Elevated Levels of Soluble Intercellular Adhesion Molecule-1 in the Coronary Circulation of Patients With Coronary Organic Stenosis and Spasm

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The cell surface expression of intercellular adhesion molecule-1 (ICAM-1) is upregulated following activation during inflammatory responses, mediating both cell migration and activation. The involvement of inflammation in unstable angina is suggested by the presence of activated circulating leukocytes. To examine whether plasma soluble ICAM-1 (sICAM-1) levels increase in the coronary circulation of patients with coronary organic stenosis and coronary spasm, plasma sICAM-1 levels were measured in the coronary sinus (CS) and the aortic root (Ao) simultaneously in 10 patients with 90% or more coronary narrowing and coronary spasm (coronary spastic angina (CSA) with organic stenosis), in 11 patients with coronary spasm and no significant coronary narrowing (CSA without organic stenosis), in 16 patients with stable exertional angina, and in 13 control subjects. The plasma sICAM-1 levels (ng/ml) in the CS increased in CSA with organic stenosis (230±26) as compared with CSA without organic stenosis (158±14), stable exertional angina (130±9) and control subjects (121±10) (p<0.01). The levels in the Ao also increased in CSA with organic stenosis (208±24) as compared with CSA without organic stenosis (140±13), stable exertional angina (130±11) and control subjects (121±10) (p<0.01). Furthermore, the plasma sICAM-1 levels were higher in the CS than in the Ao only in CSA with organic stenosis. These results suggest that activation of leukocytes occurs through the induction of ICAM-1 in the coronary circulation in the patients with CSA with organic stenosis. (Jpn Cir J 2000; 64: 170–176)

Key Words: Coronary circulation; Coronary organic stenosis; Coronary spastic angina; Soluble intercellular adhesion molecule-1 (sICAM-1)

Coronary artery spasm has been implicated in the pathogenesis of not only variant angina but also unstable angina and acute myocardial infarction. However, the precise mechanism(s) by which coronary spasm leads to coronary thrombosis, resulting in unstable angina or acute myocardial infarction, remains unknown. We have reported that the plasma levels of fibrinopeptide A is a sensitive marker of thrombin generation, and plasminogen activator inhibitor activity, an indicator of the impairment of fibrinolysis, increased in patients with coronary spasm. It has been thought that coronary spasm occurs at the site of organic stenosis of a major coronary artery. However, coronary spasm occurs in angiographically normal coronary arteries as well as in coronary arteries with organic stenosis. There is now increasing evidence that coronary plaque rupture or erosion and superimposed thrombus is the most important mechanism of acute coronary syndrome and acute inflammation caused by leukocyte adhesion in the coronary circulation plays a pivotal role in the genesis of this syndrome. The strong adhesion between the leukocytes and stimulated endothelial cells is mediated through the binding of leukocyte integrin adhesion receptors to the endothelial intercellular adhesion molecule-1 (ICAM-1), which is a major ligand on endothelial cells for adherence of activated polymorphonuclear leukocytes. A soluble form of ICAM-1 has been detected in human serum and the soluble ICAM-1 (sICAM-1) levels increased in conditions in which ICAM-1 expression on the cell membrane has also been shown to be increased. Recently, we reported that sICAM-1 release is increased, especially in the coronary circulation, in unstable angina without coronary spasm. The purpose of the present study is to compare sICAM-1 levels in the coronary circulation in patients with coronary spastic angina (CSA) with organic stenosis, CSA without organic stenosis, stable exertional angina, and in control subjects. We measured the plasma sICAM-1 levels in the coronary sinus (CS) and the aortic root (Ao) simultaneously in 4 patient groups.

Methods

Patient Groups

Fifty patients who underwent diagnostic coronary angiography were divided into 4 groups: (1) CSA with organic stenosis, (2) CSA without organic stenosis, (3) stable exertional angina, and (4) controls. All of the 21 patients with
coronary spasm had episodes of spontaneous angina at rest with electrocardiographic ST-segment elevation in more than one lead of the standard 12-lead ECG without definite evidence of myocardial necrosis by enzymatic techniques, and they had experienced spontaneous anginal attacks at least twice during the week prior to the catheterization. In all 21 patients coronary spasm was angiographically demonstrated in the left coronary artery during spontaneous attacks or attacks induced by intracoronary injection of acetylcholine. Ten of them had 90% or more coronary organic stenosis of the left coronary artery and coronary spasm was shown in the left coronary artery (CSA with organic stenosis). Patients with CSA with organic stenosis between 25% and less than 90% were excluded from this study. The CSA with organic stenosis group consisted of 10 patients (9 men and 1 woman; mean age, 61 ± 9 years, range, 51–80). The CSA without organic stenosis group consisted of 11 patients (9 men and 2 women; mean age, 57 ± 8 years, range, 46–72). The stable exertional angina group consisted of 16 patients (10 men and 6 women; mean age, 63 ± 5 years, range, 51–71) who had typical exertional angina, no episodes of rest angina within 1 year, and had 90% or more coronary organic stenosis of the luminal diameter in the left coronary artery. The control group consisted of 13 patients (9 men and 4 women; mean age, 57 ± 9 years, range, 48–71) who had no significant coronary artery stenosis (<25% of luminal diameter), and no coronary spasm was demonstrated after intracoronary injection of acetylcholine. The 4 groups were matched for age and gender. Cardiac catheterization and coronary angiography were performed within 1 week of admission. Anti-anginal drugs, including long-acting nitrates, calcium channel blockers, β-blockers, and aspirin, were usually started on the first day of admission, and were not discontinued in the patients of the CSA with organic stenosis group who were diagnosed before coronary angiography. In the CSA without organic stenosis group, the stable exertional angina group and the control group all the medications except sublingual nitroglycerin were stopped at least 24 h before the study. At the time of the study, no patients had acute inflammation, autoimmune disease, cancer or congestive heart failure. No patients took nonsteroidal anti-inflammatory drugs or steroids. Patients with a history of myocardial infarction within 1 year of the onset of coronary artery spasm were excluded from the study because we have previously observed that sICAM-1 levels were significantly higher in acute myocardial infarction during the acute phase and for at least 4 weeks from the onset than in stable exertional angina and the control group. The study protocol was approved by the ethics committee of the hospital, and written informed consent was obtained from each patient and his or her family.

Procedures for Catheterization and Blood Sampling

Coronary angiography was performed using the Judkins technique with 6F JL4, 6F JR-4 and 6F Pig Tail catheters (Cordis) and contrast material (ioxaglate, Guerbet SA) in the morning when the patients were fasting. A 6F Goodale-Lubin catheter (USCI) and a 6F JL4 catheter were used for blood sampling. The 6F Goodale-Lubin catheter for CS blood sampling was inserted through the right antecubital vein and was advanced into the CS. Its position was confirmed by injecting a small amount of contrast medium. Immediately after this procedure, coronary angiograms were taken in the right and left anterior oblique positions with adequate angulation to allow clear visualization of the left and right coronary arteries, respectively. All patients were given 50 units/kg of heparin with the insertion of the Judkins catheter. After control left and right coronary angiography, or after the relief of coronary spasm by intracoronary injection of nitroglycerin or isosorbide dinitrate (ISDN) when spontaneous attack of coronary spasm occurred, blood samples for sICAM-1 were collected from the CS and Ao simultaneously at the same speed. At the time of blood sampling, the first 3 ml of blood was discarded, and an additional 2.7 ml of blood for sICAM-1 assay was immediately drawn into a tube containing 0.3 ml of sodium citrate (0.13 mol/L, pH 7.5). The blood samples were immediately centrifuged at 3000 rpm for 10 min at 4°C, and the divided plasmas were stored at −80°C until assay. The assay was performed within 1 month of collection. When coronary spasm did not occur spontaneously in the patients suspected to have coronary spasm, we provoked it with intracoronary acetylcholine. When acetylcholine was injected into the coronary artery, a tripolar electrode catheter (USCI) was placed in the right ventricular apex through the right femoral vein and was connected to a temporary pacemaker set at a rate of 50 beats/min. Incremental doses of acetylcholine were injected into the left coronary artery (50, 100 μg) and subsequently into the right coronary artery (20, 50 μg) until coronary spasm was induced or the maximal doses were reached in all patients with coronary spasm and in the control subjects. Coronary spasm induced by this method usually resolves spontaneously within 2–3 min. Coronary spasm was defined as total or subtotal occlusion of the epicardial coronary arteries associated with signs of myocardial ischemia such as chest pain and ischemic ST-segment changes. Blood samples were taken again simultaneously from the CS and Ao when coronary spasm was provoked. Ten minutes after the completion of the intracoronary injection of acetylcholine, when the systemic hemodynamic parameters and the coronary arterial diameter on angiograms had returned to the baseline level, an intracoronary bolus injection of ISDN (1000–4000 μg) was given to relieve the coronary spasm and coronary angiography was performed in all study patients in multiple projections. We estimated the degree of organic stenosis after the intracoronary injection of ISDN.

sICAM-1 Assay by Enzyme-Linked Immunosorbent Assay (ELISA)

Plasma sICAM-1 levels were measured by commercially available ELISA kits (R&D Systems Europe, Oxford, UK). The plates were precoated using the following procedure: monoclonal antibody 14C11 (10 mg/ml in 0.1 mol/L bicarbonate buffer) was added to a 96-well microtiter ELISA plate (Nunc Immunoplates, Life technologies, Paisley, UK) at 50 ml/Well and stored at 4°C overnight. Wells were washed twice with phosphate-buffered saline-Tween (PBS-T) and blocked with 100 ml of 1% casein-PBS-T at room temperature for 2 h. Plates were washed 3 times.

For measuring the samples, anti-ICAM-1 monoclonal antibody BBIG-11 labeled with horseradish peroxidase (R&D Systems Europe) and sICAM-1 standards and samples were added (100 ml/well) to the wells and incu-
Table 1 Characteristics of the Study Groups

<table>
<thead>
<tr>
<th></th>
<th>CSA with organic stenosis (n=10)</th>
<th>CSA without organic stenosis (n=11)</th>
<th>SEA (n=16)</th>
<th>Control (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Mean±SEM</td>
<td>61±9</td>
<td>57±28</td>
<td>63±5</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>51–80</td>
<td>46–72</td>
<td>51–71</td>
</tr>
<tr>
<td>Male/female</td>
<td>9/1</td>
<td>9/2</td>
<td>10/8</td>
<td>9/4</td>
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<tr>
<td>Previous myocardial infarction (n)</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Blood pressure ≥160 and/or 95 mmHg (n)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Smokers (n)</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Diabetes mellitus (n)</td>
<td>4</td>
<td>2</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Obesity (BMI ≥25) (n)</td>
<td>2</td>
<td>2</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Serum total cholesterol (mg/dl)</td>
<td>201±10</td>
<td>187±14</td>
<td>204±10</td>
<td>216±10</td>
</tr>
<tr>
<td>Serum HDL cholesterol (mg/dl)</td>
<td>48±9</td>
<td>39±3</td>
<td>38±3</td>
<td>46±2</td>
</tr>
<tr>
<td>Serum triglyceride (mg/dl)</td>
<td>196±50</td>
<td>184±35</td>
<td>171±20</td>
<td>158±18</td>
</tr>
<tr>
<td>Extent of coronary vessel stenosis ≥75% (n)</td>
<td>6</td>
<td>0</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>1-vessel</td>
<td>6</td>
<td>0</td>
<td>7</td>
<td>0</td>
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<td>2-vessel</td>
<td>2</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>3-vessel</td>
<td>2</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Medication used before catheterization (n)</td>
<td>10</td>
<td>11</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>Long-acting nitrates</td>
<td>10</td>
<td>11</td>
<td>11</td>
<td>3</td>
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<tr>
<td>Calcium antagonists</td>
<td>10</td>
<td>11</td>
<td>16</td>
<td>6</td>
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<tr>
<td>β-blockers</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Aspirin</td>
<td>10</td>
<td>0</td>
<td>16</td>
<td>1</td>
</tr>
</tbody>
</table>

Data presented are mean value ± SEM or number. HDL, high-density lipoprotein; CSA, coronary spastic angina; SEA, stable exertional angina.

Statistical Analyses

Values for plasma sICAM-1 levels and age, serum total cholesterol, serum high-density lipoprotein (HDL) cholesterol, serum triglyceride levels were given as mean ±SEM. The plasma sICAM-1 levels, age, serum total cholesterol, serum HDL cholesterol, and serum triglyceride levels among the 4 groups were compared by one-way analysis of variance (ANOVA). When these results were statistically significant, they were analyzed by Bonferroni’s multiple comparison test. The two-tailed paired Student’s t-test was used in the analysis of the difference between the sICAM-1 levels of the CS and those of the Ao, and in the analysis of changes between before and after coronary spasm in CSA with organic stenosis and CSA without organic stenosis groups. The clinical characteristics of the 4 groups shown in Table 1, except age, serum total cholesterol, serum HDL cholesterol, and serum triglyceride, were compared by the chi-square test. Probability levels of less than 0.05 were considered to be statistically significant.

Results

Characteristics of the Study Group and Angiographic Data

Table 1 shows the clinical characteristics of the 4 groups; there were no significant differences in the following variables: age, gender, hypertension, smoking, diabetes mellitus, obesity, serum total cholesterol, serum HDL cholesterol, and serum triglyceride levels. When the significant organic stenosis is defined to be 75% or more of luminal diameter of the coronary artery, 6 had one-vessel disease, 2 had two-vessel disease, and 2 had three-vessel disease of the 10 patients with CSA with organic stenosis. Four patients in the CSA with organic stenosis group and 4 in the CSA without organic stenosis group experienced spontaneous anginal attacks during the catheterization before the provocation of coronary spasm. In the other 6 patients in the CSA with organic stenosis group and the other 7 patients in the CSA without organic stenosis group, coronary spasm was induced by intracoronary acetycholine. Furthermore, the acetycholine-induced coronary spasm resolved spontaneously without the use of nitroglycerin in all these patients. Of the 16 patients with stable exertional angina, 7 had one-vessel disease, 4 had two-vessel disease, and 5 had three-vessel disease. There were no significant differences in the angiographic characteristics of the extent of coronary vessel disease between the CSA with organic stenosis group and the stable exertional angina group. A history of previous myocardial infarction was noted in 1 patient in the CSA with organic stenosis group, and in 3 patients in the stable exertional angina group. There were no significant differences in the standard medications, including long-acting nitrates, calcium antagonists, β-blockers, and aspirin, between the CSA with organic stenosis group and the stable exertional angina group. However, none of the patients in the CSA without organic stenosis group received β-blockers or aspirin.

Plasma sICAM-1 Levels in the Coronary Circulation

The mean plasma sICAM-1 levels (ng/ml) in the CS were significantly higher (p<0.01) in the CSA with organic stenosis group than in the CSA without organic stenosis, stable exertional and control groups (230±26 vs 158±14, 130±9, and 121±10). The levels were not significantly different among the CSA without organic stenosis, stable...
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The mean plasma sICAM-1 levels (ng/ml) in the Ao were also significantly higher (p<0.01) in the CSA with organic stenosis group than in the other 3 groups (208±24 vs 149±13, 130±11, 121±10). The levels were also not significantly different among the CSA with organic stenosis, stable exertional and control groups (Fig 2). Furthermore, the mean value was significantly higher (p<0.01) in the CS than in the Ao in the CSA with organic stenosis group. On the other hand, there were no significant differences in the values between the CS and Ao in the CSA without organic stenosis group, the stable exertional group, and the control group. Therefore, the mean plasma sICAM-1 level of the CS-Ao difference (ng/ml) was significantly higher (p<0.05) in the CSA with organic stenosis group than in the other groups (22±6 vs 9±5, and 0±3, 0±4) (Fig 3). The plasma sICAM-1 levels in the CS and Ao, and the mean plasma sICAM-1 level of the CS-Ao difference (ng/ml) remained unchanged in 5 patients in the CSA with organic stenosis group and in 6 patients in the CSA without organic stenosis group whose these levels were measured before and after the spasm attacks provoked by the intra-

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coronary injection of acetylcholine (228±36 to 219±29 in the CS and 213±31 to 212±29 in the Ao, and 15±5 to 7±4 in the CS-Ao difference in the CSA with organic stenosis group, 176±20 to 173±17 in the CS and 162±20 to 163±21 in the Ao, and 14±8 to 10±8 in the CS-Ao difference in the CSA without organic stenosis group). The 7 patients whose plasma sICAM-1 levels in the CS were 220ng/ml or more had anginal attacks every day and severe attacks before the cardiac catheterization.

In-Hospital Prognosis
Six patients in the CSA with organic stenosis group and 1 patient in the CSA without organic stenosis group had recurrent episodes of angina at rest despite therapy including intravenous nitrates and heparin in addition to the administration of anti-anginal drugs and aspirin after the cardiac catheterization. The plasma sICAM-1 levels in the coronary sinus in 4 of these 7 patients were ≥220 ng/ml or more. Furthermore, 3 patients in the CSA with organic stenosis group required immediate coronary revascularization to control symptoms resistant to therapy: 2 were treated with urgent angioplasty and 1 had urgent coronary artery bypass grafting.

Discussion
Coronary spasm occurs not only in coronary arteries with organic stenosis but also in angiographically normal coronary arteries, and has been implicated in the pathogenesis of acute coronary syndrome, including unstable angina and acute myocardial infarction. Mazzone et al showed that patients with unstable angina had an increased expression of granulocyte and monocyte CD11b/CD18 adhesion receptors and indicated that an inflammatory reaction takes place within these patients’ coronary trees. ICAM-1 is a counter-receptor for the β2 leukocyte integrins LFA-1 (CD11a/CD18) and MAC-1 (CD11b/CD18), and their engagement results in leukocyte adhesion and transmigration through endothelial cells. ICAM-1/LFA-1 interactions are thought to be particularly important for leukocyte transmigration. Furthermore, we have previously demonstrated that sICAM-1 release is increased, especially in the coronary circulation, in unstable angina without coronary spasm. In the present study, we demonstrated increased levels of sICAM-1, which reflects expression of ICAM-1, on vascular endothelium within the coronary circulation in the CSA with organic stenosis group as compared with the CSA without organic stenosis, stable exertional angina and control groups. These facts indicate that inflammation occurs more actively in the coronary circulation of patients with CSA and organic stenosis.

Forman et al reported a patient with variant angina complicated by sudden death in whom mast cell infiltration was found at the site of angiographic demonstration of coronary spasm. Kohchi et al described adventitial infiltration of inflammatory cells involving autonomic nerve fibers in 12 patients with CSA with organic stenosis in the waxing phase. These pathological observations provide a link between an active atherosclerosis process and an inflammatory response in patients with coronary spasm. Furthermore, pathological examination of human atherosclerosis has shown increased expression of ICAM-1 on endothelial cells, smooth muscle cells, and macrophages in the atherosclerotic plaques and endothelium of adventitial vessels adjacent to the plaques. Other reports indicate the importance of ICAM-1 in the pathophysiology of acute myocardial ischemia. The blood sICAM-1 levels have also been evaluated in patients with atherosclerosis, ischemic heart disease, and acute coronary syndrome. It has been reported that sICAM-1 levels were higher in patients with acute myocardial infarction and unstable angina than in those with stable exertional angina or in the control subjects. Therefore, it is suggested that sICAM-1 levels may be related to the pathophysiology of acute coronary syndrome, which is supported by the data from the present study that showed that sICAM-1 release was increased in the coronary circulation of patients with CSA.
Soluble ICAM-1 in Coronary Spasm With Organic Stenosis

and organic stenosis, the cause of acute coronary syndrome.

Recently, an in vitro study demonstrated that fibrin deposition on vascular endothelial cells leads to leukocyte accumulation and extravasation through the induction of ICAM-1, which was estimated from the increased ICAM-1 expression on vascular endothelial cells and increased sICAM-1 in culture medium. We have demonstrated that coronary spasm increases fibrinopeptide A, which is a marker of thrombin generation, and may lead to fibrin formation in the coronary artery involved. However, pacing-induced ischemia in patients with stable exertional angina does not increase fibrinopeptide A. Therefore, fibrin also triggers the increase of sICAM-1 in the coronary circulation with coronary spasm. The present data show that the elevation of sICAM-1 is greater in the CSA with organic stenosis group than in the CSA without organic stenosis group.

On the other hand, the selectins belong to an identified family of cell surface glycoproteins that have important roles as adhesion molecules and appear to be the initial adhesion molecules that influence the properties of leukocytes at the initiation of the inflammatory process. Although all the selectins retard the movement of leukocytes in the microvasculature and cause them to roll, P-selectin is a membrane protein stored in both the α-granules of platelets and in the Weibel-Palade bodies of endothelial cells, and is rapidly involved in mediating leukocyte rolling after ischemia and reperfusion. It is considered that the increase in plasma soluble P-selectin may reflect the total P-selectin upregulation on the stimulated endothelial cells and activated platelets. We have previously demonstrated that the plasma soluble P-selectin levels were increased significantly in the coronary circulation after coronary spasm in patients with coronary spastic angina, but remained unchanged after pacing-induced myocardial ischemia in those with stable exertional angina. From these facts, it is suggested that coronary spasm induces an acute inflammatory reaction and thrombin generation in the coronary circulation and may lead to fibrin and thrombus formation and ultimately to acute coronary syndrome. In the present study, sICAM-1 levels remained unchanged before and after coronary spasm in both the CSA with organic stenosis and the CSA without organic stenosis groups. Within a very short time (seconds to minutes) after ischemia and reperfusion P-selectin is translocated to the cell surface without the need for new protein synthesis. However, ICAM-1 expression increased from 4 h post-fibrin formation with sustained elevated expression at 48 h. These facts, and the disparity in sensitivity for inflammation, may be related to the difference in changes of P-selectin and sICAM-1 between the present study and our previous study. Miwa et al have reported that sICAM-1 is trapped in the coronary circulation at baseline and released into the coronary circulation following coronary spasm and reperfusion. Because all the patients with CSA in the present study had a high level of disease activity, sICAM-1 may have been already fully released into the coronary circulation before induction of coronary spasm and its levels may have not increased in the coronary circulation after induced coronary spasm.

Increased sICAM-1 levels have been observed in type 2 diabetic patients, in patients with hyperlipidemia, and in smokers, which indicates that increased ICAM-1 is associated with the activation of the atherogenic process in these patients. In the present study, there was no significant difference among the 4 study groups in the number of the patients with diabetes mellitus or who were smokers, or in the level of serum total cholesterol, HDL-cholesterol and triglyceride, or other atherogenic risk factors. Thus, the increased sICAM-1 level is thought to be related to the pathophysiology itself of organic stenosis and coronary spasm.

The present study indicates that activation of leukocytes took place within the coronary circulation only in the CSA with organic stenosis groups as compared with the other 3 groups. Activation of leukocytes enhances platelet aggregation, and the products of platelet activation aid neutrophil accumulation at inflammatory sites. Furthermore, the leukocytes, once stimulated, release a variety of potentially toxic and vasoactive substances; in particular, the lipoygenase-derived metabolites of arachidonic acid, leukotrienes C4, D4, and E4. These substances have been shown to induce coronary vasoconstriction and decrease coronary flow in a variety of preparations. Therefore, the increase of sICAM-1 in the coronary circulation of patients with CSA and organic stenosis may induce further coronary spasm. In fact, 3 large studies and our previous study of the long-term prognosis of patients with coronary spasm have shown that the important factor affecting prognosis for patients with coronary spasm is the degree and severity of coronary artery disease. In conclusion, CSA with organic stenosis may induce leukocyte activation, platelet aggregation, and further coronary spasm, and ultimately may lead to coronary thrombosis.

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