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Involvement of Inflammation in Acute Coronary Syndromes Assessed by Levels of High-Sensitivity C-Reactive Protein, Matrix Metalloproteinase-9 and Soluble Vascular-Cell Adhesion Molecule-1

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Abstract

Objectives. Inflammation is important in the development of atherosclerosis. Matrix metalloproteinases (MMPs) and interferon-γ which participate in collagen degradation are pathological factors in plaque vulnerability as an important mechanism underlying acute coronary syndrome. This study investigated whether inflammation is related to the onset of acute coronary syndrome.

Methods. This study included 56 patients with acute coronary syndrome (ACS group), 104 patients with chronic coronary artery disease (S group), and 38 control subjects with no evidence of ischemic heart disease (C group). High-sensitivity C-reactive protein (hs-CRP), MMP-9, and interferon-γ were measured in peripheral blood samples. Soluble adhesion molecules (VCAM-1, ICAM-1) were also measured as inflammatory markers.

Results. The hs-CRP level was significantly higher in the ACS group (44.5 mg/*l*) than in the S group (2.1 mg/*l*) and the C group (0.6 mg/*l*) ($p < 0.0001$). The MMP-9 level was also significantly higher in the ACS group (333.8 ng/m*l*) than in the S group (110.8 ng/m*l*) and the C group (72.0 ng/m*l*) ($p < 0.0001$). The VCAM-1 level was significantly higher in the ACS group (506.5 ng/m*l*) than in the C group (448.8 ng/m*l*) ($p < 0.05$). The ICAM-1 level and the interferon-γ level did not differ between the groups. There was a significant positive correlation between the level of hs-CRP and the level of the collagen degradation product MMP-9 ($r = 0.52$) in all subjects.

Conclusions. These results suggest that plaque destabilized by MMP-9 produced in response to inflammation participates in the mechanism of acute coronary syndrome.

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Key Words

■**Coronary artery disease (acute coronary syndrome)** ■**Plaque**

INTRODUCTION

Inflammation is important in the development of atherosclerosis. Adhesion molecules, such as vascular-cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), cytokines and chemokines promote the progression of this disease. Matrix metalloproteinases (MMPs) and interferon-γ which participate in collagen degradation are pathological factors in plaque vul-

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nerability as an important mechanism underlying acute coronary syndromes[1].

Systemic inflammation increases the production of cytokines, oxidized low-density lipoproteins (LDL) and lipopolysaccharide (endotoxin). Pathogens such as chlamydia and viruses stimulate macrophages, T lymphocytes and other types of inflammatory cells in atherosclerotic lesions to release atherogenic reactants, such as adhesion molecules, cytokines and chemokines[2].

Systemic inflammation also activates T lymphocytes and macrophages, inflammatory cells residing in plaque. T lymphocytes release interferon-γ, which inhibits smooth muscle cell proliferation and decreases collagen synthesis. Interferon-γ also increases the production of MMPs by macrophages, leading to degradation of the extracellular matrix of the fibrous cap of plaque. The fibrous cap weakens, finally resulting in plaque disruption[3].

Consequently, patients with vulnerable plaques may show elevated peripheral blood levels of interferon-γ and MMPs. Therefore, MMP-9 and interferon-γ levels were measured in patients with acute coronary syndrome. The blood levels of high-sensitivity C-reactive protein (hs-CRP) were measured to examine the relationship between inflammation and the onset of acute coronary syndrome. Soluble adhesion molecules (VCAM-1, ICAM-1) were also measured as inflammatory markers.

SUBJECTS AND METHODS

Study population

This study included 56 patients with acute coronary syndromes admitted within 6 hr after symptom onset (ACS group, 48 with acute myocardial infarction and 8 with unstable angina), 104 patients with chronic coronary artery disease (S group, 46 with old myocardial infarction and 58 with stable angina), and 38 control subjects with no evidence of ischemic heart disease (C group). The following clinical characteristics of the subjects were recorded: age, sex, presence or absence of hypertension, diabetes mellitus, hyperlipidemia and smoking. All subjects gave written informed consent before enrollment in the study.

Measurement of inflammatory markers

Hs-CRP, MMP-9 and interferon-γ levels were measured in peripheral blood samples. Soluble adhesion molecule (VCAM-1, ICAM-1) levels were also measured as inflammatory markers. Hs-CRP was measured by nephelometric assay (Dade Behring). MMP-9 was measured by enzyme immunoassay (Fuji Pharmaceutical Industries). VCAM-1 and ICAM-1 were determined by enzyme-linked immunosorbent assay (R&D Systems), and interferon-γ was measured by enzyme immunoassay (Bio Source).

Statistical analysis

Continuous data are expressed as mean ± SD. The Kruskal-Wallis test was used to compare clinical characteristics between the three groups. Any significant difference underwent the Mann-Whitney *U*-test with Bonferroni's correction. Differences in hs-CRP, MMP-9, soluble VCAM-1 and soluble ICAM-1 levels between the three groups were compared by one-way analysis of variance. Significant differences were tested by Fisher's protected least significant difference. p values of less than 0.05 were considered to indicate statistical significance.

RESULTS

Baseline characteristics of patients

Mean age was similar in the ACS group (64.9 ± 9.5 years), the S group (60.1 ± 10.2 years), and the C group (59.6 ± 9.7 years). The proportion of men was also similar in the three groups (ACS group, 78.6%; S group, 77.9%; C group, 73.7%). The proportion of patients with hyperlipidemia was higher in the ACS group (60.7%) and the S group (68.3%) than in the C group (28.9%), but the proportions of patients with other risk factors, such as hypertension, diabetes mellitus, and smoking did not differ significantly (**Table 1**).

Inflammatory markers

The hs-CRP level was 44.5 mg/*l* in the ACS group, significantly higher than 2.1 mg/*l* in the S group or 0.6 mg/*l* in the C group ($p < 0.0001$). The MMP-9 level was 333.8 ng/m*l* in the ACS group, significantly higher than 110.8 ng/m*l* in the S group or 72.0 ng/m*l* in the C group ($p < 0.0001$; **Fig. 1**). The VCAM-1 level was 506.5 ng/m*l* in the ACS group, significantly higher than 448.8 ng/m*l* in the C group ($p < 0.05$, ACS group vs C group). The ICAM-1 was not different between the three groups (**Fig. 2**). The interferon-γ level was low in all three groups and did not differ significantly.

All subjects showed a significant positive correlation between the level of hs-CRP and the level of the collagen degradation product MMP-9 ($r = 0.52$,

Table 1 Baseline characteristics of patients

	ACS group (*n*=56)	S group (*n*=104)	C group (*n*=38)
Age (yr, mean±SD)	64.9±9.5	60.1±10.2	59.6±9.7
Male	44 (78.6)	81 (77.9)	28 (73.7)
Coronary risk factors			
Hypertension	28 (50.0)	67 (64.4)	25 (65.8)
Diabetes mellitus	16 (28.6)	29 (27.9)	4 (10.5)
Hyperlipidemia	34 (60.7)*	71 (68.3)*	11 (28.9)
Smoking	20 (35.7)	53 (51.0)	14 (36.8)

( ): %. *$p<0.05$ vs C group.
ACS group: With acute coronary syndrome. S group: With chronic coronary artery disease. C group: Without evidence of ischemic heart disease.

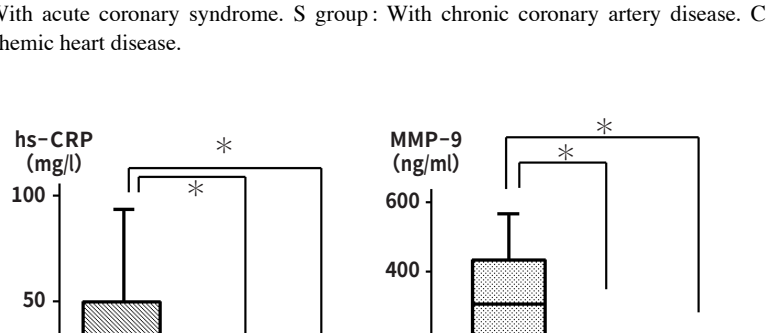

Fig. 1 Levels of high-sensitivity C-reactive protein and matrix metalloproteinase-9 in the ACS group, S group and C group
The levels of hs-CRP (*left*) and MMP-9 (*right*) were significantly higher in the ACS group than in the S and C groups. The box represents the interquartile range (between the 25th and 75th percentiles); the median is shown as a horizontal bar within each box. The bars outside each box show the range of 95% of all values.
*$p<0.0001$.
hs-CRP = high-sensitivity C-reactive protein; MMP-9 = matrix metalloproteinase-9.
Explanation of the groups as in Table 1.

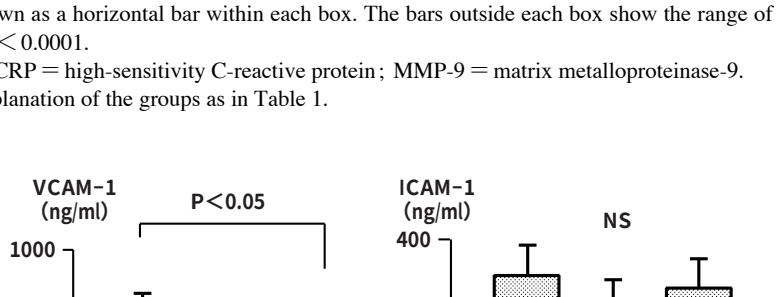

Fig. 2 Levels of vascular-cell adhesion molecule-1 and intercellular adhesion molecule-1 in the ACS group, S group and C group
The level of VCAM-1 (*left*) was significantly higher in the ACS group than in the C group. The level of ICAM-1 (*right*) did not differ between the groups. The box represents the interquartile range (between the 25th and 75th percentiles); the median is shown as a horizontal bar within each box. The bars outside each box show the range of 95% of all values.
VCAM-1 = vascular-cell adhesion molecule-1; ICAM-1 = intercellular adhesion molecule-1.
Explanation of the groups in Table 1.

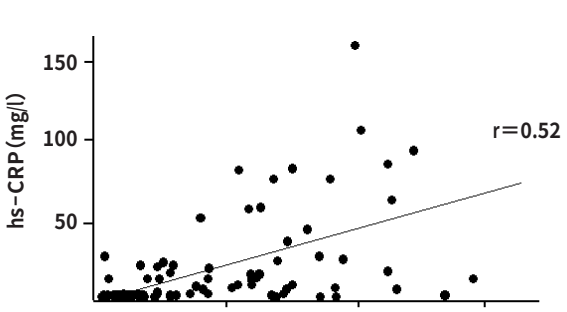

Fig. 3 Scatterplot of high-sensitivity C-reactive protein and matrix metalloproteinase-9 values in all subjects
The levels of hs-CRP and MMP-9 were significantly correlated ($r = 0.52$).
Abbreviations as in Fig. 1.

$p < 0.0001$; **Fig. 3**).

DISCUSSION

Acute coronary syndromes such as unstable angina and acute myocardial infarction are linked to disruption of plaque. Since elevated concentrations of CRP, serum amyloid A and other acute-phase proteins occur in the blood of patients with unstable angina, the involvement of inflammation has received considerable attention in acute coronary syndromes[4]. Inflammatory markers found in increased concentrations in patients with acute coronary syndrome include acute-phase proteins and cytokines, such as CRP, serum amyloid A, plasminogen activator inhibitor-1, MMPs, interleukin-6 and interleukin-8[4–8].

MMPs degrade collagen and other components of extracellular matrix and so are important in the destabilization of plaque and the onset of acute coronary syndrome. Our study showed that hs-CRP and MMP-9 levels were significantly higher in the ACS group than in the other groups. This elevation of MMP-9 levels may be associated with increased production of MMP, probably in activated macrophages or vascular smooth muscle cells in plaque prone to rupture. Vulnerable plaque is characterized by a large lipid core, a thin fibrous cap covering the plaque, sparse smooth muscle cells and aggressive macrophage infiltration[2]. Constitutive expression of MMP occurs[9]in vascular smooth muscle cells in normal arteries, and MMP expression is increased in vascular smooth muscle cells in atherosclerotic arteries.

Recently, a relationship has been found between blood levels of MMPs and acute coronary syndrome, but mostly in small studies. Serial changes occur in the peripheral blood levels of MMPs in patients with acute coronary syndrome[6]. Great cardiac vein-aortic root differences in plasma MMP-9 levels are elevated in patients with acute coronary syndrome, but no significant differences were detected in the plasma MMP-9 levels in the aortic root[10]. Our study of 208 patients showed that peripheral blood levels of MMP-9 in patients with acute coronary syndrome were significantly higher than those in patients with chronic coronary artery disease and control subjects. We also demonstrated a significant positive correlation between hs-CRP levels and MMP-9 levels in all subjects. These findings suggest that inflammation is important in the onset and progression of acute coronary syndromes.

MMPs are produced by endothelial cells and smooth muscle cells, as well as many other types of cells including neutrophils, macrophages and tumor cells. MMP-9 levels are increased in the coronary circulation of patients with acute coronary syndrome[10]. These findings indicate that MMP-9 is formed in the coronary circulation. Therefore, increased MMP-9 levels in acute coronary syndrome patients are related to plaque rupture and do not result from systemic inflammation.

Our study showed that hs-CRP levels were higher in the coronary artery disease group than in the control group. These findings may indicate that the hs-CRP level is useful for identifying chronic inflammation and may improve risk management in patients with atherosclerotic disease. The hs-CRP concentration is correlated with the risk of myocardial infarction and predicts the risk of coronary events[11]. Other studies have shown that elevated CRP concentration on admission for myocardial infarction is related to increased risk of unstable angina[12]and a CRP concentration of more than 5 mg/*l* is associated with increased mortality[13].

Our study found no significant difference in either the hs-CRP level or the MMP-9 level between patients with acute myocardial infarction and unstable angina. This observation suggests that elevated concentrations of hs-CRP and MMP-9 are somehow related to the development of acute coronary syndromes and do not result from tissue damage associated with acute myocardial infarction.

VCAM-1 level was significantly elevated in the ACS group, suggesting that patients with acute coronary syndrome have an increased risk of ather-

osclerosis. The expression of adhesion molecules such as VCAM-1 and ICAM-1 is induced by inflammatory cytokines, including interleukin-1 and tumor necrosis factor, and by endotoxins. These molecules are implicated in the local infiltration of inflamed tissue by various types of leukocytes. Oxidized LDL is thought to be very important role in the development of atherosclerosis. Lysophosphatidylcholine, considered the major active component of oxidized LDL, acts on cultured endothelial cells, selectively enhances the expression of VCAM-1, ICAM-1 and P-selectin on the cell surface, and strengthens the adhesion of monocytes to endothelium[14,15]. These adhesion molecules are thus thought to promote atherosclerosis.

Study limitations

This study had several limitations. Not all serum inflammatory marker levels differed significantly between the study groups. The serum level of interferon-γ was often below the detection limit, so assay sensitivity should be improved by the development of high-sensitive assays. Levels of interferon-γ in the coronary sinus blood (rather than the peripheral blood) should also be measured. Another limitation was that inflammatory marker levels were measured after the onset of symptoms in the ACS group, and we could not determine whether elevation of these markers was a cause or an effect of coronary events. However, because the patients with acute coronary syndrome were admitted within 6 hr after symptom onset, time-related effects on inflammatory marker levels were considered minimal.

CONCLUSIONS

Acute coronary syndromes are associated with significantly increased levels of hs-CRP, VCAM-1 and MMP-9 as compared with chronic coronary artery disease and control subjects with no evidence of ischemic heart disease. Our results suggest that plaque destabilized by MMP-9 produced in response to inflammation participates in the mechanism of acute coronary syndromes.

要　　約

急性冠症候群の発症と炎症との関連: 高感度C反応性蛋白とマトリックス・メタロプロテアーゼ-9および可溶性Vascular-Cell Adhesion Molecule-1の測定による検討

野本　和幹　　大口　純人　　渡辺　郁能　　久代登志男　　上松瀬勝男

目　的: 動脈硬化の進展には炎症が大きな役割を果たしている．急性冠症候群発症の機序の一つであるプラークの不安定化には，コラーゲン分解に関与するマトリックス・メタロプロテアーゼ(MMP)やインターフェロンγなどの物質が関係していることが病理学的に考えられている．本研究の目的は，急性冠症候群の発症と炎症との関連を明らかにすることである．

方　法: 当院に来院した発症6時間以内の急性冠症候群(急性群)56例，慢性冠動脈疾患(慢性群)104例，虚血性心疾患を有さない対照患者(対照群)38例を対象とし，末梢血の高感度C反応性蛋白，MMP-9およびインターフェロンγを測定した．さらに，炎症関連物質として可溶性接着分子(VCAM-1，ICAM-1)も測定した．

結　果: 高感度C反応性蛋白は，それぞれ急性群44.5 mg/*l*，慢性群2.1 mg/*l*，対照群0.6 mg/*l*で，急性群は慢性群と対照群に比較して有意に高値を示した($p < 0.0001$)．MMP-9も急性群333.8 ng/m*l*，慢性群110.8 ng/m*l*，対照群72.0 ng/m*l*で，急性群は慢性群と対照群に比較して有意に高値を示した($p < 0.0001$)．VCAM-1は急性群506.5 ng/m*l*，対照群448.8 ng/m*l*で，急性群は対照群に比較して有意に高値を示した($p < 0.05$)．ICAM-1とインターフェロンγは各群間で差は認められなかった．全例で検討すると，高感度C反応性蛋白値とMMP-9値との両値間に$r = 0.52$の有意な相関がみられた．

結　論: 急性冠症候群の発症には，炎症に基づくMMP-9の産生を介したプラークの不安定化が関与していることが示唆された．

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