Increased Plasma Soluble Adhesion Molecules; ICAM-1, VCAM-1, and E-selectin levels in patients with slow coronary flow

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Cell Adhesion Molecules (CAMs)

CAMs: proteins located on the cell surface involved in the binding with other cells or the extracellular matrix (ECM) in the cell adhesion process.

Families of CAMs

1. Immunoglobulin superfamily (IgSF CAMs)
   - NCAM-1, ICAM-1, VCAM-1
2. Selectin
   - E-selectin, L-selectin, P-selectin
3. Cadherins
   - E-cadherin, P-cadherin, N-cadherin
4. Integrins
   - Integrins

E = Endothelium, L = Leukocyte, P = Platelet, N = Neuron

Slow Coronary Flow (SCF) Phenomenon

SCF: an angiographic observation characterized by angiographical normal or near-normal coronary arteries with delayed opacification of the distal vasculature.

Inflammation

Major contributing factors to...
- cardiovascular events
- initiation & progression of atherosclerosis
- atherosclerotic plaque development & rupture
- aortic aneurysm formation
- angiogenesis
- ischemia/reperfusion damage
- variant angina

Adhesion molecules & Inflammation process

Vascular adhesion molecules play a primary role in inflammatory process.


VCAM-1, ICAM-1 & E-selectin are expressed on the endothelial cell membrane & mediate the adhesion & transmigration of leukocytes to vascular endothelium


Endothelial activation & inflammation may be present in patients with SCF and may contribute to the pathogenesis of SCF phenomenon.

The aim of this study

To evaluate plasma soluble adhesion molecules; ICAM-1, VCAM-1 and E-selectin as possible indicators of endothelial activation or inflammation, in patients with slow coronary flow.

Methods

Study population

Patients

Angiography
Exercise stress test
Myocardial perfusion scintigraphy

Group I
- 11, 6 (48 ± 9 yrs.)
- Chest pain (6)
- Positive (7) / Equivocal (4) results of noninvasive screening test
- normal coronary artery
- slow coronary flow

Group II (Control group)
- 11, 9 (50 ± 8 yrs.)
- normal coronary artery
- no slow coronary flow
- no structure cardiac/systemic disease
- normal echocardiogram
- normal exercise stress test

Exclusion criteria

Coronary artery ectasia
Inflammatory/Immunologic disease
Local/systemic infection
History of infection (< 3 months)
History of MI
LV dysfunction
LV hypertrophy
Uncontrolled HT & malignancy

Both Groups
- Normal WBC count
- Normal erythrocyte sedimentation rate
- Normal fibrinogen level
Coronary angiography (without nitroglycerine)

- Epicardial coronary arteries diameters
- Thrombolysis in Myocardial Infarction frame count (TIMI frame count)
- Coronary artery flow rate

**Documentation of slow coronary flow**

- Thrombolysis in Myocardial Infarction frame count (TIMI frame count)
- A method for the assessment of coronary artery flow.
- A simple, reproducible, objective and quantitative index of coronary blood flow

The number of cineframes required for a contrast agent to reach standardized distal coronary landmarks indicates the time that the contrast spends to reach the periphery.

Cineangiographic frames = 30 frames/second

**The first & last frames used for TIMI frame counting**

**First frame:** Dye fully enters the artery
**Last frame:** Dye first enters the end-point branch off the target artery.

**Anatomic landmarks used for TIMI frame counting in the LAD**

- Proximal Cuff: The most proximal branch of the LAD
- Most distal branch nearest the apex of the left ventricle
- The end-point branch for the LAD is the most distal branch nearest the apex of the left ventricle.
- LAD is longer than the other coronary artery.

**Corrected TIMI frame count for LAD = TIMI frame count / 1.7**

**Anatomic landmarks used for TIMI frame counting in the LCx**

**Anatomic landmarks used for TIMI frame counting in the RCA**
Diagnostic criteria for slow coronary flow

Average TIMI frame count = total TIMI frame count (LAD+LCx+RCA) /3

Normal coronary flow:
Patients with a corrected TIMI frame count ≤ 2 SD of the published normal range.

Slow coronary flow:
Patients with a corrected TIMI frame count > 2 SD of the published normal range.

Blood sampling & Measurement of adhesion molecules

Group I & II
(Fasting state)
Centrifuged at 3000 g (15 min)
ELISA kits
Stored at -70 °C

Statistical analysis

- Comparisons of categorical and continuous variables between two groups were performed using Chi-square test & unpaired t test
- The correlation between the levels of adhesion molecules and TIMI frame count was assessed by the Pearson correlation test
- Sample power analysis: 95% power
- P < 0.05
- Continuous variables: mean ± SD
- Categorical variables: %

Comparison of baseline characteristics of patients with SCF & control subjects

<table>
<thead>
<tr>
<th></th>
<th>Control subjects (n = 20)</th>
<th>Patients with SCF (n = 17)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± S.D.)</td>
<td>50 ± 8</td>
<td>48 ± 9</td>
<td>ns</td>
</tr>
<tr>
<td>Gender (F/M)</td>
<td>6/11</td>
<td>9/11</td>
<td>ns</td>
</tr>
<tr>
<td>Heart Rate (BPM)</td>
<td>78 ± 17</td>
<td>91 ± 16</td>
<td>ns</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>128 ± 19</td>
<td>131 ± 21</td>
<td>ns</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>78 ± 12</td>
<td>76 ± 12</td>
<td>ns</td>
</tr>
<tr>
<td>HT</td>
<td>8/20 (40%)</td>
<td>6/17 (35%)</td>
<td>ns</td>
</tr>
<tr>
<td>DM</td>
<td>2/20 (10%)</td>
<td>2/17 (12%)</td>
<td>ns</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>11/20 (55%)</td>
<td>8/17 (47%)</td>
<td>ns</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>195 ± 23</td>
<td>198 ± 28</td>
<td>ns</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>127 ± 15</td>
<td>130 ± 17</td>
<td>ns</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>43 ± 7</td>
<td>41 ± 8</td>
<td>ns</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>168 ± 21</td>
<td>168 ± 20</td>
<td>ns</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>9/20 (45%)</td>
<td>7/17 (41%)</td>
<td>ns</td>
</tr>
</tbody>
</table>

Comparison of TIMI frame count of patients with SCF & control subjects

<table>
<thead>
<tr>
<th></th>
<th>Control subjects (n = 20)</th>
<th>Patients with SCF (n = 17)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIMI frame count</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAD</td>
<td>28 ± 7</td>
<td>52 ± 12</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LCx</td>
<td>26 ± 6</td>
<td>45 ± 9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>RCA</td>
<td>27 ± 8</td>
<td>48 ± 11</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Average TIMI frame count</td>
<td>27 ± 7</td>
<td>48 ± 10</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
Comparison of epicardial coronary artery diameters of patients with SCF & control subjects.

<table>
<thead>
<tr>
<th>Epicardial coronary artery diameters (mm)</th>
<th>Control subjects</th>
<th>Patients with SCF</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAD</td>
<td>3.29 ± 0.47</td>
<td>3.25 ± 0.44</td>
<td>0.7</td>
</tr>
<tr>
<td>LCx</td>
<td>2.98 ± 0.42</td>
<td>2.96 ± 0.43</td>
<td>0.8</td>
</tr>
<tr>
<td>RCA</td>
<td>3.15 ± 0.49</td>
<td>3.11 ± 0.46</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Comparison of plasma soluble adhesion molecule levels of patients with SCF & control subjects.

<table>
<thead>
<tr>
<th></th>
<th>Control subjects</th>
<th>Patients with SCF</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICAM-1 (ng/ml)</td>
<td>242 ± 113</td>
<td>545 ± 198</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>VCAM-1 (ng/ml)</td>
<td>918 ± 336</td>
<td>2040 ± 634</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>E-selectin (ng/ml)</td>
<td>52 ± 8</td>
<td>67 ± 9</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Plasma soluble ICAM-1 levels of patients with slow coronary flow & control subjects.

![ICAM-1 levels graph](image)
P < 0.001

Plasma soluble VCAM-1 levels of patients with slow coronary flow & control subjects.

![VCAM-1 levels graph](image)
P < 0.001

Plasma soluble E-selectin levels of patients with slow coronary flow & control subjects.

![E-selectin levels graph](image)
P < 0.001

Correlation of plasma soluble ICAM-1 level & average TIMI frame count.

![Correlation graph](image)
r = 0.550, p < 0.001

SCF: slow coronary flow
NCF: normal coronary flow
Correlation of plasma soluble VCAM-1 level & average TIMI frame count.

\[ r = 0.569, p < 0.001 \]

Correlation of plasma soluble E-selectin level & average TIMI frame count.

\[ r = 0.443, p = 0.006 \]

Correlation between plasma adhesion molecules

<table>
<thead>
<tr>
<th>Adhesion molecule</th>
<th>( r )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICAM-1 &amp; VCAM-1</td>
<td>0.906</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ICAM-1 &amp; E-selectin</td>
<td>0.852</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>VCAM-1 &amp; E-selectin</td>
<td>0.862</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

No significant correlation between lipid parameters & average TIMI frame count.

No significant association between SCF & coronary risk factors, such as DM, HT, hyperlipidemia, smoking and gender.

Discussion

Normal coronary system
- Large epithelial vessel
- Intramyocardial vessel (microcirculation) (S.E. Epstein et al., 1985)

Major source of coronary vascular resistance

Slow Coronary Flow
Coronary blood flow inversely related to microvascular resistance and coronary microvascular endothelial dysfunction has been implicated in slow coronary flow. A.A. Tambe et al., 1972

LV & RV biopsy studies have demonstrated small vessel diseases as fibromuscular hyperplasia, medial hypertrophy, myointimal proliferation and endothelial degeneration in patients with SCF. E. Mangieri et al., 1996; M. Mosseri et al., 1986
Dipyridamole infusion could relieve the slow dye progression.

E. Mangieri et al., 1996

Not only structural disease of small vessels, but also functional microvascular dysfunction might contribute to SCF phenomenon.

Increased Endothelin-1 levels & insufficient NO response in coronary sinus blood after atrial pacing were detected in Patients with SCF supporting the presence of endothelial dysfunction.

Pekdemir et al., 2004

Patients with isolated coronary artery ectasia have slow coronary flow phenomenon.

Senen et al., 2004

Endothelial activation & inflammation have been reported to be important precursors to atherosclerosis initiation and progression.


Chronic inflammatory process involves leukocyte activation & adhesion to vascular endothelium.

Leukocyte adhesion to endothelial cells and transmigration are controlled through the expression and shedding of adhesion glycoproteins on the endothelial and leukocyte surfaces.

Immunoglobulin superfamily
- ICAM-1 & VCAM-1 → mediate adhesion & transmigration of leukocytes.

Selectin family
- E-selectin → mediate leukocytes rolling

Cellular adhesion molecule-regulated process of leukocyte recruitment

Endothelial cell dysfunction (impaired endothelium-dependent vasorelaxation in arterioles)

Brachial artery flow-mediated dilatation is impaired in patients with slow coronary flow in the absence of any cardiac risk factors.

Sezgin et al., 2003

There is significant relationship between TIMI frame counts for major epicardial coronary arteries & endothelium-dependent vasodilatation in the brachial artery.

Beltrame et al., 2003

Mibebradil can improve a coronary blood flow, suggesting the presence of microvascular spasm in Pt with SCF.

J.F. Beltrame et al., 2004

The presence of an increased resting coronary vasomotor tone in coronary resistance vessels in Pt with SCF.

Beltrame et al., 2003

Calcium T-channel blocker

Bieber et al., 2003

Patients with isolated coronary artery ectasia have slow coronary flow phenomenon.

Senen et al., 2004
A significant inverse correlation between flow-mediated vasodilatation of the brachial artery & circulating plasma soluble ICAM-1 and VCAM-1 in healthy subjects

Lupattelli et al., 2000

Patients with variant angina have elevated levels of circulating plasma soluble E-selectin & ICAM-1, suggested that endothelial activation may be present in the coronary circulation in these patients

Mina et al., 1997

Increased levels of plasma soluble adhesion molecules have also been considered as markers of endothelial injury or activation.

G. Lupattelli, et al., 2000

Previously studies

Delayed vessel opacification has been reported to be produced in dogs and rabbits with intracoronary endothelin-1 injection.

H. Kurihara et al., 1989, S.W. Larkin et al., 1989

Thromboxane A2 released across the coronary vascular bed has been shown to be increased in Pt. with SCF.

M. Di Donato et al., 1987

Role of inflammation in pathogenesis of SCF

Inflammatory cells infiltrating vascular tissues release cytokines, proteases and ROS and may trigger vasoconstriction, thrombus formation, neointimal growth, angiogenesis and tissue remodeling.

R. Lasala et al., 1993, J.D. Nielsen et al., 1998

Damaged/activated endothelial cells can secrete vasoconstrictor factors (endothelin-1 & thromboxane A2)

M. Di Donato et al., 1987, M. Yanagisawa et al., 1988

Increased levels of circulating plasma soluble ICAM-1, VCAM-1, and E-selectin in Pt. with SCF had significant higher than control subjects

• Plasma soluble adhesion molecule levels were found to be significantly correlated with average TIMI frame count

This study

Endothelial activation or inflammation at microvascular level may play a role in the pathogenesis of slow coronary flow.

Study limitation

• Coronary angiography is not the most sensitive technique for detecting the presence of coronary atherosclerosis.

• The determination of systemic levels of soluble adhesion molecules does not directly indicate their origin.

Conclusion

Endothelial activation & inflammation in the coronary circulation

Increased levels of circulating plasma soluble ICAM-1, VCAM-1, and E-selectin in Pt. with SCF

Raised levels of soluble adhesion molecules may be an indicator of endothelial activation & inflammation
Methods for diagnosing coronary artery insufficiency

- **Exercise stress tests**: a test involved walking on a treadmill or pedaling a stationary bike at increasing levels of difficulty, while ECG, HR, and BP are monitored.

- **Myocardial perfusion scintigraphy (MPS)**: using single photon emission computed tomography (SPECT) in the diagnosis and management of angina and MI.

### Noninvasive screening tests

#### Index of coronary blood flow

- **Grade 0**: no flow
- **Grade 1**: penetration without perfusion
- **Grade 2**: partial perfusion
- **Grade 3**: complete perfusion

#### Coronary angiography

- **Indication**
  - Chest pain
  - Positive/equivocal results of noninvasive screening tests for MI in Patients with SCF

Iohexol were used as a contrast agent in all patients.

Coronary angiography is mainly used in patients with angina to assess the extent and severity of the disease.
Enzyme-Linked Immunosorbent Assay (ELISA) test

ELISA: a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample.

**Enzyme-Linked Immunosorbent Assay (ELISA) test**

**Sandwich ELISA**

- **Step 1**: Coat the microplate with coating Ab. Block & wash.
- **Step 2**: Add standards, samples & controls & incubate. Analyte is bound by the immobilized coating Ab. Aspirate & wash.
- **Step 3**: Add biotinylated detection Ab & incubate. A second epitope on the analyte is bound, forming the coating Ab-analyte-detection Ab “sandwich” complex. Aspirate & wash.
- **Step 4**: Add streptavidin-HRP & incubate. It binds the biotinylated detection Ab. Aspirate & wash.
- **Step 5**: Add substrate & incubate. The substrate is converted to a blue colored reaction product by HRP in proportion to the amount of bound analyte.
- **Step 6**: Add stop solution & read the reaction & converts the blue solution to yellow.

**Leukocyte adhesion cascade**

- **endothelium**
- **Blood vessel**
- **Leukocyte**
- **Activated leukocyte**
- **Margination**
- **Rolling**
- **Adhesion**
- **Transmigration between endothelial cells into surrounding tissue**
- **Surrounding tissue where inflammatory response began**

**Dipyridamole**

**Mibefradil**

**Mibefradil Calcium T-channel Blocker**

Mibefradil inhibits irreversible opening of slow calcium channels in the coronary arteries.