Matrix metalloproteinase (MMP), an extracellular matrix degrading enzyme, plays a crucial role in the breakdown of the fibrous cap of plaque and subsequent rupture in the pathogenesis of acute coronary syndrome (ACS). The MMP family has been identified in the shoulder regions of human atherosclerotic plaque, and is more frequently expressed in the coronary plaque of patients with ACS than with stable angina pectoris (SAP). MMP-9 (92-kDa gelatinase) is associated with atherosclerotic arterial remodeling and is actively synthesized in vulnerable plaque. MMP-9 affects plaque stability in association with various inflammatory cytokines. Elevated plasma levels of MMP-9 in the peripheral blood have been shown in patients with ACS and are associated with severe coronary stenosis and cardiovascular mortality. In addition, the plasma MMP-9 level is elevated in the coronary circulation of patients with ACS, indicating that the production of MMP-9 may be enhanced in ACS.

MMP release, however, may also occur in the interstitium of ischemic myocardium in the early period after a myocardial infarction (MI) as well as from coronary plaque. The serum MMP concentration is associated with left ventricular remodeling after MI and in particular, an elevated plasma MMP-9 level is associated with infarct size. Hence, whether the main source of increased plasma MMP-9 is the culprit coronary plaque or the infarcted myocardium still remains unclear.

The aim of this study was to determine the major source of plasma MMP-9 in patients with early phase acute MI (AMI) by measuring the plasma levels of MMP-9 in the coronary artery as well as a peripheral artery of patients with AMI and in those with SAP during percutaneous coronary intervention (PCI) using a distal protection device. We also investigated in situ MMP-9 expression in coronary artery plaque aspirated from patients with AMI and compared it with samples obtained by directional coronary atherectomy (DCA) in patients with SAP.

Methods

Patients
We studied 23 consecutive patients with AMI within 24 h from the onset of chest pain (6 women; age range 44–87 years, 64±12 years) and 10 consecutive patients with SAP as the control (5 women; age range 50–71 years, 66±10 years, p=0.61 vs AMI). The diagnosis of AMI was determined on the basis of chest pain lasting longer than 30 min with ST-segment elevation >2 mm in at least 2
contiguous leads of the electrocardiogram, and with more than a 3-fold increase in serum creatine kinase level. Patients with SAP were those who complained of chest pain on exertion with evidence of myocardial ischemia in whom critical coronary stenosis was confirmed by coronary arteriography. All subjects underwent PCI under distal protection with the PercuSurge GuradWire System (Medtronic AVE). Patients with AMI were transferred to the catheterization laboratory and PCI was performed within 1 h of admission. Patients unsuitable for the distal protection procedure because of triple vessel disease, cardiogenic shock or any other serious conditions were excluded. Patients with renal failure, liver function abnormality, and those with a systemic inflammatory disease were also excluded. Oral antiplatelet therapy (200 mg/day aspirin and 200 mg/day ticlopidine) was begun after PCI unless contraindicated. The Ethics Committee of the Kansai Rosai Hospital approved the study protocol and all patients gave written informed consent before cardiac catheterization.

**Distal Protection Device**

A balloon-type temporary occlusion and aspiration system for the coronary artery (PercuSurge GuardWire™ System) was used for distal protection during PCI and blood sampling from the coronary artery. Detailed specifications of the system are described elsewhere. In brief, it consists of a guidewire with a distal occlusion balloon (GuardWire Plus), an aspiration catheter (Export), MicroSeal Adapter and EZ Flator inflating system. Guard Wire Plus comprises a 0.014-inch nitinol hypotube with an inflatable internal lumen, which is connected to the occlusion balloon (3–6 mm in diameter) at the end. Export Aspiration catheter is a 0.071-inch monorail-type aspiration catheter incorporating a 0.040-inch lumen that requires an 8Fr guide catheter. GuardWire Plus is connected to the MicroSeal Adapter and EZ Flator, which adjust the size of the distal occlusion balloon under low pressures less than 1 atm. This distal protection and aspiration system enabled us to take selective blood samples from the PCI site in the coronary artery.

**PCI With Distal Protection**

Prior to PCI each patient was given 10,000 units and additional heparin was administered during the procedure to maintain the activated clotting time >250 s. First, the culprit lesion was crossed with a 0.014-inch angioplasty guidewire and then the Export Aspiration catheter was advanced to the culprit lesion for extensive aspiration while advancing and retracting the catheter (initial aspiration: Initial). The SAP patients underwent the same procedure, although none had a total occlusive lesion. Next, the PercuSurge GuardWire was advanced beyond the lesion, parallel with the coronary guidewire. Direct stenting and/or balloon angioplasty was performed under distal protection. (E) Second aspiration (Second blood sampling) to collect material released from the disrupted plaques. (F) Final coronary arteriogram after deflation of the distal occlusion balloon shows restored coronary flow.

![Fig 1. Serial coronary arteriograms showing the intervention procedure. (A) Complete occlusion of the left anterior descending coronary artery. (B) Coronary guidewire crossing the culprit lesion, followed by thrombus aspiration with an aspiration catheter (Initial blood sampling). (C) Coronary arteriogram immediately after the initial aspiration. Thrombolysis In Myocardial Infarction 3 flow grade was obtained in 21 of 23 cases (91%). (D) PercuSurge GuardWire™ also crossing the lesion, parallel with the coronary guidewire. Direct stenting and/or balloon angioplasty was performed under distal protection. (E) Second aspiration (Second blood sampling) to collect material released from the disrupted plaques. (F) Final coronary arteriogram after deflation of the distal occlusion balloon shows restored coronary flow.](image-url)
Blood samples were obtained from the femoral artery (FA) at the beginning of PCI and during the first and second aspirations from the coronary artery (Fig 2). Blood from the coronary artery was directly collected into the collection bottle without filtration. In 7 AMI patients, a thermodilution catheter was inserted via the internal jugular vein after the PCI, and samples from the coronary sinus (CS) were collected simultaneously using a 5F Multi-purpose catheter (Goodman). The blood was immediately mixed with sodium EDTA, centrifuged at 3,000 rpm for 5 min, separated and stored at –80°C. Plasma levels of MMP-9 were measured by one-step sandwich enzyme immunoassay using 2 monoclonal antibodies (Daiichi Fine Chemical).20

Immunohistochemistry

Subgroups of AMI and SAP patients were selected for immunohistochemistry. The AMI group comprised 20 patients (3 women; age range 44–82 years) from whom coronary plaque was successfully obtained during the emergency PCI. These patients underwent aggressive intracoronary aspiration using an aspiration catheter (Resque™ Thrombectomy System, Boston Scientific), and thrombi that included fragments of the coronary plaque were obtained. The SAP group comprised 10 patients (4 women; age range 55–65 years) from whom coronary plaque samples were while they underwent elective DCA.

MMP-9 Staining

Specimens were placed in tissue fixative (Histochoice, Hedwin, Baltimore, MD, USA). After overnight fixation, they were embedded in paraffin and sectioned at 4-μm intervals. Tissue sections were deparaffinized with xylene followed by immersion in a graded alcohol series. They were washed 3 times for 5 min each in phosphate-buffered saline (PBS) and blocked with bovine serum albumin for 60 min. Specimens were then incubated with primary antibodies against MMP-9 (Fuji Chemical, Tokyo, Japan) overnight at 4°C. After being washed in PBS, they were incubated with biotinylated rabbit anti-mouse IgG for 60 min at room temperature. Specimens were then washed with PBS, stained with horseradish peroxidase-conjugated streptavidin, and finally incubated with substrate solution for 1–15 min. The tissue sections were also stained with hematoxylin-eosin. MMP expression was semiquantitatively graded as 0 if there was no staining in any visual field, 1 if <25% cells were stained, 2 if <50% cells were stained and 3 if >50% cells and extracellular matrix were positive for staining. Grading of immunohistochemistry staining was performed by pathologists unaware of the patients' backgrounds.

Statistical Analysis

Data are expressed as mean±SE, unless otherwise indicated. One-way factorial analysis of variance (ANOVA) followed by Sheffe’s post hoc test was used for intergroup comparisons. Two-factor ANOVA or unpaired t-test was applied to evaluate the difference between the groups. Mann-Whitney’s U test was used to compare the staining grade. A probability value of less than 0.05 was considered statistically significant.

Results

Plasma MMP-9 Measurement

AMI vs SAP  Plasma MMP-9 levels were elevated at any sampling point in patients with AMI, whereas they fell within the normal range of 38±13 ng/ml20 in patients with
SAP (Fig 3). MMP-9 levels in the coronary artery, both Initial and Second, were significantly higher in patients with AMI than in those with SAP (Initial, 141.6±23.3 ng/ml vs 37.2±6.3 ng/ml, p<0.01; Second, 194.9±27.3 ng/ml vs 54.2±12.0 ng/ml, p<0.01) although there was no statistical difference regarding the MMP levels in the systemic circulation, in FA, between patients with AMI and those with SAP.

**Variation of MMP-9 Level Among the Sampling Points**

MMP-9 levels in Initial were significantly higher than those in the FA (141.6±23.3 ng/ml vs 81.9±19.2 ng/ml, p<0.05) in patients with AMI, and they further increased in Second (194.9±27.3 ng/ml, p<0.0001 vs FA) (Fig 4). MMP-9 levels in the FA were also elevated in some patients with AMI and they remained high throughout the PCI procedure in those patients. In contrast, MMP-9 levels remained within the normal range in patients with SAP throughout the PCI procedure (Fig 4). MMP-9 levels in the CS were similar to those in the FA despite the elevation in the coronary artery, particularly in Second (Fig 5).

**Immunohistochemistry**

Hematoxylin-eosin staining of the plaque samples aspirated from patients with AMI revealed both thrombi and atheromatous tissue, in which many macrophages strongly positive for MMP-9 were present (Fig 6). In contrast, specimens from patients with SAP had MMP-9 negative to moderately positive cells (Fig 6). The cell-associated staining grade for MMP-9 was 0 in 2 samples (10%), 2 in 3 samples (15%), and 3 in 15 samples (75%) in patients with AMI. Compared with 0 in 8 samples (80%), 1 in 1 sample (10%), 2 in 1 sample (10%), and no samples showing grade 3 from patients with SAP. This semiquantitative grading demonstrated that MMP-9 expression was significantly augmented in the specimens from patients with AMI (p<0.0001).

**Discussion**

The present study demonstrates for the first time that the plasma levels of MMP-9 are significantly higher in the coronary artery than in the systemic circulation (FA and CS) in patients with AMI. Mechanical disruption of the culprit plaque by PCI induces a further elevation of the MMP-9 level in the coronary artery. These elevations were not observed in patients with SAP. In addition, immunohistochemistry revealed augmented MMP-9 expression in the coronary plaque from patients with AMI. Given these findings, the increase in the plasma MMP-9 level in patients with AMI is mainly attributable to the rupture of the culprit plaque in the coronary artery, rather than to the production from necrotic myocardium downstream.

Earlier immunohistochemistry studies on human coronary artery specimens obtained by directional atherectomy revealed localization of MMP-9 in the coronary artery plaque, associated with ischemic heart disease. Larger numbers of MMP-9-positive macrophages were contained within atherectomy specimens from patients with unstable angina pectoris than in those with SAP. MMP-9 was shown to be actively synthesized by macrophages and
smooth muscle cells in the atherosclerotic lesions of the coronary artery in patients with unstable angina pectoris. These reports uniformly indicated augmented in vivo MMP-9 localization in the atheromatous plaque and its potential role in plaque vulnerability. Thus, atherosclerotic plaque may be the major source of MMP-9 released into the circulation. However, MMP-9 release and activation were also demonstrated in the ischemic myocardial interstitium in the early post-MI period in animals. Thus, the necrotic myocardium could also be the predominant source of MMP-9. Previous blood sampling from the CS was unable to exclude the production of MMP by necrotic myocardial tissue. In the present study, however, selective blood sampling using the PercuSurge distal protection device together with CS sampling demonstrated in vivo the elevation of plasma MMP-9 levels in the coronary artery in patients during the acute phase of an AMI. Recently, Funayama et al also reported elevated MMP-9 levels in the human coronary artery but they did not refer to the influence of PCI nor did they simultaneously measure the MMP-9 levels in the CS blood.

Local blood concentration or dilution may affect the measured values, and elevation of circulating plasma MMP-9 levels alone does not directly prove in situ expression in coronary artery plaque. Hence, we immunohistochemically demonstrated augmented expression of MMP-9 and associated inflammatory cells in the coronary artery plaque obtained from patients with AMI. Immunohistochemistry, as well as the blood samples, indicated that culprit plaque and associated inflammatory cells contained large amounts of MMP-9, which would be released into the coronary circulation by mechanical disruption.

Interestingly, patients with AMI who demonstrated TIMI 0 flow in the first coronary arteriogram showed relatively higher levels of MMP-9 in the initial aspiration samples than those who demonstrated TIMI 1–3 flow (165.7±30.6 ng/ml vs 86.8±21.0 ng/ml, p=0.12). It is interesting to speculate that MMP-9 content may vary depending on the local thrombus burden. Of note, MMP-9 may induce thrombus formation through degradation of the tissue factor pathway inhibitor, as reported for porcine coronary arteries. Further increases in the MMP-9 level in the second aspiration samples taken in our study indicate that mechanical disruption of the culprit plaque in AMI causes MMP-9 release into the coronary circulation, which may also further aggravate thrombus formation during coronary intervention.

Recently, plasma MMP-9 has been identified as a novel indicator of cardiovascular mortality in patients with coronary artery disease. Together with the data from our study, this suggests that plasma MMP-9 may not only be a risk marker, but also an indicator of atherosclerotic plaque vulnerability in patients with ischemic heart disease. Nonetheless, our data failed to show a difference between the AMI and SAP groups in MMP-9 levels in the FA, suggesting that care should be taken to interpret systemic biomarkers for coronary artery disease.

Study Limitations

The study population was relatively small, although the elevation of the MMP-9 level reached statistical significance. A larger population including a variety of re-flow conditions would be of interest. Because all blood samples were obtained only during a PCI procedure performed in the acute phase, these results can not refer to the subsequent clinical course of AMI patients. In the immunohistochemistry study, aspirated specimens were compared with DCA samples. It would be desirable to use the same DCA method to obtain tissue specimens from patients with AMI, but as this is seldom performed in the clinical setting of AMI, we used fragmented coronary artery plaque obtained by the aspiration technique.
Plasma MMP-9 in AMI

Conclusion

Analysis of blood samples from a peripheral artery, the coronary artery and the CS during PCI, together with immunochemical staining of plaque, indicate that MMP-9 is mainly released into the coronary circulation, not from the myocardium, but from the culprit coronary artery plaque in patients with AMI.

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References