Sauter M, Notohamiprodjo S, et al. Recombinant GPI-anchored TIMP-1 stimulates growth and migration of peritoneal mesothelial cells. *PLos One*. 2014;7(4):e33963.
24. Huxley TH. *Collected Essays of Thomas Huxley: Discourses Biological and Geological Part Eight*. Albany, NY: Kessinger Publishing; 1894.