Clinical significance of matrix metalloproteinases activity in acute myocardial infarction

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ABSTRACT. Matrix metalloproteinases (MMP) degrade myocardial fibrillar collagen in acute myocardial infarction (MI) patients. Their activity is tightly controlled in normal myocardium by a family of closely related tissue inhibitors known as TIMP. An imbalance in their activity might contribute to post-MI remodeling. Plasma levels of MMP-1, TIMP-1 and MMP-1/TIMP-1 complex were measured, using relevant ELISA kits, in 24 (22 males-2 females), acute MI patients with a mean age 59 ± 14 years. Blood samples were taken on admission (0h),and 3h, 6h, 9h, 18h, 24h, 36h, 48h, 3rd, 4th, 5th, 7th, 15 th, 30 th days after MI. All patients underwent coronary arteriography with ventriculography for estimation of left ventricular ejection fraction (LVEF) and extent of coronary artery diseases, and echocardiographic study for measuring end-diastolic diameter (EDD). Ten patients with an LVEF < 45%, an EDD > 47.5mm, and heart failure symptoms were included in group A and compared against 12 patients with an LVEF > 45% an EDD < 47.5mm in group B.Mean plasma concentrations of MMP-1 were higher by 21% in group A (1.3 ± 0.2 ng/mL) compared to group B (1 ± 0.1 ng/mL) over the total study period. TIMP-1 plasma concentrations showed very little difference between the 2 groups, (704 ± 213 ng/mL *versus* 691 ± 165 ng/mL, (6%)) Finally, plasma concentrations of MMP-1/TIMP-1 complex were lower by -36% in group A with a mean value of 2.7 ± 0.6 ng/mL *versus* 3.7 ± 0.5 ng/mL in group B. Mean values for the differences were significant at time points 0, 6, 18, 24 and 48 hours for MMP-1 (p < 0.036), and on 48h and the 4th day for MMP-1/TIMP-1 complex (p < 0.031). Moreover, a good correlation was found between plasma concentrations of creatine kinase (CK) and MMP-1 at 18h (r = 0.422, p = 0.041) and on the 4th day (r = 0.67, p = 0.046), and TIMP-1 on the 4th day (r = 0.67, p = 0.047). Additionally, mean values for LVEF were 35.8 ± 8.8% in group A *versus* 51.2 ± 1.8% (p = 0.00014) in group B. Also, the EDD in-group A was 52.1 ± 6.9 mm *versus* 42.9 ± 3.2 mm in group B (p = 0.00013). In acute MI patients, increased MMP-1, with no change in TIMP-1, is associated with left ventricular dysfunction and dilatation, suggesting that increased collagenolytic activity contributes to loss of LV function.

Keywords: matrix metalloproteinases, acute myocardial infarction, extracellular matrix

Left ventricular dysfunction and remodeling after myocardial infarction (MI) are thought to be among the first steps to progression to heart failure, the degree of remodeling predicting morbidity and mortality [1-6].

The combination of cellular and extracellular events that occur during the post-MI period results in changes in left ventricular geometry, and has been called infarct expansion [3-6] Previous studies have demonstrated that a structural determinant of infarct expansion is the extracellular collagen matrix (ECCM) remodeling [1-6]. The matrix metalloproteinases are a family of proteolytic enzymes, that contribute to the left ventricular remodeling process [7, 8].Endogenous MMP activity is controlled by tissues inhibitors of MMP (TIMP) [7, 8]. Another role of the TIMP that is independent of modulating MMP actional states, is through their effects on cell growth. TIMP-1 and TIMP-2 have been shown to stimulate a growth response in fibroblast cell cultures in a concentration–dependent manner [9]. Past clinical studies have demonstrated alterations in MMP and TIMP after MI [10, 11]. Recent experimental data have provided evidence for a cause-and-effect relationship between MMP-induced- and the post-MI myocardial-remodeling process [7, 12-14].

The purpose of this study was to examine whether a differential expression of plasma concentration occurs between matrix metalloproteinase-1 (MMP-1) and its natural tissue inhibitor (TIMP-1) in patients with acute MI. This unequal expression of plasma concentrations affects left ventricular chamber dimensions and global contractile performance.

MATERIALS AND METHODS

Study population

The study population consisted of 24 patients (22 males, 2 females, mean age 59±14 years) who had been admitted to our department of Cardiology in Laiko Hospital, with a first attack of acute MI, and 12 healthy subjects as controls (8 males, 4 females, mean age 43±14), who were studied in order to estimate normal levels of collagenolytic enzymes. The control subjects had no past history or any evidence of cardiovascular disease, hypertension or diabetes mellitus. The present study did not include any patients or control subjects with a history of neoplastic, hepatic, or infectious disease, severe renal failure, serum creatinine > 2.5mg/dL or any surgical procedure in the preceding 6 months. The local scientific ethics committee approved the study and each participant gave their informed written consent.

The diagnosis of acute MI was confirmed by typical, prolonged chest pain, accompanied by serial changes on the standard, 12-lead electrocardiogram (ECG), or significant (two-fold greater than the normal range) increases in markers of myocardial damage creatine kinase (CK) or its isoenzyme MB (CK-MB).

All patients were admitted less than 6h from the onset of chest pain (mean 4.2h±0.6h). Depending upon the indications, patients were thrombolyzed using alteplase, and were treated with angiotensin-converting enzyme inhibitor (ACEI) *(table 1)*. All patients received a combination of standard medication including nitrates, beta-adrenergic blockers, calcium antagonists, and low molecular weight heparin and antiplatelet drugs such as aspirin or clopidogrel.

Table 1
Baseline characteristics

	Group A	Group B	Control group
Patient number	10	12	12
Age (years):			
Mean ± SD	**55 ± 13**	**57 ± 11**	**43 ± 14**
Range	37-70	41-77	31-77
Sex:			
Male/Female	10/0	10/2	8/4
Infarct location:			
Anterior/Inferior	9/1	6/6	
Extent of coronary artery disease:			
0 vessel disease	0	0	
1 vessel disease	1 pt	7 pts	
2 vessel disease	5 pts	4 pts	
3 vessel disease	3 pts	-	
No catheterization	1 pt	1pt	
Total Cholesterol:			
> 200 mg%	6 pts	10 pts	
< 200 mg%	4 pts	2 pts	
Thrombolysis:			
Yes	9 pts	5 pts	
No	1 pt	7 pts	
Angiotensin converting enzyme inhibitor			
Yes	8 pts	5 pts	
No	2 pts	7 pts	

Two out of the 24 patients died on the 4th post-infarction day, one from acute pulmonary edema due to rupture of the chordae tendinae followed by acute mitral regurgitation and death, and the other as a result of extension of the acute MI from the inferior towards the lateral and anterior wall, followed by cardiogenic shock and ventricular fibrillation. These two patients are not included in the study. In sixteen patients, the infarct location was the anterior wall, and in eight, the inferior wall.

Patients were assigned to two groups according to the normal lower limits of LVEF = 45%, and the mean value (47.5mm) of EDD measured at papillary muscle level by two-dimensional echocardiography. Ten patients with an ejection fraction of < 45%, heart failure symptoms (third heart sound (S_3) and basal rales on auscultation, mild congestion on chest X-ray) during the hospitalization in the coronary care unit, and left ventricular end-diastolic diameter (LVEDD) > 47.5 mm were included in group A. This group was compared to the remaining 12 patients with an LVEF > 45%, without heart failure symptoms, and LVEDD < 47.5 mm (group B).

Hemodynamic and echocardiographic evaluation

On 6th post-MI day, twenty out of the 22 patients (two patients refused) underwent coronary arteriography to estimate the extent of coronary artery disease and single-plane ventriculography in the right anterior oblique (RAO) view in order to estimate ejection fraction [15] and regional wall movement abnormalities in the anteriobasal, and posteriobasal segments [16]. We evaluated LVEDD, on the 6th and 30th days [17, 18], using a Hewlett Packard Sonos 1000 ultrasound system with a 2.5 MHz transducer.

Serum plasma concentrations of MMP-1, TIMP-1 and MMP-1/TIMP-1 complex

Blood samples were collected on admission (0 hours), at 3h, 6h, 9h, 12h, 18h, 24h, 36h and 48 hours and on the 3th, 4th, 5th, 7th, 15 th and 30 th day after the acute MI. They were centrifuged for 10 minutes at 3000 rpm and stored at -20^{o} to 30^{o}C until the assay (within 6 months).

Plasma concentrations of MMP-1, TIMP-1, and MMP-1/TIMP-1 complex antigen levels were measured using the relevant ELISA kits provided by Amersham International plc, Buckingham, UK. The matrix metalloproteinase-1 human ELISA system code 2610 [19] measured total human MMP-1, both free and that complexed with TIMP-1.The lower limit of detection was 0.5 ng/mL and the coefficients of variation of the intra-assay/ inter-assay were 6.7% and 12.4% respectively. The immuno-assay system for TIMP-1, code 2611 [20], recognize total human TIMP-1, free TIMP-1 and that complexed with MMP-1. The lower detection limit and the intra-assay/inter-assay variation coefficients were 45 ng/mL, 10.5% and 13.7% respectively. Also, the immuno-assay systems for MMP-1/TIMP-1 complex, code 2612 [21], recognize activated MMP-1 that had subsequently been complexed with the specific inhibitor TIMP-1. It did not recognize free, active MMP-1, TIMP-1, or Pro MMP-1 or MMP-1/TIMP-1 complex from other species. The lower detection limit was 1.5 ng/mL, and the intra-assay/inter-assay variation coefficients were 8.2% and 12.7% respectively. The immuno-

assay is based on a two-side ELISA sandwich format. Concentrations in ng/mL were determined by interpolation from a standard curve for each collagenolytic enzyme. Blood samples for assay of CK and its isoenzyme MB were obtained at the same time points, and measured using Medicon, Olympus and Bayer ELISA Kits respectively.

Statistical analysis

We employed analysis of variance (ANOVA repeated measurements) to assess time-dependent alterations in serum MMP-1, TIMP-1, and MMP-1/TIMP-1 complex concentrations. If the analysis indicated a statistically significant change, between subjects and within subjects, then the difference between the mean values was compared by paired and unpaired Student's t-test and the Mann-Whitney test for the same period of time. Blood values are given as mean ± SD. $p < 0.05$ was considered statistically significant.

RESULTS

The clinical and demographic characteristics of both groups of patients are presented in *table 1*. There were no significant differences as regards age and sex. However, clear baseline differences existed in blood plasma cholesterol levels, extent of coronary artery disease, site and size of infarct, number of patients having been thrombolysed (n = 14) and treated with ACEI (n = 13).

Time course of MMP-1, TIMP-1, MMP-1/TIMP-1 complex plasma concentration changes

MMP-1

Figure 1 shows the time-dependent changes in plasma concentrations of MMP-1 in both groups of acute MI patients. Group A expressed higher levels of MMP-1 plasma concentrations, average percentage increase (21%) for the total study period, while the mean±SD value was 1.3±0.2 ng/mL in group A *versus* 1±0.1 ng/mL in group B. Furthermore, at certain time points (6h, 18h, 24h, 48h), the mean values of the differences between the groups, were statistically significant ($p < 0.006$).

TIMP-1

Figure 2 outlines the time-dependent changes in TIMP-1 plasma concentrations. Note that both curves for TIMP-1 plasma concentrations are characterized by fluctuations of mean values throughout the study period, while the mean average plasma concentrations were 704±213 ng/mL in group A *versus* 691±165 ng/mL in group B, and the percentage difference between them was 6%.

MMP-1/TIMP-1 complex

Figure 3 demonstrates the time-dependent changes in MMP-1/TIMP-1 complex plasma concentrations for each time point in both groups of acute MI patients. Note that the MMP-1/TIMP-1 complex, reflecting activated MMP-1 and expressed as TIMP–1 component, shows a lower average expression in group A compared to group B (-38%) over the total study period, while the mean ± SD values were 2.7±0.6 ng/mL *versus* 3.7±0.5 ng/mL in both groups, respectively. Furthermore, at 48h and on the 4th day, the mean values for the differences between groups were statistically significant, with $p < 0.031$ and $p < 0.0052$ respectively.

Healthy subjects

Mean ± SD plasma values for these subjects were, MMP-1 1.2±0.2 ng/mL, TIMP-1 222±69 ng/mL and MMP-1/TIMP-1 complex 1.3±0.3 ng/mL. Statistical analysis between the mean values for MMP-1, TIMP-1, MMP-1/TIMP-1 complex, and the corresponding mean values for normal healthy subjects showed no statistical significance for MMP-1, while TIMP-1 and MMP-1/TIMP-1

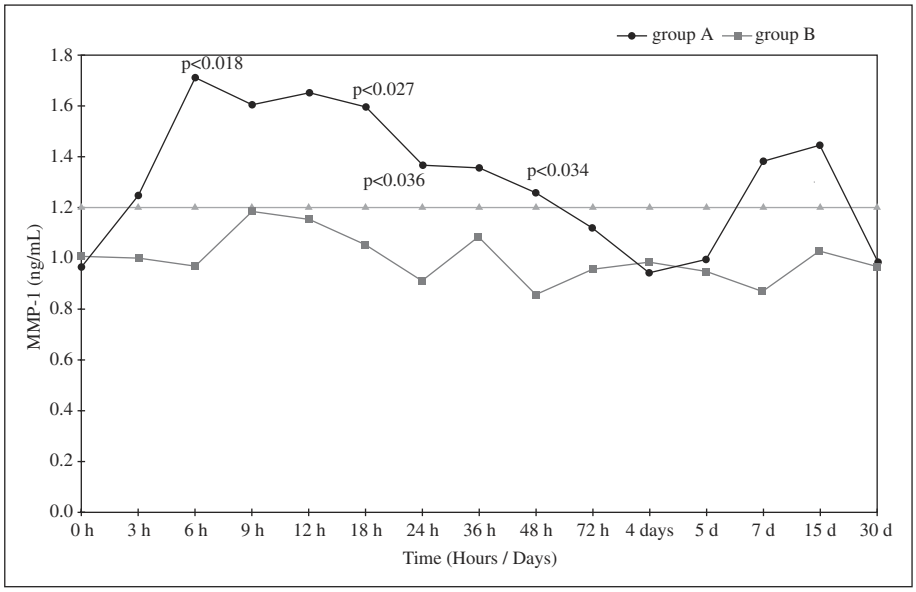

Figure 1

Mean changes inMMP-1 as a function of time in acute MI patients allocated to group A, compared to group B.
Note the significant increase inMMP-1 activity in the first 48 hours after the MI. The straight line represents mean value of MMP-1 in healthy subjects.

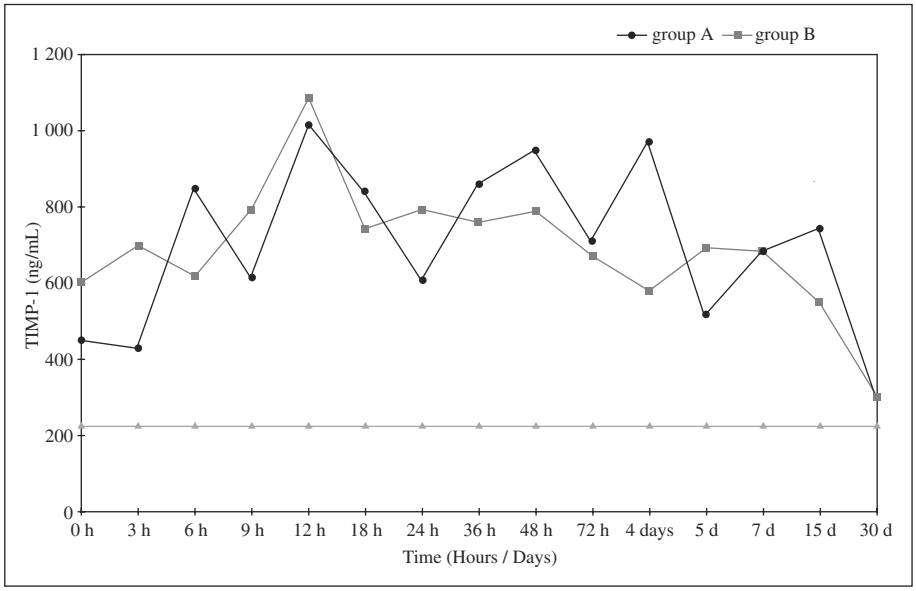

Figure 2
Mean changes in TIMP-1 as a function of time in acute MI patients allocated to group A, compared to group B. The straight line represents mean values of TIMP-1 in healthy subjects.

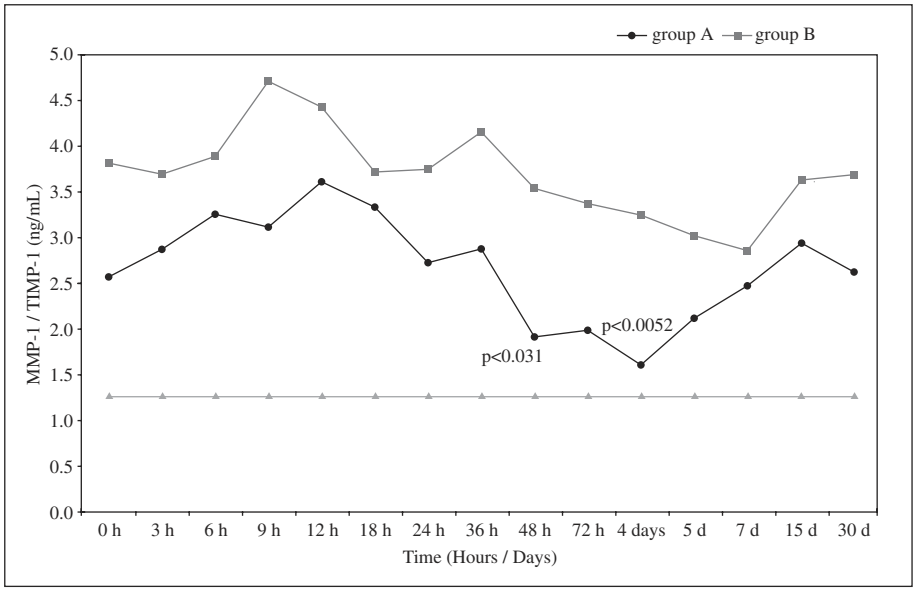

Figure 3
Mean changes in MMP-1/TIMP-1 complex as a function of time in acute MI patients allocated to group A, compared to group B. Note the lower mean values for MMP-1 / TIMP-1 complex throughout the study period, and the significant changes on 48h and the 4th day ($p < 0.031$ and $p < 0.052$). The straight line represents the mean values of MMP-1/ TIMP-1 complex of healthy subjects.

were statistically significant with $p < 0.048$ and $p < 0.049$ respectively at most time points.

Left ventricular ejection fraction

Characteristics are the mean values for ejection fraction *(figure 4)* between the two groups of acute MI patients. Group A had an ejection fraction of 35.8±8.8%, whereas group B had an ejection fraction of 51.2±1.8%. This -43% percentage reduction in the ejection fraction in group A compared to group B was statistically significant ($p < 0.00014$).

Left ventricular dimensions

Echocardiographic study on the 6th day, showed a mean value for LVEDD of 52.2±6.9 mm in group A, whereas group B patients demonstrated a mean value for LVEDD of 42.9±3.2 mm *(figure 5)*. This 17% difference between the two groups was statistically significant ($p < 0.001319$). Moreover, echocardiographic study on the 30th day, showed a value for LVEDD of 53.2±7.4mm for group A *versus* 43.3 ± 2.4mm for group B. This 18% difference was also statistically significant ($p < 0.0009$).

Creatine kinase

Additionally, *figure 6* demonstrates the mean values for the changes in CK (IU/mL), as a function of time, in both groups of acute MI patients. Note the significance of changes between time points 6h to the 5th day ($p < 0.024$). Also, a good correlation was found between plasma con-

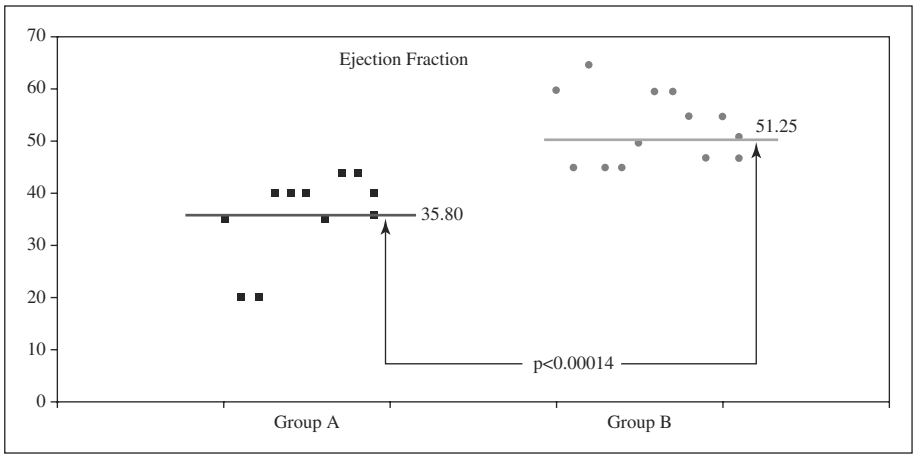

Figure 4

Mean value for ejection fraction between group A and group B.
Note the characteristic (43%) statistically significant ($p < 0.00014$) reduction of ejection fraction.

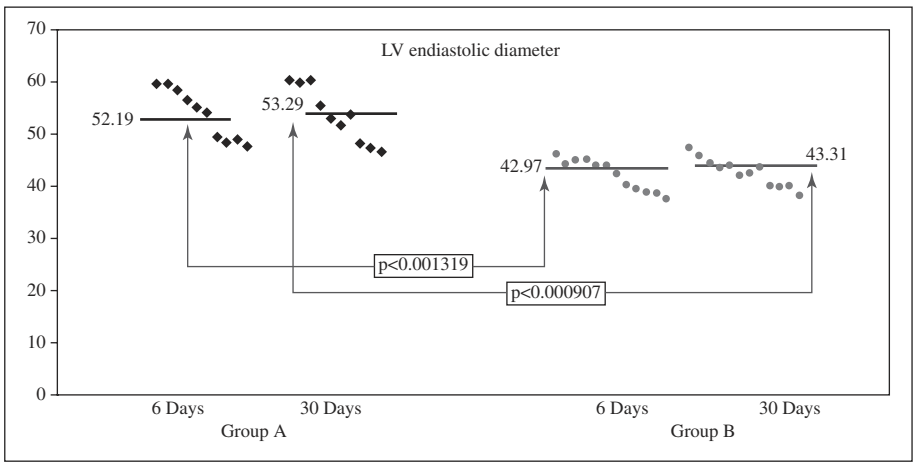

Figure 5

Mean value for left ventricular end diastolic diameter measured at the 6th and at 30th day, by echocardiographic study, in group A and group B.
Note the left ventricular chamber dimensions, which were unchanged in the two echocardiographic measurements for both groups.

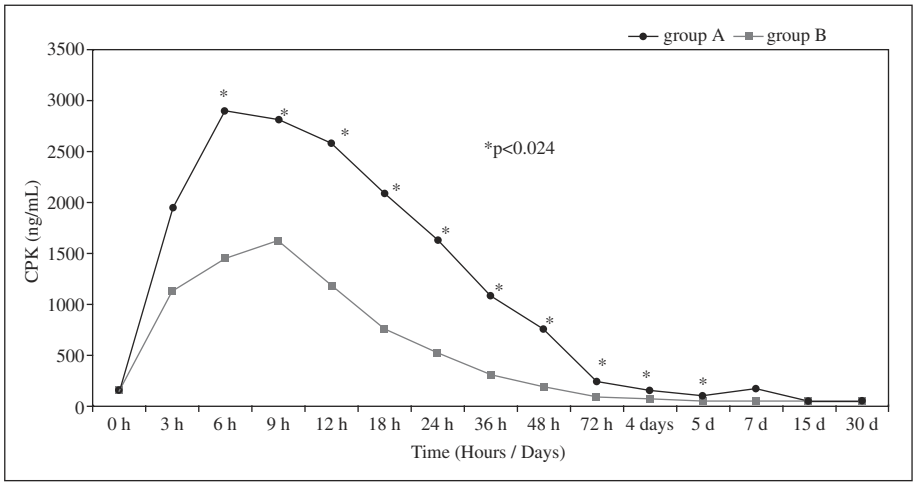

Figure 6

Mean changes in creatine kinase IU/mL as a function of time in acute MI patients allocated to group A, and group B. Note that the significance ($0.0027 < p < 0.034$) changes from 6h to 5 days.

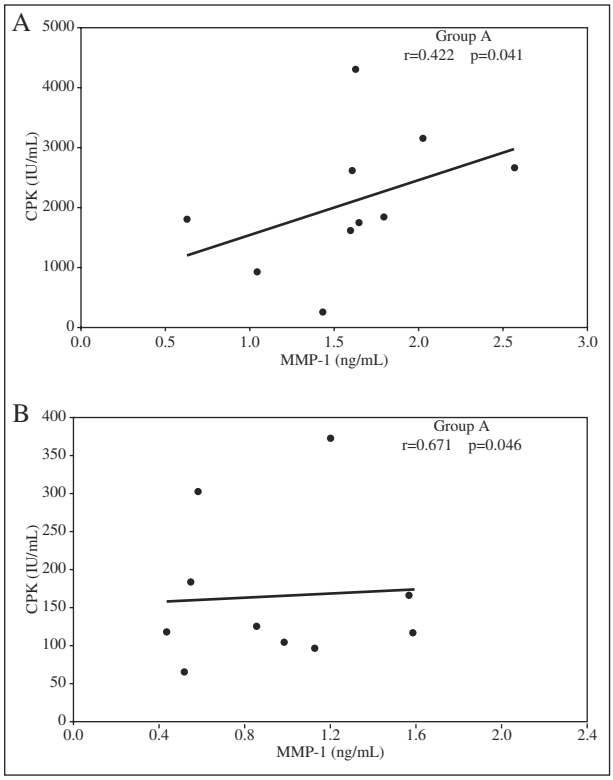

Figure 7
Graphs showing a positive correlation between plasma concentrations of MMP-1 and CK at 18h (r = 0.422, p = 0.041) (**A**), and on the 4th day (r = 0.67 p < 0.046) (**B**), in acute MI patients.

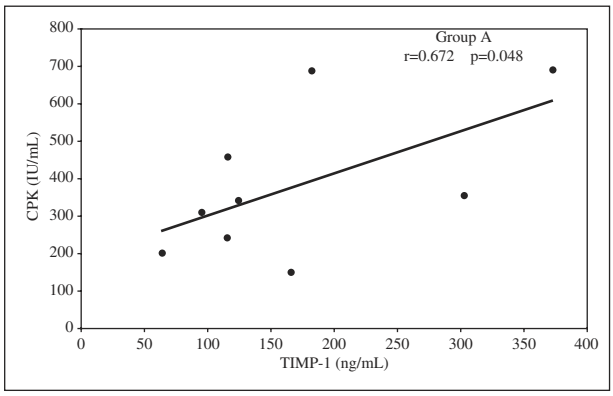

Figure 8
Correlation between plasma concentrations of TIMP-1 and CK on the 4th day in acute MI patients (r = 067 p < 0.047).

centrations of CK and MMP-1 at 18h (r = 0.422, p = 0.041) (*figure 7a*), and on the 4th day (r = 0.67 p < 0.046) (*figure 7b*), and of TIMP-1 on the 4th day (r = 0.67 p < 0.047) (*figure 8*).

DISCUSSION

The findings of our study demonstrated two patterns of collagenolytic enzyme activity (collagenolysis) in acute MI patients.

Pattern I collagenolysis of group A was characterized by higher expression of MMP-1, by different expressions of TIMP-1, and by lower expression of the MMP-1/TIMP-1 complex. In contrast, pattern II collagenolysis of group B was represented by lower expression of MMP-1, by equivalent expression of TIMP-1, and by higher expression of the MMP-1/TIMP-1 complex.

These quantitative differences in expression of collagenolytic enzyme plasma concentrations, between the two groups of acute MI patients, were accompanied by two kinds of post-MI left ventricular function. More specifically, pattern I collagenolysis, seen in group A, was followed by left ventricular dysfunction and a greater left ventricular chamber dimensions, whereas pattern II collagenolysis seen in group B, was associated with normal left ventricular function and smallerleft ventricular chamber dimensions. Additionally, at certain time points the mean values for the differences in MMP-1 and MMP-1/TIMP-1 complex plasma concentrations, between group A and group B, were statistically significant.

Furthermore, the mean values for the differences in ejection fraction and LVEDD, between both groups, were statistically significant. These changes might be attributable, at least in part, to the differential expression levels of collagenolytic enzymes, as well as to other parameters of left ventricular dysfunction such as location and size of infarction.

Relevant to our findings are recent reports indicating that MMP-1 plays a role in post-MI left ventricular remodeling. Hirohara *et al.* showed a time-dependent change in serum MMP-1 levels after acute MI that may contribute to post-MI ventricular remodeling by promoting the degradation of the extracellular matrix [10]. Additionally, Soejima *et al.* [22] have reported a negative correlation, between MMP-1 plasma levels at 7 day and 2 weeks, and left ventricular ejection fraction. Furthermore, Hojo *et al.* [23] showed a significant correlation between maximum peripheral blood mononuclear cell MMP-1 levels and maximum plasma C-reactive protein levels, and left ventricular end diastolic volume index. So our results are generally in agreement with their findings, except for the fact that we could not find any correlation, between changes in MMP-1, TIMP-1, and MMP-1/TIMP-1 complex plasma concentrations and changes in LVEF. The only correlation was between plasma concentrations of MMP-1 at 18h and day 4 and TIMP-1 at day 4, and plasma concentrations of CK. The correlation between MMP-1, TIMP-1 and MMP-1/TIMP-1 complex and the clinical parameters LVDD, and EF was estimated by performing multivariate analysis. No significant correlation was observed.

Also, one other important finding emerging from our echocardiographic study was the fact that left ventricular chamber dimension changes occurred early, on the 1st or 2nd day post-MI. Furthermore, the very early initiation of ACEI in acute MI patients, minimized post-infarction left ventricular dilatation, through a reduction in metalloproteinase enzyme plasma concentrations. This mode of action of ACEI may be considered as an alternative mechanism responsible for their beneficial effects on left ventricular expansion in the chronic phase [24].

It is well known, that cardiac myocytes and the fibrillar extracellular collagen matrix (ECCM) play a critical role in determining cardiac performance [25, 26]. In particular, ECCM provides structural support and integrity to the myocardium [27], and facilitates the conversion of myocyte contraction into pump function. The integrity of the original ECCM is thought to play an important role in determining the extent of dysfunction after myocardial

infarction [28], whereas, parallel to myocyte cell death is damage of the existing ECCM [29, 30].

Degradation of ECCM follows the activation of MMP that are secreted, as zymogen [31]. Recent reports have documented the time-dependent activation of MMP after ischemia or acute MI. MMP activation can occur within minutes of ischemia, with significant increases occurring as early as 15 minutes, and peaking 1 to 2 days after MI [9, 32, 33]. Different mechanisms have been implicated in MMP activation. It is believed that the early (< 48 hours) MMP activation is associated with zymogen activation, whereas subsequent (> 48 hours) increases are associated with newly synthesized protein [32-35].

In our results, the curve for the time-dependent changes of MMP activation *(figure 1)* showed an early (< 24 hours) and a late (5^{th}-30^{th} days) increase in mean values for MMP-1 plasma concentrations. This early increase is due to activation of latent, pro-MMP, to active MMP by the urokinase plasminogen activator (uPA) plasmin system [36]. Also, the late increase in MMP-1 activation may be due to the newly synthesized protein resulting from macrophage infiltration of the infarcted myocardial area [32, 33, 35].

Many studies have shown that remodeling of ECCM plays a major role in left ventricular remodeling [37, 38]. Differential regional remodeling of the ECCM contributes significantly to global left ventricular structural dysfunction after acute MI [37, 38], and plays a pivotal role in infarct expansion [37], infarcted dilation [12, 37], and progressive global left ventricular dilatation [38, 39]. The function of MMP during the healing and remodeling process of the left ventricle after acute MI has been clarified in studies using broad range MMP inhibitors and genetically modified mice. Rohde *et al.* [12] first demonstrated that *in vivo* MMP inhibition attenuates early, left ventricular dilatation, four days after experimental, acute MI in mice. Also Creemers *et al.* [40], have studied the effects of a broad range MMP inhibitor on left ventricular remodeling and infarct healing, one and two weeks after acute MI in mice. In this study, infarcted mice allocated to ilomast treatment, showed a significant decrease in left ventricular dilatation after MI, as measured by echocardiography.

Also, other studies have shown a fine balance between matrix metallproteinases that degrade ECCM and endogenous tissue inhibitors (TIMP) that inhibits MMP, [41] and maintains normal remodeling and function, while an imbalance in their expression can result in adverse remodeling [41-44]. The functional role of TIMP-1 in the control of left ventricular geometry and cardiac function was recently studied in TIMP-1-deficient mice [45]. Echocardiography in these mice demonstrated an 18% increased in left ventricular end diastolic volume, and a 38% increase in cardiac mass at four months of age. Reduced myocardial collagen content probably accounted for the increased left ventricular dilatation. These results suggest that constitutive TIMP-1 expression contributes to the maintenance of normal left ventricular myocardial structure [45]. Furthermore, several transgenic models have been constructed that disrupt normal myocardial MMP and TIMP levels [7, 45, 46]. Kim *et al.* [46] demonstrated that cardiac-restricted, over expression of MMP-1 resulted in changes in the myocardial ECCM structure, which was accompanied by alterations in left ventricular function. Finally, in a rat MI model, MMP-1 mRNA levels increased soon after MI, but this increase was not associated with a concomitant increase in TIMP-mRNA levels [7].

The MMP-1/ TIMP-1 complex is formed in a stoichiometric, 1:1 molar ratio and forms an important endogenous system for regulating MMP activity. Its plasma levels might express the power of endogenous MMP inhibitory control of human myocardium TIMP-1 to form a complex with several MMP [47]. In the present study, we found a discrepancy between MMP-1 levels and MMP-1/TIMP-1 complex levels, while TIMP-1 levels fluctuated. The lower MMP-1/ TIMP-1 levels found in group A compared to group B in acute MI patients might be explained by the loss of TIMP-mediated inhibitory control that has been reported recently in post-MI remodeling [7], in dilated cardiomyopathy [48], and in an *in vitro* system of ischemia and reperfusion [49].

Taken together, the above data suggest that the increased MMP expression and activation, coupled with the loss of endogenous MMP inhibitory control occurring early in the post-MI period can potentially contribute to post-MI left ventricular remodeling and dysfunction.

Study limitations

We could not compare plasma concentrations of collagenolytic enzymes occurring in acute MI patients with comparable MI involving the same wall, because of the small size of the study population.

A larger patient population and a longer period of observation are required to clarify the exact role of the differential expression of collagenolytic enzymes in the pathophysiology of post-infarction cardiac remodeling, and to establish correlations, between the patterns of collagenolysis and progressive cardiac enlargement.

CONCLUSION

In acute MI patients, increased MMP-1, with no change in TIMP-1, is associated with left ventricular dysfunction and dilatation, suggesting that increased collagenolytic activity contributes to loss of left ventricular function.

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