The Heart Surgery Forum #2008-1152
12 (4), 2009 [Epub August 2009]
doi: 10.1532/HSF98.20081152 Online address: http://cardenjennings.metapress.com

Circulating Matrix Metalloproteinase 3 due to Myocardial Ischemia

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ABSTRACT

Background: Experimental data suggest that matrix metalloproteinases (MMPs) such as MMP-3 have a central role in the remodeling period after a myocardial infarction (MI). The aim of this study was to use an experimental small-animal model to investigate the fluctuation in MMP-3 levels occurring in vivo after an acute MI.

Methods: We studied 13 New Zealand white rabbits weighing between 3 and 4 kg. After anesthetizing the animals, we performed a tracheotomy and induced an acute MI in 10 of the animals by occluding the left anterior descending coronary artery for 45 minutes. The remaining 3 rabbits constituted the control group. Three hours after reperfusion, blood samples were taken for biomedical analyses.

Results: Three hours after the artificially induced acute MI, serum MMP-3 levels were decreased by almost 50%. Cardiac troponin I (cTnI) concentrations were increased greatly (90-fold) after MI, further validating the efficiency of our experimental in vivo model of acute MI.

Conclusion: Combining the data, we demonstrated that acute MI caused an early reduction in MMP-3 levels. The range of MMP-3 reduction is limited compared with other factors predicting MI, such as cTnI, which increases its usefulness. We demonstrated, however, that plasma fluctuation in MMP-3 levels could be used as a supplementary independent predictor of cardiovascular events in patients with stable coronary artery disease. This acute MI model used in our controlled setting proved to be a reliable and safe method for conducting in vivo studies.

INTRODUCTION

Myocardial infarction (MI) leads to complex architectural alterations involving the infarcted and noninfarcted myocardium, cardiac remodeling, and heart failure. Adverse cardiac remodeling, including cardiac myocyte elongation, alterations in the extracellular matrix, wall thinning, an increase in end-diastolic diameter, and a decrease in ejection fraction, may occur despite the use of such agents as angiotensin-converting enzyme inhibitors or β-adrenergic blockers [Greemers 2001; Romanic 2001]. Matrix metalloproteinases (MMPs) are a family of zinc-dependent enzymes comprising more than 25 members divided into several classes according to their specificity for various extracellular matrix (ECM) substrates [Spinale 2002]. Of the MMPs, the role of stromelysins such as MMP-3 after an MI is of particular interest because MMP-3 can act on a wide range of myocardial substrates and activate several other MMPs [Romanic 2001]. MMP-3 is capable of degrading a wide range of ECM components, including collagens III, IV, V, and IX, elastin, proteoglycans, laminin, and fibronectin, and can activate other MMPs, as well as its own proenzyme, pro–MMP-3 [Samnegard 2006]. Pro–MMP-3 can be activated by a variety of proteinases, such as plasma kallikrein, plasmin, neutrophil elastase, and cathepsin G, which are activated by inflammatory cytokines, hormones, growth factors, and cell surface proteins [Okada 1989] (Figure 1).

Experiments have shown that MMP-3, also known as stromelysin 1, is an efficient activator of the latent form of MMP-9. The initial rates of activation of pro–MMP-9 vary linearly with stromelysin 1 concentration, and the slopes of the lines correlate with the values of measured kinetic parameters [Ogata 1992; Lu 2000]. In vitro and in vivo studies have generated various and conflicting results, and questions have arisen regarding whether a transient change

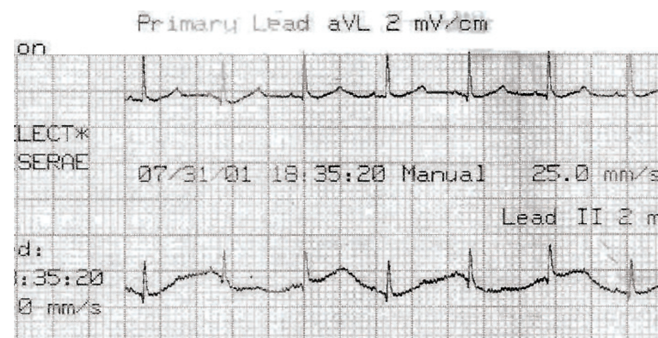

Figure 1. Cascade of matrix metalloproteinase 3 (MMP-3) and MMP-9 activation.

Presented in part at the 18th WSCTS World Congress, World Society of Cardiothoracic Surgeons, April 30 to May 3, 2008, Kos, Greece.

Received December 20, 2008; accepted March 24, 2009.

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Table 1. Values for Blood Parameters of the Control Group (n = 3)*

Variable	Descriptive Statistics			
	Minimum	Maximum	Mean	SD
pO_2, mm Hg	99	359	250.33	135.149
pCO_2, mm Hg	44.30	96.00	62.8667	28.76392
pH	7.20	7.40	7.3000	0.10000
HCO_3^-, mEq/L	19.80	34.60	25.667	7.92233
K^+, mmol/L	3.10	3.40	3.3000	0.17321
Na^+, mmol/L	141	145	142.67	2.082
Hemoglobin, g/L	9.20	9.80	9.5667	0.32146
Lactate, mmol/L	1.10	1.60	1.3333	0.25166
cTnI (before), ng/mL	0.30	0.50	0.3667	0.11547
cTnI (after), ng/mL	0.30	1.10	0.6333	0.41633
MMP-3 (before), ng/mL	1.00	2.50	1.8667	0.77675
MMP-3 (after), ng/mL	1.00	2.50	1.8667	0.77675

*cTnI indicates cardiac troponin I; MMP-3, matrix metalloproteinase 3.

in serum MMP-3 concentration occurs during the acute phase of MI. Regarding the existing literature related to in vivo experiments involving rabbits as an experimental small-animal study model, our PubMed search revealed only a single study that reported an alteration in MMP-3: a study of rabbit cardiac myocytes [Romanic 2001].

In view of the above information, the purpose of this study was to investigate in vivo fluctuations in MMP-3 levels in plasma after an acute MI in the early phase. We used a small animal as an experimental study model, because, according to the literature, similar studies that have been made with a Langendorff heart preparation and the addition of bank blood can adversely affect any changes in MMPs [Lorell 1988; Yellon 1992].

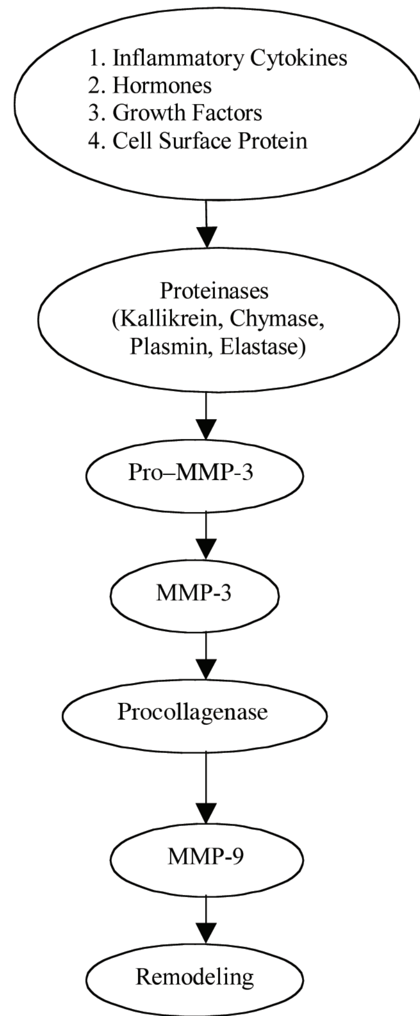

Figure 2. ST elevation on the electrocardiogram during the first minute after the occlusion of the left anterior descending coronary artery.

MATERIALS AND METHODS

Experimental Protocol

Our study sample consisted of 13 New Zealand white rabbits (weight, 3-4 kg). Acute MI was induced in 10 of the rabbits by ligating the left anterior descending coronary artery. The 3 remaining animals constituted the control group.

All animals received proper care in the experimental surgery laboratory of our institution in compliance with the Principles of Laboratory Animal Care, formulated by the National Society for Medical Research, and the Guide for the Care and Use of Laboratory Animals, prepared by the National Academy of Sciences and published by the National Institutes of Health [Committee on Care and Use of Laboratory Animals 1996].

Rabbit MI Model

Rabbits were anesthetized intramuscularly with a mixture of ketamine and xylazine (50 mg/kg and 25 mg/kg, respectively). We performed a tracheotomy under aseptic conditions and then intubated the rabbits. The rabbits were ventilated with weight-matched tidal volumes and a fraction of inspired oxygen (FIO_2) of 100%.

A 4-lead electrocardiogram was recorded throughout the procedure. Monitoring of blood pressure and heart rate was accomplished through a femoral artery cutdown. Before the operation blood samples were taken from the ear artery catheter for analysis of the pH, pCO_2, pO_2, and other variables, including glucose, urea, uric acid, total protein, albumin, aspartate aminotransferase, alanine aminotransferase, MMP-3, and cardiac troponin I (cTnI).

A middle sternotomy was performed. After intravenous injection of 300 IU of heparin, we temporarily occluded the left anterior descending coronary artery for 45 minutes to cause an acute MI without preconditioning [Iliodromitis 1997]. The onset of ischemia was verified by an ST elevation on the electrocardiogram. The occlusion was released 45 minutes later, and the flow in the vessel was restored. Blood samples were taken 3 hours after reperfusion for biomedical analyses. The control group was subjected to the entire

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Table 2. Values for Blood Parameters of the Case Group (n = 10)*

Variable	Descriptive Statistics			
	Minimum	Maximum	Mean	SD
pO_2, mm Hg	222	568	413.50	115.989
pCO_2, mm Hg	18.40	53.00	33.9500	12.88127
pH	7.26	7.60	7.3990	0.12688
HCO_3^-, mEq/L	17.20	27.60	21.0800	3.11334
K^+, mmol/L	2.43	5.80	3.5630	0.97155
Na^+, mmol/L	131	149	140.60	5.777
Hemoglobin, g/L	7.60	11.30	9.4900	1.14935
Lactate, mmol/L	1.60	6.30	3.9700	1.39845
cTnI (before), ng/mL	0.30	0.50	0.3400	0.06992
cTnI (after), ng/mL	73.00	108.50	89.7400	14.46991
MMP-3 (before), ng/mL	3.70	9.00	6.9300	1.55353
MMP-3 (after), ng/mL	1.00	8.00	3.2600	2.07536

*cTnI indicates cardiac troponin I; MMP-3, matrix metalloproteinase 3.

sternotomy procedure, monitoring, and sampling without the occlusion of the left anterior descending coronary artery.

A lethal dose of ketamine and xylazine was injected through the ear vein, followed by 10 mL of cardioplegic solution to arrest the heart in diastole. The heart was removed from the chest for further histologic examination.

Laboratory Methods

Biomedical analysis was performed in an automated analyzer (Olympus AO 600; Tokyo, Japan) with the use of Olympus reagents.

cTnI values were estimated by immunoassay with an AxSYM apparatus (Abbott Laboratories, Abbott Park, IL, USA). The assay is based on microparticle enzyme immunoassay technology [Abbott Laboratories 2001]. Rabbits were selected as the experimental model because Okubo et al [2000] first reported cTnI values in myocardial contusions in rabbits, and the levels of monophosphorylated cTnI in the heart areas of humans and rabbits are similar [Ardelt 1998].

MMP-3 was measured in the serum of rabbits with a commercially available enzyme-linked immunosorbent assay (ELISA) (Biotrak; GE Healthcare Life Sciences, Buckinghamshire, UK). In brief, rabbit serum and quality-control materials (controls and standards) were incubated in microtiter wells of ELISA plates coated with antirabbit MMP-3 antibodies. To detect the formed complex, we labeled a second antibody to rabbit MMP-3 with peroxidase and incubated it in the wells. The excess of unbound enzyme-labeled antibody was removed by washing and aspiration. A specific substrate for peroxidase was incubated in the wells. A colored product developed, and we terminated the reaction by adding an acid solution. The color intensity in each well was read at 450 nm with a spectrophotometer. The concentration of MMP-3 in each sample was determined by interpolation from the standard curve produced in the same experiment [Ardelt 1998].

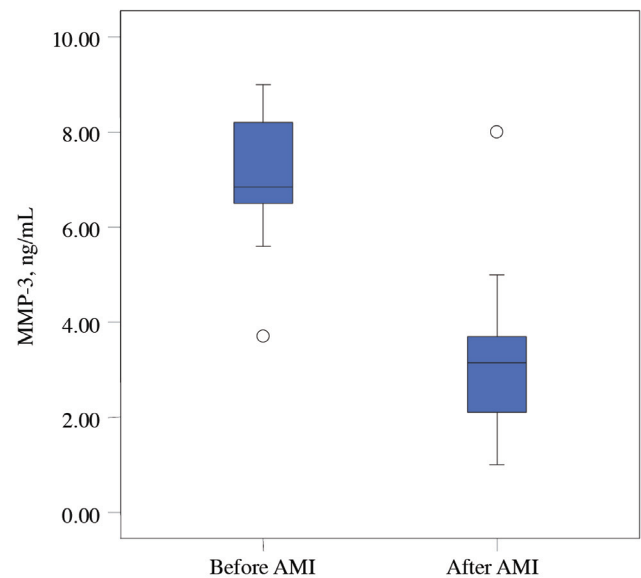

Figure 3. Matrix metalloproteinase 3 (MMP-3) values before and after acute myocardial infarction (AMI). Data are presented as mean ± SEM. Whiskers represent the range of the data.

Associations between blood variables were evaluated by means of the Mann-Whitney *U* test and the Wilcoxon *W* test, as appropriate. Values are expressed as the mean ± SEM. Differences with *P* values <.05 were considered statistically significant.

RESULTS

All of the rabbits subjected to acute MI survived the entire experimental protocol (approximately 6 hours). Figure 2 shows an electrocardiogram of the infarcted rabbit heart. The MMP-3 levels and other variables were assessed in serum samples taken before the acute MI and at 3 hours after reperfusion. The results (summarized in Tables 1 and 2 for the control and case groups, respectively) show the stable condition of the experimental animals. According to the statistical analysis, a significant decrease in serum MMP-3 concentration was observed 3 hours after reperfusion in the animals subjected to an acute MI (Figure 3). In addition, despite the extensive acute MI and 90-fold higher cTnI values (Figure 4), the measured values for pO_2, pCO_2, pH, HCO_3^-, K^+, Na^+, and hemoglobin were all at acceptable levels (Table 3). Table 4 shows that the differences in cTnI and MMP-3 values before and after the acute MI were statistically significant.

DISCUSSION

A wealth of clinical and experimental data suggests that MMPs have a central role in post-MI remodeling and that MMP inhibition after acute MI causes a reduction in left ventricular remodeling without inhibiting neovascularization [Lindsey 2002].

Table 3. Statistical Analysis*

Test Statistic	Variable											
	pO_2	pCO_2	pH	HCO_3^-	K^+	Na^+	Hb	Lactate	cTnI (before)	cTnI (after)	MMP-3 (before)	MMP-3 (after)
Mann-Whitney *U*	5.000	6.000	8.500	9.500	15.000	12.500	12.000	0.500	13.500	0.000	0.000	8.000
Wilcoxon *W*	11.000	61.000	14.500	64.500	70.000	67.500	67.000	6.500	68.500	6.000	6.000	14.000
Z	−1.690	−1.521	−1.100	−0.931	0.000	−0.426	−0.510	−2.454	−0.311	−2.535	−2.539	−1.198
Asymptotic significance (2-tailed)	.091	.128	.271	.352	1.000	.670	.610	.014	.756	.011	.011	.231
Exact significance (2 × 1-tailed)†	.112	.161	.287	.371	1.000	.692	.692	.007	.811	.007	.007	.287

*Group variable: group. Hb indicates hemoglobin; cTnI, cardiac troponin I; MMP-3, matrix metalloproteinase 3.
†Not corrected for ties.

In the present study, we focused on serum levels of MMP-3 and observed a significant decrease after MI. This decrease, according to our study design, pertains to the early (3 hours) period of acute MI. This finding is in contrast with the well-documented induction of MMP-3 that other groups have observed on the second post-MI day [Okada 1989; Ardelt 1998; Samnegard 2006]. Specifically, a previous study that used the Langendorff experimental model evaluated MMP-3 expression in rabbit cardiac myocytes. MMP-3 expression was detected in the ischemic tissue 2 days after coronary artery occlusion and remained up-regulated throughout the 14-day time course, whereas MMP-3 expression was absent in the control samples. The results demonstrated that both MMP-3 and MMP-9 were induced following induction of hypoxia [Romanic 2001]. A second study, which partially confirmed our findings in the present study, demonstrated that serum MMP-3 levels are lower in the acute stage of MI than during the recovery phase. Thus, one can speculate that MMP-3 is more important in determining the composition and turnover of the fibrous cap than in weakening it and hence promotes atherosclerosis progression rather than plaque rupture [Samnegard 2006]. Several studies have observed MMP-3 induction after an acute MI [Okada 1989; Romanic 2001; Samnegard 2006]. In the majority of these studies, MMP-3 induction was observed on or after the second day after an acute MI, leaving a 2-day time window at the acute phase of the ischemic injury.

Because acute MI was induced in our study in a healthy coronary artery via a 45-minute period of ligation and the MMP-3 level was measured 3 hours later, the early decrease in the MMP-3 level cannot be correlated with fibrous cap characteristics; rather, it should be correlated with early MMP-3 excretion patterns of ischemia-reperfusion. Combining the data derived from our study with that of Samnegard and colleagues [Samnegard 2006], we find it obvious that an early reduction in MMP-3 is followed by a gradual induction of MMP-3 on the second post-MI day.

In another study, Wu et al [2005] demonstrated that the baseline plasma MMP-3 concentration could independently predict cardiovascular events in patients with stable coronary artery disease.

The cTnI concentration increases detectably in the serum within 4 to 6 hours after the onset of chest pain, reaches peak

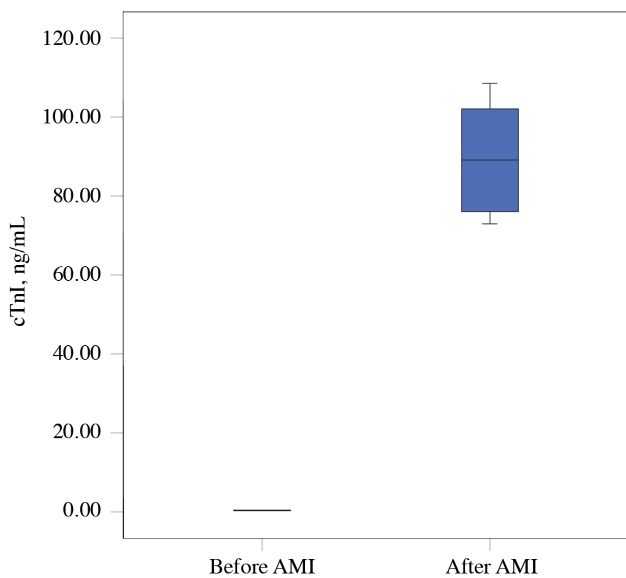

Figure 4. cTn I values before and after acute myocardial infarction (AMI).

Table 4. Statistical Analysis*

	Test Statistic	
	cTnI (after) − cTnI (before)	MMP-3 (after) − MMP-3 (before)
Z	−2.803†	−2.803‡
Asymptotic significance (2-tailed)	.005	.005

*Wilcoxon signed rank test. cTnI indicates cardiac troponin I; MMP-3, matrix metalloproteinase 3.
†Based on negative ranks.
‡Based on positive ranks.

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concentrations in approximately 12 hours, and remains elevated for 3 to 10 days following an acute MI. The temporal pattern of cTnI release following an infarction thus extends across the diagnostic windows of both creatine kinase isoenzyme MB and lactate dehydrogenase [Abbott Laboratories 1997]. The measurement of cTnI levels in serum currently is used as a routine test to detect cardiac injury after trauma [Okubo 2000].

A second finding in our study deserves special attention. cTnI concentrations were found to be greatly increased after MI. This significant increase in cTnI concentrations (90-fold higher) further validates the efficiency of our in vivo experimental animal model of acute MI.

According to the literature, the process of the development of acute MI has been studied mainly through a Langendorff heart preparation with the addition of bank blood. In our study, we preferred the use of an in vivo experimental rabbit model because of its advantages over other possible small-animal models for studying cardiovascular diseases [Marian 1999; Sanbe 2005]. The heart rate of the rabbit is similar to that of the human heart, and this fact is of importance because a faster heart rate negatively influences the refractory period associated with the occurrence of arrhythmia [Boyett 1978]. Furthermore, the manner in which Ca^{2+} is handled during contraction/relaxation and the alteration in Ca^{2+} flux during heart failure in rabbits more accurately reflect those of the human system [Bers 2002]. In support of these suppositions, the predominant myosin isoform in the rabbit myocardium is β-myosin heavy chain, which has an approximately 98% homology to human β-myosin heavy chain [Marian 1999].

In conclusion, serum MMP-3 levels decreased by almost 50% in the acute phase of MI, 3 hours after an artificially induced acute MI. In other studies, the MMP-3 increase that is observed 2 days later appears to be the second phase of the MMP-3 cardiac-secretion pattern and the beginning of the cardiac remodeling process. Further carefully designed studies involving selective sampling techniques and localizing methods might help to distinguish the source of this MMP-3 induction from the infarct, per infarct, or even from extracardiac sources. This model of acute MI involving sternotomy and coronary artery ligation in the controlled setting of the rabbit (as evidenced by myocardial cTnI levels more than 90-fold higher than normal) proved to be a reliable and safe method for conducting in vivo studies and should be of help in future research.

ACKNOWLEDGMENT

The authors gratefully acknowledge the excellent assistance of Katsaraki Aphrodite. for the statistical analysis of the biochemical variables measured in the study.

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