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Peripheral Blood Levels of Matrix Metalloproteases-2 and -9 Are Elevated in Patients With Acute Coronary Syndromes

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Objectives. **This study was sought to investigate whether peripheral blood levels of matrix metalloproteases (MMPs) are affected in patients with acute coronary syndromes (ACS).**

Background. **Synthesis of MMPs has been reported in coronary atherosclerotic lesions in patients with unstable angina (UA), suggesting a pathogenic role of MMPs in the development of ACS.**

Methods. **Using sandwich enzyme immunoassay, serum MMP-2 and plasma MMP-9 were measured in 33 patients with ACS (22 with acute myocardial infarction [AMI], 11 with UA), 17 with stable effort angina (EA) and 17 normal control subjects.**

Results. **Serum MMP-2 in patients with UA and AMI on day 0 was two times greater than that in control subjects, and patients with EA showed higher MMP-2 levels than those in control subjects. Plasma MMP-9 in patients with UA and AMI on day 0 was elevated by threefold and twofold versus that in control subjects, respectively. In patients with UA and AMI who underwent medical treatment (n = 11 and 13, respectively), MMP-2 elevation was sustained until day 7. In patients with UA, MMP-9 elevation on day 0 was followed by a gradual decrease toward the control range up to day 7. Some patients with AMI showed a transient MMP-9 elevation with a peak on day 3, whereas in others, MMP-9 levels were significantly elevated on day 0 and remained higher than those in control subjects up to day 3.**

Conclusions. **Serial changes in serum MMP-2 and plasma MMP-9 were documented in patients with ACS. These findings provide an insight into the molecular mechanism of plaque destabilization.**

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Rupture of plaques with superimposed thrombosis is now considered to be the main cause of the acute coronary syndromes (ACS) that range from unstable angina (UA) to Q wave acute myocardial infarction (AMI) (1,2). Several pathophysiologic mechanisms are involved in the process of plaque rupture, including inflammation, rheologic factors, circumferential wall stress and vasoconstriction, as well as destabilizing changes in the plaque tissues (1,2). Matrix metalloproteases (MMPs) are critical for vascular remodeling by regulating degradation of the extracellular matrix (ECM) (3). Recently, there is increasing evidence that 72-kDa and 92-kDa type IV collagenases (MMP-2 and MMP-9, respectively), which act specifically on the basement membranes and partially degraded collagen, play a pathogenic role in the development of the atherosclerotic plaques. It has been shown (4,5) that MMP-2 is constitutively expressed in vascular smooth muscle cells (VSMCs) in normal arteries and that in addition to increased MMP-2 expression, atherosclerotic plaques prone to rupture show induction and activation of MMP-9 in VSMCs and infiltrating macrophages. Furthermore, MMP-2 and MMP-9 are considered to play a role in the regulation of migration and proliferation of VSMCs in atherosclerotic lesions by acting specifically on basement membrane components that modulate the cell-to-cell communication with activated surrounding cells, such as inflammatory cells, endothelial cells and VSMCs (3).

It has been reported (6,7) that concentrations of MMPs are elevated not only in affected tissue and body fluid but also in peripheral blood in some patients with cancer, liver cirrhosis or rheumatoid arthritis. These findings raise the possibility that patients with vulnerable atherosclerotic plaques would show elevated peripheral blood levels of MMPs. Therefore, using sandwich enzyme immunoassay, we measured serum MMP-2 and plasma MMP-9 in patients with ACS, including UA and AMI.

Methods

Patients. This study enrolled 33 patients with ACS (22 with AMI [mean ± SD age 62 ± 9 years, 15 men]; 11 with UA

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Abbreviations and Acronyms

ACS = acute coronary syndromes
AMI = acute myocardial infarction
CK = creatine kinase
CRP = C-reactive protein
EA = effort angina
ECG = electrocardiogram, electrocardiographic
ECM = extracellular matrix
MMP = matrix metalloprotease
UA = unstable angina
VSMCs = vascular smooth muscle cells

[mean age 61 ± 10 years, 7 men]), 17 with stable effort angina (EA) (mean age 62 ± 11 years, 12 men) and 17 normal volunteers (control subjects: mean age 54 ± 11 years, 10 men) (Table 1). The normal volunteers had no past history or evidence of cardiovascular disease, hypertension or diabetes mellitus. The present study did not include patients or control subjects with a history of neoplastic, hepatic, infectious or autoimmune disease; peripheral atherosclerotic disease; or any surgical procedure in the preceding 6 months. Written informed consent was obtained from each subject.

Patients with AMI was defined as the presence of typical prolonged chest pain accompanied by serial changes on the standard 12-lead electrocardiogram (ECG) or significant (2-fold more than the upper normal range) increase in creatine kinase (CK). This study included 22 patients with AMI admitted to the coronary care unit within 3 to 9 h after the onset of chest pain, of whom 9 with indications for direct percutaneous transluminal coronary angioplasty or intravenous thrombolytic therapy underwent these interventional treatments. The remaining 13 patients (AMI-M group: mean age 64 ± 7 years, 9 men) were admitted to the hospital >6 h after the onset of symptoms and underwent medical treatment with combinations of standard medications, including oral nitrates, beta-adrenergic blocking agents, calcium antagonists and aspirin, and intravenous nitrate or heparin, or both. The diagnosis of patients with UA was defined as anginal pain at rest occurring during the preceding 24 h with transient significant ischemic ST segment or T wave changes, or both, without significant (1.5-fold more than the upper normal range) increases in CK. Patients in whom ST segment elevation or new abnormal Q waves developed during the observation period were not included. In all patients with UA, intensive medical treatment as previously described was started on day 0 (the admission day) if patients had not already taken medicines, and the clinical symptoms and signs of myocardial ischemia were controlled after admission. The EA group included patients who complained of angina on effort without evidence of recent deterioration or rest pain in the previous 6 months.

Blood sampling and enzyme immunoassay. Blood samples were drawn from the peripheral vein on admission. In the UA and AMI-M groups, serial blood samples were also collected on days 1, 3 and 7 after admission. For plasma preparation, 2Na-EDTA (final 0.1%) was added to whole blood. After centrifugation, serum and plasma samples were frozen and stored at −80°C until use. Sandwich enzyme immunoassay was performed for measuring concentrations of serum MMP-2 and plasma MMP-9 using commercial available kits with monoclonal antibodies against each substance (6,7) according to the

Table 1. Clinical Characteristics

	Control Group (n = 17)	EA Group (n = 17)	UA Group (n = 11)	AMI Group (AMI-M group) [n = 22 (13)]
Age (yr)	54 ± 11	62 ± 11	61 ± 10	62 ± 9 (64 ± 7)
Men/women	10/7	12/5	7/4	15/7 (9/4)
Hypertension	0	7	6	11 (7)
Diabetes mellitus	0	3	3	7 (4)
Smoker	3	7	6	11 (7)
Hypercholesterolemia (total chol >220 mg/dl)	1	6	3	10 (4)
History of preceding EA	—	—	6	11 (5)
Previous myocardial infarction	—	3	3	5 (3)
Coronary angiography	—	17	9*	20 (11)
No. of ≥75% stenosed vessels		2.1 ± 1.0	1.8 ± 0.8	2.1 ± 0.8 (2.0 ± 0.9)
Medication used				
Nitrates	—	16	11	21 (13)
Beta-blockers	—	4	8	6 (3)
Calcium antagonists	—	12	10	17 (10)
Aspirin	—	15	10	19 (12)
Heparin	—	—	11	20 (12)

*Coronary angiography was carried out within 4 weeks after stabilization of symptoms. Data presented are mean value ± SD or number of patients. Ages were not different among the four groups, and there was no significant difference in the other characteristics among the groups with stable effort angina (EA), unstable angina (UA), acute myocardial infarction (AMI) and AMI with conventional medical treatment (AMI-M).

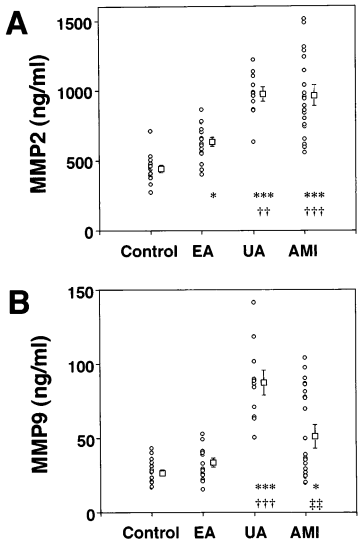


Figure 1. Serum MMP-2 (**A**) and plasma MMP-9 (**B**) levels were measured in normal volunteers (Control) and patients with stable EA, UA or AMI. Peripheral blood was drawn from a peripheral vein of each patient on admission. **Squares and bars** = mean value ± SE. *$p < 0.05$ and ***$p < 0.001$ versus control group. ††$p < 0.01$ and †††$p < 0.001$ versus EA group. ‡‡$p < 0.01$ versus AMI group.

manufacturer's instructions (Fuji Chemical Industries Ltd., Takaoka, Japan).

Statistical analysis. Results are expressed as mean value ± SD, except in the figures, where SE values are shown. One-way factorial analysis of variance followed by the Sheffê F test was used for intergroup comparisons. A p value <0.05 was considered statistically significant.

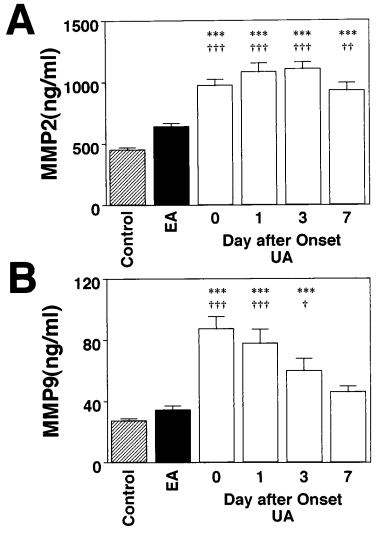
Results

Baseline characteristics. There were no significant differences in average age, ratio of male to female or prevalence of major risk factors among the normal control subjects and three patient groups (Table 1). Medications did not differ among the groups. Intravenous heparin was given to all patients in both the UA and AMI groups. The number of angiographically significant stenosis did not differ among the EA, UA and AMI groups. The clinical characteristics of the AMI-M group were not different from those of the other patients with AMI.

Peripheral blood levels of MMP-2 and MMP-9. Figure 1 demonstrates the peripheral blood levels of MMP-2 and MMP-9 in the normal volunteers and patients with EA, UA and AMI on admission. In the control group, serum MMP-2 and plasma MMP-9 levels were 443 ± 102 and 27 ± 8 ng/ml, respectively. These levels were similar to those reported previously in normal volunteers (6,7). Serum MMP-2 in the UA and AMI groups (976 ± 158 and 962 ± 273 ng/ml, respectively) was significantly higher than that in the control ($p < 0.001$ and $p < 0.001$, respectively) or EA group (634 ± 125 ng/ml, $p < 0.01$ and $p < 0.001$, respectively). MMP-2 levels in the EA group were also higher than those in the control group ($p < 0.05$). The UA group showed significantly higher plasma MMP-9 levels (87 ± 26 ng/ml) than those in not only the control group ($p < 0.001$) but also the EA and AMI groups (34 ± 11 and 49 ± 28 ng/ml, $p < 0.001$ and $p < 0.001$, respectively). Although the average of MMP-9 levels in the AMI group was significantly higher than that in the control group ($p < 0.05$), it was apparent that the AMI group included two distinct subgroups: Six patients demonstrated significantly higher MMP-9 levels than did control subjects and patients with EA, and seven had MMP-9 levels similar to those in the control subjects and patients with EA.

Serial changes in MMP-2 and MMP-9 levels in patients with ACS. Serial changes in MMP-2 and MMP-9 levels were assessed in the patients with UA and AMI who underwent conventional medical treatment to avoid the potential effects of interventional or thrombolytic therapies. During the observation period, all patients with UA were well controlled and did not experience the symptoms or signs of exacerbation of myocardial ischemia. No AMI-M group patients showed clinical findings of reinfarction or postinfarction angina. In the patients with UA (Fig. 2A), elevation of serum MMP-2 was sustained from day 0 to day 7 by about twofold versus that in the control and EA groups. Plasma MMP-9 levels on day 0 were higher in the UA group by about threefold and twofold than in the control and EA groups, respectively, and a gradual decrease toward the control range was observed up to day 7 (Fig. 2B). As shown in Figure 3A, AMI-M group patients demonstrated a twofold sustained elevation of MMP-2 versus that in the control and EA groups. It was apparent that the two subgroups showing distinct time courses of plasma MMP-9 changes were included in the AMI-M group (Fig. 3B). In seven patients, MMP-9 levels were equivocal to those in the control and EA groups on day 0 and then were transiently increased,

Figure 2. Serial changes in serum MMP-2 (**A**) and plasma MMP-9 (**B**) in patients with UA who underwent conventional medical treatment. **Columns** = mean values; **bars** = SE. ***$p < 0.001$ versus control group. †$p < 0.05$, ††$p < 0.01$ and †††$p < 0.001$ versus EA group.



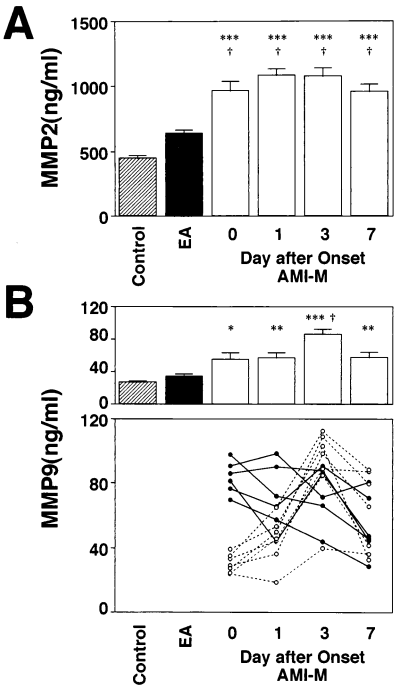


Figure 3. Serial changes in serum MMP-2 (**A**) and plasma MMP-9 (**B** [**top panel**]) in patients with AMI who underwent conventional medical treatment (AMI-M). Symbols as in Figure 2. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ versus control group. **B** (**bottom panel**), Individual data for AMI-M group patients. Six AMI-M group patients (**solid circles**) showed a sustained elevation of MMP-9 levels from day 0 until day 3, whereas in the other seven patients (**open circles**) MMP-9 levels were equivocal to those in the control and EA groups on day 0 and were then transiently increased, with a peak on day 3.

with a peak on day 3. In the six other patients, significant MMP-9 elevations (twofold increase vs. control group) were seen on day 0, and the levels remained higher than those in control subjects up to day 3. There were no significant differences in MMP-2 and MMP-9 levels on day 0 between the AMI-M group and the rest of the AMI group.

Serum CK and CK-MB isoform levels had no significant correlation with either serum MMP-2 or plasma MMP-9 levels at any time point in patients with UA or AMI, and neither maximal MMP-2 nor MMP-9 levels were associated with maximal CK or CK-MB isoform levels (data not shown). Also, maximal levels of these MMPs had no correlation with blood C-reactive protein (CRP) on day 0 or maximal CRP levels (data not shown).

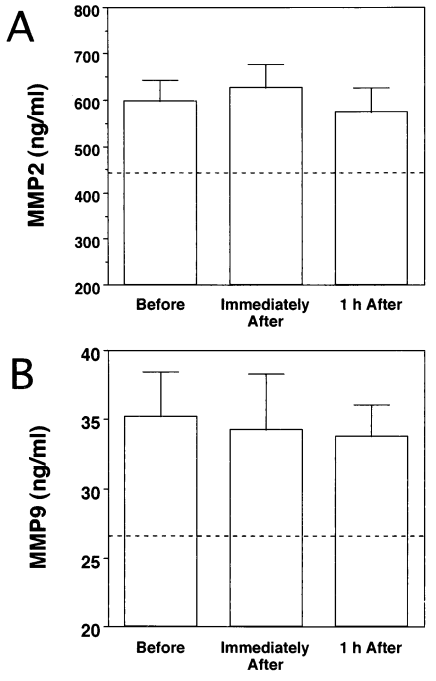
Myocardial ischemia and MMP-2 and MMP-9 levels. In patients with EA, serum MMP-2 and plasma MMP-9 levels were measured before, immediately after and 1 h after the treadmill exercise test. MMP-2 and MMP-9 levels did not change during the exercise test (Fig. 4), although exercise-induced angina and typical ischemic ECG changes were documented.

Discussion

The present study showed that increases in serum MMP-2 levels were sustained up to day 7 in both UA and AMI group patients and that plasma MMP-9 was transiently elevated in patients with UA and AMI, although the time course of MMP-9 was different between the two groups. To our knowledge, present report is the first to show that peripheral blood levels of MMP-2 and MMP-9 were increased in patients with ACS.

MMPs play an important role in ECM remodeling during all phases of atherosclerosis. The ECM constitutes the bulk of most advanced atherosclerotic plaques. Therefore, ECM metabolism in atherosclerotic lesions is considered to favor the overall net accumulation rather than degradation of matrix components. However, focal accumulation of cells that overexpress activated forms of MMPs may promote local destruction of ECM in atheroma, leading to plaque destabilization and rupture (3). Constitutive expression of MMP-2 has been shown (4) in VSMCs in normal arteries, and MMP-2 expression was increased in VSMCs in atherosclerotic arteries. Furthermore, MMP production by VSMCs is associated with the shift of these cells to the modulated phenotype typical of atheroma (8). The present study demonstrated higher MMP-2 levels in patients with EA than in control subjects and, furthermore, that patients with ACS had sustained MMP-2 elevations that were greater than those in patients with EA.

Figure 4. Serum MMP-2 (**A**) and plasma MMP-9 (**B**) were measured before, immediately after and 1 h after the treadmill exercise test in patients with stable EA. A symptom-limited exercise test was performed, and anginal pain accompanied with typical ischemic ECG changes occurred in all the patients. **Dashed lines** = average levels in normal volunteers (serum MMP-2: 443 ± 102 ng/ml; plasma MMP-9: 27 ± 8 ng/ml); other symbols as in Figure 2.



Taken together, increases in serum MMP-2 may be associated with progression and destabilization of coronary atherosclerosis or phenotypic changes in VSMCs in such lesions. Induction of MMP-9 expression as well as interstitial collagenase and stromelysin has been shown (4,5) in both VSMCs and accumulating macrophages in atherosclerotic plaques, particularly in the shoulder and core of plaques prone to rupture. These observations raised the possibility that these MMPs are strongly associated with the molecular mechanism of the onset and development of ACS. Accordingly, the transient elevation of MMP-9 levels in patients with UA may be associated with the increased expression of MMP-9, probably in activated macrophages or VSMCs in the plaque prone to rupture. It is noteworthy that MMP-9 levels declined after admission in patients with UA in whom clinical symptoms and signs were stabilized by medical treatment. This observation might reflect the stabilization of the sources of MMP-9. Two distinct patterns of the time course of MMP-9 levels were observed in patients with AMI: On day 0, six patients had elevated MMP-9 levels, whereas the other seven patients did not. It is plausible that the significance of the contribution of MMPs to the pathogenesis of AMI is widely varied in each case because other factors, such as a variety of mechanical and hemodynamic forces, rheologic factors and vasoconstriction, could also precipitate and trigger disruption of vulnerable plaques (1,2). The delayed increase or sustained elevation in MMP-9 seen on day 3 may be due to induced MMP-9 in the infarcted myocardium because delayed activation of MMP-9 has been reported (9) in the rat myocardium at ~day 4 after experimental infarction.

All MMPs require activation from precursors to attain enzymatic activity. The antibodies available do not distinguish the active form of these enzymes from their proenzyme forms; and areas that contain MMPs in the plaques also contain tissue inhibitors of metalloproteases, molecules that can prevent the matrix-degrading action and activation of MMPs. Therefore, we believe that increased MMP immunoreactivity does not necessarily correspond to its augmented enzymatic activity. We cannot deny the possibility that ischemia may cause leakage of MMPs from the vessel wall or myocardium. However, it is unlikely to be a main factor because no elevation of MMP-2 or MMP-9 was observed in the patients with EA immediately and 1 h after exercise-induced ischemia, even though these patients showed significant ischemic evidence during the exercise test. It is also not likely that leakage due to myocardial necrosis was the main source of MMPs because MMP levels were not correlated with serum CK or CK-MB isoform in the patients with AMI. Finally, it is possible that peripheral macrophages and leukocytes might be a source of elevated MMPs because monocytes in the systemic circulation can be activated in patients with ACS (10). Although there was no correlation between CRP, a marker of systemic inflammation, and MMP levels, this does not necessarily deny the possibility that elevated MMP levels could indicate cell activation in plaques. Further investigations are needed to address these issues.

Conclusions. Serial changes in serum MMP-2 and plasma MMP-9 were documented in patients with ACS and provide an insight into the molecular mechanism of plaque destabilization of coronary atherosclerotic lesions.

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References

1. Libby P. Molecular basis of the acute coronary syndromes. Circulation 1995;91:2841–50.
2. Falk E, Shah PK, Fuster V. Coronary plaque disruption. Circulation 1995;92:657–71.
3. Dollery CM, McEwan JR, Henney AM. Matrix metalloproteases and cardiovascular disease. Circ Res 1995;77:863–8.
4. Galis ZS, Sukhova KG, Lark MAW, Libby P. Increased expression of matrix metalloproteases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. J Clin Invest 1994;94:2493–503.
5. Brown DL, Hibbs MS, Kearney M, Loushin C, Isner JM. Identification of 92-kDa gelatinase in human coronary atherosclerotic lesions. Association of active enzyme synthesis with unstable angina. Circulation 1995;91:2125–31.
6. Fujimoto N, Mouri N, Iwata K, Ohuchi E, Hayakawa T. A one-step sandwich enzyme immunoassay for human matrix metalloprotease 2 (72-kDa gelatinase/type IV collagenase) using monoclonal antibodies. Clinica Chimica Acta 1993;221:91–103.
7. Fujimoto N, Hosokawa N, Iwata K, Shinya T, Okada Y, Hayakawa T. A one-step sandwich enzyme immuno assay for inactive precursor and complexed forms of human matrix metalloprotease 9 (92 kDa gelatinase/type IV collagenase, gelatinase B) using monoclonal antibodies. Clinica Chimica Acta 1994;231:79–88.
8. Sasaguri Y, Murahashi N, Sugama K, Kato S, Hiraoka K, Satoh T, Isomoto H, Morimatsu M. Development-related changes in matrix metalloprotease expression in human aortic smooth muscle cells. Lab Invest 1994;71:261–9.
9. Cleutjens JPM, Kandala JC, Guarda E, Guntak RV, Weber KT. Regulation of collagen degradation in the rat myocardium after infarction. J Mol Cell Cardiol 1995;27:1281–92.
10. Jude B, Agraou B, McFadden EP, et al. Evidence for time-dependent activation of monocytes in the systemic circulation in unstable angina but not in acute myocardial infarction or in stable angina. Circulation 1994;90:1662–8.