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Serum MMP-9 Activity as a Diagnosing Marker for the Developing Heart Failure of Post MI Patients

Gwo-Ping Jong[1, 4], Tsochiang Ma[2], Pesus Chou[4], Mu-Hsin Chang[1], Chieh-His Wu[3], Ping-Chun Li[5], Shin-Da Lee[6], Jer-Yuh Liu[7], Wei-Wen Kuo[3], and Chih-Yang Huang[7]

[1]*Division of Cardiology*
Armed Force Taichung General Hospital
Taichung
[2]*Department of Health Service Management*
[3]*Department of Biological Science and Technology*
China Medical University
Taichung
[4]*Community Medical Research Center and Institute of Public Health*
National Yang-Ming University
Taipei
[5]*Division of Cardiovascular Surgery*
China Medical University Hospital
Taichung
[6]*School of Physical Therapy*
[7]*Institute of Biochemistry*
Chung-Shan Medical University
Taichung, Taiwan, R.O.C.

Abstract

Myocardial infarction (MI) is the result, in mostly cases, of the destabilization and rupture of atherosclerotic lesions. The destruction of cardiac tissue resulting from myocardial ischemia could further result in heart failure. It has been suggested that plaque instability may be mediated by matrix metalloproteinase (MMP) family. Studies have identified increased MMP-2 and MMP-9 in human platelets, and acute myocardial infarction patients with elevated MMP-2 and MMP-9 levels. However, the alteration of MMP-2 and MMP-9 from post MI left ventricle remodeling to heart failure remains to be clarified. The purpose of this study is to investigate the serum concentrations and activities of MMP-2 and MMP-9 in the developing heart failure from post MI patients. Twenty eight patients with MI without heart failure (Killip FC I) (group A; compensated) and twenty seven MI patients with heart failure (Killip II-III) (group B; decompensated) were collected to evaluate the serum levels and activities of MMP-2 and MMP-9 by ELISA and Zymography, respectively. It was observed that the both serum levels and activities of MMP-9 significantly increased ($P < 0.01$) in decompensated group compared to compensated group, but there was no significant difference of serum MMP-2 levels and activities between two groups. The highly elevated serum MMP-9 concentration of decompensated patients is not related with inflammatory or localized infarct area of myocardium and the real mechanisms remain to be revealed. We suggest that the increase of MMP-9 levels and activity may be used as a new marker to diagnose the development of heart failure in patients with post MI, and provide the therapeutic implications in the future.

Key Words: myocardial infarction, heart failure, MMP-2, MMP-9

Corresponding author: Dr. Chih-Yang Huang, Institute of Biochemistry, Medical College, Chung-Shan Medical University, Taichung 402, Taiwan, R.O.C. Tel: 886-4-24730022 ext. 11682, Fax: 886-4-23248195, E-mail: chuang1@csmu.edu.tw
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Introduction

Many patients who experience a myocardial infarction (MI) may undergo left ventricular (LV) remodeling (10, 17). Post MI LV remolding can result in LV chamber dilatation as well as hypertrophy and fibrosis of noninfarcted myocardium (11). Severity degrees of LV remolding are associated with increased risk for development of heart failure (15).

Accompanying cardiac myocyte cell death in the setting of MI is damage to the existing extracellular matrix (ECM) of the heart, in particular to collagens (11, 15). The cardiac ECM provides structural support and integrity to the myocardium and facilitates the conversion of myocyte contraction into pump function (4, 21, 24, 26). The integrity of the original ECM is thought to play an important role in determining the extent of remolding after MI and matrix metalloproteinases (MMPs) play crucial roles in regulation of ECM.

Recently, several studies have revealed that elevated levels of MMP-2 and MMP-9 are associated with cardiovascular diseases (3, 7, 13, 20). Fernandez-Patron *et al*. (7) identified MMP-2 and MMP-9 in human platelets, and suggested that the MMP-2 or MMP-9 system may have an important role in regulation of platelet-platelet and platelet-vessel wall interactions. Beatriz-Alvarez *et al*. (13) found the serum levels of MMP-2 and MMP-9 is related to unstable plaque. Kai *et al*. (3) also found that 33 patients with unstable angina or acute myocardial infarction showed serum MMP-2 level 2-fold and plasma MMP-9 level 2 to 3-fold higher than those in 17 healthy control subjects. Rosenberg *et al*. (20) further demonstrated that MMP-9 activity increases from the first 12 hours after the ischemic event up to the fifth day afterward, and MMP-2 increases after the fifth day post-ischemia.

Additionally, our previous results also showed that cardiac pro-matrix metalloproteinases (MMP)-2, -9 and active MMP-2 were markedly increased in chicken with natural outbreak dilated cardiomyopathy (DCM) and the cold induced DCM, and this particularly occurred in the right ventricular (27). At the same time, the elevated serum MMPs in patients with mild to moderate dilated cardiomyopathy were found as well (22). Moreover, the activation of MMPs and the concentration of metalloproteinases 2 and 9 are increased in plasma of patients with heart failure, suggesting the MMP activity is directly or indirectly associated with ventricular dilatation and heart failure (1, 2).

However, the differences in ECM regulatory system, especially activation of MMP-2 and MMP-9 from post MI LV remodeling to heart failure remain to be clarified in human. In this study, MMP-2 and MMP-9 have been measured in two sets of patients with a recent MI.

Materials and Methods

Subjects

This study is a perspective study. Between February 2002 and December 2003, a total 55 patients with acute MI admitted for coronary revascularization within 12 hours of symptom onset. Good explanations were made to each patient about the benefit, possible effects of direct angioplasty; all signed consent forms. Acute MI was defined as typical chest pain, ST segment elevation > 0.1 mV in more than 2 leads in 12-lead electrocardiography and a rise in both creatine kinase and its isoenzyme MB to more than twofold the upper limit of the normal range. Successful reperfusion was demonstrated by primary coronary angioplasty in all patients. All patients were treated with appropriate doses of beta-blockers, ACE inhibitors, aspirin and statins within 12 hours of admission and continued on this medication thereafter. The eligible patients were allocated into Killip I (Group A, compensated) and Killip II & III (Group B, decompensated) at the entrance according to the patient's clinical condition (28).

Determination of MMP2 and MMP9

One hour before coronary angioplasty, blood samples were drawn by a trained phlebotomist *via* a venipuncture of an antecubital vein with the patient in the supine position. The blood samples were drawn and immediately used for biochemical autoanalyzer. The other part of blood samples were separated by refrigerated centrifuge 3000 rpm for 15 min at 4°C within1 hour of drawing and subsequently frozen in liquid nitrogen and stored at -80°C until use. Serum MMP2 and MMP9 were determined with a commercially available non-extraction enzyme-linked immunosorbent assay (ELISA), using Biotrak assay systems (Amersham, Arlington Heights, IL, USA) for MMP-2 and Quantikine (R&D Systems, Minneapolis, MA, USA) for MMP-9, validated for use with human serum. ELISAs were performed according to the instructions of the manufacturer. The target proteins were immunosorbented by enzyme-conjugated antibody before adding the corresponding substrates for color development. Protein contents were determined by comparing the relative absorbency of the samples to that of known amounts of standard by a microplate reader (Model: RS01, Kansin Instruments. CO, LTD, Sunnyvale, CA, USA).

Gelatin Zymography Protease Assay

Gelatin zymography analysis was carried out by loading 10 µl sample of serum (1/40) on 0.1%

Table 1. Clinical characteristics of the patients at post MI without HF (compensated) and those with HF (decompensated)

	decompensated (n = 28)	compensated (n = 27)	*P* value
Age	58 ± 12	56 ± 13	NS
Male (%)	21 (75)	19 (70)	NS
Current smoking (%)	10 (36)	11 (41)	NS
Hypertension (%)	13 (46)	12 (44)	NS
Diabetes mellitus (%)	5 (18)	7 (26)	NS
Dyslipidemia (%)	4 (14)	5 (19)	NS
Familiar history of AMI	3 (11)	5 (19)	NS
Previous AMI	2 (7)	1 (4)	NS
Body mass index	23.4 ± 3.8	24.5 ± 4.5	NS
LAD Lesion by angiography	16 (57)	15 (56)	NS
Complete white blood count	9622 ± 2450	9844 ± 1972	NS
Neutrophil count	7627 ± 924	7230 ± 805	NS
hs-CRP (mg/dl)	1.05 ± 0.21	1.27 ± 0.35	NS
Sodium at admission	138 ± 5	137 ± 6	NS
Creatinine at admission	1.2 ± 0.8	1.4 ± 1.1	NS
CPK (IU/l)	1874 ± 1824	4386 ± 2015$^{\#\#}$	0.042
CK-MB (IU/l)	108 ± 95	455 ± 285$^{\#\#}$	0.023
Mean of vessel disease	1.8 ± 0.6	2.2 ± 0.7	NS
LVEF during hospitalisation	42 ± 14	34 ± 12	0.038

Data are expressed as mean ± SD. $^{\#\#}P < 0.05$, represents statistic significance between compensated and decompensated patients. AMI, acute myocardial infarction, HF, heart failure, LVEF, left ventricular ejection fraction.

gelatin - 8% SDS-PAGE. Electrophoresis was run at 150 V for 2.5 h. Enzymes on the gels were renatured by washing twice in a 2.5% Triton X-100 solution with shaking for 30 min. The gels were then incubated with a reaction buffer (50 ml) containing 40 mM Tris-HCl (pH 8.0), 10 mM $CaCl_2$, and 0.01% NaN_3 at 37°C for 16 h before staining with 0.25% Coomassie brilliant blue R-250 for 30 min. Quantitative analysis was carried out after discoloring the stain in a discoloring solution (875 ml H_2O, 50 ml methanol, and 75 ml acetic acid). A randomly chosen human breast cancer biopsy extract was used as the marker. Expression of 92 kd (MMP-9) and 72 kd (MMP-2) gelatinase in the serum were determined using the Kodak Scientific Imaging Systems SP700 (Eastman Kodak Company, Rochesterm, NY, USA).

Creatine Kinase Measurement

The creatine kinase (CK) reagent (Kit #442635) (KANTO Chemical Co., Inc, Tokyo, Japan) was used to determine the serum CK activity. Changes in absorbance were determined by a spectrophotometer (Beckman, CS-7, Beckman Instruments, Fullerton, CA, USA) and concentrations were calculated by the SYNCHRON CX System (Hitachi 7170, Tokyo, Japan).

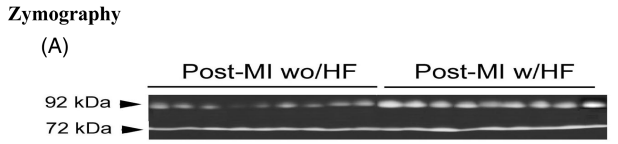
Statistical Analysis

All data are presented as means ± SD. ANOVA was used to analyze the statistics of the serum concentrations and avtivities of MMP-2 & MMP-9. Student's unpaired *t* test was used to compare the differences between groups of patients. A *P* value < 0.05 was considered statistical significance.

Results

Clinical Characteristics

Except the significant higher cardiac enzyme [creatine kinase (CK) and creatine kinase - MB form (CK-MB)] of group B (decompensated with acute MI) and lower left ventricular ejection fraction than those of group A (compensated with acute MI), there were no significant differences between these two groups, regarding age, gender, current smoking, hypertension, diabetes mellitus, dyslipidemia, previous MI, familiar history of MI, body mass index, complete white blood count, high sensitive CRP, total count of neutrophil, fasting glucose, sodium at admission, creatinine at admission, left anterior descending coronary artery (LAD) lesion ratio and extension of coronary atherosclerosis (Table 1).



(B)

	Post-MI wo/HF (n = 28)	Post-MI w/HF (n = 27)
MMP 9 (O.D.)	1.00 ± 0.23	2.99 ± 0.30**
MMP 2 (O.D.)	1.00 ± 0.09	1.15 ± 0.11

Fig. 1. (A) Zymography analysis of serum matrix-metalloproteinase-2 (MMP-2) and MMP-9 of the subjects. (B) The optical density (O.D.) of zymography was quantitated using a Phospho Imager. The data shown are from three independent experiments and are expressed as mean ± SD. $^{**}P < 0.01$, represents statistic significance between compensated (group A; MI without heart failure) and decompensated patients (group B; MI with heart failure). MI, myocardial infarction, HF, heart failure.

Variation of Serum Matrix-Metalloproteinase-2, -9 (MMP-2, 9) Activities between Decompensated and Compensated Patients with Acute MI

No significant differences were observed in the optical densities of serum MMP-2 (post MI without HF, 1.00±0.09, n = 28; post MI with HF, 1.15± 0.11, n = 27) (Fig. 1A and 1B). The optical density of MMP-9 of decompensated patients was significantly higher than that of compensated patients (2.99±0.30 *vs*. 1.00±0.09; $P < 0.01$) (Fig. 1A and 1B). In addition, we found the elevated MMP-9 activity in decompensated patients is unrelated with LAD lesion ratio ($P = 0.26$) and the total count of neutrophil ($P = 0.34$). However, the MMP-9 levels differ between compensated and decompensated patients with comparable CPK concentrations were found.

Variation of Serum Matrix-Metalloproteinase-2, -9 (MMP-2, 9) Levels between Decompensated and Compensated Patients with Acute MI

Mean MMP-2 levels showed no significant difference in compensated patients and decompensated patients (1248.2±366.7 ng/ml *vs*. 1477.3±518.4) (Fig. 2B). MMP-9 levels of decompensated patients were significantly higher than that of compensated patients (2641.3±462.4 ng/ml *vs*. 1046.2±413.8; $P < 0.01$) (Fig. 2A). In addition, we found the elevated MMP-9 concentration in decompensated patients with acute MI is unrelated with LAD lesion ratio ($P = 0.44$) and the total count of neutrophil ($P = 0.48$).

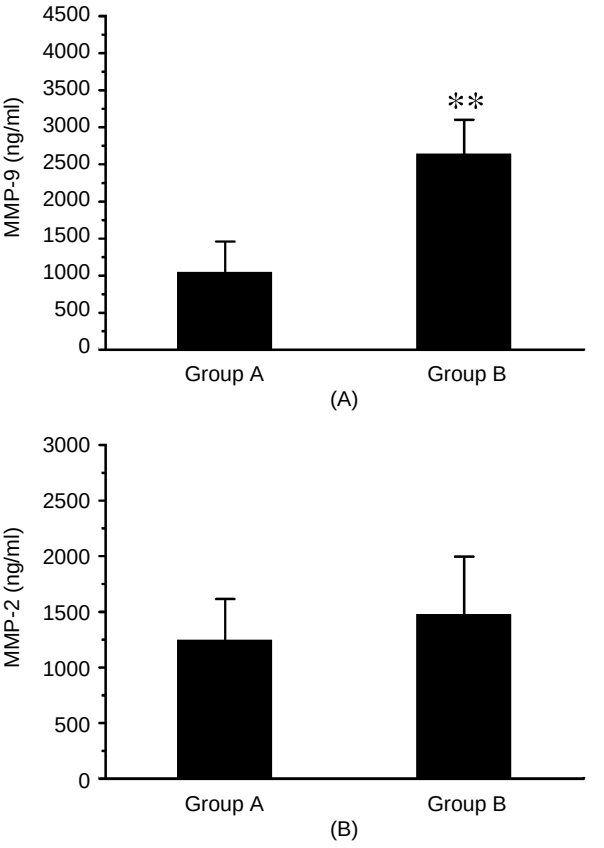


Fig. 2. ELISA analysis of serum (A) MMP-9 and (B) MMP-2 concentrations in patients with compensated (group A; MI without heart failure) versus decompensated patients (group B; MI with heart failure). The data shown are from three independent experiments and are expressed as mean ± SD. $^{**}P < 0.01$, represents statistic significance between compensated and decompensated patients.

Discussion

This present study demonstrates that MMP-9 may play a pathophysiologic role during the early phase of acute MI to heart failure. The principal finding is that serum concentration and activities of MMP-9 were elevated within 12 hours in decompensated patients as compared to compensated patients.

Studies reported, the role of MMPs in acute MI demonstrates differential regulation of these proteins during early stage (6, 12, 14, 16, 19). MMP-2 has been shown to modulate the reperfusion injury in a myocardial ischemia and reperfusion rat model (5). However, our results demonstrate no significant differences between patients of acute MI with and without heart failure.

The increase in plasma MMP-9 concentrations has been previous ascribed to an increased expression of MMP-9 in ruptured atherosclerotic plaques (13). However, in patients with acute plaque rupture but

without signs of myocardial damage (heart failure), MMP-9 levels were elevated only in blood from the coronary sinus, while concentrations in peripheral blood remained unchanged. This indicates that the absolute amount of MMP-9 released by the ruptured plaque is not sufficient for detection in peripheral venous blood sample. Additionally, many studies reported that increased expression of soluble interstitial MMP-9 in early stage after acute MI (9, 20). All these data suggest that the elevated plasma concentrations of MMP-9 are likely due to an enhanced release from the infracted myocardium. Of interest, patients with left anterior descending (LAD) coronary artery blockage are linked with highly myocardium at risk and the ultimate infarct size during acute coronary occlusion (8, 18). However, we found no association between elevated MMP activities or levels with LAD lesion ratio in two groups. Furthermore, it is well documented that both neutrophil (NE) and matrix metalloproteinase-9 (MMP-9) have a synergistic effect in inflammatory injury (23) and even suggested that circulating neutrophil leucocytes is the major source of the MMP-9 secreted into the circulation (25). Moreover, we found the elevated MMP-9 activities or levels in decompensated patients is not related with the level of high sensitivity CRP and total count of neutrophil leucocytes, either. The related mechanisms of elevated MMP-9 need to be revealed in the near future. However, we found the MMP-9 levels differ between compensated and decompensated patients with comparable CPK concentrations (data not shown), thus the elevated levels of MMP-9 could represent a secondary phenomenon.

It is concluded that abnormal increased plasma MMP-9 activities and levels could be seen in decompensated patients of MI as compared to compensated patients. The increase of MMP-9 activities and levels not only can be used as the marker of diagnosing the developing heart failure in patients of post MI, but also can provide some explanation of the pathogenesis from post MI to heart failure. In the clinical application, we believe the implication of MMP-9 inhibitors can be used as a potential strategy for the therapeutic approach that will reduce their influence in heart failure.

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