In-Vivo Higher Plasma Levels of Platelet-Derived Growth Factor and Matrix Metalloproteinase-9 in Coronary Artery at the Very Onset of Myocardial Infarction with ST-Segment Elevation

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Objective: Platelet-derived growth factor (PDGF) induces matrix metalloproteinase (MMP), which is regarded as a biomarker of plaque rupture or vulnerability. The aim of this study is to investigate those interactions in human coronary arteries at the onset of ST-segment elevation myocardial infarction (STEMI).

Methods: Thirty-two patients with STEMI who underwent primary percutaneous coronary intervention (PCI) were enrolled in this study. Plasma levels of PDGF-BB and MMP-9 were measured from infarct-related artery (IRA) and from femoral artery (FA) during PCI.

Results: Plasma levels of PDGF-BB and MMP-9 in the IRA were significantly higher than those in the FA (PDGF-BB: median 3130 pg/ml, IQR (interquartile range): 2020 to 4375 pg/ml vs. median 2605 pg/ml, IQR: 1305 to 3290 pg/ml, p <0.01, MMP-9: median 49 ng/ml, IQR: 35 to 100 ng/ml vs. median 42 ng/ml, IQR: 27 to 78 ng/ml, p = 0.04, IRA and FA, respectively).

Conclusions: This in vivo study demonstrated that PDGF-BB with MMP-9 seems to play a role in coronary plaque instability in acute phase of STEMI.

Keywords: platelet-derived mediator, coronary artery plaque, myocardial infarction

Introduction

Platelet-derived growth factor (PDGF) is potent mitogen and chemo-attractant for vascular smooth muscle cells (SMC). Also, linking of PDGF to matrix metalloproteinase (MMP), which is involved in a degradation of extracellular matrix proteins leading to the migration of SMC into the intima and to the rupture of plaques has been reported in animal studies. However, those interactions in human in vivo studies have not been fully elucidated.

Therefore, we have examined whether this concept from animal study may be extended to in vivo human study using biomarkers including PDGF-BB and MMP-9 in circulating blood samples from infarct-related coronary artery (IRA), which was occluded by massive thrombus after coronary plaque rupture, in acute phase of ST-segment elevation myocardial infarction (STEMI).

Materials and Methods

Patients

The present study included 32 patients with STEMI (within 12 hours from symptom onset). STEMI was defined as persisting chest pain, with new ST-segment elevation in more than 2 contiguous leads. Myocardial damage was evaluated by an elevation of creatine phosphokinase-myocardial band (CK-MB) during the hospital stay. Exclusion criteria were as follows: autoimmune disease, liver or kidney disease, malignancy, cardiogenic shock, or overt heart failure.

All patients were pretreated immediately before the revascularization with aspirin 200 mg and clopidogrel 300 mg orally, intravenous heparin was administered and anticoagulation was monitored to maintain an activated clotting time of 250 to 300 seconds. No patient was treated with glycoprotein IIb/IIIa receptor antagonists. Coronary angiography was performed and the culprit artery was defined as a vessel with 90% stenosis to total occlusion by visual estimation.

Percutaneous coronary intervention (PCI) with thrombus aspiration was performed according to standard techniques. After the procedure, aspirin 100 mg daily, clopidogrel 75 mg daily and conventional medicines for myocardial infarction including a beta-blocker, an...
angiotensin-converting enzyme inhibitor, and statins were prescribed.

Patients with normal coronary artery by coronary angiography without myocardial infarction or angina pectoris were recruited as control. Blood samples from coronary artery and femoral artery were obtained during the coronary angiography of those control patients. This study was approved by the Institutional Review Board and informed consent was obtained from all patients.

Collection of blood
The blood sample at the site of the occlusion was taken from a first aspiration, and after the procedure, another sample was taken from the femoral artery. Also, samples were collected 48 hours after PCI from the femoral artery. Blood was collected on ice using ethylenediaminetetraacetic acid as an anticoagulant and centrifuged at 4°C at 3000 x g in 10 min within 30 min after being obtained from the vessels.

Biomarkers and biochemical analysis
Plasma concentration of PDGF-BB and MMP-9 were measured by enzyme-linked immunosorbent assay. CK and CK-MB were measured every 8 h for 24 h to determine maximum CK and CK-MB level. Plasma and serum aliquots were stored at –80°C until analysis.

Data analysis and statistics
Data analyses were performed using the StatView system (SAS Institute, Cary, North Carolina, USA). Continuous variables are presented as mean ± SD if the variables are

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### Table 1 Baseline patients characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 8)</th>
<th>STEMI (n = 32)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>67 ± 10</td>
<td>65 ± 12</td>
<td>0.69</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>6 (75)</td>
<td>22 (69)</td>
<td>0.73</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164 ± 9</td>
<td>162 ± 9</td>
<td>0.54</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>61 ± 11</td>
<td>61 ± 12</td>
<td>0.88</td>
</tr>
<tr>
<td>Body mass index</td>
<td>22 ± 3</td>
<td>23 ± 3</td>
<td>0.54</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>128 ± 15</td>
<td>150 ± 40</td>
<td>0.15</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>69 ± 15</td>
<td>88 ± 26</td>
<td>0.05</td>
</tr>
<tr>
<td>Heart rate (min)</td>
<td>70 ± 17</td>
<td>73 ± 19</td>
<td>0.71</td>
</tr>
</tbody>
</table>

### Table 2 Procedural characteristics

<table>
<thead>
<tr>
<th></th>
<th>STEMI (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target vessel LAD/LCX/RCA, n</td>
<td>14/4/14</td>
</tr>
<tr>
<td>TIMI flow grade 0 or 1/2/3, n</td>
<td>24/2/6</td>
</tr>
<tr>
<td>Thrombus, n (%)</td>
<td>26 (81)</td>
</tr>
<tr>
<td>Total ischemic time (min)</td>
<td>260 ± 180</td>
</tr>
<tr>
<td>Door to aspiration time (min)</td>
<td>86 ± 40</td>
</tr>
<tr>
<td>Final TIMI flow grade 0/1/2/3, n</td>
<td>0/3/29</td>
</tr>
<tr>
<td>Stent implantation, n (%)</td>
<td>30 (94)</td>
</tr>
<tr>
<td>Intra-aortic balloon pump, n (%)</td>
<td>3 (9)</td>
</tr>
</tbody>
</table>

STEMI: ST-segment elevation myocardial infarction; LAD: left anterior descending; coronary artery; LCX: left circumflex artery; RCA: right coronary artery; TIMI: thrombolysis in myocardial infarction; BP: blood pressure; PCI: percutaneous coronary intervention; CABG: coronary artery bypass grafting; LVEF: left ventricular ejection fraction; CK: creatine kinase; Cr: creatinine; CRP: C-reactive protein; LDL-C: low-density lipoprotein cholesterol; TC: total cholesterol; HDL-C: high density lipoprotein cholesterol.
normally distributed, and if the variables are not normally distributed, those variables were expressed as median and interquartile range (IQR): median (25th and 75th percentile). Wilcoxon’s signed-rank test or Student’s t test was used for group comparison of continuous variables with non-normal or normal population distributions, respectively. Categorized values were compared with Chi-square test. Values of $p < 0.05$ were considered significant.

**Results**

**Patient characteristics**

Baseline characteristics of the 32 study patients and 8 normal control subjects are summarized in the Table 1. Control patients were valvular heart disease ($n = 3$), dilated cardiomyopathy ($n = 4$) and atrial septal defect ($n = 1$). The left ventricular ejection fraction (EF) and the serum high-density lipoprotein (HDL)-cholesterol level of STEMI were significantly lower than those of the normal control group. Procedural characteristics are summarized in the Table 2. The total ischemic time was $260 \pm 180$ min.

**Baseline levels of biomarkers**

1. **Control vs. STEMI**

In coronary artery, levels of PDGF-BB (median 3130 pg/ml, IQR: 2020 to 4375 pg/ml) and MMP-9 (median 49 ng/ml, IQR: 35 to 100 ng/ml) in STEMI were significantly higher than those in control (PDGF-BB: median 1940 pg/ml, IQR: 875 to 2215 pg/ml, $p = 0.01$, MMP-9: median 21 ng/ml, IQR: 17 to 28 ng/ml, $p < 0.01$).

In femoral artery, levels of PDGF-BB (median 2605 pg/ml, IQR: 1305 to 3290 pg/ml) and MMP-9 (median 49 ng/ml, IQR: 27 to 78 ng/ml) in STEMI were significantly higher than those in control (PDGF-BB: median 1715 pg/ml, IQR: 1245 to 2215 pg/ml, $p < 0.01$).
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IQR: 821 to 2105 pg/ml, p = 0.03, MMP-9: median 19 ng/ml, IQR: 15 to 25 ng/ml, p < 0.01) (Figs. 1a and 1b).

(2) Femoral artery vs. coronary artery

In the control group, there is no difference between the levels of biomarkers in systemic and coronary circulation (Table 3).

In STEMI patients at baseline, the levels of PDGF-BB and MMP-9 in IRA were significantly higher than those in systemic levels of these markers (PDGF-BB: median 2605 pg/ml, IQR: 1305 to 3290 pg/ml vs. median 1415 pg/ml, IQR: 821 to 2105 pg/ml, p = 0.03, MMP-9: median 42 ng/ml, IQR: 27 to 78 ng/ml vs. median 21 ng/ml, IQR: 15 to 25 ng/ml, p < 0.01, femoral level and IRA level respectively) (Figs. 2a and 2b).

(3) The biomarkers in femoral artery 48 h after PCI in STEMI

The levels of PDGF-BB and MMP-9 in femoral artery decreased 48 h after PCI compared with those at baseline (PDGF-BB: median 2605 pg/ml, IQR: 1305 to 3290 pg/ml vs. median 1415 pg/ml, IQR: 1050 to 1820 pg/ml, p < 0.01, MMP-9: median 42 ng/ml, IQR: 27 to 78 ng/ml vs. median 21 ng/ml, IQR: 14 to 34 ng/ml, p < 0.01, baseline vs. 48 h, respectively) (Figs. 2a and 2b).

Discussion

To our knowledge, this is the first report to examine in vivo plasma level of PDGF-BB with MMP-9 in infarct-related coronary artery and systemic circulation in acute phase of STEMI. The principal findings of this study are: (1) the levels of PDGF-BB and MMP-9 in IRA were significantly higher than those in femoral artery at the acute phase of STEMI; (2) the level of PDGF-BB and MMP-9 significantly decreased from baseline to 48 h after PCI.

It has been reported that receptor of PDGF is involved in the development of advanced atherosclerotic lesions, and the PDGF receptor-β is implicated in angiogenesis or fibrous cap thickening, i.e., in other words, stable plaque that lead to occlusive lesion. Endothelial injury followed by platelet adhesion, aggregation, and ensuing secretion of PDGF as a “response to injury” triggered smooth muscle cell proliferation and the formation of an occlusive lesion. The lesion has some inflammatory cells, thrombus and myxomatous extracellular matrix. The PDGF induce MMPs, including type IV collagenase (MMP-2 and MMP-9) in a variety of cell types, and PDGF and tumor necrosis factor in combination can synergistically up-regulate MMP-9 in
vascular smooth muscle cells. The porcine study demonstrated that local expression of MMP-9 promotes intravascular thrombus formation through increased tissue factor expression and the enhanced expression of MMP-9 at the shoulders of atherosclerotic lesion has been linked to plaque rupture. Therefore, the present data that higher plasma levels of PDGF and MMP-9 in IRA seem to be valid results that is in accord with above studies. Under dispute over the source of PDGF, thrombus in IRA may also contribute higher PDGF level in IRA in the present study, since it has been reported that α-granules in active platelet release PDGF in animal study. However, as described above, PDGF induces MMPs and MMP promotes intravascular thrombus including platelets, indicating PDGF is up-stream than thrombus formation by MMP in this cascade. Moreover, in 51% of human STEMI cases, older thrombi were present, which suggests an important message that more than half of platelets in thrombosis in IRA are not activated platelets that release PGDF or other cytokines. In other words, part of the PDGF or MMP seems to be released from intra-atheroma and platelet in thrombus itself also may gradually participate in plaque instability and thrombosis formation.

In the present study, plasma level of both PDGF-BB and MMP-9 significantly decreased from baseline to 48 h after PCI, indicating both biomarkers were participating in very acute phase of coronary plaque rupture.

**Study limitations**

This study represents a single-center experience with a limited number of patients. No distal protection devise was used during obtain samples from IRA but first aspiration sample from occluded site (TIMI 0) by thrombosis. Regarding the medication that prescribed before catheterization including aspirin and clopidogrel, it may not influence between the levels of IRA and femoral artery in the same patient. Heistad mentioned that it is important to acknowledge that our understanding of the cellular biology of the unstable plaque remains speculative, because there are no good experimental models of plaque destabilization.

**Conclusions**

This human in vivo study demonstrated that PDGF-BB with MMP-9 seems to play a role in coronary plaque instability in acute phase of STEMI.

**Acknowledgment**

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**Disclosure Statement**

All authors have no conflict of interest.

**References**